UNIVERSITE DE FRANCHE-COMTE BESANÇON

Faculté de Médecine et de Pharmacie

Ecole Doctorale Homme, Environnement, Santé

Doctorat de l'Université de Franche-Comté

Mention : Sciences de la Vie et de la Santé

Option : Aspects Moléculaires et Cellulaires de la Biologie

Présentée et soutenue publiquement par

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le 26 mars 2010

Voie de signalisation NF-kappaB dans la régulation de la transcription du VIH-1 et du HCMV dans les macrophages

Jury

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UNIVERSITY OF FRANCHE-COMTE, BESANÇON FRANCE

Faculty of Medicine and Pharmacy

Graduate School "Human, Environment, Health"

Doctorate

Discipline : Life and Health Sciences

Major : Cell and Molecular Biology

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NF-kappaB signaling in transcriptional regulation of HIV-1 and HCMV in macrophages

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بسم الله الرحمن الرحيم

A

Saint Prophéte Muhammad (Que la paix et la bénédiction d'Allah soient sur Lui)

To

Holy Prophet Muhammad (May Allah's peace and blessings be upon Him)

Remerciement

C’est au terme de ces quelques années que vient le temps pour moi d’adresser mes remerciements à toutes les personnes qui ont contribué à ce travail. Le travail de thèse présenté dans ce mémoire a été effectué au sein de l’unité de recherche UPRES EA3186 Agents Pathogènes et Inflammation, de l’Université de Franche-Comté, Besançon, France.

Je souhaite tout d’abord adresser mes chaleureux remerciements à mon directeur de thèse , Monsieur le Pr. Georges Herbein, pour m’avoir accueilli dans son équipe, conseillé, aidé et guidé dans mon travail tout au long de ces quatre années.

Je suis également très reconnaissant envers Messieurs les Pr Olivier Rohr, Dr Pierre Jalinot, Dr Christian Davrinche et Pr Philippe Saas qui m’ont fait l’honneur de consacrer du temps à l’évaluation de cette thèse et de venir, parfois de très loin, juger mon travail.

Je remercie également le Dr Alain Coaquette de m’avoir apporté son soutien et ses conseils lors de mes premiers pas dans le laboratoire de virologie. Merci à Audrey pour tous les bons conseils qu’elle a eu la gentillesse de me donner.

Mes chaleureux remerciements à l’ensemble des techniciens et secrétaires du laboratoire de virologie pour leur présence et leur aide. Merci à Wasim et Quentin pour vos conseils et aides lors des “manips” ainsi que pour l’écriture de ce manuscrit. Merci également à tous ceux qui m’ont aidé un jour ou l’autre au cours de cette thèse

Je voudrais aussi remercier la Higher Education Commission, Pakistan d’avoir apporté le soutien financier nécessaire à la réalisation de ce travail et la SFERE pour son aide dans les démarches administratives.

Enfin, merci à mon épouse et mes enfants Aena et Aysha pour leur amour inconditionnel et leur sacrifice et patience pendant ces quatre ans. *Please forgive me for all the dinners you had without me when I was working on “odd” experiments*. Je remercie tous les membres de ma famille et tout particulièrement ma mère pour leur soutien et leurs prières. Tout celà fut si important pour moi, et pour tout ça, merci…

Resumé

Le facteur de transcription NF-kappaB (NF-κB) constitue une famille de cinq protéines qui régule l'expression d'un grand nombre de gènes impliqués dans diverses fonctions biologiques, notamment l'immunité, l'inflammation, le développement, et l'apoptose. Les membres de la famille NF-κB comprennent les protéines p65, RelB, c-Rel, p50 et p52, qui forment des homo- et des hétérodimères. La facteur de transcription NF-κB est normalement séquestré dans le cytoplasme, en association avec les membres de la famille IκB (inhibitor of kappa B). La phosphorylation et l'ubiquitination médiée par IKK (IκB Kinase) conduisent à la dégradation de IκB et à la translocation de NF-κB dans le noyau où la régulation transcriptionnelle des gènes cibles a lieu. L'activation de IKK est tributaire entre autres des protéines adaptatrices TRAF et RIP. Ainsi la voie de signalisation NF-κB se compose des dimères NF-κB, des protéines IκB, du complexe IKK et des protéines adaptatrices.

L'activation de la voie de signalisation NF-κB est fréquente au cours des infections virales. NF-κB étant un composant essentiel de la réponse immunitaire innée antivirale, il participe à la réaction de l'hôte contre les agents pathogènes. Les virus ont développé des stratégies pour moduler la voie de signalisation NF-κB en leur faveur notamment pour faciliter leur réplication, empêcher l'apoptose des cellules infectées et favoriser l'échappement à la réponse immunitaire. Par ailleur les virus ont incorporé des sites de fixation de NF-κB dans leurs promoteurs. Ainsi l'activation de NF-κB résulte en une transactivation des promoteurs viraux et en une transcription virale augmentée. En effet de nombreux virus et certains protéines virales activent ou inhibent la voie de signalisation NF-κB pour créer un environnement favorable au développement du cycle viral dans la cellule hôte.

Dans la première partie de notre étude, nous nous sommes intéressés au rôle du facteur de transcription NF-κB dans la transcription du cytomégalovirus humain (HCMV) en infectant des macrophages dérivés de monocytes sanguins (MDMs). Les macrophages jouent un rôle important dans la pathogénèse liée à HCMV et l'effet de l'infection virale sur la signalisation cellulaire dans ces cellules reste peu documenté. Nous avons montré que les souches virales AD169 (souche de laboratoire) et HCMV-DB (souche issue d'un isolat clinique) pouvaient se multiplier dans des cultures de cellules primaires MDMs mais que le titre viral demeurait inférieur à celui observé dans des cellules plus permissives telles que les fibroblastes de la lignée MRC5. Etant donné que le facteur de transcription NF-κB joue un rôle essentiel dans la réplication virale notamment par la transactivation du promoteur viral très précoce IE et du gène tardif viral, nous avons étudié l'activation et la composition du complexe NF-κB dans des cellules MDMs et des fibroblastes MRC5 infectés par HCMV. Par des techniques de retard sur

gel (EMSA) et de colorimétrie (Microwell colorimetric NF-κB assay), nous avons montré que l'infection par HCMV entraînait une activation du complexe p52/Bcl-3 dans les cellules MDMs alors qu'il activait le complexe classique NF-κB p50/p65 dans les fibroblastes de la lignée MRC5. L'utilisation des cellules monocytoides U937 transfectées par un vecteur pCMV-Luc, vecteur d'expression de la luciférase sous la dépendance du promoteur viral du gène très précoce EA (MIEP), nous a permis de montrer que le complexe p52/Bcl-3 activait le promoteur viral MIEP. Par la technique d'immunoprécipitation de chromatine (technique ChIP), nous avons mis en évidence l'interaction entre le complexe p52/Bcl-3 et le MIEP dans les cellules MDMs infectées par le HCMV. Ainsi, l'activation du complexe NF-κB p52/Bcl-3 dans les cellules MDMs pourrait être à l'origine de la réplication limitée d'HCMV dans ces cellules.

Dans la deuxième partie de notre étude, nous avons étudié le rôle de NF-κB dans la transcription du VIH-1 dans les cellules MDMs lors de la co-infection par le virus de hepatite C (VHC) et le VIH-1. L'infection par VIH favorise l'évolution de l'hépatite C chronique et augmente la charge virale VHC, mais le rôle d'VHC sur la réplication virale du VIH reste mal connu. Les cellules mononuclées périphériques (PBMC) sont permissives aux virus VIH et VHC et peuvent constituer un réservoir extra-hépatique de virions. Il a été montré que la transcription de NF-κB est activée lors de l'infection par VHC et VIH-1. Nous nous sommes proposés d'étudier le rôle de NF-κB sur la transcription du VIH-1 dans les cellules MDMs lors d'une co-infection VHC/VIH-1. Des études préliminaires conduites dans notre laboratoire ont montré que la charge virale VIH-1 était plus élevée dans les cellules PBMC et MDMs provenant de sujets co-infectés que chez les sujets mono-infectés. Nous avons observé, *in vitro*, que les protéines Nef du VIH-1 et Core du VHC activent le complexe p50/p65 de NF-κB dans les cellules MDM. Grâce à une technique reposant sur l'expression de la luciférase, nous avons montré que les deux protéines activaient la transcription du LTR de VIH-1. Nous avons également demontré que les protéines Nef et Core activent la réplication du VIH-1 dans les cellules promonocytaires chroniquement infectés U1. De plus, ces protéines stimulaient également le réplication du VIH-1 dans des macrophages primaires infectés par VIH-1. Par conséquent, les deux protéines Nef et Core pourraient favoriser la formation de réservoirs cellulaires contenant les deux virus.

L'ensemble de ces études nous a permis de mieux comprendre le rôle de NF-κB dans la transcription virale au sein des macrophages.

Mots clés: NF-κB, Bcl-3, transcription, macrophages, HCMV, VIH, VHC, protéine Nef, protéine Core

Abstract

The mammalian nuclear factor-κB (NF-κB) is a family of five DNA-binding proteins that regulates expression of a large number of genes involved in diverse biological functions including immunity, inflammation, development, and apoptosis. Members of NF-κB family include p65 (RelA), RelB, c-Rel, p50 (NF-κB1) and p52 (NF-κB2), which are found as homo- and heterodimers. The transcription factor NF-κB is normally sequestered in the cytoplasm in association with the members of the inhibitor of kappa B (IκB) family. IκB kinase (IKK) mediated phosphorylation, ubiquitination, and degradation of IκB frees NF-κB to translocate to the nucleus to regulate the transcription of target genes. Activation of IKK is dependent upon intracellular adapter proteins such as TRAF and RIP. Thus NF-κB pathway consists of NF-κB dimers, IκB proteins, IKK complex and intracellular adapter proteins.

Activation of NF-κB is a common feature during viral infections. NF-κB is an essential component of innate antiviral immune response and is a part of the protective reaction of the host against pathogens. Viruses have evolved strategies to modulate NF-κB signaling pathway for their own benefit especially to facilitate their replication, prevent apoptosis of infected cells and evasion of immune responses. In addition a number of viruses contain NF-κB binding sites in their promoters. Thus activation of NF-κB results in the transactivation of viral promoters, thus enhancing viral transcription and replication. In fact several viruses and a number of viral proteins have been reported either to stimulate or inhibit NF-κB activation to create an environment for successful viral life cycle in the host cell.

In the first part of our study we studied the role of NF-κB in the transcription and replication of HCMV in primary human monocyte-derived macrophages (MDMs). Monocytes/macrophages are key cells in the pathogenesis of human cytomegalovirus (HCMV) infection, but the *in vitro* rate of viral production in MDMs is considerably lower than in fibroblasts. Considering that the NF-κB signaling pathway is potentially involved in the replication strategy of HCMV through efficient transactivation of the major immediate-early promoter (MIEP), efficient viral replication, and late gene expression, we investigated the composition of the NF-κB complex in HCMV-infected MDMs and fibroblasts. Preliminary studies showed that HCMV could grow in primary MDMs culture but that the viral titer in culture supernatants was lower than that observed in the supernatants of more permissive MRC5 fibroblasts. EMSA and microwell colorimetric NF-κB assay demonstrated that HCMV infection of MDMs increased p52 binding activity without activating the canonical p50/p65 complex. Moreover, Bcl-3 was up-regulated and was demonstrated to associate with p52, indicating p52/Bcl-3 complexes as the major component of the NF-κB complex in MDMs. Luciferase

assays in promonocytic U937 cells transfected with an MIEP-luciferase reporter construct demonstrated MIEP activation in response to p52 and Bcl-3 overexpression. Chromatin immunoprecipitation assay demonstrated that p52 and Bcl-3 bind the MIEP in acutely HCMV-infected MDMs. In contrast, HCMV infection of MRC5 fibroblasts resulted in activation of p50/p65 heterodimers. Thus, activation of p52/Bcl-3 complexes in MDMs and p50/p65 heterodimers in fibroblasts in response to HCMV infection might explain the low-level growth of the virus in MDMs vs efficient growth in fibroblasts.

In the second part of our study we studied the role of NF-κB in the transcription and replication of HIV-1 in macrophages during HIV-1/ hepatitis C virus (HCV) coinfection. HIV-1 infection favors the progression of HCV disease and enhances the viral load of HCV but the effect of HCV on replication of HIV-1 is not well studied. Macrophages are permissive to HCV and HIV-1 infection so can constitute extra-hepatic reservoir of these viruses. As transcription factor NF-κB is activated during HIV-1 and HCV infection we studied the role of NF-κB in the transcription of HIV-1 in MDMs. Preliminary studies from our laboratory demonstrate higher levels of HIV-1 viral load in MDMs isolated from the peripheral blood of HIV-1/HCV coinfected subjects in comparison with HIV-1 mononinfected patients. To assess the potential role of HIV-1 Nef and HCV core proteins in this phenomenon, we studied their respective role in regard to NF-κB activation and HIV-1 replication in primary macrophages. Following the treatment of primary macrophages with exogenous HIV-1 Nef and HCV core proteins, we observed activation of NF-κB in primary macrophages which consist of p50/p65. Consistently, degradation of IκBα, and phosphorylation of IKKα, IKKβ was observed in response to both HIV-1 Nef and HCV core protein. In addition, HIV-1 Nef and HCV core proteins stimulated synergistically the HIV-1 long terminal repeat (LTR), and subsequently enhanced HIV-1 replication in both chronically infected promonocytic U1 cells and acutely HIV-1 infected MDMs. Therefore, our results indicate that HIV-1 Nef and HCV core proteins synergize to enhance NF-κB activation and HIV-1 replication in primary macrophages and thereby could fuel the progression of the HIV-1 disease in HIV/HCV coinfected patients.

All together, our results have important implication in terms of viral persistence and formation of viral reservoirs in macrophages during chronic viral infections.

Keywords: NF-κB, Bcl-3, Transcription, Macrophages, HCMV, HIV, HCV, Nef, Core protein

Table of Contents

List of Figures

List of Tables

Abbreviations

AD169:	High-passage laboratory strain AD169
AIDS:	Acquired immunodeficiency syndrome
Akt :	Protein kinase B
AP-1:	Activator of protein-1
APOBEC3:	Apolipoprotein B mRNA-editing enzyme catalytic polypeptide like editing complex 3
CBP:	CREB binding protein
CCR:	CC chemokine receptor
CD:	Cluster of differentiation
CDK:	Cyclin-dependent kinase
ChIP:	Chromatin immunoprecipitation
CKII :	Casein kinase II
CREB:	cAMP response element binding
CTD:	C-terminal domain
CXCR:	CXC chemokine receptor
EBNA:	Epstein-Barr nuclear Ag
EBV:	Epstein-Barr virus
EGFR :	Epidermal growth factor receptor
FasL:	Fas ligand
HAT:	Histone acetyltransferases
HBV:	Hepatitis B virus
HCMV:	Human cytomegalovirus
HDAC:	Histone deacetylase
HDAC:	Histone deacetylase
HIV-1:	Human immunodeficiency virus type 1
HLA:	Human leukocyte antigen
hpi:	Hours postinfection
HPV:	Human papillomavirus
HSV-1 :	Herpes simplex virus -1
HTLV:	Human T-cell leukemia virus
HVS :	Herpesvirus saimiri
IFN:	Interferon
IKK:	IκB kinase
IL:	Interleukin
IκB:	Inhibitor of kappaB
JNK:	c-Jun N-terminal kinase
kDa:	Kilo Dalton

LPS: Lipopolysaccharides

LTR: Long terminal repeat

Lys : Lysine

MAPK: Mitogen-activated protein kinase

MDMs: Primary human monocyte-derived macrophage

MHC: Major histocomatibility complex

MIEP: Major immediate-early promoter

MOI: Multiplicity of infection

NEMO: NF-κB essential modulator

NFAT: Nuclear Factor of Activated T cells

NF-κB: Nuclear factor-κB

nt: Nucleotide

nuc: Nucleosome

ORF: Open reading frame

PBMCs: Peripheral blood mononuclear cells

PBS: Phosphate buffered saline

PI3K: Phosphatidylinositol-3-kinase

RHD: Rel homology domain

RIP: Receptor- interacting protein

RIP: Receptor- interacting protein

RT: Reverse transcriptase

Ser : Serine

siRNA: Small interfering RNA

SIV: Simian immunodeficiency virus

Sp-1: Stimulator protein-1

STAT: Signal transducer and activator of transcription

TAD: Transactivation domain

TAR: Transactivation Response element

TF: Transcription factor

TLRs: Toll like receptors

TNF: Tumor necrosis factor

TNFR: Tumor necrosis factor receptor

TRAF: Tumor necrosis factor receptor-associated factor

WT: Wild type

β-TrCP: β-transducin repeat-containing protein

1

Introduction

Macrophages are heterogeneous cells of mononuclear phagocyte system that is widely distributed in the body. Mononuclear phagocytes include blood monocytes, tissue macrophages, connective tissue histiocytes, dendritic cells in lymph nodes and spleen, Langerhans cells of skin, Kupffer cells of liver and microglial cells of brain (Ross and Auger, 2002). They perform an indispensable role for clearance and inactivation of invading pathogens including viruses. Macrophages perform multiple functions during viral infections. Macrophages are able to eliminate the viruses from the circulation through phagocytosis after blood-borne infection. They are also responsible for secreting potent antiviral factors for example interferons which can restrict viral replication and chemokines which recruits leukocytes at the inflammatory site. In addition, macrophages perform antigen presentation function for effective immune response against viral infections (Zink et al., 2002). Importantly, macrophages act as a site of viral replication, infectious reservoir and a vehicle for viral dissemination for a number of viruses especially causing chronic infections including human cytomegalovirus (HCMV) and human immunodeficiency virus type 1 (HIV-1). HCMV DNA has been detected in the monocytes and tissue macrophages. HCMV DNA and antigens have been detected in monocytes during acute HCMV infection and differentiation of monocytes into macrophages triggers production of infectious virus (Hanson and Campbell, 2006). The macrophage is the predominant infiltrating cell type found in HCMV infected organs (Sinzger et al., 1996). HIV-1 infects macrophages which resist apoptosis and viral lysis resulting in formation of viral reservoirs. Macrophages are the earlier targets of HIV-1 which can directly transmit the virus to T cells and produce cytokines which are important for HIV pathogenesis. Tissue and brain macrophages are productively and latently infected by HIV-1 in infected patients. Similarly, the primary human monocyte-derived macrophage (MDMs) are productively permissive for *in vitro* viral replication (Cassol et al., 2006).

Nuclear factor -κB (NF-κB) is a family of transcription factors which is involved in multiple biological processes including immunity, apoptosis, development, proliferation, inflammation and innate as well as adaptive immune response. It consists of five members in mammals: c-Rel, RelA (p65), RelB, NF-κB1 (p50 and its precursor p105), and NF-κB2 (p52 and its precursor p100) and are found in various homo- and heterodimeric complexes. Normally, NF-κB dimers are cytoplasmic bound by the members of the inhibitor of kappa B (IκB) family which prevent their nuclear translocation. Phosphorylation and ubiquitination of IκB by IκB kinase (IKK) results in proteosome-dependent elimination of IκB and nuclear translocation of NF-κB and activation of transcription from NF-κB target genes (Hayden and Ghosh, 2008). The

IκB family consists of seven known members: IκBα, IκBβ, IκBγ, IκBε, Bcl-3, and the precursor proteins p100 and p105 (Hayden and Ghosh, 2004). Bcl-3 is an oncoprotein found in the nucleus and is a direct regulator of transcription. It functions either as transactivator or repressor of transcription through NF-κB site in context specific manner. Unlike other IκBs, it contains a well-defined transactivation domain and is not proteolytically degraded upon cell stimulation. Bcl-3 has preferential binding capacity with p50 and p52 homodimers and activates transcription through a ternary complex with nuclear co-regulators such as JAB1, Tip60, and Bard1 (Dechend et al., 1999). NF-κB activation has been reported in response to a number of stimuli including viruses and their proteins (Hiscott et al., 2006).

NF-κB activation is hallmark of virus infection and is a fundamental component of immediate early immune response against the pathogens. Although NF-κB is considered as a host's protective reaction against pathogens but viruses have developed strategies to hijack this important cellular function to exploit it for their own benefit either through transactivation of viral transcription or prolonging the host cell life. A number of viruses including HCMV and HIV have been reported to incorporate NF-κB binding sites in their transcriptional response elements and exploit cellular NF-κB to drive their own transcriptional events (Gilmore and Mosialos, 2003). Activation of NF-κB results in the transactivation of viral promoters, thus enhancing viral transcription. HCMV infection activates the NF-κB signaling pathway (Kowalik et al., 1993; Yurochko and Huang, 1999; Yurochko et al., 1995) and major immediate-early promoter (MIEP) of HCMV contains four NF-κB binding sites. NF-κB and upstream IKK2 have been demonstrated as requirements for efficient transactivation of the major immediate-early promoter (MIEP), late gene expression, and viral replication of HCMV (Caposio et al., 2007a; Caposio et al., 2007b; DeMeritt et al., 2004; DeMeritt et al., 2006). Similarly, HIV-1 long terminal repeat (LTR) contains two NF-κB binding sites and NF-κB is activated in response to HIV-1 infection as well as in response to viral proteins for example Nef and Vpr (Varin et al., 2005; Varin et al., 2003). HIV-1 replication is inhibited following inhibition of NF-κB pathway by multiple approaches. Inhibition of NF-κB by dominant-negative IκBα (Kwon et al., 1998), RelA-associated inhibitor (Takada et al., 2002) and IKK inhibitors (Asamitsu et al., 2008) has been reported to inhibit HIV-1 replication.

NF-κB exists in different dimers and at least 12 complexes have been reported. Similar NF-κB stimuli generate distinct intracellular signal transduction pathways in different cell types which can activate distinct NF-κB complexes (Beg and Baldwin, 1994; Lernbecher et al., 1993) and binding of different dimers to NF-κB binding sites at comparable levels of recruitment can

support different levels of transcription (Lin et al., 1995b; Saccani et al., 2003). Thus, the presence of distinct NF-κB dimers in different cell types might contribute to varied levels of viral replication in the different cell types. We hypothesized that this mechanistic versatility might be involved in the varied transcription of HCMV in different cell types. In addition cell signaling pathways in response to distinct stimuli can be culminated to activate NF-κB and activate transcription from NF-κB responsive viral promoters. Thus we hypothesize that NF-κB activation in response to HIV-1 Nef protein and HCV core protein can results in activation of transcription from HIV-1 LTR in macrophages of HIV-1/HCV coinfected patients. Because inhibitors of IKK/ NF-κB have been proposed as therapeutic tools for controlling HCMV (Caposio et al., 2007b; Prosch et al., 2002) and HIV replication (Asamitsu et al., 2008; Takada et al., 2002), it is important to study the pathways leading to persistent replication of HCMV and HIV-1 in macrophages.

Specific objectives of the study:

HCMV transcription in macrophages

- Determination of composition of NF-κB complexes in MDMs and fibroblasts in response to HCMV infection
- To study transcriptional regulation of MIEP in MDMs by NF-κB

HIV-1 transcription in macrophages during HIV-1/HCV coinfection

- NF-κB activation and composition in MDMs in response to HIV-1 Nef and HCV core proteins
- Effects of HIV-1 Nef and HCV core proteins on HIV-1 replication in chronically infected U1 cells and acutely infected MDM.

2

Review of Literature

I. NF-κB Signaling

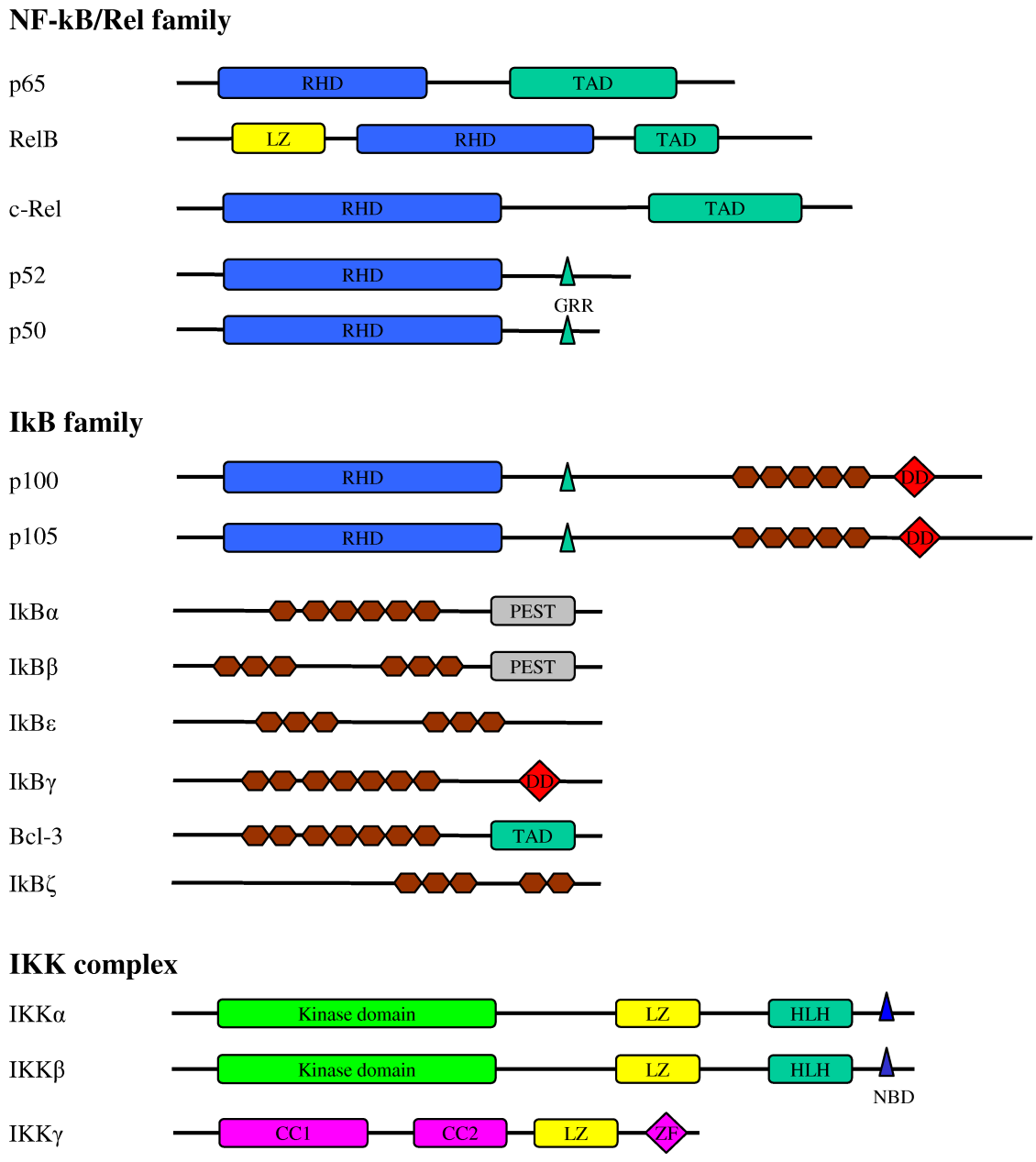
NF-κB is a family of five DNA-binding proteins that regulates expression of a large number of genes involved in diverse biological functions including immunity, inflammation, development, and apoptosis. It is found as homo- and heterodimers and is normally sequestered in the cytoplasm in association with the members of the inhibitor of kappa B (IκB) family. IκB kinase (IKK) mediated phosphorylation, ubiquitination, and degradation of IκB frees NF-κB to translocate to the nucleus to regulate the transcription of target genes. Transcriptional activity of NF-κB proteins is further regulated by a number of post-translational modifications. Activation of IKK is dependent upon intracellular adapter proteins TRAF (TNF receptor associated factor) and RIP (receptor- interacting protein). Thus NF-κB signaling pathway consists of NF-κB dimers, IκB proteins, IKK complex and intracellular adapter proteins.

1. Components of NF-κB signaling

NF-κB family contains five members in mammals: p65 (RelA), RelB, c-Rel, p50 (NF-κB1) and p52 (NF-κB2) [Fig. 1]. NF-κB family members share a Rel homology domain (RHD) in their N-terminal for DNA binding and for the formation of various homo- and heterodimeric complexes. NF-κB proteins differ in structure of their C-terminal domain and mode of synthesis. RelA/p65, RelB, and c-Rel contain transactivation domain (TAD) which is necessary for NF-κB dependent transcriptional activity and are synthesized as mature form. On the other hand, NF-κB1/p50 and NF-κB2/p52 lack TAD and are processed by partial proteolysis from their precursor p105 and p100, respectively. Processing of p105 to p50 is a constitutive process while p100 processing to p52 is tightly regulated and inducible (Xiao et al., 2006). Homodimers of p50 and p52 are repressors of transcription unless associated with a TAD-containing subunit (Hayden and Ghosh, 2008; Mankan et al., 2009).

IκB family consists of structurally related proteins containing multiple ankyrin repeats to interact with RHDs to prevent nuclear localization of Rel subunits (Fig. 1). NF-κB dimers are associated with one of three typical IκB members in cytoplasm; IκBα, IκBβ, IκBε which undergo stimulus induced degradation. In addition precursor proteins p105 and p100 may act as IκBs. The atypical IκB proteins include IκBζ, IκBNS (IκB-delta), and Bcl-3 that are not generally expressed in resting cells but are induced following activation to mediate their function in the nucleus. Bcl-3 is an oncoprotein that contains two transactivation domains upstream and downstream of ankyrin repeats and can activate transcription through NF-κB sites. Bcl-3 preferentially binds with p50 and p52 homodimers and activates transcription through a

ternary complex with nuclear co-regulators such as JAB1, Tip60, and Bard1. In addition alternative splicing of NFKB1 gene results in production of a protein identical to its C-terminal called IκBγ but its functions are not clear yet (Ghosh and Hayden, 2008).



RHD, Rel homology domain; TAD, transactivation domain; LZ, leucine zipper domain; ANK, ankyrin-repeat; GRR, glycine-rich region; PEST, proline-, glutamic acid-, serine- and threonine-rich; DD, death domain; HLH, helix-loop-helix domain; CC1/2, coiled-coil domains; LZ, leucine zipper; ZF, zinc finger domain; NBD, NEMO-binding domain.

Figure 1: Members of the NF-κB signaling pathway (NF-κB, IκB and IKK protein families).

IKK family consists of IKKα, IKKβ, NEMO (NF-κB essential modulator)/ IKKγ, and IKKi (also called IKKε) and TBK1 (TANK-binding kinase) [also called NAK (NF-κB activated kinase) and T2K (TRAF2-associated kinase)]. IKKα, and IKKβ, contain a kinase domain at N-terminal while protein interaction motifs [leucine zipper (LZ) and a helix-loop-helix (HLH) domain] at C-terminal (Fig. 1). IKKβ, IKKγ are responsible for activation of canonical NF-κB pathway in response to proinflammatory cytokines e.g. TNF (tumor necrosis factor), IL-1 (interleukin-1), and Toll-like receptors (TLRs). Both proteins share sequence similarity and phosphorylate IκB in response to TNF and other NF-κB activating stimuli. On the other hand, IKKα is responsible for activation of non-canonical pathway and has the potential to attenuate IKKβ signaling. IKKi and TBK1 activate transcription factors IRF-3 and IRF-7 through phosphorylation at key C-terminal residues resulting into their homodimerization and nuclear import. IRF-3 and IRF-7 are important for induction of several proinflammatory and antiviral genes such as type I interferon genes following viral infections (Clement et al., 2008; Hacker and Karin, 2006).

Activation of cytoplasmic kinases including IKKs are activated in response to signals initiated by a number of NF-κB inducing receptors including TLRs, T-cell receptors and tumor necrosis factor receptor (TNFR) superfamily. These receptors signal through scaffolding proteins which converge to activation of IKK complex. TNFR superfamily signal through TRAF and RIP family members. There are seven members each of TRAF and RIP family. TRAF proteins share a C-terminal TRAF domain and TRAF2-7 share N-terminal domain which function as E3 ubiquitin ligases. TRAF2, TRAF5, and TRAF6 have been reported as positive regulators while TRAF3 as negative regulator of NF-κB activation. TRAF family members are involved in activation of both canonical and non-canonical pathway while canonical pathway additionally require RIP (Hayden and Ghosh, 2008).

2. NF-κB signaling pathways

2.1. Canonical pathway

Canonical NF-κB pathway is activated in response to a broader array of stimuli including endogenous and exogenous ligands as well as diverse physical and chemical agents (Gilmore, 2010). Activation of canonical pathway results in the translocation of p50/p65 heterodimers which are sequestered in the cytoplasm by the inhibitory family IκB in unstimulated cells (Fig. 2A).

NF-κB is activated in response to signals initiated by a number of signaling pathways including TNFR superfamily. TNFR1 and TNFR2 receptors signal through scaffolding proteins which converge to activation of IKK complex which in turn target IκB. At least 30 members of TNFR superfamily have important roles in inflammation, immunity, cell proliferation, and apoptosis. Binding of TNFα with TNFR1 results in translocation of TNFR1 to lipid rafts which act as a platform for other signaling proteins including TRADD, RIP1 and TRAF2 resulting in activation of IKK complex and NF-κB activation (Legler et al., 2003). In fact TRAF2 and TRAF5 are together require for NF-κB activation as NF-κB activation in TRAF2 as well as TRAF5 knockout mice is intact but TRAF2/5 double knockout mice show impaired NF-κB activation (Tada et al., 2001). RIP1 functions as an adapter protein to recruit IKK complex through binding to NEMO (Zhang et al., 2000).

Signaling downstream of RIP and TRAF occurs through TAK1 (transforming growth factor β-activated kinase 1). After binding of TNFα with TNFR1, TRAF2 mediated Lys-63-linked polyubiquitination of RIP1 result in recruitment and activation of TAK1. Ubiquitin-mediated activation of TAK1 leads to activation of IKK complex, which consists of two catalytic kinases IKKα, and IKKβ along with a regulatory subunit IKKγ. IKKβ plays central role in NF-κB activation and is required for phosphorylation of IκBα at Ser-32 and Ser-36 and of IκBβ on Ser-19 and Ser-23. Although IKKα has been reported to be dispensable for canonical pathway, it can mediate IκBα phosphorylation and can play important functions in this pathway through non- IκB substrates. Activation of IKK requires IKKα, and IKKβ phosphorylation in T loop serines through unidentified upstream IKK kinases, autophosphorylation or both. IKKβ is phosphorylated at Ser-177 and Ser-181 while IKKα is phosphorylated at Ser-176 and Ser-180. IKKγ is required for activation of NF-κB and most likely increases the IKK kinase activity through facilitation of T loop phosphorylation (Hacker and Karin, 2006; Hayden and Ghosh, 2008).

Activated IKK complex phosphorylates the IκB resulting in subsequent Lys-48-linked polyubiquitination by ubiquitin ligase complex containing the β-transducin repeat-containing protein (β-TrCP). IκB is then degraded by 26S proteosome resulting into release of p65 containing NF-κB dimers to translocate to nucleus of which p50/p65 is most prevalent. NF-κB dimers interact with κB sites (5' GGGRNWYYCC 3' where N- any base, R- purine, W- adenine or thymine, and Y- pyrimidine) in promoters or enhancers of target genes and modulate their transcription through recruitment of transcriptional coactivators and corepressors (Hayden and Ghosh, 2008).

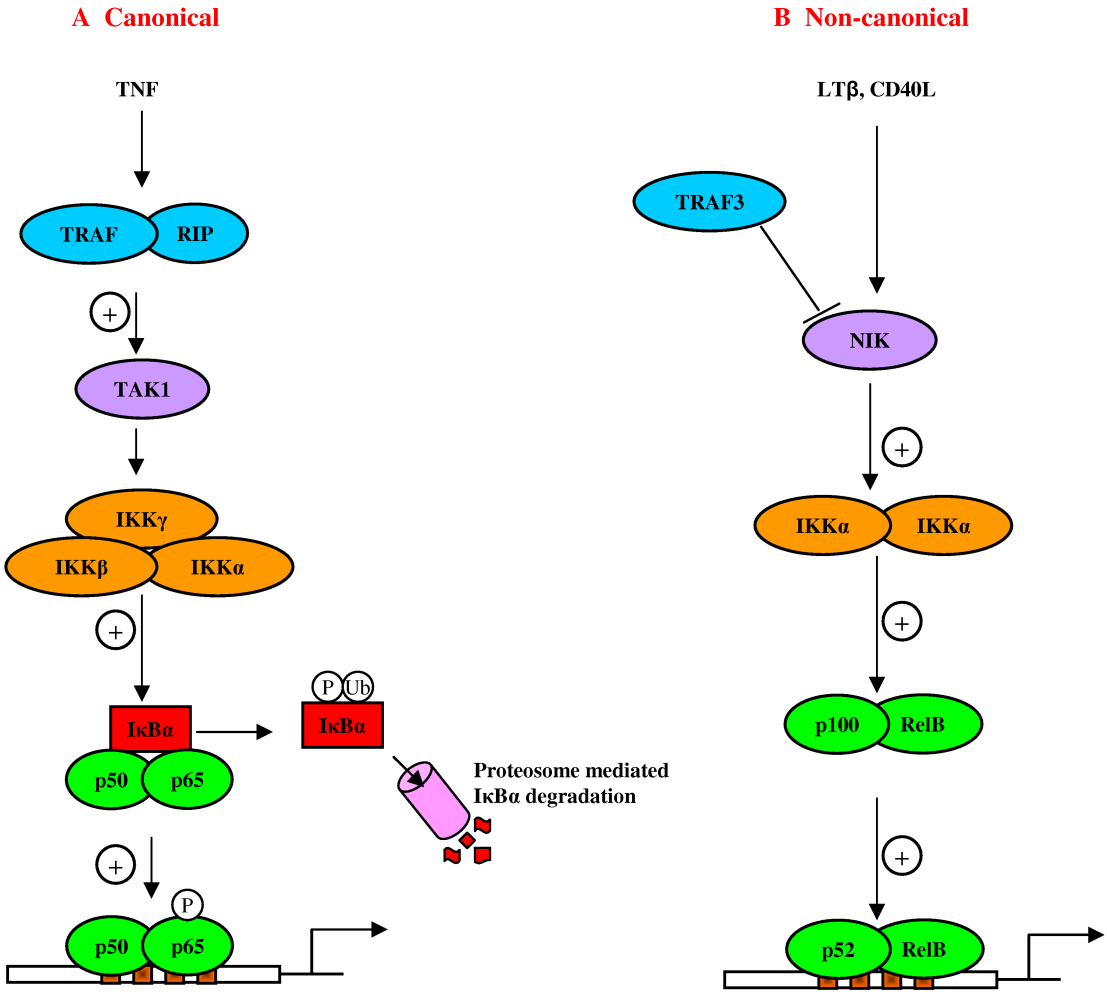


Figure 2: Canonical and non-canonical pathway of NF-κB

2.2. Non-canonical pathway

Non-canonical pathway is activated by processing of p100 to p52 resulting in release of p52/RelB dimers (Fig. 2B). This pathway is independent of both IKKβ and IKKγ, which are central regulators of canonical NF-κB pathway, and depends primarily on IKKα and NIK (Dejardin et al., 2002). Important similarities have been observed in the phenotype of mouse knockouts or mutants of specific signaling molecules of non-canonical pathway. Mice lacking *nfkb2* gene encoding p100, lacking the genes lymphotoxin (LTα, LTβ) and its receptor LTβR, and mice carrying a mutation in NIK show defect in secondary lymphoid organs (Xiao et al., 2006).

Induction of non-canonical pathway in response to TNFR family members such as LTβ receptor (LTβR) results into an elevated level of NF-κB inducing kinase (NIK) protein (Xiao et al., 2001b). The mechanism of NIK activation has been found to be rescuing the basally

translated NIK protein from undergoing TRAF3 mediated degradation rather than activation of NIK at translational or transcriptional level (Liao et al., 2004; Qing and Xiao, 2005). NIK activates IKKα (Senftleben et al., 2001) and act as a docking molecule for the recruitment of IKKα to p100 through two C-terminal serines, 866 and 870, of p100 (Xiao et al., 2004). IKKα is found as homodimer and activated IKKα induces site specific phosphorylation and ubiquitination of multiple serines in N- (serines 99, 108, 115 and 123) and C-terminal regions (serine 872) of p100, resulting into β-TrCP mediated ubiquitination of p100 and subsequent processing by the 26S proteosome to p52 (Xiao et al., 2006). Constitutive processing of p100 to p52, which occurs at a low level in a cell type-specific manner, is independent of β-TrCP but require IKKα (Qing and Xiao, 2005). In fact partial proteolysis of p100 removes C-terminal ankyrin repeats domain to generate p52. Moreover p100 functions to stabilize RelB as both are found associated in the cytosol but RelB is not targeted by other IκB family members. Processing of p100 by NIK and IKKα results into nuclear translocation of RelB-containing dimers (Solan et al., 2002).

In addition to LTβR, other stimuli of non-canonical pathway include B-cell activating factor (BAFF), CD40 ligand, TNF-like weak inducer of apoptosis (TWEAK) and RANKL (Xiao et al., 2006).

2.3. Interplay of canonical and non-canonical pathways

Both canonical and non-canonical pathways are considered to be independent but one of important feature of NF-κB inducers is to co-induce both canonical and non-canonical NF-κB pathways. As both pathways may regulate many distinct and overlapping genes, induction of both pathways by same inducer represents another level of complexity in regulation of NF-κB target genes. Although LTβR is a known inducer of alternative pathway, it leads to activation of canonical pathway as well resulting in increased binding of p50/p65 heterodimer (Dejardin et al., 2002). As p65 transactivates *nfkb2* promoter (Lombardi et al., 1995), activation of canonical pathway may feed non-canonical pathway by positive transcriptional regulation of *nfkb2* for induction of p100 protein and in turn production of p52. It was observed that treatment of p65- and IKKβ-deficient cells with LTβR display a lower level of p100 and p52 proteins than that of LTβR-activated WT cells (Dejardin et al., 2002). Similarly it has been reported that steady-state expression of RelB is regulated by the canonical pathway and constitutive p65 activity (Basak et al., 2008).

Canonical and non-canonical pathways are interconnected at multiple signaling steps. TRAF3, which has been reported to be a negative regulator of non-canonical pathway (Liao et al., 2004), has recently been reported to suppresses canonical pathway as well through stabilization of NIK (Zarnegar et al., 2008). Similarly p100, which is the precursor protein of p52, has been reported as a fourth IκB protein to associate with a fraction of p50/p65 heterodimers through pathways that are regulated by IKKα (Basak et al., 2007).

Another phenomenon that is being accumulated in literature is the regulation of NF-κB transcription by exchange of dimers. Each NF-κB dimer regulates a number of promoters while many other promoters are redundantly regulated by more than one dimer. NF-κB dimers bind either to same κB binding site or different sites in the same promoter. As each dimer supports different level of transcription, exchange of dimers over time provides another mechanism for the regulation and fine tuning of NF-κB transcription. LPS (lipopolysaccharide) activation of dendritic cells has been reported to induce rapidly activated dimers including p50/p65 but are gradually replaced by slowly activated p52/RelB heterodimers (Saccani et al., 2003). Temporal change of NF-κB activity during the course of stimulation might be the result of NF-κB inhibition by re-synthesis of IκB or activation of IKKα. In addition LTβR and LPS stimulation induces sequential activation of canonical and alternative pathways which are independent from each other. Rapid and transient activation of p65 was followed by sustained and protein-synthesis dependent p52/RelB dimers (Mordmuller et al., 2003; Muller and Siebenlist, 2003). Thus activation of alternative pathway at late stages of NF-κB response leads to sustained NF-κB transcription by avoiding negative feedback pathways.

2.4. Role of Bcl-3 in NF-κB mediated transcription

Bcl-3 is a candidate proto-oncogene and is a member of IκB family. Unlike other IκBs, it does not sequester NF-κB complexes in cytoplasm, found in the nucleus, and is not proteolytically degraded upon cell stimulation. It contains TAD and regulates NF-κB dependent transcription. Bcl-3 preferentially binds with transcriptionally repressive p50 and p52 homodimers thus transforming them into transcriptionally active p50/Bcl-3 and p52/Bcl-3 complexes (Bours et al., 1993; Fujita et al., 1993). As p50 and p52 lack TAD and intrinsic ability of transactivation, Bcl-3 possibly confers transactivation property to these homodimers. Both N- and C-terminal contain transactivation sequences and are required for transactivation function of Bcl-3 (Palmer and Chen, 2008). Bcl-3 has been reported to activate transcription through a ternary complex with nuclear co-regulators such as JAB1, Tip60, and Bard1 (Dechend

et al., 1999). Another mechanism of transcriptional activation might be the removal of repressive p50 and p52 homodimers by Bcl-3, thus allowing the transcriptionally active NF-κB dimers into scene (Ghosh and Hayden, 2008). Under certain circumstances, Bcl-3 has been reported to repress NF-κB dependent transcription. Bcl-3 can stabilize p50 homodimers on the kB binding sites preventing access of transcriptionally active heterodimers. Bcl-3 delays turnover of DNA-bound repressive p50 homodimers by inhibition of p50 ubiquitination and subsequent degradation of DNA-bound p50 homodimers. Bcl-3 expression thus inhibits NF-κB transcription and results in TLR tolerance in macrophages (Carmody et al., 2007). Thus Bcl-3 can either activate or inhibit NF-κB dependent transcription in context specific manner.

The dual role of Bcl-3 in NF-κB dependent transcription is thought to be modulated by post-translational modifications especially phosphorylation. Bcl-3 is subject to multiple phosphorylation events that may modulate its ability to bind with p50 and p52 and its transactivation function (Bundy and McKeithan, 1997; Viatour et al., 2004b). Bcl-3 phosphorylation by GSK3 was reported to modulate its function by triggering its degradation through the proteosome pathway (Viatour et al., 2004a). In addition transcriptional activation by Bcl-3 has been reported to depend upon nuclear localization and ubiquitination of Bcl-3. Deubiquitinating enzyme CYLD negatively regulates Bcl-3 ubiquitination and prevents nuclear localization of Bcl-3 and p50/Bcl-3 and p52/Bcl-3 dependent transcription (Massoumi et al., 2006). Although some but not all HATs known to acetylate transcription factors, increase Bcl-3 transcription (Viatour et al., 2004b) direct acetylation of Bcl-3 by HATs has not been demonstrated.

3. Negative feedback mechanisms and termination of NF-κB activity

Activation of NF-κB is followed by NF-κB-induced negative feedback mechanisms which results in distinct change in the nature of the NF-κB activity during the course of stimulation. Important mechanisms for termination of NF-κB activity include IκBα re-synthesis and A20 expression; genes encoding both are positively regulated by NF-κB. Newly synthesized IκBα sequester NF-κB in the nucleus and translocates them back to cytoplasm (Hoffmann et al., 2002; Sun et al., 1993). A20 is a cytoplasmic zinc finger-domain containing deubiquitinating enzyme which is important in limiting inflammation through termination of NF-κB activation (Song et al., 1996). It is induced in response to TNF through activation of NF-κB and A20 deficient mice are hypersensitive to lipopolysaccharide and TNF and fail to terminate TNF-induced NF-κB responses (Lee et al., 2000). A20 is a specific deubiquitinating enzyme for Lys-63-linked polyubiquitinated signaling molecules of NF-κB including TRAF6 and RIP2 (Lin et

al., 2008). Examples of other NF-κB suppressing enzymes with deubiquitinating activity include Cezanne and Cyld (Renner and Schmitz, 2009).

However these negative feedback mechanisms do not explain how DNA-bound, active NF-κB dimers are suppressed and physically removed. It has been suggested that promoter-bound NF-κB subunit p65 is degraded through ubiquitination-proteosome system (Saccani et al., 2004). Although specific Ubiquitin E3 ligases involved are not known but several protein including SOCS-1 (Suppressor of cytokine signalling-1), COMMD1 (Copper Metabolism MURR1 Domain containing-1), and PDLIM2 seem playing a role. SOCS-1 is a part of the ECS ubiquitin ligase complex and its over-expression decreases p65 stability (Ryo et al., 2003). Recently it has been reported that ubiquitination and degradation of p65 by SOC-1 containing ubiquitin ligase (ESSSOCS1) require COMMD1 (Maine et al., 2007). Negative regulation of NF-κB is also mediated by PDLIM2 which acts as a nuclear ubiquitin E3 ligase targeting the p65 subunit and promoting p65 polyubiquitination and subsequent proteasome mediated degradation (Tanaka et al., 2007). In addition IKKα has been reported as negative regulator of NF-κB and inflammation possibly though nuclear degradation of p65 (Lawrence et al., 2005; Li et al., 2005b). Such degradation of chromatin-bound NF-κB molecules thus may result into an exchange of NF-κB dimers at target genes (Saccani et al., 2003).

4. Regulation of NF-κB activity by post-translational modifications

NF-κB activity is highly and specifically regulated at multiple levels. Among these, post-translational modifications of Rel proteins are important regulatory mechanisms that regulate the activity of NF-κB signaling pathway (Perkins, 2006). The most important and prevalent post-translational modification is phosphorylation which is often required for other modifications regulating NF-κB activity (Viatour et al., 2005). Other modifications include acetylation (Calao et al., 2008), ubiquitination (Chen, 2005), sumoylation (Mabb and Miyamoto, 2007), nitrosylation (Marshall et al., 2004), and isomerization (Ryo et al., 2003) of specific amino acid residues. The functional consequences of these modifications may vary in a context-dependent manner.

4.1. Phosphorylation of NF-κB proteins

Modification of proteins by phosphorylation is an important mechanism of post-translational gene regulation. Phosphorylation of p65 is the best characterized post-translational modification so far. These modifications affect affinity of p65 for κB binding site, IκB binding and/or recruitment of transcriptional coactivators/repressors. Consequence of such modifications

varies from transcriptional transactivation to repression of gene expression depending upon the cell type and stimulus.

Nine phosphoacceptor sites are known in RelA which include six serine and three threonine residues. Among these three are found in N-terminal RHD while six other are located in C-terminal TAD (Table 1). Phosphorylation of two serine residues found in N-terminal RHD (Ser-311 and Ser-276) promotes interaction of p65 with transcriptional coactivator CBP [cAMP response element binding (CREB) binding protein] resulting into transcriptional activation. Ser-311 is phosphorylated by zetaPKC in response to TNF-α and results into interaction of p65 with CBP and its recruitment to κB-dependent promoters (Duran et al., 2003). Ser-276 is phosphorylated in the cytoplasm by protein kinase A (PKA) promoting interaction of p65 with CBP (Zhong et al., 1998). Moreover, Ser-276 is a target of mitogen- and stress- activated protein kinase-1 (MSK1) in the nucleus followed by CBP recruitment (Vermeulen et al., 2003). While unphosphorylated p65 interacts with histone deacetylases (HDACs), phosphorylation induced interaction of p65 with CBP displaces inhibitory p50-HDAC1 complex from κB sites (Zhong et al., 2002). Analysis of a knock-in mice expressing mutant S276A form of p65 shows that Ser-276 phosphorylation is important for only subset of NF-κB regulated genes and recruitment of HDACs by unphosphorylated p65 can affect the expression of genes positioned near NF-κB binding sites through epigenetic mechanisms (Dong et al., 2008).

Ser-536 found within the C-terminal TAD has also been reported to be phosphorylated by multiple kinases including IKKα, IKKβ, IKKε, TBK1 [TANK binding kinase 1] (Buss et al., 2004b), and RSK1 [ribosomal S6 kinase1] (Neumann and Naumann, 2007). Mutation of Ser-536 to alanine has been reported to inhibit the binding of CBP with p65 (Chen et al., 2005). Thus phosphorylation events in N-terminal RHD and C-terminal TAD may change the conformation of p65 promoting its interaction with CBP.

On one hand phosphorylation of p65 result in activation of NF-κB while on the other hand phosphorylation at distinct sites may play role in termination of NF-κB response. Phosphorylation of p65 at Ser-468 controls its COMMD1-dependent ubiquitination and proteasomal elimination from a subset of NF-κB target genes (Geng et al., 2009). Therefore phosphorylation of p65 at distinct sites may act as a switch between ability of p65 to drive or inhibit NF-κB dependent transcription. Phosphorylation of other NF-κB proteins including p50 (Ser-337) and RelB (Ser-368, Ser-552, and Thr-84) has also been reported (Table 1).

Table 1: Phosphorylation of NF-κB proteins

Protein	Residue	Kinase	Function	Reference
p65	Ser-276	PKA	↑ transactivation	(Zhong et al., 1998)
		MSK-1	↑ transactivation	(Vermeulen et al., 2003)
	Ser-311	zetaPKC	↑ transactivation	(Duran et al., 2003)
	Thr-254	Unknown	↑ nuclear translocation	(Ryo et al., 2003)
	Ser-536	IKKα, IKKβ, IKKε	↑ transactivation	(Buss et al., 2004b)
		TBK1	↓ nuclear export	(Buss et al., 2004b)
		RSK1	↑ nuclear turnover	(Bohuslav et al., 2004)
		Akt	↑ transactivation	(Viatour et al., 2005)
	Ser-468	GSK-3β	↓ transactivation	(Buss et al., 2004a)
		IKK β	↓ transactivation	(Schwabe and Sakurai, 2005)
		IKKε	↑ transactivation	(Mattioli et al., 2006)
	Ser-529	CKII	↑ transactivation	(Wang et al., 2000)
	Ser-535	CaMKIV	↑ transactivation	(Bae et al., 2003)
	Thr-435	Unknown	↓ transactivation	(Yeh et al., 2004)
	Thr-505	ATR/ChK1	↓ transactivation	(Rocha et al., 2005)
p50	Ser-337	PKA	↑ DNA binding	(Guan et al., 2005)
RelB	Ser-368	Unknown	↑ dimerization	(Maier et al., 2003)
	Ser-552	Unknown	↑ degradation	(Marienfeld et al., 2001)
	Thr-84	Unknown	↑ degradation	(Marienfeld et al., 2001)

4.2. Reversible acetylation of NF-κB proteins

The packaging of DNA into chromatin plays important role in gene regulation by interfering with transcription factor accessibility. Chromatin compaction status is dependent upon acetylation state of histones. Acetylation of specific lysine residues in N-terminal of nucleosomal histones by histone acetyltransferases (HATs) results in chromatin decompaction facilitating transcription factor accessibility to DNA and thus usually results into gene activation. In contrast deacetylation of histones by HDACs results in chromatin compaction and repression of transcription. NF-κB gene expression is also regulated by a number of coactivators possessing HAT activity including p300, CBP, PCAF and Tip60 and corepressors containing HDAC activity including HDAC and sirtuins family (Calao et al., 2008).

Acetylation of histones surrounding NF-κB regulated genes modulates NF-κB responsive transcription. Histones H3 and H4 have been reported to be acetylated during activation of NF-κB responsive genes by TNF-α (Edelstein et al., 2005; Lee et al., 2006a). Activation of LTR of HIV-1 is linked to recruitment of CBP and hyperacetylation of histones H3 and H4 (Thierry et

al., 2004). Moreover, corepressor proteins HDAC1 and HDAC2 negatively regulates NF-κB regulated IL-8 gene (Ashburner et al., 2001).

In addition to amino-terminal of histones that surround NF-κB-responsive genes, several NF-κB family members are direct target of HATs and HDACs (Table 2). Phosphorylation of p65 acts as a switch between its ability to interact with HATs and HDACs, thus p65 is post-translationally modified by these enzymes as well. RelA is acetylated by p300/CBP while deacetylated by HDAC-3 and SIRT1. Its acetylation has been reported at 7 specific points; Lys-122, -123, -218, -221, -310, -314, and -315 (Calao et al., 2008; Chen and Greene, 2004; Neumann and Naumann, 2007).

Table 2: Acetylation and deacetylation of NF-κB proteins

Acetylation				
Protein	Residue	Enzyme	Function	Reference
p65	Lys-310	CBP/ p300	↑ transactivation	(Chen et al., 2002)
	Lys-221	CBP/ p300	↑ DNA binding ↓ IκB interaction	(Chen et al., 2002)
	Lys-218	CBP/ p300	↓ IκB interaction	(Chen et al., 2002)
	Lys-122, -123	p300, PCAF	↓ DNA binding	(Kiernan et al., 2003)
	Lys-314, -315	p300	↑ or ↓ expression of subset of genes	(Buerki et al., 2008)
p50	Lys-431, -440, -441	CBP/ p300	↑ DNA binding	(Furia et al., 2002)
Deacetylation				
p65	Lys-122, -123	HDAC-3	↑ DNA binding	(Kiernan et al., 2003)
	Lys-310	SIRT1	↓ transactivation	(Yeung et al., 2004)

Acetylation of Lys-221, and possibly Lys-218, impairs the assembly with IκBα and thus enhances RelA binding to the κB enhancer. Lys-310 acetylation has been shown to activate transcriptional activity with no effect on binding to DNA and IκB (Chen et al., 2002). Prior phosphorylation of Ser-276 has been shown to be required for assembly of phospho-p65 with CBP and acetylation of Lys-310. Phosphorylation of Ser-536, although not obligatory required, increases acetylation of Lys-310 (Chen et al., 2005). Possible mechanism of Lys-310 acetylation after Ser-536 phosphorylation might be IKKα-mediated derepression of SMRT (silencing mediator for retinoic acid and thyroid hormone receptor) –HDAC3 corepressor complex (Hoberg et al., 2006) or Akt-mediated phosphorylation of p300 (Liu et al., 2006) allowing p300 to acetylate p65. IKKα has also been reported to directly phosphorylate CBP to increase its p65 binding and HAT activity thus increasing NF-κB regulated transcription (Huang et al., 2007). Acetylation of p65 at Lys-122, and -123 by p300 and PCAF (p300/CBP-associated factor)

facilitates its IκBα-mediated nuclear export while HDAC3 was able to deacetylate p65 (Kiernan et al., 2003). Acetylation of p65 at Ser-314 and -315 does not affect nuclear-cytoplasmic shuttling and the DNA binding but the expression of specific sets of genes was activated or repressed (Buerki et al., 2008). Thus multiple acetylation events on distinct residues of p65 represent another level of regulation of NF-κB transcription by posttranslational modifications. However the exact mechanism by which reversible acetylation of p65 regulates NF-κB transcriptional activity remains to be investigated.

II. Modulation of NF-κB signaling pathway during viral infections

Activation of NF-κB is a characteristic feature during viral infections. As NF-κB is an essential component of innate antiviral immune response, it is considered as a protective reaction of the host against pathogens. Viruses have evolved strategies to modulate NF-κB signaling pathway for their own benefit especially to facilitate their replication, prevent virus-induced apoptosis, evasion of immune responses, and contribution in viral pathogenesis. A number of properties of NF-κB make it an ideal target for invading pathogens; it is activated within minutes from stimulation, does not require protein synthesis and at least 150 cellular genes and several viruses contain binding sites in their promoters. In fact multiple viruses and their proteins have been reported either to stimulate or inhibit NF-κB activation to create an environment for successful viral life cycle in the host cell.

1. Activation of NF-κB signaling pathway during viral infections

A large number of RNA (Table 3) as well as DNA viruses (Table 4) have been reported to activate NF-κB pathway either directly by viral products or indirectly through activation of immune response for example cytokines. Viruses and viral products including viral proteins and viral dsRNA are responsible for NF-κB activation through targeting of multiple members of NF-κB pathway including cell surface receptors, intracellular adapter proteins, IKK complex, IκB, and NF-κB proteins themselves (Fig. 3).

In many cases binding of viral particle with its receptor on cell surface initiates signal transduction pathway which activates cellular transcription factors including NF-κB. Human cytomegalovirus (HCMV) envelope glycoproteins gB and gH interact with cell surface receptors to activate NF-κB (Yurochko and Huang, 1999; Yurochko et al., 1997a). Similarly envelope glycoprotein gD of herpes simplex virus -1 (HSV-1) activates NF-κB after binding with cellular receptor, the herpesvirus entry mediator A [HVEM] (Patel et al., 1998). Interaction of Epstein-Barr Virus (EBV) glycoprotein gp350 with cellular receptor CD21 activates NF-κB and IL-1β synthesis (D'Addario et al., 1999). Similarly engagement of CD4 by HIV-1 envelope glycoprotein gp120 result in the activation of NF-κB through IκB-IKK pathway (Bossis et al., 2002).

In addition, viruses interact with downstream scaffolding proteins to activate NF-κB. Viral proteins of a number of herpesviruses contain TRAF binding motif and activate NF-κB through interaction with TRAFs (Brinkmann and Schulz, 2006). LMP-1 protein of EBV interacts with different members of TRAFs through TRAF binding motif PxQxT (Devergne et

al., 1998; Devergne et al., 1996). Although contradictory to each other, three different studies have reported the requirement of TRAF2, 3 and 6 for NF-κB activation by LMP-1 (Guasparri et al., 2008; Luftig et al., 2003; Xie et al., 2004). The human herpesvirus 8 (HHV-8, also called Kaposi's Sarcoma herpesvirus, KSHV) encoded viral FLICE inhibitory protein (v-FLIP)/K13 binds with TRAF2 resulting in activation of IKK complex and NF-κB (Guasparri et al., 2006). Moreover, v-FLIP binds with IKK complex and RIP for activation of NF-κB (Liu et al., 2002). Another HHV-8 protein K15 associates with TRAF-1, -2 and -3 and activates NF-κB pathway (Brinkmann et al., 2003). Herpesvirus saimiri (HVS) protein StpC interacts with TRAF2 and NIK to activate NF-κB pathway (Sorokina et al., 2004). StpC interacts with TRAF6 as well through its TRAF6-binding domain PxExxE which results in ubiquitination of TRAF6 and activation of NF-κB (Chung et al., 2007). Another HVS protein STP-A11 interacts with TRAF6 and upregulate NF-κB activity (Jeong et al., 2007). HSV-1 U(L)37 tegument protein interacts with TRAF6 through its TRAF6-binding domain which is required for NF-κB activation (Liu et al., 2008). In addition to herpesviruses, Rotavirus capsid protein, VP4, and its N-terminal cleavage product VP8* contain conserved PxQxT sequence, which are essential for binding to cellular TRAFs and for NF-κB activation (LaMonica et al., 2001). Moreover NF-κB activation by Hepatitis C virus (HCV) core protein occurs through TRAF2/6 as the dominant negative forms of TRAF2/6 significantly blocked NF-κB activation by HCV core protein (Yoshida et al., 2001). HCV Core protein can potentiate TNF-alpha induced NF-κB activation through TRAF2 (Chung et al., 2001).

Other downstream target of viral proteins is IKK complex. The human T-cell leukemia virus type-1 (HTLV-1) tax protein directly activates IKK complex through interaction with NEMO (Chu et al., 1999). In fact NEMO brings Tax to IKKα and β for their activation (Xiao and Sun, 2000). More recently it has been reported that Tax physically recruits IKKα to p100, triggering phosphorylation-dependent ubiquitination and processing of p100 (Xiao et al., 2001a). In addition v-FLIP of HHV-8 binds with IKKα, IKKβ, and IKKγ for activation of IKK complex (Field et al., 2003; Liu et al., 2002).

Casein kinase II (CKII), a cellular serine-threonine protein kinase, incorporated in HCMV virion possesses potent IκB kinase activity allowing for direct phosphorylation of IκBα following virion entry into infected cells (Nogalski et al., 2007). The HTLV-1 Tax protein can interact with IκB proteins through their ankyrin motifs. This interaction either disrupts IκB-NF-κB complex or recruits p100 or p105 proteins to proteosome to accelerate their cleavage to

active forms (Kfoury et al., 2005). Hepatitis B virus (HBV) X protein (HBx) activates NF-κB and interacts directly with IκBα (Weil et al., 1999).

Finally some viral protein for example Tax can bind to homology domain of p50, p52, p65 and c-Rel and favors dimer formation. In addition viral proteins can favor p65 activation through post-translational modifications. Tax recruits the transcriptional coactivators CBP/p300 to p65/RelA, thereby favoring NF-κB activation (Kfoury et al., 2005). Respiratory syncytial virus infection activates NF-κB through p65 phosphorylation at Ser-276 by MSK1 (Jamaluddin et al., 2009). Another mechanism of NF-κB activation is the upregulation of p65 and p105/p50 promoter activity through Sp1 induction by HCMV glycoproteins gB and gH (Yurochko et al., 1997a).

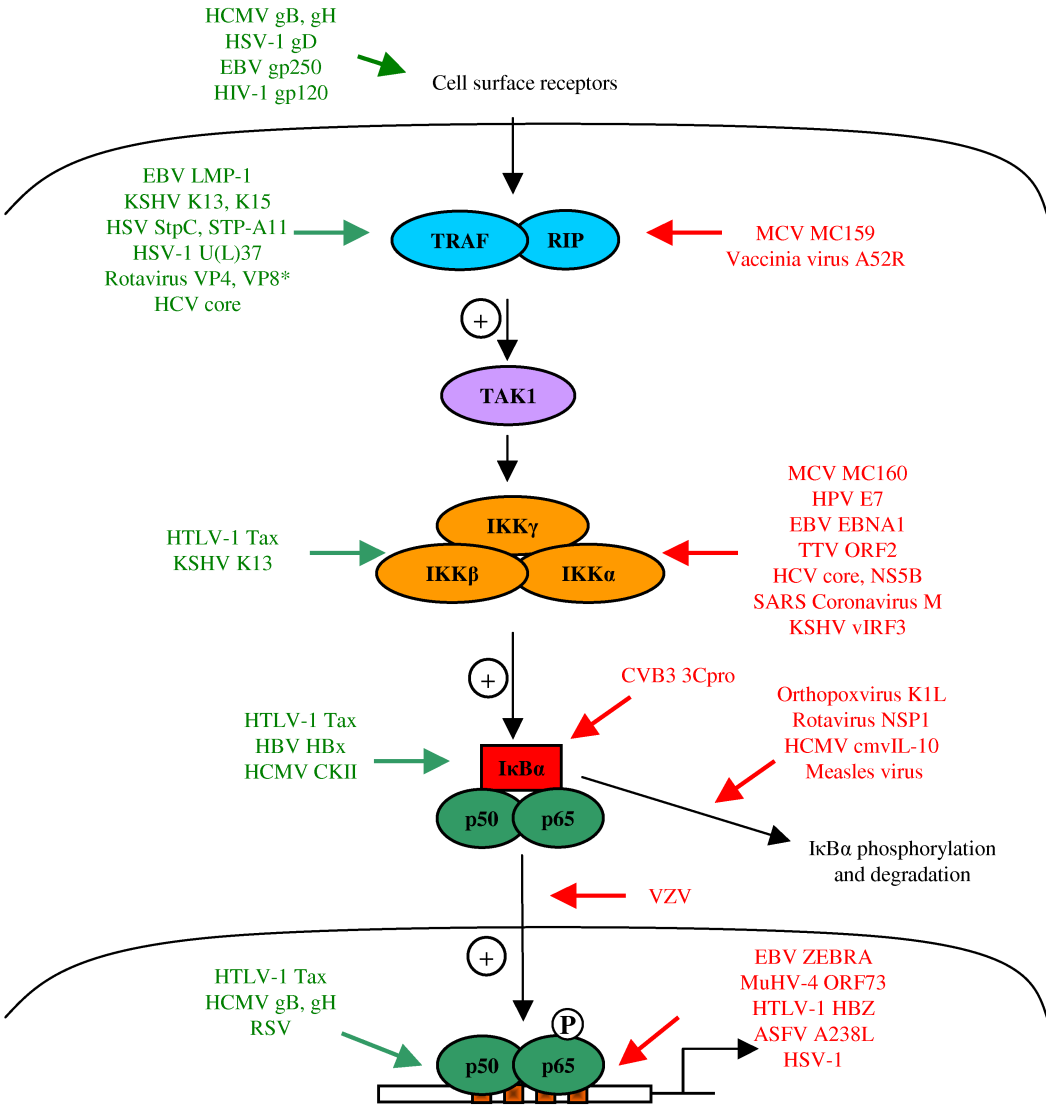


Figure 3: Strategies used by viruses to target NF-κB signaling pathway

Table 3: Activation of NF-κB by RNA viruses

Family	Virus	Viral protein	Reference
Arteriviridae	Porcine arterivirus	--	(Lee and Kleiboeker, 2005)
Coronaviridae	SARS coronavirus	Nuclcocapsid (N)	(Liao et al., 2005b)
		Non-structural protein-1	(Law et al., 2007)
		Spike protein	(Wang et al., 2007)
Filoviridae	Ebola virus	Viral matrix & glycoprotein	(Martinez et al., 2007)
Flaviviridae	Dengue Virus	NS5	(Medin et al., 2005)
	Japanese encephalitis virus	--	(Chang et al., 2006)
	HCV	Core protein	(You et al., 1999)
		NS3	(Hassan et al., 2007)
		NS4B	(Li et al., 2009)
	West Nile flavavirus		(Kesson and King, 2001)
	African swine fever virus	IAP	(Rodriguez et al., 2002)
Orthomyxoviridae	Avian influenza Virus	--	(Lee et al., 2005)
	Human influenza A Virus	--	(Sun et al., 2005a)
		Hemagglutinin	(Pahl and Baeuerle, 1995)
Picornaviridae	Encephalomyocarditis virus	--	(Moran et al., 2005)
	Rhinovirus	--	(Zhu et al., 1996)
	Coxsackievirus	--	(Esfandiarei et al., 2007)
	Enterovirus 71	--	(Tung et al., 2010)
Paramyxoviridae	Measles virus	--	(Harcourt et al., 1999)
	Human metapneumovirus	--	(Bao et al., 2007)
	Newcastle disease virus	--	(Ten et al., 1993)
	Respiratory syncytial virus	--	(Garofalo et al., 1996)
		M2-1	(Reimers et al., 2005)
	Sendai virus	--	(Hiscott et al., 1989)
	Parainfluenza virus	--	(Sabbah and Bose, 2009)
Retroviridae	HIV-1	--	(Bachelerie et al., 1991)
		gp160	(Chirmule et al., 1994)
		gp120	(Alvarez et al., 2005)
		Nef	(Varin et al., 2003)
		Vpr	(Varin et al., 2005)
		Tat	(Westendorp et al., 1994)
	HTLV-I	Tax	(Ballard et al., 1988)
	HTLV-II	Tax	(Mori and Prager, 1996)
	Moloney Murine Leukemia Virus	--	(Pak and Faller, 1996)
Rhabdoviridae	Rabies virus	--	(Nakamichi et al., 2005)
	Vesicular stomatitis virus	--	(Boulares et al., 1996)
Reoviridae	Reovirus	--	(Connolly et al., 2000)
	Blue tongue virus	--	(Mortola and Larsen, 2009)
Togaviridae	Sindbis Virus	--	(Lin et al., 1995a)
Unassigned	Hepatitis delta virus	LHDAg	(Park et al., 2009)

Table 4: Activation of NF-κB by DNA viruses

Family	Virus	Viral protein	Reference
Adenoviridae	Adenovirus	E1A	(Shurman et al., 1989)
		E3/19K	(Pahl et al., 1996)
		Ad2/5 Fiber	(Tamanini et al., 2006)
Hepadnaviridae	HBV	HBx	(Twu et al., 1989)
		e antigen	(Yang et al., 2006)
		LHBs	(Hildt et al., 1996)
		MHBst	(Meyer et al., 1992)
Herpesviridae	HCMV	IE1	(Sambucetti et al., 1989)
		gB, gH	(Yurochko et al., 1997a)
	EBV	LMP	(Hammarskjold and Simurda, 1992)
		EBNA-2	(Scala et al., 1993)
		EBERs	(Samanta et al., 2006)
	Herpesvirus saimiri	HVS13	(Yao et al., 1995)
		STP-A11 (non-can)	(Cho et al., 2007)
		StpC	(Sorokina et al., 2004)
	Human herpesvirus 6	--	(Ensoli et al., 1989)
	HSV -1	--	(Gimble et al., 1988)
		ICP27	(Hargett et al., 2006)
	HSV -2		(Yedowitz and Blaho, 2005)
	HHV-8	K1	(Prakash et al., 2005)
		K15	(Brinkmann et al., 2003)
Papillomaviridae	Human Papillomavirus-like particles	--	(Yan et al., 2005)
	HPV type 16	E6	(James et al., 2006)
Parvoviridae	Kilham rat virus	--	(Zipris et al., 2007)
	Parvovirus B19	NSI	(Moffatt et al., 1996)
Polyomaviridae	Simian virus 40 (SV40)	Small T-antigen	(Johannessen et al., 2003)
Poxviridae	Shope fibroma virus	vPOP	(Dorfleutner et al., 2007)
	Vaccinia virus ankara	--	(Guerra et al., 2004)

The worst exploitation of NF-κB pathway is the incorporation and use of NF-κB binding sites in the promoters of different classes of viruses. Activation of NF-κB results in the transactivation of viral promoters, thus enhancing viral transcription. A number of viruses has been reported to depend upon NF-κB through NF-κB binding sites (Gilmore and Mosialos, 2003). Examples include adenovirus (Williams et al., 1990), avian leukosis virus (Bowers et al., 1996), bovine leukemia virus (Brooks et al., 1998), HCMV (Sambucetti et al., 1989), EBV (Sugano et al., 1997), HBV (Kwon and Rho, 2002), HIV-1 (Griffin et al., 1989), HSV-1 (Patel et al., 1998), JC Virus (Ranganathan and Khalili, 1993), human papillomavirus (HPV) type 16

(Fontaine et al., 2000), simian immunodeficiency virus [SIV] (Bellas et al., 1993) and simian virus 40 (Kanno et al., 1989). Although NF-κB binding sites have not been reported in the genome, it has been shown that NF-κB pathway is required for influenza virus replication and genomic RNA synthesis (Kumar et al., 2008; Ludwig and Planz, 2008) and Theiler's murine encephalomyelitis virus (TMEV) replication (Kang et al., 2008). As NF-κB induce antiviral response, role of NF-κB activation in the inhibition of viral replication have also been reported. Inhibition of HBV replication by MyD88 protein has been reported to be mediated through NF-κB (Lin et al., 2007). Similarly some studies have reported negative role of NF-κB in HCMV replication (Eickhoff et al., 2004; Eickhoff and Cotten, 2005).

Some viruses result in persistent low-level NF-κB activation which helps them to maintain their persistent infection for example HIV-1 (DeLuca et al., 1999) and HCMV (Khan et al., 2009) infection of myeloid cells. Persistent NF-κB activation due to chronic viral infection can promote inflammation and progression to cancer (Karin, 2006).

Moreover viruses can use NF-κB for prolonging host cell life by preventing virus-induced apoptosis. Enhanced host cell life gives viruses the time to grow and to produce viral progeny. Murine encephalomyocarditis virus (EMCV) virulence in mice has been linked with NF-κB mediated inhibition of apoptosis. Mice knockout for p50 protein (p50 -/-) show increased apoptosis of EMCV infected cells and survive an EMCV infection that readily kills normal mice. The mice survival was tightly correlated to the animals' ability to clear the virus from the heart *in vivo* (Schwarz et al., 1998). Binding of HSV-1 glycoprotein gD with the receptor HVEM results in NF-κB activation and protection against apoptosis (Sciortino et al., 2008). Coxsackievirus B3 (CVB3) activates NF-κB transcription factor via a PI3K/Akt pathway to improve host cell viability (Esfandiarei et al., 2007).

2. Inhibition of NF-κB signaling pathway during viral infections

As NF-κB can mount an antiviral response, many viruses interfere with its activation to evade and neutralize immune response (Table 5). Viruses utilize multiple strategies to inhibit NF-κB through targeting of NF-κB signaling at multiple steps including intracellular scaffolding proteins, IKK complex, IκB degradation and NF-κB nuclear translocation (Fig. 3).

Table 5: Viral inhibitors of NF-κB pathway

	Virus	Viral protein	Reference
DNA viruses			
Circoviridae	Tarque teno virus	ORF2	(Zheng et al., 2007)
Coronaviridae	SARS coronavirus	M protein	(Fang et al., 2007)
Herpesviridae	Varicella-zoster virus (VZV)	--	(Jones and Arvin, 2006)
		IE63	
	HSV	--	(Amici et al., 2006)
	EBV	ZEBRA	(Dreyfus et al., 1999)
		EBNA1	(Valentine et al., 2010)
	HHV-8	vIRF3	(Seo et al., 2004)
		K1	(Lee et al., 2002)
	CMV	IE86	(Taylor and Bresnahan, 2006)
Papillomaviridae	Papillomavirus	E7	(Spitkovsky et al., 2002)
Polydnaviridae	Microplitis demolitor bracovirus	H4 and N2	(Thoetkiattikul et al., 2005)
Poxviridae	Vaccinia virus	A52R	(Bowie et al., 2000)
		M2L	(Gedey et al., 2006)
		K1L	(Shisler and Jin, 2004)
	Cow pox virus	--	(Oie and Pickup, 2001)
		006KO	(Mohamed et al., 2009)
	Myxoma virus	MNF	(Camus-Bouclainville et al., 2004)
		M013	(Rahman et al., 2009)
	Molluscum contagiosum virus	MC159	(Murao and Shisler, 2005)
		MC160	(Nichols and Shisler, 2006)
	Poxvirus	N1L	(DiPerna et al., 2004)
	Parapoxvirus orf virus	ORFV024	(Diel et al., 2010)
RNA viruses			
Flaviviridae	African swine fever virus	A238L	(Revilla et al., 1998)
	HCV	NS5A	(Park et al., 2002)
		NS5B	(Choi et al., 2006)
		NS3/4A	(Karayiannis, 2005)
		Core	(Joo et al., 2005)
Paramyxoviridae	Measles virus	--	(Dhib-Jalbut et al., 1999)
	Sendai virus	C and V proteins	(Komatsu et al., 2004)
	Mumps virus	S and H protein	(Wilson et al., 2006)
	Canine distemper virus	--	(Friess et al., 2005)
Picornaviridae	Coxsackievirus	CVB3	(Zaragoza et al., 2006)
	Foot and mouth disease virus	Leader proteinase (L(pro))	(de Los Santos et al., 2007)
	Poliovirus	3C Protease	(Neznanov et al., 2005)
Reoviridae	Rotavirus Reovirus	NSP1	(Sherry, 2009)
Retroviridae	HIV-1	Vpu	(Bour et al., 2001)

Example of viral targeting of scaffolding protein upstream of IKK include viral protein A52R of vaccinia virus which acts as a dominant negative form of MyD88 to abrogate IL-1 and TLR-4 mediated NF-κB activation (Bowie et al., 2000). In addition molluscum contagiosum virus (MCV) MC159 interacts with TRAF2 and inhibits late events of TNFα induced NF-κB activation. Both function of MC159 seems correlated as mutant MC159 protein which was unable to bind with TRAF2, could not inhibit TNF-mediated NF-κB activation (Murao and Shisler, 2005). Another protein of this virus MC160 prevents IKK complex formation through down regulation of IKKα expression (Nichols and Shisler, 2006). HPV oncoprotein E7 also attenuates NF-κB activation by targeting the IKK complex resulting in impaired IκBα phosphorylation and degradation (Spitkovsky et al., 2002). Similarly EBV EBNA1 inhibits phosphorylation of IKKα/β resulting in reduced phosphorylation of IκBα and p65 and reduced NF-κB activity (Valentine et al., 2010). Torque teno virus (TTV) ORF2 protein physically interacts with IKKβ and IKKα and suppresses NF-κB activity through inhibition of IκB protein degradation (Zheng et al., 2007). HCV proteins NS5B (Choi et al., 2006) and Core (Joo et al., 2005), SARS coronavirus M protein (Fang et al., 2007) and HHV-8 vIRF3 (Seo et al., 2004) have also been reported to target IKK for suppression of NF-κB.

Another target of viruses is the degradation of IκB protein, main NF-κB-inhibitor. Measles virus has been reported to inhibit phosphorylation and degradation of IκB resulting in repression of NF-κB dependent genes in virus-infected cells (Dhib-Jalbut et al., 1999). Cowpox virus and other members of the orthopoxvirus genus (Oie and Pickup, 2001) and vaccinia virus K1L (Shisler and Jin, 2004) inhibit the induction of NF-κB-regulated gene expression by interfering with the process of IκBα degradation. Similarly cmvIL-10 of HCMV inhibit NF-κB activation through reduced degradation of IκB (Nachtwey and Spencer, 2008). HIV-1 protein Vpu (Bour et al., 2001) and rotavirus nonstructural protein NSP1 (Graff et al., 2009) interfere with the β-TrCP-dependent ubiquitination and degradation of phosphorylated IκBα resulting into reduction of NF-κB activity. Some viruses target the steps downstream of IκB degradation. African swine fever virus encodes a viral homolog of IκB protein containing ankyrin repeats similar to those of cellular IκB. Once the cellular IκB is degraded, this viral protein inhibits p50/p65 binding to its target sequences in the DNA thus impairing NF-κB activation (Revilla et al., 1998). During CVB3 infection viral protease 3Cpro result in cleavage of IκB which in turn inhibits NF-κB and increases apoptosis but decreases viral replication (Zaragoza et al., 2006).

Varicella-zoster virus (VZV) has been reported to inhibit NF-κB pathway by sequestering of p50 and p65 proteins in the cytoplasm (Jones and Arvin, 2006). Similarly,

ZEBRA protein of EBV can interact with p65 inhibiting NF-κB activity in T cells (Dreyfus et al., 1999). Dysregulation of the NF-κB response during HSV-1 infection has been reported to be mediated by a virus-induced block of NF-κB recruitment to the promoter of the IκBα gene. Authors show that HSV-1 redirects NF-κB recruitment to the promoter of ICP0, an immediate-early viral gene with a key role in promoting virus replication (Amici et al., 2006). Some viruses target p65 in the nucleus resulting in its degradation. Murid herpesvirus-4 (MuHV-4) encoded ORF73 protein results into poly-ubiquitination and subsequent proteasomal-dependent nuclear degradation of p65 and inhibits NF-κB transcriptional activity (Rodrigues et al., 2009). The HTLV-1 bZIP factor (HBZ) degrades p65 through increasing the expression of the PDLIM2 gene and suppress the classical NF-κB pathway, and not the alternative pathway (Zhao et al., 2009). African swine fever virus protein A238L can inhibit p65 acetylation and p300 transactivation as well (Granja et al., 2006).

3. Role of Bcl-3 during viral infections

Bcl-3 is activated in response to a number of viral infections and seems to affect viral as well as cellular gene expression. Activation of Bcl-3 in mouse trigeminal ganglia in response to HSV-1 reactivation stimulus potentially results in upregulation of ICP0 transcription, which is an important viral event for initiation of HSV-1 reactivation from latency (Tsavachidou et al., 2001). Bcl-3 is activated during HCMV infection of macrophages which regulates transcription from MIEP of HCMV (Khan et al., 2009). In certain cases, Bcl-3 results in repression of viral transcription. HTLV-1 Tax protein enhances Bcl-3 expression which functions as a repressor of LTR-mediated transcription through interactions with TORC3 [The transducers of regulated CREB activity-3] (Hishiki et al., 2007). In addition Tax induced Bcl-3 displaces p300 from HTLV-1 promoter resulting in repression of Tax-mediated transcription (Kim et al., 2008).

In addition to its role in regulation of viral transcription, Bcl-3 is potentially involved in viral pathogenesis. Differential overexpression and nuclear localization of Bcl-3 has been reported in HPV infected oral cancer (Mishra et al., 2006) and mouse mammary tumor virus c-rel-induced mammary tumors (Romieu-Mourez et al., 2003), and in EBV induced nasopharyngeal carcinoma (Thornburg et al., 2003). Bcl-3 is involved in upregulation of genes important in oncogenesis. HBx of HBV upregulates Bcl-3 which in turn activates cyclin D1 through p52/Bcl-3 complexes (Park et al., 2006). Induction of p50 homodimer/Bcl-3 complexes by EBV LMP1 CTAR-1 results in epidermal growth factor receptor expression (Thornburg and Raab-Traub, 2007). Bcl-3 expression due to LMPI1 has been reported to be dependent upon NF-κB activation (Nakamura et al., 2008) and STAT3 [Signal Transducer and Activator of

Transcription] (Kung and Raab-Traub, 2008). Bcl-3 is also activated during T-cell leukemia/lymphoma (ATLL) due to HTLV-1 infection which in turn transcriptionally regulates parathyroid hormone-related protein (PTHrP) promoter P2 (Nadella et al., 2007). Respiratory syncytial virus induces Bcl-3 expression in A549 cells and antagonizes the NF-κB signaling pathways by inducing HDAC1 recruitment to the IL-8 promoter (Jamaluddin et al., 2005).

III. Human Cytomegalovirus

1. Introduction

HCMV is a species-specific herpesvirus that silently infects a large population of the world and results in persistent infection for the life of the host associated with frequent shedding of the virus. Epidemiological studies show that HCMV is universally distributed among human population with higher prevalence in developing countries. Horizontal transmission of HCMV occurs through direct contact with infectious material and viral infection is high in situations where contact with body fluid occurs from persons excreting virus for example between sexual partners and contact with children. Transmission through airborne route or aerosol has not been reported. Transmission of virus also occurs through transfusion of blood and blood products and transplantation of cells and organs from seropositive donors. Vertical transmission from mother to fetus or newborn play important role in maintenance of viral infection in the population and occurs via the placenta, during delivery, and by breast feeding. The most common route of transmission from mother-to-infant is breast milk (Mocarski et al., 2007).

In the normal host, HCMV does not cause clinical disease after primary infection but virus becomes latent which can be reactivated latter during life. However it can causes febrile illness with features of mononucleosis, which is occasionally serious. HCMV cause acute disease in the absence of an effective immune response especially in acquired immunodeficiency syndrome (AIDS) patients, immunocompromised solid-organ and bone marrow allograft recipients. Approximately 20-40% adults with AIDS develop CMV disease characterized by retinitis, esophagitis, and colitis (Britt, 2008; Mocarski et al., 2007).

HCMV rarely cause clinical disease in normal term neonates infected during delivery or after birth from maternal source but is symptomatic in congenital in *utero* infection. Symptoms include intrauterine growth retardation, jaundice, hepatosplenomegaly, thrombocytopenia, and hepatitis along with neurological involvement. Most of non-CNS symptoms are self limiting but damage to CNS and organ of perception is permanent resulting in birth defects such as mental retardation, impaired intellectual performance, hearing loss, and impaired vision. Moreover, HCMV is the potential cause of morbidity in low-birthweight premature infants (Landolfo et al., 2003; Mocarski et al., 2007).

Association of HCMV infection with the development of cardiovascular disease including atherosclerosis, arterial restenosis, and transplant vascular sclerosis (TVS) has been described. The development of vascular disease is associated with chronic inflammation

followed by proliferation of smooth muscle cells with possible contribution of inflammatory mediators and growth factors produced by HCMV infected cells (Streblow et al., 2008). Recently, HCMV infection have been detected in inflamed tissue of patients with inflammatory diseases including inflammatory bowel disease, rheumatoid arthritis, Sjögren's syndrome, dermato-and polymyositis, psoriasis, Wegener's granulomatosis, ulcerative colitis, and Crohn's disease (Soderberg-Naucler, 2008). In addition potential role of HCMV in malignancy has been suggested following detection of viral proteins and DNA in certain type of cancers (Michaelis et al., 2009b). Certain mechanism including modulation of cell cycle, resistance to apoptosis, promotion of tumor invasion and proliferation, and chromosome damage (Michaelis et al., 2009a) have been proposed but the exact role of HCMV in the pathogenesis of malignancy is unknown.

2. Structure of Virion

The virion of HCMV is 150-200 nm in diameter and has complex structure reflecting the large number of proteins encoded by this virus. It contains 230-kbp double-stranded linear DNA genome enclosed in the icosahedral nucleocapsid which is approximately 125-nm in diameter. Nucleocapsid in enclosed by a proteinaceous layer defined as the tegument or matrix which in turn is surrounded by a lipid bilayer envelope containing a large number of viral glycoproteins. Cell culture infection of HCMV produces two other defective particles, in addition to infectious particles. These include dense bodies (DB) and non-infectious enveloped particles (NIEP). Dense bodies are composed of tegument enclosed by lipid bilayer but lack DNA and capsid. Non-infectious enveloped particles are enveloped defective/ immature capsids and lack viral genome (Mocarski et al., 2007). The structure of virion is shown in Fig. 4.

Icosahedral nucleocapsid is composed of at least six core proteins; the major capsid protein (MCP), the minor capsid protein (TRI1), minor capsid protein binding protein (TRI2), the smallest capsid protein (SCP), a portal protein (PORT) and precursor of capsid assembly protein (pAP) encoded by UL86, UL85, UL46, UL48.5, UL104, and UL80.5 genes, respectively. The major capsid protein is most abundant and is the major building block of viral nucleocapsid (Britt, 2008; Mocarski et al., 2007). Tegument is an amorphous layer which keeps an association between surrounding nucleocapsid and envelope through specific interactions with nucleocapsid and envelope components. Tegument is composed of at least 27 virus encoded proteins, most of them are phosphorylated and highly immunogenic. Most abundant tegument proteins are members of UL82 family including pp65 (lower matrix protein), pp71 (upper matrix protein), pp150 (large matrix phosphoprotein) and large tegument protein (LTP)

encoded by UL83, UL82, UL32, and UL48 genes, respectively (Britt, 2008; Mocarski et al., 2007). Tegument proteins play important role in viral life cycle including viral entry, gene expression, immune evasion, assembly and egress (Kalejta, 2008).

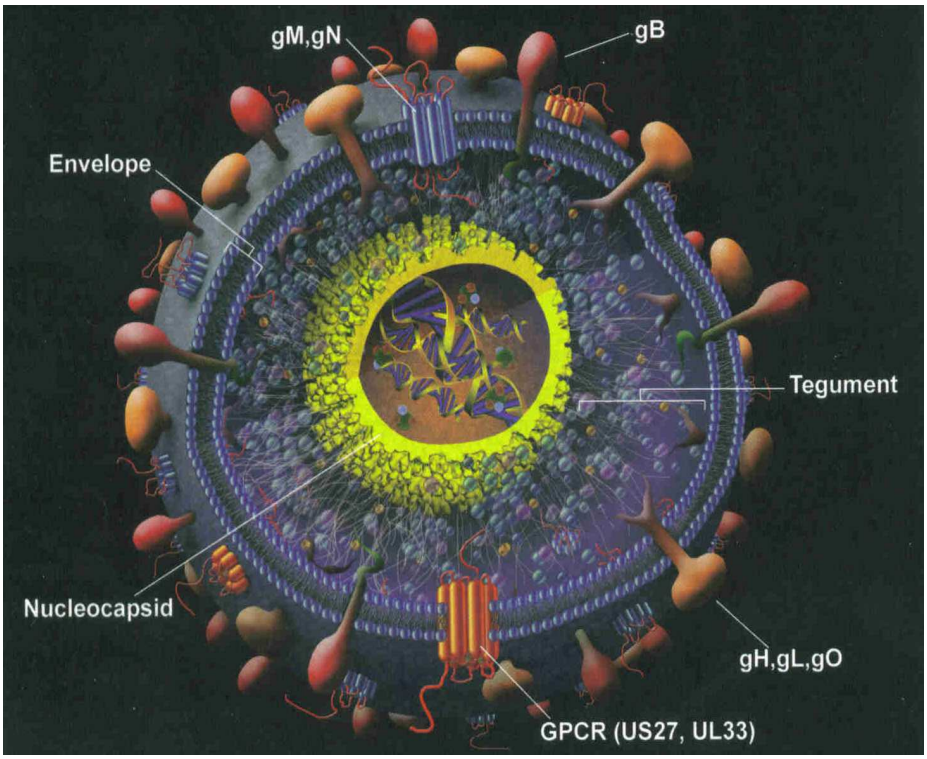


Figure 4: Structure of HCMV virion particle. Reproduced from (Streblow et al., 2006).

Lipid bilayer found outside the tegument is derived from host cell endoplasmic reticulum- Golgi intermediate compartment (ERGIC) containing at least 20 viral glycoproteins. Functionally critical and abundant glycoproteins have been found to exist as disulfide-linked complexes termed gcI (gB), gcII (gM:gN), and gcIII (gH:gL:gO). All these proteins are necessary for viral replication and function as essential players in viral entry into host cells, cell-to-cell spread, and virion maturation. In addition they initiate signal transduction pathways in the host cells which optimize the cellular environment for efficient viral replication. Glycoprotein B (gB), found as homodimer in gcI, is essential for initial contact with the host cell through heparan sulfate proteoglycan (HSPG) binding. It interacts with other cell receptors as well including epidermal growth factor receptor (EGFR), Toll-like receptor 2 (TLR-2), and integrins. Moreover it is important for viral entry and cell-to-cell virus transmission. Glycoprotein M is most abundant glycoprotein of the virion and makes up complex gcII in association with gN. This complex is essential for viral replication and might be important for initial binding of the virus with host cell through interaction with heparan sulfate proteoglycan. The complex gcIII, compose of gH, gL, and gO, has suggested role in viral entry through fusion of the virus with

cell membrane. Importantly, gB, gH and gN have been reported as the targets of CMV neutralizing antibodies. In addition HCMV contains many minor glycoproteins including virus encoded G-protein coupled receptor homologs. One of these encoded by US28 is worth mentioning as it activates signaling pathways and is a virulence factor for HCMV-associated vascular disease and malignancy. Clear function for minor glycoproteins in viral replication remains elusive (Britt and Boppana, 2004; Mocarski et al., 2007).

In addition certain host cell proteins have been reported to be incorporated into virion including kinases, actins, tubulin, annexins, actin and vimentin. Over 70 host proteins have been identified by mass spectrometry (MS) based proteomics approach (Varnum et al., 2004). Some of these proteins may suggest novel strategies utilized by the virus for successful replication cycle in the host cell for example CKII found in HCMV virion is able to phosphorylate IκB and can result in NF-κB activation and HCMV replication (Nogalski et al., 2007). However validity of the results is sometime questioned on the issues of virion preparation purity and functional significance.

The HCMV genome is the largest of all herpesviruses and consists of two domain called long and short genome segments (L and S). Each segment is composed of central unique region [thus unique long (UL) and unique short (US)], with repeat regions at the end of each segment. Repeat regions found at the either end of genome are called terminal repeat [thus terminal repeat long (TRL) and terminal repeat short (TRS)] while repeat regions found at the junctions of UL and US are termed internal repeat (thus internal repeat long IRL and internal repeat short IRS). Thus general organization of HCMV genome is TRL-UL-IRL-IRS-US-TRS. Each long and short segment can be inverted relative to each other resulting in four different infectious genome isomers in viral progeny (Murphy and Shenk, 2008) but the functional significance of isomerization in unknown. Analysis of viral genome indicates that it contains around 200 ORFs, and multiple *cis*-acting elements which control viral transcription and replication. One cis-acting element, oriLyt, has been mapped between UL57 and UL69 genes in the middle of UL region (Borst and Messerle, 2005). The core region of oriLyt contains transcription factor binding sites, multiple repeat elements, and the sites at which RNA-DNA structures form. Other cis-acting elements include multiple transcriptional control regions including major IE promoter (MIEP) enhancer and minor enhancer-promoter controlling the US3 IE gene. MIEP enhancer is located in UL segment and controls the transcription of IE72 and IE86 very early in the replication cycle (Mocarski et al., 2007; Murphy and Shenk, 2008).

3. Viral replication cycle

The HCMV virion enters the cell through direct fusion resulting in deposition of nucleocapsid into cytoplasm. Nucleocapsid is transported to the nucleus and viral DNA enters into the nucleus. Expression of viral gene consists of three classes of proteins including immediate-early (IE), delayed-early (DE) and late (L). HCMV transcription and translation is directed by host cell machinery where host cell RNA polymerase II and associated basal transcription apparatus is responsible for viral transcription. Virion assembly occurs in the nucleus and nucleocapsid is enveloped in ERGIC membranes followed by release through exocytosis (Fig. 5).

3.1 Viral entry and uncoating

HCMV binds and enters efficiently in wide range of cell types. As viral replication is restricted to few cell types, a post-entry block is thought to restrict viral replication in non-permissive cell types. Viral entry is the result of a series of events involving viral envelop glycoprotein complexes (gcI, gcII, and gcIII) and cellular receptors that culminate in fusion of the virion envelope with the cellular plasma membrane by a pH-independent mechanism. Early event of virus-cell interaction consists of low-affinity binding of viral glycoprotein gB to heparan sulfate proteoglycan resulting in attachment of the virus with cell surface (Compton et al., 1993). The subsequent interaction of viral glycoproteins with other receptors turns the week adhesion of the viral particles into a more stable binding. Epidermal growth factor receptor (EGFR) and integrin αvβ3 has been reported as an HCMV receptor and coreceptor, respectively (Wang et al., 2005; Wang et al., 2003). HCMV glycoproteins gB and gH independently bind to EGFR and integrin αvβ3, respectively, to initiate viral entry and signaling. So interaction of HCMV glycoproteins with their receptors generates an intracellular signal transduction pathway leading to the alteration of cellular gene expression. Final step of entry leads to deposition of nucleocapsid and tegument protein in the cytoplasm followed by their rapid translocation to the nucleus through microtubules and release of DNA to nucleus (Landolfo et al., 2003; Mocarski et al., 2007).

3.2 Viral gene expression

HCMV transcription and translation is directed by host cell machinery where host cell RNA polymerase II, associated basal transcription apparatus, and host transcription factors are responsible for viral transcription. Viral gene expression occurs as cascade of transcriptional

events that result in synthesis of three categories of viral proteins termed immediate-early (IE), delayed-early (DE) and late (L).

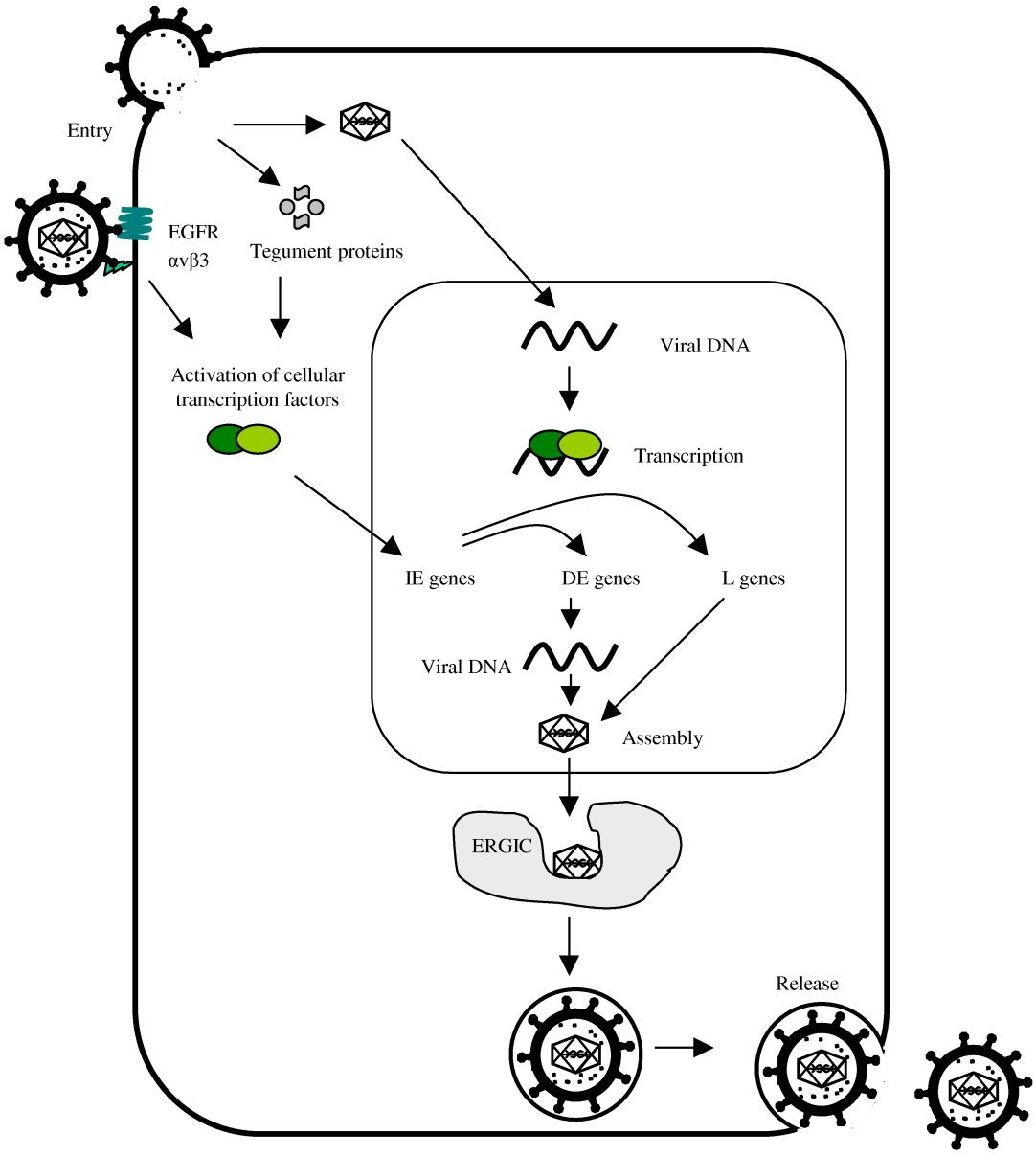


Figure 5: HCMV replication cycle

IE gene expression starts immediately after entry of virus without need of any viral gene product. Viral genome associates with ND10 immediately after uncoating where expression of IE gene transcription starts. ND10 components Daxx, PML, ATRX are able to repress IE transcription and pp71 has been reported to rescue Daxx mediated repression through prevention of Daxx-mediated HDAC recruitment to viral DNA. The IE genes mapped on HCMV genome include IE1/IE2 (UL122-123), UL36-37, IRS1/TRS1, and US3 each of which encode for a number of transcripts and gene products (Fig. 6). The expression of IE1 and IE2 is controlled by

major immediate-early promoter (MIEP), activity of which varies depending upon the cell type and stage of cellular differentiation. MIEP is strong in differentiated cells but is repressed in undifferentiated cells, thus is important in latency and reactivation of HCMV (Sinclair and Sissons, 2006). MIEP contains binding sites for a number of transcription factors and efficiency of IE gene expression is considered the basis for cellular permissiveness to HCMV. The IE gene products are necessary for viral replication through expression of subsequent viral genes. In addition they play key role in the pathogenesis of HCMV disease as they regulate a large number of cellular genes as well. IE1-72 protein transactivates a variety of viral and cellular promoters including its own promoter. IE2-86 is responsible switch from IE to DN gene expression as it activates DN and L genes but autorepress its own promoter (Meier and Stinski, 2006). Tegument proteins play important role for expression of IE genes while IE gene products are required for subsequent expression of DE and L genes. Transcription of DE genes starts by 6 hours postinfection (hpi) and continues through 18-24 hpi.

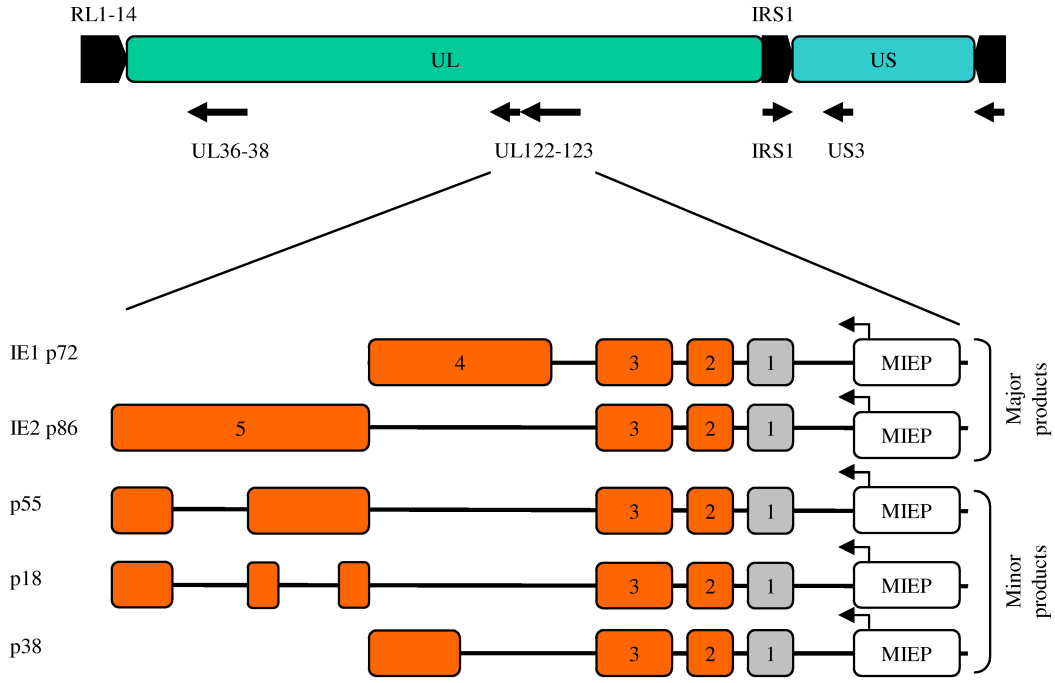


Figure 6: Organization of IE locus in the HCMV genome and IE products

DE genes mostly encode for non-structural proteins which are important for regulation of viral DNA synthesis and capsid formation and immune evasion. In addition they alter the cellular environment for replication. UL112-113 encoded protein result in the initiation of DNA replication and UL54 encoded for viral DNA polymerase. The L proteins are the last to come in the game. These proteins play mainly structural roles and focus on capsid maturation, DNA

encapsidation, virion maturation, and egress from the cell. The transcription of L proteins starts 24 hpi and requires prior viral DNA replication (Landolfo et al., 2003; Mocarski et al., 2007).

Viral DNA replication begins later than 16 hpi and requires expression of specific viral proteins and participation of a number of host proteins. HCMV does not encode deoxyribonucleotide biosynthesis enzymes and relay exclusively on the host dNTPs supply for its DNA replication. Consequently, expression of enzymes involved in nucleotide metabolism is increased. Virus does not shut off cellular RNA and protein synthesis, instead stimulates transcription and translation but completely deregulate the cell cycle. At least in fibroblasts, replication is stopped and cellular DNA synthesis ceases stopping host DNA synthesis machinery from competing with the virus for dNTPs. Viral DNA synthesis depends on transcriptional activation in the oriLyt region. Six core replication proteins are required for synthesis of DNA including the UL54-encoded polymerase catalytic subunit (POL) with the UL44-encoded polymerase processivity (PPS) subunit (POL:PPS complex), the UL57-encoded single strand DNA binding protein (SSB), and the heterotrimeric helicase-primase (HP) consisting of U105-encoded HP1, UL70-encoded HP2, and UL102-encoded HP3. Accumulation of transcriptional transactivators IE1-p72, IE2-p86, and the UL84 protein result in the initiation of transcription across oriLyt which contains transcription factor binding sites. The integrity of this cis-acting element is required for efficient viral replication since mutations of this region dramatically impair the initiation of viral DNA synthesis (Landolfo et al., 2003; Mocarski et al., 2007).

3.2.A. Structure of immediate-early gene promoter

MIEP is composed of core promoter, enhancer, unique region, and modulator regions (Fig. 7). Core promoter contains a TATA box between -28 and -222, a cis-repression sequence (crs) between -13 and +1, and an Inr between +1 and +7. In case of extensive mutation of crs, recombinant virus is unable to replicate while there is significant reduction in viral replication in case of mutation at the position -10 and -9 (Isomura et al., 2008). Enhancer is central in coordinated regulation of MIEP activity. HCMV enhancer of ~540bp consists of a distal component (between –550 and app -300) and a proximal component (between –300 and –39 relative to transcription start site +1). It seems that both components function jointly for efficient MIEP activation as mutant viruses deleted at distal enhancer replicates slowly (Meier and Pruessner, 2000). The proximal component is absolutely required for viral replication as enhancerless virus does not replicate (Isomura et al., 2004). Unique region (UR) contain binding sites for cellular repressor proteins, such as CCAAT displacement protein (CDP), special AT-

rich sequence binding protein 1 (STAB-1), and pancreatic-duodenal homobox factor-1 (PDX-1). UR repressor function represses the transcription of UL127 promoter present at the UR-modulator junction. Modulator is the large component that flanks 3' end of the UL127 ORF which has been reported to augment transcription from MIEP but mutant viruses with deleted modulator replicates similarly (Stinski and Petrik, 2008).

The activity of MIEP enhancer is the outcome of interplay between a number of cellular and viral factors which regulate MIEP either in a positive or negative way. Enhancer activity is affected by cell type, the stage of cellular differentiation and diverse signal transduction pathways. The degree of cellular permissiveness to the virus thus is thought to be dependent upon balance of levels of activators and repressors of MIEP transcription.

MIEP enhancer contains binding sites for a number of cellular transcription factors and number of each type of binding site differs between different CMV species. Multiple binding sites for several transcription factors ensure cooperative or synergistic interaction among same or different transcription factors. Cellular transcription factors including NF-κB, AP-1 (Activator of protein-1), CREB/ATF (Activating transcription factor), Sp1, serum response factor (SRF), ELK-1, and retinoic acid/ retinoid X receptor bind to cognate cis-acting elements in the enhancer to stimulate transcription from the core promoter. These transcription factors are thought to strengthen RNA polymerase II transcription and recruit coactivators with the ability to enhance transcriptional initiation and elongation either cooperatively or an in independent manner (Meier and Stinski, 2006). In addition MIEP contains binding sites for some repressor proteins such as Yin Yang 1 (YY1), Ets-2 repressor factor (ERF), methylated DNA-binding protein (MDBP), modulator-recognition factor (MDF), and Gfi-1. These repressor proteins interact physically and recruit HDACs to silence MIEP in non-permissive cells (Sinclair, 2009).

Transient transfection experiments proved NF-κB and CREB/ATF sites as critical components of MIEP enhancer. Mutation of one type of site in the enhancer had no effect on transcription from MIEP and viral transcription in fibroblasts. Mutation of all NF-κB or all the CREB/ATF binding sites had no or negligible effect on viral transcription (Benedict et al., 2004; Gustems et al., 2006; Keller et al., 2003). It seems that other there is functional redundancy between different types of binding sites as complete elimination of MIEP enhancer abolished the viral replication completely. In fact cooperative interaction between multiple transcription factors is required for efficient IE transcription. Recently, it was demonstrated that Elk-1 and SRF binding sites are required for MIEP transcription and viral replication and can compensate for inactivation of NF-κB binding sites (Caposio et al., 2010). In addition, it was reported that

NF-κB, NF-1, AP-1, Sp-1 (stimulator protein-1) and CREB/ATF binding sites in proximal enhancer cumulatively activate MIE transcription. The most significant and independent effect on the MIE promoter was observed for CREB site while the other sites had a minor independent effect. The combination of the different transcription factor DNA binding sites was significantly stronger than multiple duplications of the CREB site (Lashmit et al., 2009). It is possible that the loss of binding sites is compensated or dominated by transactivation function of viral proteins.

In addition to cellular transcription factors MIEP activity is also regulated by a number of viral (tegument and IE proteins). Virion transactivator or VTA (pp71) in association with ppUL35 is incorporated in virion and strongly stimulates MIEP expression and viral replication upon entry into the cell (Schierling et al., 2004). Another tegument proteins ppUL26 can also influence MIEP expression. Moreover MIEP activity is increased in response to positive feedback by IE gene product IE2-72 (Mocarski et al., 2007).

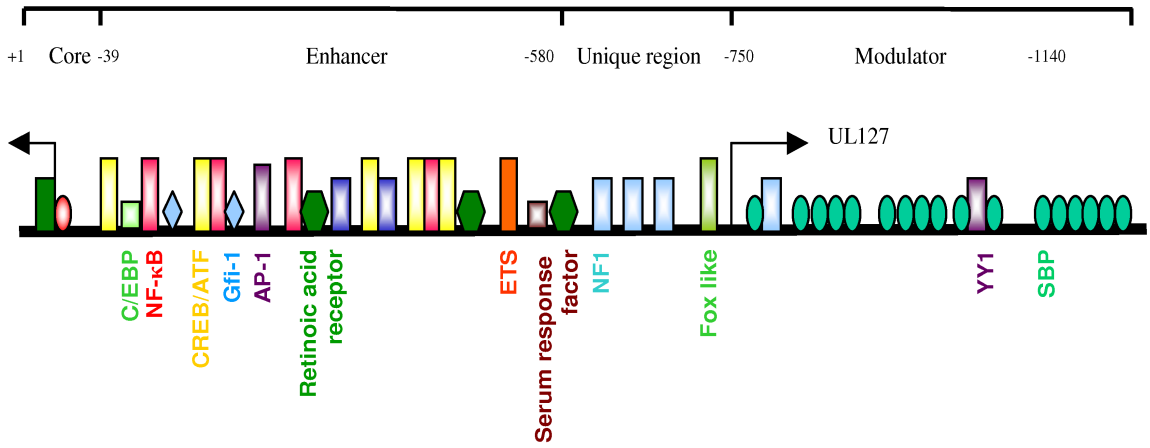


Figure 7: Structure of MIEP

3.2.B. Regulation of MIEP by chromatin structure

Transcriptional regulation of cellular genes is regulated by structure of chromatin through numerous post-translational modifications on distinct histones. Composition and pattern of these modifications give rise to “histone code” which determines the overall structure of chromatin and binding of regulatory proteins (Strahl and Allis, 2000). Generally speaking, active chromatin is associated with acetylation of certain lysine residues in histones H3 and H4 while methylation at lysine 9 of histone H3 is associated with inactive/ compact chromatin as it recruits heterochromatin protein 1 (HP1). Acetylation of histones is mediated by a family of enzymes called histone acetyltransferases (HATs) while HDACs are responsible for deacetylation of histones.

Like cellular gene expression, viral gene expression is also regulated by their chromatin structure. HCMV genome is also associated with histones when it reaches the nucleus. Thus chromatin structure of cytomegalovirus genome modulates its gene expression and productive infection (Sinclair, 2009). Inhibition of HDACs with Trichostatin A (TSA) and valproic acid was reported to increase viral protein synthesis and viral replication (Michaelis et al., 2004; Tang and Maul, 2003). It was suggested that HDACs are recruited to the ND10 bodies and it was shown that MCMV IE1 binds with ND10 component Daxx reversing HDAC-mediated repression of viral gene expression (Tang and Maul, 2003). Another study showed that knockdown of Daxx resulted in increased MIEP expression and MIEP was associated with markers of transcriptionally active chromatin indicating that chromatin modification around the viral MIEP is the underlying mechanism of Daxx mediated repression of MIEP transcription (Woodhall et al., 2006). In fact histones with markers of compact chromatin are associated with viral genome immediately after infection but inhibition of HDAC can overcome this phenomenon (Groves et al., 2009). In addition infection at high MOI can overcome Haxx-HDAC mediated MIEP repression indicating that virus is equipped with some viral proteins to repress it. UL82 gene product pp71 has been reported to degrade Daxx resulting in reversal of HDAC-mediated MIEP repression (Saffert and Kalejta, 2006). IE1 has also been reported to antagonize HDACs to facilitate HCMV replication (Nevels et al., 2004). Recently, it was shown that histone modifications regulate HCMV gene expression throughout the replication cycle. At the start of infection, histone H3 at IE promoter is acetylated while methylated at early and late promoters. Latter on all viral promoters are modified with activating acetylations (Cuevas-Bennett and Shenk, 2008).

3.2.C. Silencing/desilencing of MIEP during latency and reactivation

After a primary infection, HCMV persists throughout life time of the host in latent form. Silencing and desilencing of MIEP during latency and reactivation is also regulated by chromatin remodeling of MIEP by post-translational modifications of histones. In fact MIEP chromatin structure upon infection will determine the permissiveness of cells to productive infection. HCMV MIEP is repressed in non-permissive cells due to recruitment of HDACs by repressor transcription factors resulting in transcriptionally repressive compact chromatin. It was reported that MIEP associates with acetylated histones in permissive cells while with heterochromatin protein-1 (HP1) in non-permissive cells (Murphy et al., 2002). Consistently, HDAC inhibitors treatment of non-permissive cells induces MIEP transcription and viral permissiveness while superexpression of HDAC3 in permissive cells reduces MIEP activity and

viral infection (Murphy et al., 2002). It has been demonstrated that MIEP is associated with HP1 and not with acetylated histones in latently infected CD34+ and monocytes of naturally infected subjects. Differentiation of these DC progenitors cells to mature DCs result in reactivation of latent virus, lost of HP1, histones bound to MIEP are acetylated, and HDAC-1 is downregulated (Reeves et al., 2005; Wright et al., 2005).

During latency, lytic viral infection is suppressed and a few latency associated transcripts are expressed. One of these is latency-associated transcript (LUNA) which encodes a small protein of yet unknown function. Unlike MIEP, LUNA promoter predominantly associates with acetylated histones in CD34+ cells after experimental infection (Sinclair, 2009). Thus chromatin structure explains repression of MIEP while expression of latency associated genes in undifferentiated myeloid cells.

3.3 Virion assembly, maturation, egress, and release

First step in virion assembly is the interaction of precursor (pAP) of assembly protein (AP) with MCP in the cytoplasm which culminates in translocation of MCP to the nucleus. Procapsid is formed around the pAP by five capsid proteins MCP, TRI1, TRI2, SCP, and PORT in the nucleus. Subsequently, assembled procapsids undergo a series of changes forming a DNA-containing nucleocapsids. Proteolytic cleavages catalyzed by the precursor (pPR) of maturational protease (PR; assemblin) are important for this maturation. Finally AP is separated from MCP and pAP, AP, and PR are removed from the nucleocapsid. Encapsidation of DNA occurs through recognition of HCMV cis-acting element (packaging or pac sites) by the encapsidation proteins. Proteins implicated in encapsidation process include UL51, UL52, UL56, UL77, UL89, UL93, UL95, UL97, UL103, and UL104 gene products (Gibson, 2006; Mocarski et al., 2007).

Process of viral egress consists of two stages including primary envelopment/ deenvelopment and secondary reenvelopment stage controlled by tegument proteins. Primary envelopment occurs at the inner nuclear membrane followed by deenvelopment at the outer nuclear membrane releasing the nuclear capsid into the cytoplasm. The said process is controlled by products of two viral genes UL50 and UL53. In addition host encoded protein kinases as well as the viral protein kinase (VPK; pUL97) are also implicated. Tegument proteins are added to virion when it passes through the cytoplasm before secondary reenvelopment which occurs at ERGIC membranes. The secondary reenvelopment and maturation step is controlled by UL32 encoded viral protein pp150. Small amount of some host proteins are also included in the

tegument during reenvelopment. Vesicles thus produced transport the nucleocapsid to the plasma membrane for release through exocytosis. The characteristic cytoplasmic inclusions which appear during HCMV infection is the result of Golgi alterations during late stage of viral replication (Mocarski et al., 2007).

4. HCMV cellular tropism

4.1 HCMV target cells *in vivo* and *in vitro*

HCMV infection is strictly restricted to humans but range of its target cell types is broad. HCMV infection can not be studies in animals, however analysis of patient samples and autopsy material using histological and immunohistochemical techniques has identified *in vivo* targets of the virus in the natural host. The presence of infected cells has been demonstrated in various cells of ectodermal, mesodermal, and endodermal origin in virtually all organs (Sinzger et al., 2008). Detection of viral replication in multiple organs has been reported during severe intrauterine HCMV infection (Bissinger et al., 2002). *In vivo* cell types infected with HCMV include macrophages, endothelial cells, epithelial cells, smooth muscle cells, fibroblasts, connective tissue cells, stromal cells, neuronal cells, and hepatocytes (Sinzger et al., 1999a; Sinzger et al., 1995; Sinzger et al., 1993). Liver, gastrointestinal tract, lungs, retina and brain are predominant sites of clinical manifestations of HCMV infection in immunocompromised hosts (Sinzger et al., 2008).

HCMV infects a large number of cell types *in vitro* as well. Susceptible cell types including fibroblasts (Furukawa et al., 1973), vascular smooth muscle cells (Tumilowicz et al., 1985), retina pigment epithelial cells (Tugizov et al., 1996), kidney epithelial cells (Heieren et al., 1988; Tanaka et al., 1984), placental trophoblast cells (Halwachs-Baumann et al., 1998), hepatocytes (Sinzger et al., 1999a), neuronal and glial brain cells (Poland et al., 1990), vascular endothelial cells (Ho et al., 1984), monocyte-derived dendritic cells (Riegler et al., 2000), monocyte-derived macrophages [MDMs] (Ibanez et al., 1991), human embryo-chondrocytes (Wang et al., 2008), human embryo-myoblasts (Wang et al., 2008), and human marrow stromal cell (Wang et al., 2008). The above stated cells types support full viral replication cycle resulting in production and release of progeny. Moreover, most of them acquire a cytomegalic appearance during the late replication phase. The cell types unable to support viral replication include lymphocytes and granulocytes (Sinzger et al., 2008). The cell type most widely used to grow HCMV is the fibroblast, which produces high titers of infectious virus after *in vitro* infection. Other primary cells are permissive for HCMV replication but the rate of viral production in most

of these cell types is considerably lower than in fibroblasts. However, endothelial cell cultures are susceptible for certain viral strains and allow long term propagation of the virus through passage of cell-free supernatant of infected culture (Digel and Sinzger, 2006). The cellular and/or viral factors responsible for difference in the viral productivity by different cell types are poorly known.

Cell tropism of a virus is dependent upon viral as well as cellular factors. Long term propagation of clinical isolates in fibroblast cultures selects fibroblast-tropic stains (which are low endotheliotropic). In contrast, long term propagation of the same viral isolates in endothelial cells retains endothelial cell tropism as well as fibroblast tropism (Sinzger et al., 1999b). Such differentially propagated isolate pairs show genomic differences in restriction fragment length analyses indicating a genetic contribution in the HCMV tropism (Sinzger et al., 1999b). Endothelial cell propagated strains have also been reported to grow better in macrophages as well (Sinzger et al., 2006). Viral genes important for tropism may function through positively or negatively influencing viral replication cycle, antiviral factors or host cell life. In addition cellular differentiation is also required for HCMV gene expression and successful life replication of the virus. Cells non-permissive for HCMV replication (for example monocytes) become permissive for viral replication after differentiation (macrophages). The process of differentiation activates a number of signal transduction pathways in the cells which determines activation of MIEP, chromatin structure of MIEP, and in turn permissiveness of cells.

4.2 Role of monocyte/macrophages in HCMV pathogenesis

Blood monocytes and tissue macrophages play a major role in the pathogenesis of HCMV infection by serving as target cells in infected organs, as disseminators of the virus throughout the host, or as sites of CMV latency (Michelson, 1997). Monocytes are the primary cell type infected in the blood during acute HCMV infection, as determined by the detection of HCMV DNA and antigens (Sinclair and Sissons, 1996). In addition they are the predominant infiltrating cell type found in infected organs (Sinzger et al., 1996). Infected blood monocytes, likely derived from infection of bone marrow progenitor cells, may disseminate HCMV to other permissive cells of the host. The relatively nonpermissive monocytes and bone marrow progenitor cells harbor latent HCMV DNA (Kondo et al., 1996) and reactivation of IE expression (Taylor-Wiedeman et al., 1994) and infectious virus (Soderberg-Naucler et al., 1997b) has been reported following differentiation of monocytes into macrophages. However, *in vivo* and *in vitro* studies have shown that monocytes are not productive for viral replication and

are abortively infected (Ibanez et al., 1991; Taylor-Wiedeman et al., 1994). On the other hand, macrophages, the differentiated counterparts of monocytes, are long-lived cells and are productively infected in patients with disseminated HCMV disease (Sinzger et al., 1996). So undifferentiated monocytes are proposed to act as a site of HCMV latency followed by active replication in differentiated macrophages (Hanson and Campbell, 2006). Thus monocyte/ macrophages may play role in persistence of virus through latency in monocytes and persistent infection of macrophages.

4.3 HCMV replication in macrophages

Immunohistochemical analyses of tissue sections of various HCMV infected tissues demonstrate viral proteins representing all stages of permissive HCMV infection in tissue macrophage indicating that these cells support complete viral replication cycle (Sinzger et al., 1996). *In vitro* HMCV replication of monocytes is not productive and is limited to early events of gene expression but these cells become permissive to viral replication once differentiate into macrophages (Ibanez et al., 1991; Lathey and Spector, 1991; Taylor-Wiedeman et al., 1994). Similarly differentiated macrophages support MCMV replication as well (Hanson et al., 1999). Thus viral replication in macrophages is dependent upon state of macrophage differentiation.

In fact particular differentiation pathways used for generation of macrophages have significant effects on characteristics of viral replication (Jarvis and Nelson, 2002). A number of monocyte-derived macrophage (MDM) culture systems have been generated to study HCMV replication. Some of these systems were based on production of cytokines from cocultured activated T cells which results in differentiation of monocytes to macrophages. One of method was based on cocultivation of monocytes with concanavalin A (Con A) stimulated autologous nonadherent cells (Ibanez et al., 1991). It was reported that HCMV replication in this culture system was dependent upon presence of CD8+ T cells and production of IFN-γ and TNF-α (Soderberg-Naucler et al., 1997a). Another system used allogenic stimulation of PBMC (peripheral blood mononuclear cells) to induce monocyte differentiation (Soderberg-Naucler et al., 2001). Cellular depletion and cytokine neutralization experiments revealed that this system was dependent upon CD4+ and CD8+ T cells and the cytokines IFN-γ and IL-2 (Soderberg-Naucler et al., 2001). Addition of above said cytokines to monocyte culture was sufficient to generate HCMV permissive MDM. These findings are relevant as elevated levels of IFN-γ and TNF-α has been detected in sera of HCMV patients. Thus cytokines production following T cell activation might be necessary for HCMV replication in macrophages *in vivo*. As TNF stimulates

HCMV IE enhancer/promoter (Prosch et al., 1995; Prosch et al., 2002) and reactivates murine CMV from latency (Hummel et al., 2001; Simon et al., 2005), through activation of NF-κB p50/p65 complex, it is tempting to speculate that HCMV takes the advantage for its growth in these permissive MDM cultures from activation of NF-κB p50/p65 heterodimer in response to TNF. Kaufman et al. observe that NF-κB p50 and p52 are constitutively expressed in human monocytic cells (U937 and THP-1) but nuclear expression of p65 require cellular activation by TNF-α or PMA [phorbol 12-myristate-13-acetate] (Kaufman et al., 1992).

4.4. Determinants of macrophage tropism

Cell tropism of a virus is dependent upon viral as well as cellular factors. HCMV gene expression and successful life cycle of the virus in the host cell is dependent upon state of cellular differentiation. Monocytes are non-permissive for HCMV replication but become permissive for viral replication after differentiation to macrophages. The process of differentiation from monocytes to macrophages is based on a number of structural and functional changes which may affect HCMV replication directly and indirectly. As molecular events in the differentiation of monocytes into macrophages are not fully known, knowledge of cellular factors required for HCMV gene expression remains obscure. In fact differentiating signals activate a number of pathways which determines the cell-type specific gene expression.

Possibly differentiation signals induces transcription factors that are required for successful HCMV gene expression for example NF-κB. Differentiation of monocytes into macrophage results in p65 activation and nuclear translocation (Ammon et al., 2000; Conti et al., 1997). More recently it has been demonstrated that treatment of non-permissive cells with differentiation inducer renders them permissive for IE gene expression and HCMV replication in NF-κB dependent manner (Kitagawa et al., 2009). It has been reported that the expression of corepressors YY1 and ERF was unchanged during differentiation of DC progenitor cells to mature DCs but HDAC-1 was downregulated (Wright et al., 2005). Thus differentiation state-dependent control of viral gene expression in macrophages seems to be the result of interplay between transcriptional activators and repressors.

Search of macrophage tropic HCMV genes is constrained due to availability of permissive macrophage cell culture *in vitro* but MCMV genes associated with replication in macrophages have been identified (Hanson and Campbell, 2006). M36 has been proven as a general determinant of macrophage tropism through its antiapoptotic function and independent of cellular differentiation. A M36 mutant virus replicated well in fibroblasts and endothelial

cells but its replication was significantly impaired in differentially permissive macrophage cell lines and primary macrophages by sensitizing infected macrophages to apoptosis (Menard et al., 2003). Consistently, the M36 mutant virus has impaired dissemination to target organs as compared to WT virus (Hanson and Campbell, 2006). In addition, viral genes M139 to M141 have also been reported to be required for viral replication in macrophages in differentiation-dependent manner. The viral products of these genes do not have an antiapoptotic role but function through mutual interaction and stability (Hanson et al., 2001; Menard et al., 2003). Deletion of either M140 or M141 resulted in impaired MCMV replication in macrophages and spleen tissue but not in liver tissue indicating their role in macrophage tropism *in vivo* (Hanson et al., 2001). Recently, it was reported that deletion of M140 resulted in reduced MCP (M86) and tegument protein M25 and a significant reduction in the number of viral capsids in the nucleus of macrophages (Hanson et al., 2009). Another MCMV gene M45 has been demonstrated to be required for replication in endothelial cells and macrophage cell line IC-21 through its antiapoptotic function (Brune et al., 2001) but its HCMV homolog UL45 does not protect cells from apoptosis and is dispensable for replication in endothelial cells (Hanson and Campbell, 2006).

5. NF-κB signaling pathway and HCMV

5.1. Activation of NF-κB by HCMV

NF-κB has been reported to be stimulated in response to HCMV infection (Kowalik et al., 1993). HCMV infected fibroblasts showed activation of p50 and p65 at RNA level while decrease in IκBα protein level. On the other hand RNA level of IκBα was increased possibly due to presence of NF-κB binding site in IκB promoter indicating a negative feed back mechanism (Kowalik et al., 1993; Yurochko and Huang, 1999). Transactivation of p50 and p65 promoters by HCMV is mediated through Sp-1 which is strongly activated upon HCMV infection and its binding sites are present in p50 and p65 promoters. The viral proteins IE1-72, IE2-55, and IE2-86 physically interact with Sp1 and cooperate with Sp1 to increase p50 and p65 promoter transactivation (Yurochko et al., 1995; Yurochko et al., 1997b). Similarly murine cytomegalovirus IE1 protein activates NF-κB through transactivation of p50 promoter (Gribaudo et al., 1996). Further studies showed biphasic activation of NF-κB in response to HCMV infection (DeMeritt et al., 2004; Yurochko et al., 1995). First phase starts immediately upon infection while second phase is observed 8-12 hpi. First increase of NF-κB was showed to start in the presence of proteins synthesis inhibitors indicating that it represents the release of

pre-formed stores of NF-κB. For first increase of NF-κB, binding of virus with the cells is sufficient and *de novo* synthesis of NF-κB protein is not required while second phase requires *de novo* synthesis of p50 and p65 proteins (Yurochko et al., 1995). Treatment of cells with purified gB and gH proteins has been demonstrated to be sufficient to induce first phase of NF-κB (Yurochko et al., 1997a).

HCMV uses multiple strategies to activate NF-κB. UL55 (gB) and UL75 (gH) glycoprotein ligands have been reported as initiators of the rapid activation of NF-κB and Sp-1 during HCMV infection of fibroblasts (Yurochko et al., 1997a) and monocytes (Yurochko and Huang, 1999). UL144 gene product, which have amino acid sequence similarity with TNF receptor superfamily, activates NF-κB through TRAF-6 and tripartite motif 23 (TRIM23) protein dependent manner (Poole et al., 2009; Poole et al., 2006). Moreover HCMV US28 and HCMV M30 have also been reported to activate NF-κB (Waldhoer et al., 2002). HCMV infection stimulates IKK expression and activity which is required for HCMV-mediated NF-κB activation (Caposio et al., 2004). Another strategy used by HCMV to activate NF-κB includes inclusion of cellular serine-threonine enzyme CKII in the virion. *In vitro* kinase assay shows rapid phosphorylation of IκBα by CKII indicating that cellular kinase incorporated in virion might be important for optimal level of NF-κB immediately after infection of cells (Nogalski et al., 2007). Moreover NF-κB has been thought to be activated via production of reactive oxygen species (Speir et al., 1996). Thus HCMV glycoproteins mediate first phase of NF-κB activation followed by participation of other viral proteins in the scene to activate second phase of NF-κB activation.

5.2. Role of NF-κB signaling in HCMV replication

As NF-κB may mount antiviral immune response through activation of various host inflammatory genes (Ghosh and Karin, 2002) it seems deleterious for viral replication but many viruses including CMV have been reported to utilize NF-κB response elements into their genomes. HCMV genome contains NF-κB binding sites in it and HCMV replication has been reported to depend upon NF-κB. Initial studies reported a positive role of NF-κB in the transcription of IE genes as mutation in major immediate-early promoter (MIEP) resulted in decreased transactivation of the promoter (Sambucetti et al., 1989). Co-transfection of MCMV (Gribaudo et al., 1995) and HCMV (Lee et al., 2004) IE promoter reporter contracts with p50 and p65 plasmids showed that activation of cytomegalovirus IE gene expression is dependent upon NF-κB. Further in this direction, IKK and IκB have been shown to be required for HCMV-

mediated NF-κB activation and maximal MIEP transactivation. Inhibition of IKK1, IKK2, and IκB through transfection of dominant negative phenotype resulted in reduced HCMV-induced NF-κB activation and MIEP transactivation (Caposio et al., 2004; DeMeritt et al., 2004).

Moreover the transactivation of MIEP of HCMV by TNFα (Prosch et al., 1995), HBV HBx protein (Assogba et al., 2002), and LPS (Lee et al., 2004) is mediated by NF-κB. MIEP contains four NF-κB binding sites which binds with NF-κB with similar affinity but differ in their ability to activate the promoter by TNFα. The binding site 1, and 3 is most potent, site 2 is intermediate while site 4 which is most far away from transcription start site has not role in transactivation of MIEP (Prosch et al., 2002).

The importance of NF-κB is not only shown in reporter assay but during viral infection as well. NF-κB and IKK have been shown to be required for NF-κB mediated HCMV gene expression and productive viral replication. Inhibition of IKK2 through transfection of dominant negative phenotype of IKK (dnIKK) and chemical inhibitor AS602868 resulted in reduced expression of early as well as late HCMV genes and viral replication (Caposio et al., 2004; Caposio et al., 2007a). Similarly NF-κB inhibitor drugs blocked IE as well as late gene expression and viral replication in fibroblasts (DeMeritt et al., 2004; DeMeritt et al., 2006). Proteosome inhibitors have also been reported to block HCMV gene expression through NF-κB inhibition (Prosch et al., 2003) but another study has brought into question the role of NF-κB in proteosome-mediated HCMV inhibition (Kaspari et al., 2008). Recently, NF-κB has been proposed as a basis for permissiveness of different cells to HCMV. Hexamethylene bisacetamide, a chemical inducer of differentiation, activated NF-κB in nonpermissive human cells which enabled them to support IE1 and IE2 gene expression and consequently HCMV replication (Kitagawa et al., 2009).

Contrary to above mentioned studies, some reports have presented conflicting data on role of NF-κB in HCMV replication. The neutral role of NF-κB in MIEP transactivation and viral replication has also been reported (Benedict et al., 2004). Another study deleted the binding sites present in the MIEP and concluded that none of four binding sites are required for IE gene expression and viral replication (Gustems et al., 2006). So it remains controversial that whether NF-κB upregulation is beneficial for viral replication or not. In fact it has been reported that inactivation of NF-κB binding sites can be compensated by Elk-1 and SRF binding sites for activation of MIEP transcription and viral replication (Caposio et al., 2010). Some others have reported the inhibitory role of NF-κB in HCMV replication (Eickhoff et al., 2004; Eickhoff and

Cotten, 2005). Consistently, two studies have reported that HCMV inhibit TNFα and IL-1β mediated NF-κB activation late during infection (Jarvis et al., 2006; Montag et al., 2006).

Although the data on role of NF-κB in HCMV biology is apparently controversial but it shows that HCMV is equipped with different tools to manipulate NF-κB pathway. HCMV activates and exploits NF-κB for its own replication while too much activation of NF-κB in the host cell might trigger immune response that will be detrimental for the virus. In fact some viral proteins have been reported to inhibit antiviral innate immune response via inhibition of NF-κB. Tegument protein pp65 (Browne and Shenk, 2003) and IE2-86 (Taylor and Bresnahan, 2006) have been reported to inhibit NF-κB activation. IE2-86 can tempter the NF-κB mediated activation of cellular genes including IL-6 (Gealy et al., 2007). However, NF-κB activation by UL144 has been reported to escape from regulation by IE2-86 (Poole et al., 2008). HCMV encoded IL-10 (cmvIL-10) suppresses NF-κB resulting in inhibition of cytokine expression in monocyte (Nachtwey and Spencer, 2008). So HCMV activates NF-κB pathway to exploit it for its own transcription while inhibit NF-κB pathway to avoid immune surveillance. Thus fine tuning of NF-κB by HCMV result in an optimal level of NF-κB which allow viral replication while evade immune response.

5.3. Role of NF-κB activation in HCMV induced pathology

NF-κB activation is an important feature of several pathologies including cancer, inflammatory diseases and viral infections. HCMV-mediated NF-κB activation might play important role in pathogenesis of HCMV induced disease as NF-κB activation may also result in functional changes in infected monocytes/macrophages.

HCMV has been reported to activate NF-κB and immunoregulatory genes in primary human monocytes (Yurochko and Huang, 1999). In addition, it was shown that HCMV induces monocyte diapedesis and motility through NF-κB pathway. HCMV induces the expression of adhesion molecule through NF-κB resulting in firm adhesion of monocytes to endothelial cells (Smith et al., 2004b; Smith et al., 2007). HCMV infection of monocytes promotes their differentiation into proinflammatory macrophages and render them permissive for the replication of the original input virus following their transendothelial migration (Yurochko, 2008). Transcriptome analysis of NF-κB regulated genes in HCMV infected monocytes reveal that a significant number of genes including proinflammatory genes are regulated in NF-κB dependent manner (Chan et al., 2008b). Interestingly, transcriptome analysis of HCMV infected monocytes show that HCMV infection result a unique M1/M2 polarization phenotype in which

macrophage exhibits characteristics of a proinflammatory M1 as well as anti-inflammatory M2 macrophage. It was reported that 48% of M1 phenotype associated genes while 100% of genes associated with M2 phenotype were NF-κB dependent (Chan et al., 2008a; Chan et al., 2009).

NF-κB is important in virus-induced inflammatory response in the host. HCMV infection leads to production of a number of cytokines and chemokines through NF-κB including IL-8 (Murayama et al., 1997), IL-6 (Carlquist et al., 1999; Iwamoto and Konicek, 1997), interferon beta (Lee et al., 2006b). In addition to macrophages, NF-κB has been demonstrated a key role in the virus-induced inflammatory response in endothelial cells as well (Caposio et al., 2007b). Recently it was reported NF-κB is responsible for cyclooxygenase-2 (Cox-2) expression by HCMV chemokine UL28 indicating a possible role of NF-κB in HCMV associated inflammation and malignancies (Maussang et al., 2009).

IV. Human Immunodeficiency virus-1 (HIV-1)

1. Introduction

HIV-1 infection targets the immune system with progressive impairment of cell mediated immune response resulting in AIDS characterized by severe immune deficiency and the development of opportunistic infections. This disease is an important public health problem as it has caused death of around 25 million people since 1981. People living with HIV/AIDS around the world counts up to 33.4 millions with 22.4 millions in sub-saharan Africa (UNAIDS, 2010). Epidemiological studies show that most of the cases of HIV infection worldwide are the result of sexual transmission across a mucosal surface. The disease was firstly reported in homosexuals and HIV-1 was isolated for the first time in 1983 (Barre-Sinoussi et al., 1983). Other modes of transmission include parental transmission or transmission from mother to infant. Another mode which poses greatest risk of transmission is transfusion of contaminated blood but it is becoming a very rare event due to regular screening of the blood products before transfusion. However the injection drug users which share contaminated syringes and needles is an important risk group, especially for HIV-1/HCV coinfections (Kuritzkes and Walker, 2007).

HIV infects and kills the key cells of immune system resulting in defects in cellular immunity and progressive decline of CD4+ T cells count in peripheral blood. As a consequence of immune deficiency, opportunistic infections and malignancies associated with AIDS appear. In addition the virus establish reservoir of the virus in the CD4+ memory T cells as an integrated provirus. The course of disease varies from few months to many years which can be divided into three stages: primary (acute) infection, chronic (asymptomatic) infection, and advanced disease (AIDS). Primary infection is usually accompanied by relatively nonspecific symptoms of an acute viral illness including fever, pharyngitis, headache, arthralgias, myalgias, malaise, and weight loss. Laboratory findings include decline in absolute CD4+ count and increase in CD8+ count in peripheral blood. A prolonged asymptomatic phase of disease follows the resolution of acute phase. However viral replication and depletion of CD4+ cells continues during this phase leading to advanced stage. AIDS is characterized by opportunistic infections and malignancies if CD4+ count is less than 500 cells/mm^3. Most common opportunistic infections include oral candidiasis, pneumococcal infections, tuberculosis, reactivation of herpes simplex, and VZV. Other complications include *Pneumocystis carinii* pneumonia, Candida esophagitis, disseminated histoplasmosis and other systemic fungal infections, toxoplasma encephalitis, and cryptococcal meningitis, disseminated *Mycobacterium avium* complex infection, reactivation of

JC virus and cytomegalovirus (CMV) infection. AIDS-associated malignancies include Kaposi's sarcoma caused by infection with HHV-8, lymphomas associated with EBV infection; and cervical and anal carcinoma associated with HPV infection (Kuritzkes and Walker, 2007).

2. Structure of Virion

The virion of HIV is a spherical structure of 100-120 nm. The virion contains two copies of a single stranded RNA genome. Viral genome is highly condensed in association with nucleocapsid (NC) and viral dependent DNA polymerase, Pol, or the reverse transcriptase (RT) enclosed in cone-shaped virion core composed of the viral p24 Gag capsid (CA) protein. The core is surrounded by viral shell made up of matrix protein (MA), which in turn is surrounded by envelope. The viral envelope is originated form the host cell membrane acquired during budding. It contains external surface viral glycoproteins gp120 (SU) and gp41 transmembrane (TM) proteins while inner portion is surrounded by MA. The structure of virion is shown in Fig. 8. Accessory proteins Nef and Vif are thought to be associated with core and Vpr outside the core. The location of p6 in the virion remains to be precisely defined. Host proteins including emerin, actin, hsp70 have also been detected in the virion (Ley, 2007).

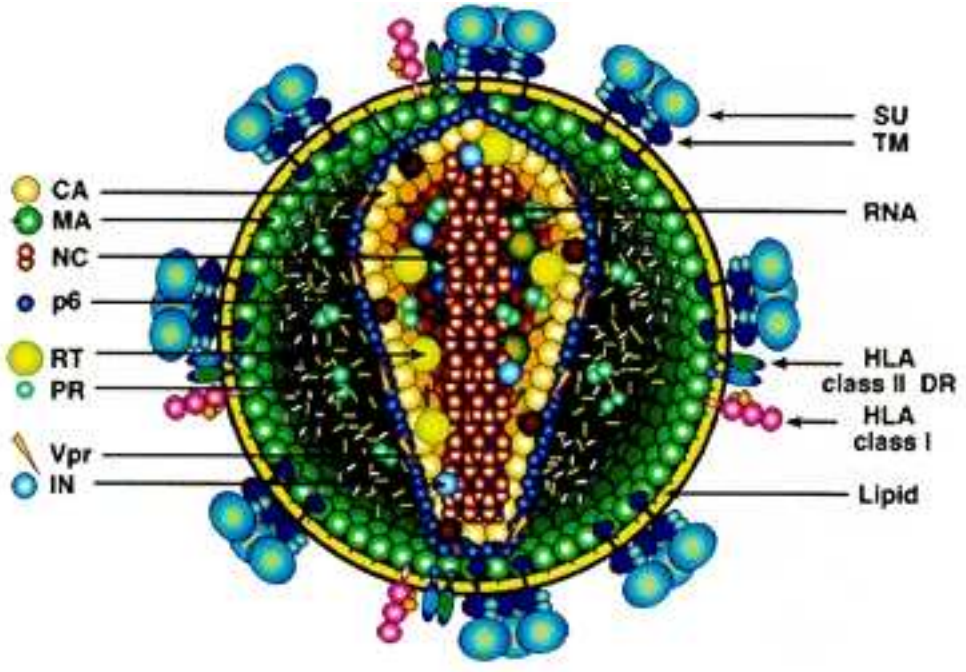


Figure 8: Structure of HIV virion particle. Reproduced from (Coffin et al., 1997).

HIV-1 genome consists of two single-stranded RNA copies containing a number of ORFs. Like other retroviruses, HIV encodes 3 structural genes *gag*, *pol* and *env* which encode

structural proteins and enzymes for productive viral infection. Unlike simple retroviruses like avian sarcoma and leukosis virus (ASLV) and murine leukemia virus (MLV), it contains several additional and overlapping ORFs of multiple functions including Tat, Rev, Nef, Vpr, Vpu and Vif. Three genes *gag*, *pol* and *env* produce three primary polyproteins precursors which are subsequently processed by viral and cellular proteases into mature viral proteins (Fig. 9). Translation product of gag gene Pr55Gag is processed into the MA, CA, NC, and p6 proteins. Gag-Pol polyprotein, Pr160Gag-Pol produces protease (PR), RT, and integrase (IN) proteins through proteolysis. Env precursor, gp160 is cleaved by a cellular enzyme to produce gp120 and gp41 proteins. Two regulatory proteins Tat, and Rev and four accessory proteins Nef, Vpr, Vpu and Vif are translated from spliced mRNA and are specific to HIV (Freed and Martin, 2007).

Tat (Trans-Activator of Transcription) is a potent trans-activator of transcription from the cellular as well as viral promoter and is essential for viral replication (Jeang et al., 1999). Tat interacts with host nuclear proteins and recruits them to Long-Terminal Repeat (LTR), to enhance the elongation of the viral transcript. Tat exerts its action through binding to a 59-nucleotide stem-loop structure, known as the Transactivation Response (TAR) element, located just downstream from the transcription start site in the LTR. Tat directly interacts with cyclin T1, which in turn recruits a cyclin-dependent kinase, CDK9, forming a complex P-TEF-b (positive transcription elongation factor b). P-TEF-b complex hyper-phosphorylates C-terminal domain (CTD) of RNA polymerase II, thereby stimulating transcriptional elongation (Romani et al., 2010).

Rev (regulator of expression of viral proteins) is a 19-kDa protein which is expressed early during infection. It is responsible for export of single-spliced and unspliced RNA from the nucleus to the cytoplasm for eventual expression of viral proteins and production of viral RNA (Li et al., 2005a).

Vpr (Viral protein R) is a 96 kDa accessory protein of HIV-1 which has multiple functions in the viral life cycle and disease progression. An increase in the rate of replication and the stimulatory effect on the HIV-1 LTR promoter was its function first reported (Cohen et al., 1990). An important role of Vpr is the active nuclear translocation of HIV-1 preintegration complex which empowers HIV-1 to infect and replicate in nondividing cells like monocytes and macrophages (Whittaker et al., 2000). In addition Vpr increases virus production by cell cycle arrest in G2 phase thus delaying cells at the point of the cell cycle where the LTR is most active. Synthetic Vpr stimulates LTR through activation of NF-κB, AP-1 and JNK [(c-Jun N-terminal kinase] (Varin et al., 2005). The ability of Vpr to activate HIV transcription is mediated by the

p300 transcriptional co-activator, which in turn enhances the ability of Vpr to activate NF-κB and the HIV enhancer (Romani and Engelbrecht, 2009).

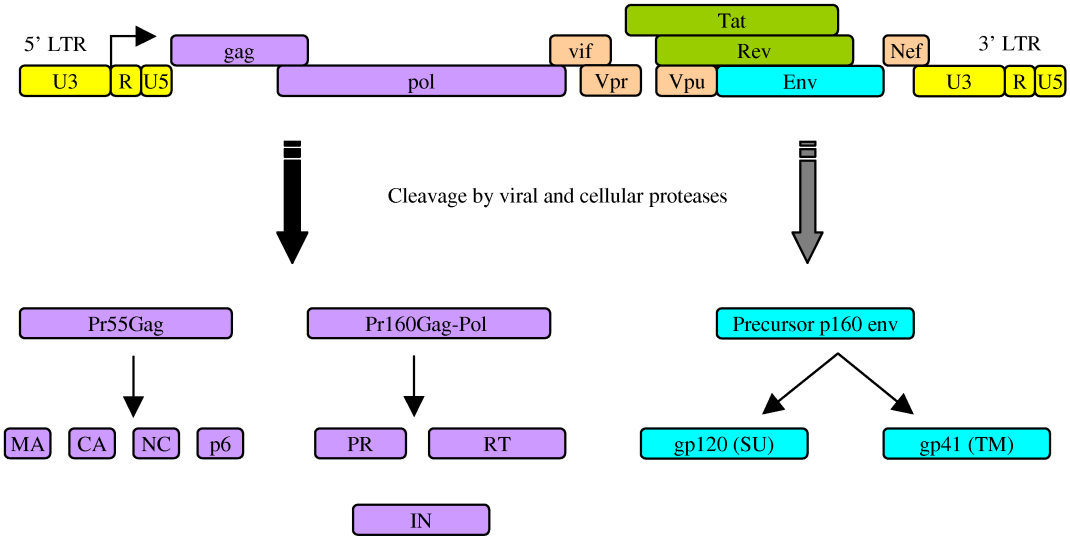


Figure 9: HIV genome and encoded proteins

Vpu (Viral protein U) is a 16-kDa unique accessory protein as it exists in HIV-1 only but not in HIV-2. It is known to degrade CD4 molecule in endoplasmic reticulum (ER) and enhance virion release from cells. Being an adaptor of the β-TrCP, Vpu employs ubiquitin–proteasome pathway to induce ubiquitination of CD4 in the ER resulting in CD4 degradation. Vpu antagonize the host restriction factors, tetherin, which specifically inhibits virion release from the cells (Neil et al., 2001; Nomaguchi et al., 2008). Moreover Vpu leads to the depression of both total and beta-catenin-associated E-cadherin levels through β-TrCP-dependent stabilization of the transcriptional repressor Snail resulting in enhanced viral release from macrophages (Salim and Ratner, 2008). In addition, it downregulates NF-κB through inhibition of β-TrCP dependent degradation of IκB and suppresses apoptosis in HIV infected T cells (Akari et al., 2001).

Vif (Viral infectivity factor) is an accessory protein of HIV which is responsible for viral budding and viral infectivity. Vif mutant virus replicates well in certain cell types including macrophages and T cells but does not replicate in some other cell lines. In fact Vif counteract a host cellular protein known as APOBEC3G (apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G) which is selectively expressed in nonpermissive cells. In the absence of Vif APOBEC3G is incorporated into the virions rendering them non-infectious. Underlying

mechanism of APOBEC3G degradation by Vif involve ubiquitin-proteasome pathway (Goila-Gaur and Strebel, 2008; Henriet et al., 2009).

Nef is a 27 kDa myristoylated protein which is expressed early during the virus life cycle and modulates several signaling pathways. Nef protein plays multiples role in the pathogenesis of HIV-1 virus through downregulation of CD4 and MHC class I molecules and activation of cell signaling (Foster and Garcia, 2007). Nef can modulate HIV-1 transcription via cellular factors that positively or negatively regulate LTR activity. Gene expression profiling demonstrates that Nef expression upregulated at least 15 transcription factors that can activate the HIV-1 LTR (Simmons et al., 2001). Activation of NF-κB, NFAT (Nuclear Factor of Activated T cells), STAT1, STAT3, JNK and AP-1 has also been observed (Khan et al., 2007).

3. Viral life cycle

3.1. Viral binding and entry

Viral entry in susceptible cells starts with adsorption of virus with cell membrane through CD4 molecule. Interaction of virion glycoprotein gp120 with CD4 receptor is required for successful viral entry and fusion (Bour et al., 1995). However it was observed that virus was unable to infect nonhuman cells even after introduction of CD4 into theses cells indicating that CD4 is not the only requirement for viral entry. Subsequent discovery of a coreceptor usage by HIV-1 resolved the issue (Deng et al., 1996; Feng et al., 1996). HIV-1 predominantly uses CXCR4 or CCR5 (CC chemokine receptor-5) as coreceptors which are members of the seven membrane-spanning CC or CXC families of chemokine receptors. Subsequently a classification system of HIV was proposed based on the coreceptor usage. Viral strains (generally Macrophage or M-tropic) that preferentially used CCR5 were termed R5, strains (generally T cell-tropic or T-tropic) that preferentially used CXCR4 were denoted X4, and dual-tropic strains that used both CCR5 and CXCR4 were named R5/X4 isolates (Berger et al., 1998). Interestingly, certain HIV and SIV isolates uses these coreceptors as primary receptors independent of CD4 receptor (Endres et al., 1996; Martin et al., 1997). Moreover, another receptor DC-SIGN (DC-specific intercellular adhesion molecule 3 [ICAM-3] grabbing nonintegrin) is expressed on subset of dendritic cells present in lymph nodes or beneath mucosal surface. Although DC-SIGN does not act as a receptor for viral entry in DCs but it has the capacity to capture the virus through binding with gp120 and efficiently transfer the virions to cells that express CD4 and chemokine receptors (Geijtenbeek et al., 2000).

In fact fusion of virus with cell membrane consists of sequential binding of gp120 with CD4 and then with a coreceptor forming a ternary CD4-coreceptor-gp120 complex and finally conformational changes occurs in gp41 which trigger membrane fusion and entry of viral core into cytoplasm. Virions are partially uncoated in the cytoplasm releasing the viral RNA genome and its reverse transcription starts (Freed, 2004; Freed and Martin, 2007).

3.2. Reverse transcription and integration

Retroviruses convert their single-stranded RNA to double stranded DNA flanked by two LTRs. This process is catalyzed by retroviral RNA dependent DNA polymerase called RT which is a heterodimer of the 66- and 51-kDa subunits. RT has two enzymatic activities; a DNA polymerase activity which can copy RNA as well as DNA template and a ribonuclease H (RNaseH) activity which degrades tRNA and genomic RNA present in RNA-DNA hybrid intermediates. Reverse transcription starts after annealing of tRNALys,3 with the primer binding site and (-)strand DNA synthesis starts from 3'-OH of the tRNA bound to primer binding site. DNA synthesis proceeds till 5' end producing RNA-DNA hybrid. Then, RNaseH breaks down the RNA portion of newly synthesized RNA- DNA hybrid intermediate resulting in ssDNA fragment called (-)strand strong-stop DNA or (-)ssDNA which is transferred to 3' end of RNA genome where it anneals using terminal repeats R. Subsequently, DNA polymerase synthesizes (-)strand DNA converting single stranded RNA to RNA-DNA double helix followed by RNaseH digestion of the RNA template. However some portions are not removed by RNaseH activity (called PPT (polypurin track) and central PPT) which serve as primer for (+)ssDNA synthesis. RNaseH removes the tRNA and the (+)ssDNA is transferred to 3' end of full length (-)strand DNA. Finally, the polymerase completes double stranded DNA helix by synthesizing (+)strand DNA (Abbink and Berkhout, 2008; Freed and Martin, 2007). RT process is an error-prone mechanism as it lacks proofreading activity and it is responsible for generating variation within retroviral populations and emergence of mutants (Svarovskaia et al., 2003).

Reverse transcription product is transported to the nucleus as a components of preintegration complex (PIC) composed of cellular and viral (IN, NC, Vpr and MA) proteins (Bukrinsky et al., 1993). RT product is integrated in the host genome after transportation of PIC to the nucleus. Process of integration is catalyzed by an enzyme called integrase. The process of integration can be divided into two sequential steps. First step is an endonucleotide cleavage called 3' processing in which two nucleotides are removed from the 3' ends of newly formed viral DNA resulting in sticky ends. Second step is called strand transfer in which viral DNA is

inserted covalently into genome of the host cell. Cellular DNA repair mechanisms including polymerization and ligation are required to complete the process (Asante-Appiah and Skalka, 1997; Delelis et al., 2008). Overview of HIV-1 replication cycle is shown in Fig. 10.

In addition to integrated provirus, retroviral DNA is also accumulated in unintegrated extrachromosomal form. Integrase inhibitors, currently in clinical trial, shift the balance from proviral integrated DNA to unintegrated DNA (Cara and Klotman, 2006). It was shown that unintegrated viral DNA in macrophage is stable for at least 30 days and also maintains biological activity with persistent viral gene transcription (Kelly et al., 2008).

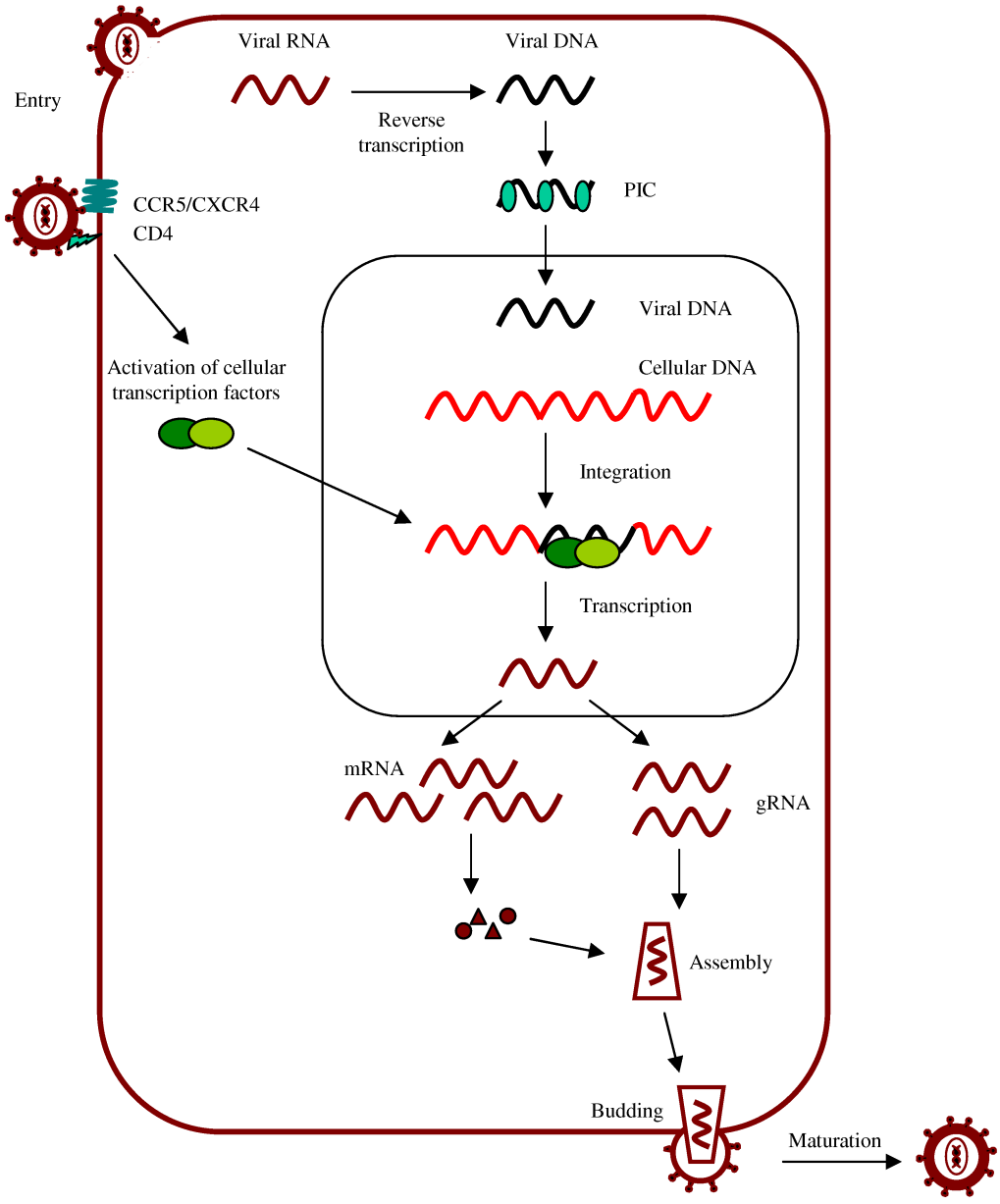


Figure 10: HIV-1 replication cycle

3.3. Viral gene expression

Viral RNA synthesis occurs from integrated DNA genome by RNA polymerase-II (RNA Pol II) enzyme under the control of LTR. Initial event of transcription from viral LTR is the participation of several general transcription factors (GTF). First among these is TFIID which binds with TATA box and adjacent core promoter. Another GTF TFIIB subsequently recognizes TFIID and recruits RNA Pol II to the promoter. Participation of another GTF TFIIH is also required as it phosphorylate the CTD of the RNA Pol II resulting in transcriptional initiation. In addition, CTD can be phosphorylated by some other kinases which include (a) the CDK7/cyclin H subunits of the CDK-activating kinase (CAK) complex associated with TFIIH, (b) the CDK8/cyclin C component of the RNA pol II holoenzyme, and (c) the CDK9/cyclin T1 subunits of the P-TEF-b (Freed and Martin, 2007). Viral protein Tat plays a key role in the viral transcription to produce high level of viral RNA through formation of more efficient RNA pol II complex. Tat interacts with a number of host cell proteins and recruits them to LTR, to enhance the elongation of the viral transcript. Tat directly interacts with cyclin T1, which in turn recruits a cyclin-dependent kinase, CDK9, to the TAR element forming a complex known as P-TEF-b. Recruitment of P-TEF-b by Tat and TAR results in hyper-phosphorylation of the CTD of RNA polymerase II, thereby stimulating transcriptional elongation (Freed, 2004; Romani et al., 2010; Turner and Summers, 1999).

3.3.A. Structure of LTR

HIV-1 LTR is composed of three domains; U3, R and U5. The R (repeat) region is a 96-nt repeat present between U3 and U5 (Fig. 11). Transcription start site is located at the junction of R and U3. LTR contains various functional regions including transactivation response element (TAR), core, enhancer and modulatory region. TAR (nt +1 to +60) is found within the R region and binds with Tat. The core promoter (nt –78 to –1), the enhancer (nt –105 to –79) and the modulatory (nt –454 to –104) region are found in U3 domain. HIV-1 LTR contains cis-acting DNA sequences for a number of cellular host proteins (Fig. 11) which regulate transcription from LTR (Freed and Martin, 2007; Pereira et al., 2000). Core promoter region contains a RNA pol II TATA box and the initiator (Inr) which initiates transcription by RNA pol II. Three Sp-1 binding sites are located in the core promoter immediately upstream to the TATA box (Harrich et al., 1989). Enhancer region contains binding sites for C/EBF family members, T3R, EBP-1, RBF-1 and two binding sites for NF-κB. Transcription factors having cognate sequences in modulatory region include but not limited to RBF-1, RBF-2, LEF-1, Ets-1, AP-1, NFAT1, NF-IL-6, and USF. Negative regulatory element (NRE; nt –340 to –184) found in

modulatory regions is thought to negatively regulate transcription from LTR. Binding sites for cellular transcriptional factors are also found in adjacent gag leader sequence (GLS) region for example Sp1, AP-1, NF-κB, NFAT, IRF-1, IRF-2, USF etc (Pereira et al., 2000; Roebuck and Saifuddin, 1999).

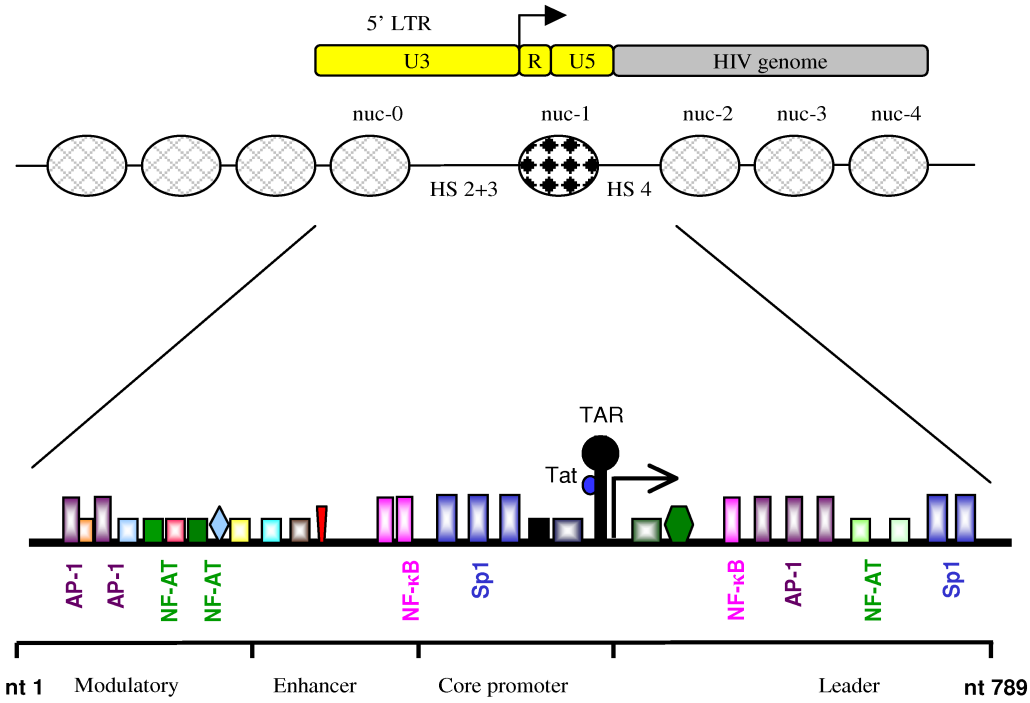


Figure 11: Structure of HIV-1 LTR

3.3.B. Transcription from LTR by cellular transcription factors

A number of transcription factors activated during HIV-1 infection including NF-κB, Sp-1 and AP-1 have their binding sites in the LTR and participate in the transcriptional regulation by recruitment of RNA Pol II on transcriptional start site. Transcription from LTR may be altered by alteration in level and functional properties of cellular transcription factors. In addition, these transcription factors have the potential to activate gene expression through cooperative interaction.

NF-κB is the major modulator of LTR transcription and inducible HIV-1 gene expression. NF-κB activates the viral transcription through two NF-κB binding sites present in the enhancer of LTR (Freed and Martin, 2007). Moreover, NF-κB mediated transcription from LTR has also been reported from sites present in GLS region (Mallardo et al., 1996). Sp-1 activates LTR transcription through three Sp-1 binding sites located in the core promoter immediately upstream to a RNA pol II TATA box (Jones et al., 1986). Mutations of these

binding sites individually or in pair resulted in minimal decreases in basal and tat-induced transcriptional activation. However, mutations of all three Sp1-binding sites resulted in a marked decrease in tat induction (Harrich et al., 1989). Cooperation between NF-κB and Sp-1 has been reported to result in activation of HIV-1 LTR (Perkins et al., 1993). In addition functional and physical synergism between Sp-1 and COUP-TF results in enhanced transcriptional activity of the HIV-1 LTR through the Sp1 element (Rohr et al., 1997). AP-1, composed of Jun homodimers or Jun/Fos heterodimers, is another transcriptional factors reported to activate LTR transcription and HIV-1 replication. Three AP-1 binding sites present in region +92 to +167 downstream to transcription start site are important for viral replication while two sites within modulatory region are unable to bind with AP-1 (Rohr et al., 2003). AP-1 physically interacts with NF-κB and synergistically transactivates the HIV-1 LTR through the NF-κB binding sites during signal-dependent activation of HIV-1 replication in latently infected U1 cells (Yang et al., 1999). NFAT family of transcription factors is sequestered in the cytoplasm but translocates to the nucleus, through dephosphorylation by calcium-activated calcineurin, after cellular activation (Crabtree, 1999). NFAT binds with NF-κB binding sites in LTR and act in synergy with NF-κB to positively activate HIV-1 transcription (Freed and Martin, 2007; Kinoshita et al., 1997). Other positive regulators of HIV transcription include NFAT5 (Ranjbar et al., 2006), SerpinB2 (Darnell et al., 2006), and CCAAT enhancer binding protein beta (Mohan et al., 2008) while Staf50 (Bouazzaoui et al., 2006) prothymosin-α (Mosoian et al., 2006), and STAT5 Delta (Crotti et al., 2007) have been reported as negative regulators of HIV transcription.

3.3.C. NF-κB regulated transcription from LTR by viral proteins

A number of viral proteins and cytokines for example TNF-α have also been reported to activate LTR-driven transcription and viral replication which might be dependent or independent of transcription factors activation. TNF-α stimulates HIV-1 replication in chronically infected promonocytic U1 cell line through activation of NF-κB and subsequent transactivation of the proviral LTR. In fact the features observed in promonocytic cells U937 and macrophages in response to exogenous HIV-1 proteins are similar to those observed following TNF-α treatment (Herbein and Khan, 2008). Exogenous Tat, Vpr and Nef are associated with an increase in the binding of transcription factors to its consensus sequence in the viral promoter resulting in activation of LTR and viral replication (Manna and Aggarwal, 2000; Varin et al., 2005; Varin et al., 2003).

Nef positively regulate HIV-1 gene expression through activation of cellular factors involved in LTR transcription including NFAT (Manninen et al., 2000), NF-κB (Varin et al.,

2003), AP-1 (Varin et al., 2003), and CDK9 (Simmons et al., 2001). However, negative impact of Nef derived miR-N367 on LTR transcription has also been reported (Omoto and Fujii, 2005). Moreover myristoylated Nef and TNF-α synergistically activate NF-κB and AP-1 in U937 cells and primary macrophages resulting in enhanced stimulation of the HIV-1 LTR, and subsequently in enhanced viral replication in both chronically infected promonocytic U1 cells and acutely HIV-1-infected primary macrophages via lipid rafts (Herbein et al., 2008). Similarly, exogenous Tat protein has been shown to amplify the activity of TNF-α (Westendorp et al., 1995) but the mechanism of action is not the same. Manna and Aggarwal have reported that $p56^{lck}$ is required for HIV-Tat-induced activation of NF-κB, AP-1, JNK while TNF-induced activation was found to be $p56^{lck}$-independent (Manna and Aggarwal, 2000). Vpr protein has stimulatory effect on the HIV-1 replication as virion-associated Vpr increases transcription from LTR promoter by delaying cells at the G2 phase of the cell cycle where the LTR is most active (Hrimech et al., 1999) and exogenous HIV-1 Vpr protein activates the transcription factors NF-κB and AP-1 and JNK in macrophages and stimulates HIV-1 LTR resulting in enhanced viral replication in the chronically infected promonocytic cells U1 and macrophages (Varin et al., 2005). Moreover, Vpr activate transcription through direct binding with LTR in sequence specific manner (Burdo et al., 2004). Similarly envelope glycoprotein gp120 activate NF-κB through IκB-IKK pathway following engagement with CD4 (Bossis et al., 2002). Recently, it was demonstrated that NF-κB dependent gp120 mediated activation of LTR transcription and HIV replication signals through procaspase 8 (Bren et al., 2009) which is in line with the essential and unexpected role of procaspase 8 in NF-κB activation (Lemmers et al., 2007; Su et al., 2005).

3.3.D. Chromatin structure and transcription of HIV

Transcription of HIV is prone to be regulated by local chromatin structure as it integrates in the host genome. Studies of chromatin organization have demonstrated the presence of two nucleosomes at the viral LTR called nuc-0 and nuc-1 (Verdin et al., 1993). Nuc-0 is found immediately upstream of the modulatory region and nuc-1 immediately downstream of the transcription start site. The positioning of nucleosomes define two nucleosome-free, open regions of chromatin in LTR called hypersensitive sites (HS) called HS2+3 and HS4. HS2+3 correspond to modulatory and enhancer/core promoter regions while HS4 to leader region downstream of the transcription start size (Fig. 11). Interestingly, nuc-1 is disrupted following TPA or TNF-α treatment (Verdin et al., 1993). Activation of HIV-1 LTR has been reported following treatment of transiently as well as stably transfected cells and acutely as well as

latently infected cell lines with HDAC inhibitors. HDAC inhibition results in disruption of nuc-1 and hyperacetylation of nuc-1 histones in the LTR (Quivy et al., 2007).

Thus nuc-1 seems to be constitutively deacetylated by HDACs in the absence of activation and acetylated by HATs following activation. Recruitment of HAT and HDACs to viral promoter is believed to be mediated by multiple host factors. YY1 recruits HDAC1 to nuc-1 decreasing LTR gene expression through maintenance of acetylation status of nuc-1 histones (He and Margolis, 2002). Other host factors known to recruit HDAC1 to LTR to repress HIV-1 gene expression include transcription factor AP-4 [Activating Protein-4] (Imai and Okamoto, 2006), C-promoter binding factor-1 [CBF-1] (Tyagi and Karn, 2007), c-Myc, Sp1 (Jiang et al., 2007), and the corepressor COUP-TF interacting protein 2 [CTIP2] (Marban et al., 2007). NF-κB is an important transcription factor which is regulated by transcriptional coactivators possessing HAT activity as well as by HDACs. The p65 subunit of NF-κB derives transcription from LTR while absence of transactivating heterodimers can result in latency. In the absence of induction, p50-HDAC1 complexes constitutively bind the latent HIV LTR and induce histone deacetylation and repressive changes in chromatin structure of the HIV LTR (Williams et al., 2006). Accordingly, synergistic activation of HIV-1 gene expression in latently infected monocytic and lymphoid cell lines has been reported with combined treatment of HDAC inhibitors and prostratrin, a non-tumor-promoting NF-κB inducer (Reuse et al., 2009). Recruitment of HATs, including CBP, P/CAF and GCN5 to the promoter and acetylation of both histones H3 and H4 occurs following activation before the onset of viral mRNA transcription (Lusic et al., 2003). C/EBP proteins recruit coactivators to LTR and physically interact with HATs suggesting its possible participation in remodeling the chromatin organization of the HIV-1 provirus (Lee et al., 2002). Several transcription factors including NF-κB, AP-1, Sp1, IRF, c-Myc, glucocorticoid receptor, NFAT, Ets-1, and TCF-1α/LEF-1 and HIV protein Tat have been reported to interact with HATs resulting in their recruitment to LTR and acetylation of nuc-1 histones (Quivy and Van Lint, 2002).

3.4. Virion assembly, budding and maturation

Assembly of structural protein occurs at the plasma membrane and Gag protein is necessary and sufficient for formation of noninfectious virus-like particles. Gag and gag-pol polyproteins are transported to the plasma membrane to form a bud which gives rise to a spherical immature particle containing the mature Env glycoproteins. The PR completes the proteolytic processing of Gag and Pol proteins resulting in cone-shaped core characteristic of

mature HIV-1 virion after budding. HIV-1 assembly occurs on endosomal membranes in the interior of infected macrophages (Freed and Martin, 2007). Recently it was reasoned that intracellular structures in which HIV assembly occurs are not endosome but are intracellular plasma membrane domain containing the tetraspanins CD81, CD9, and CD53 (Deneka et al., 2007).

4. HIV Nef protein

The *nef* gene is highly conserved in all primate lentiviruses e.g. HIV-1, HIV-2 and SIV and encodes a 25-34 kDa myristoylated protein abundantly during the early phase of HIV infection. Nef protein was firstly reported as negative regulator of transcription (Ahmad and Venkatesan, 1988), thus named as 'negative factor' but latter studies determined this protein as a major determinant of viral replication and HIV-1 pathogenesis. Rhesus macaques infected with SIV virus deficient for Nef have low viral loads and do not progress to the disease state (Kestler et al., 1991). Furthermore, the absence of intact Nef sequences in a described epidemiological cohort of HIV-1-infected patients with non-progressive-disease clearly substantiates a role for Nef in disease progression (Deacon et al., 1995; Kirchhoff et al., 1995).

Nef protein contains 206 amino acids with methionine at position 1 and glycine at position 2. Myristylation of glycine at position 2 required for almost all biological functions of Nef. One region near the amino terminal has high abundance of basic amino acids while two other regions are rich in acidic amino acids. In addition charged amino acids are accumulated in the central region of Nef. Proline rich motif present in Nef interacts with SH3 domain in multiple cellular proteins (Fig. 12).

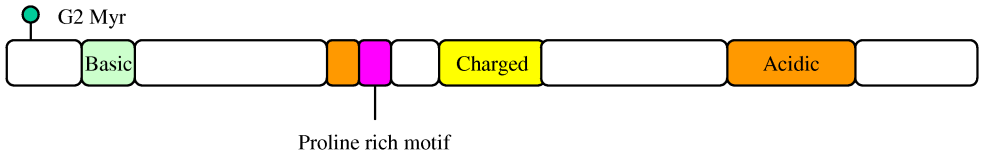


Figure 12: Structure of HIV-1 Nef

The intracellular efficiency of HIV-1 gene expression and replication is due in part to the ability of HIV-1 to co-opt host signaling pathways to activate viral transcription. Nef can modulate HIV-1 transcription via cellular factors that positively or negatively regulate LTR activity. Gene expression profiling demonstrates that Nef expression upregulated at least 15 transcription factors that can activate the HIV-1 LTR (Simmons et al., 2001). Exogenous HIV

Nef and SIV Nef protein stimulates NF-κB activation in promonocytic cells U937 and MDMs leading to sustained LTR activation (Olivetta et al., 2003; Varin et al., 2003). Moreover exogenous Nef protein activates AP-1, and induce the rapid phosphorylation of MAPK family members (i.e., ERK1/2, p38, and JNK), interferon regulatory factor 3 (IRF-3), and both the α and β subunits of the IκB kinase (IKK) complex, required for the activation of the NF-κB pathway in MDMs (Mangino et al., 2007; Varin et al., 2003). Thus the features observed in promonocytic cells and primary macrophages following exposure to exogenous Nef are very similar to those observed following TNF treatment. Both exogenous Nef and TNF activate NF-κB, AP-1 and MAPK, suggesting that they might modulate the cellular machinery in a similar way and therefore might have the same effect on HIV-1 replication in mononuclear phagocytes. In addition, myristoylated Nef and TNF-α synergistically activate NF-κB and AP-1 in macrophages resulting in enhanced stimulation of the HIV-1 LTR, and subsequently in enhanced viral replication via lipid rafts (Herbein et al., 2008). Nef expression has been reported to activate NFAT. This transcription factor has also been shown to activate HIV-1 LTR-directed transcription by interacting with an unusual binding site that overlaps with the NF-κB-responsive element (Manninen et al., 2000). Specific activation of STAT1, STAT3 and AP-1 has also been observed in MDMs (Biggs et al., 1999; Federico et al., 2001; Percario et al., 2003). Involvement of Ras in Nef-mediated activation of HIV-1 from latency has also been reported (Tobiume et al., 2002). Nef may optimize viral replication by enhancing Tat mediated gene expression from the LTR by activating signaling pathways that result in a concomitant increase in the activation of general transcription factors (Joseph et al., 2005). A recent study shows that Nef activates Tat-mediated viral transcription through a hnRNP-K-nucleated signaling complex (Wolf et al., 2008).

The HIV infection results in progressive and selective depletion of CD4+ and CD8+ T lymphocytes culminating with the formation of viral reservoir. Nef protein plays an important role in these two hallmarks. Nef seems to promote the killing of bystander cells while at the same time protecting the HIV-1-infected host cell from cell death. Nef has been reported to contribute to the depletion of T cells through a bystander effect via direct killing effect of soluble Nef or induction of apoptosis-inducing ligands on macrophages and dendritic cells (DCs). Soluble HIV-1 Nef protein possesses binding affinity to the cell surface of a variety of blood cells including uninfected CD4+T lymphocytes, CD8+T lymphocytes and macrophages. The binding of Nef to the cell surface, upon cross-linking, induces apoptotic cytolysis of the cells through a Fas independent pathway (Okada et al., 1997). The myristoylated N-terminal

region of Nef seems to have severe membrane disordering properties and when present in the extracellular medium causes rapid lysis *in vitro* of a wide range of CD4+ and CD4- T cells, suggesting a role for extracellular Nef in the depletion of bystander cells (Azad, 2000). Apoptosis of CD4+ T cells by extracellular Nef protein occurs through CXCR4 surface receptors and activation of caspase 3 (James et al., 2004). Extracellular Nef can also cause apoptosis of human neurons and primary human brain microvascular endothelial cells via activation of caspases (Acheampong et al., 2005). Nef may also play a role in bystander killing via induction of Fas ligand. Endogenous expression of Nef induces the FasL (Fas ligand) expression via interaction with the T cell receptor zeta chain to promotes the killing of bystander cells (Xu et al., 1999). Similarly, it was demonstrated that extracellular Nef up-regulates apoptosis-inducing ligands, such as TNF-α and FasL molecules, on DCs for the enhancement of CD8+ T cell apoptosis via caspase 8 activation (Quaranta et al., 2004).

Nef can prevent both Fas- and TNF-receptor-mediated deaths observed in HIV-infected T cells via interaction with the apoptosis signal regulating kinase-1 (ASK-1). Nef inhibits ASK-1, caspase 3 and caspase 8 activation, resulting in the blockade of apoptosis in HIV-infected cells (Geleziunas et al., 2001). In addition, Nef co-precipitates phosphatidylinositol-3-kinase (PI3K) in 293T cells, especially its p85 subunit, via sequences present within the N- and C-terminus of Nef (Wolf et al., 2001). This interaction leads to the activation of PI3K, which in turn phosphorylates Bad, the proapoptotic member of the Bcl-2 family. Phosphorylation of Bad results in its inactivation and subsequently inhibits mitochondrial related apoptosis. Nef-mediated inactivation of Bad is also dependent on the activation of p21-activated kinase 2 (PAK2) in T cells. In fact, Nef interacts with PAK2 via its PXXP motif (Renkema et al., 2001; Wolf et al., 2001). The Nef protection to the HIV-1-induced apoptosis in MDMs is also Bad-correlated (Olivetta and Federico, 2006). Another anti-apoptotic effect of Nef in T cells involves the interaction with the tumor suppressor protein p53 via its N-terminal extremity (amino acids 1 to 57), both in the cytoplasm and in the nucleus of the cell. This interaction results in a decrease of p53 half-life, in a decrease of p53-mediated transcriptional activity, and ultimately blocks p53-mediated apoptosis (Greenway et al., 2002). Moreover Nef can produce survival signal in myeloid cells through STAT-3 dependent pathway and also through Erk MAPK pathway dependent Bcl-XL induction (Briggs et al., 2001; Choi and Smithgall, 2004). So a model can be proposed in which extracellular Nef protein induce expression of apoptosis-inducing ligand in macrophages/ DCs for bystander T-cell killing while protecting them from apoptosis. The role

of Nef in antiapoptotic signaling in macrophages has implications in the formation of viral reservoirs that resist the current antiviral regimens.

5. HIV-1 cellular tropism

5.1. HIV-1 target cells *in vivo* and *in vitro*

Main targets of HIV-1 are CD4+ T lymphocytes, CD4+ cells of macrophage lineage and a subset of dendritic cells. A widely used classification classifies viral isolates on the basis of their tropism. The viral strains which preferably infect MDMs are termed M-tropic while which infect human CD4+ T-cells lines are named T-tropic. The viral strains which infect both macrophage and T-cell lines are called dual-tropic (Collman et al., 1992). M-tropic viruses are usually isolated from the individuals early after primary infection and remain the main virus during asymptomatic phase of HIV-1 infection (Zhu et al., 1993). On the other hand T-topic viruses are isolated late in the disease course in approximately 40% of the patients (Freed and Martin, 2007).

CD4+ T lymphocytes are the major target of HIV-1 replication (Klatzmann et al., 1984) resulting in their depletion, significant reduction in their number and immunosuppression during AIDS. Activation of T cells is believed to be required for viral replication *in vitro* and the production of infectious virus from the lymphocytes depends upon its state of activation. Activation signals from IL-2, IL-4, IL7, or IL-15, in the absence of other stimuli, have been reported to confer HIV-1 susceptibility to resting T cells (Unutmaz et al., 1999). The expression of HIV-1 co-receptor CXCR4 on PBMC and responsiveness to SDF-1α is up-regulated during T cell activation (Bleul et al., 1997). Another study shows that increased CXCR4-dependent HIV-1 fusion in activated T cells is correlated with increased CD4/CXCR4 association (Zaitseva et al., 2005). Antigen-mediated T cell activation result in transition of lymphocyte from G0 to G1 phase of cell cycle and CD28 costimulation result in increased production of IL-2 and progression through G1, S, and mitosis (Freed and Martin, 2007). CD28 costimulation itself has been reported to increase surface expression of CXCR4 (Secchiero et al., 2000). Absence of costimulatory signals arrests the cell cycle at G1a/G1b transition point which is required for productive viral replication (Korin and Zack, 1998).

Dendritic cells (DCs) are antigen presenting cells which are found in the tissue particularly those that provide an environmental interface including skin, genital and intestinal mucosa. DCs are considered first line of defense against sexually transmitted HIV-1 infection as

they are readily infected after the intravaginal inoculation of rhesus monkeys with SIV (Hu et al., 2000). In addition productive *in vitro* infection of DCs has also been reported, however it is less productive than cultures of CD4+ T cells (Piguet and Steinman, 2007). Moreover the immature DCs are more susceptible to viral infection than mature cells (Granelli-Piperno et al., 1998). In fact, the expression of different surface markers is dependent upon subset of DCs and their activation stage. Different mechanisms have been reported as a basis of restricted HIV-1 infection of mature DCs including downregulation of CCR5 (Cavrois et al., 2006) and increase of APOBEC3G (Pion et al., 2006). Moreover DCs express DC-SIGN which has the capacity to retain the virions for subsequent transfer to lymphocytes termed *trans*-infection (Geijtenbeek et al., 2000).

5.2. Role of monocyte/macrophages in HIV pathogenesis

The ability of cells of the human monocyte/macrophage lineage to host HIV-1 replication while resisting direct lysis by the virus and apoptotic cell death is believed to contribute to their ability to serve as a reservoir for viral replication in the host (Verani et al., 2005). The development of these stable antiretroviral resistant cells represents the major obstacle in achieving a complete sterile cure. Macrophages are the earlier targets of HIV-1 which has the potential to transmit the virus to T cells. Productive and latent HIV-1 replication of tissue macrophages and brain macrophages in infected patients has been reported. Accordingly, MDMs are productively permissive for *in vitro* viral replication (Cassol et al., 2006). In addition, survival signals are activated in HIV-1 infected macrophages. HIV-1 infection render macrophages resistant to TNF and cycloheximide induced apoptosis through activation of NF-κB (DeLuca et al., 1998). Absence of apoptosis in macrophages by HIV-1 was correlated with upregulation of Bcl-x(L) through NF-κB dependent mechanism (Choi and Smithgall, 2004; Guillemard et al., 2004). Activation of Bcl-2 during HIV-1 infection of macrophages has also been reported (McElhinny et al., 1995). The ability of monocytes/macrophages to migrate to organs and reside in tissues contributes in dissemination of virus to CNS, lymph nodes, lung, bone marrow, and gastrointestinal tract. The microglia may act an important HIV-1 reservoir in patients under highly active antiretroviral therapy (HAART) treatment due to inefficient transfer of drugs across blood-brain barrier. Thus viral reservoirs in macrophages may contribute to the rebound of plasma viremia after cessation of drug administration (Freed and Martin, 2007).

Progressive depletion of both CD4+ and CD8+ populations of T cells is a characteristic feature of HIV-1 infection. Direct lysis of infected T cells and apoptosis or programmed cell

death of bystander cells has been proposed as the underlying mechanism of this T cell loss in HIV-1 patients. Death of bystander cells may occurs through proapoptotic virus proteins, infected cells derived cytotoxic factors, and altered expression of cellular apoptosis regulatory proteins by lymphocytes and antigen presenting cells especially macrophages. Soluble factors, especially those produced by macrophages play an important role in the induction of cell death. Macrophages are cellular reservoirs for HIV-1, and are particularly implicated in the death of CD4+ and CD8+ T cells during HIV-1 infection (Herbein, 2006; Lum and Badley, 2003).

5.3. Replication of HIV-1 in macrophages

Macrophages and T lymphocytes are important targets of HIV replication but viral replication cycle of HIV-1 in these cell types differs considerably as the cellular milieu of macrophages is not the same as lymphocytes in terms of surface receptors, signal transduction, transcriptional factors etc. Thus viral replication in macrophages has peculiar characteristics that help it to act as a viral reservoir and site of viral persistence. HIV-1 infection of MDMs shows slow and progressive kinetics of viral replication (Collman et al., 1992). HIV-1 infected MDMs continue to produce infectious virions at a lower, but steady, rate throughout the course of culture (Marchant et al., 2006). Infection of MDMs with M-tropic virus BAL results in linear increase in HIV-1 expression overtime with a peak at day 14 postinfection (Bagnarelli et al., 1996).

HIV enters macrophage through membrane fusion via interaction of envelope protein gp120 with CD4 and CCR5/CXCR4. Macrophage tropism of R5 HIV strains is believed to link with the expression of CCR5 on macrophages. In fact the conformation of CXCR4 is expressed as monomer on the T cells while high-molecular-weight species on macrophages which does not interact with CD4 and is less efficient for viral entry (Schmidtmayerova et al., 2007). Although most laboratory adapted X4 strains are unable to replicate in macrophages, productive infection of tissue macrophages by primary X4 HIV-1 isolates has been reported (Jayakumar et al., 2005). In fact conformation of gp120 has been reported as a basis of differential capacity of X4 virus to infect macrophages (Ghaffari et al., 2005). Another report shows that susceptibility of macrophages to X4 strains depends upon its activation state (Bakri et al., 2001).

Reverse transcription of HIV-1 in macrophages is slower than T cells. In fact virus uses the cellular dNTP pool for its proviral DNA synthesis which is much limited in non-dividing macrophages (Rich et al., 1992; Schmidtmayerova et al., 2007). Nucleotide concentrations in macrophages has been demonstrated to be 130-250 fold lower than activated human CD4+ T

cells (Diamond et al., 2004). In addition Vpx has been reported critical for reverse transcription of HIV-2 in MDMs. *Vpx*-minus mutant directed the synthesis of viral DNA comparably and normally with WT virus in lymphocytic cells, no appreciable viral DNA was detected in MDMs infected with the mutant (Fujita et al., 2008). Nuclear import of newly synthesized viral DNA in macrophages is independent of mitosis and requires PIC composed of viral and cellular protein. The PIC is transported to the nucleus through nuclear pores via an active, energy-dependent mechanism (Bukrinsky et al., 1992). The viral proteins present in the PIC including IN, MA, and Vpr, contain sequences which serve as nuclear localization signals for nuclear import through importin-α/β. Recently, it was shown that nuclear import of Vpr by importin-α is crucial for HIV-1 replication in macrophages (Nitahara-Kasahara et al., 2007). Inner nuclear-envelope protein, emerin, is another important factor for efficient interaction of viral DNA with host chromatin in macrophages. Infection of macrophages lacking emerin resulted in insufficient integration (Jacque and Stevenson, 2006). Another study reported little role for emerin in dividing cells and demonstrate that this is not universally important for HIV-1 infectivity (Shun et al., 2007) indicating a possible role for emerin in macrophages only.

Transcription of HIV genome is regulated by interplay of a number of host factors which are differentially present in macrophages and T cells (Rohr et al., 2003). One of these factors which regulate HIV transcription is CCAAT/enhancer binding proteins (C/EBPs) which regulates transcription from LTR. C/EBPs protein and sites have been reported as unique macrophage-specific regulatory mechanism for HIV-1 replication as these are required for HIV-1 replication in macrophages but not CD4+ T cells (Henderson and Calame, 1997). Consistently, dominant negative isoform of C/EBPβ induced by *Mycobacterium tuberculosis*, lipopolysaccharide, or IFN-β inhibits HIV-1 transcription (Honda et al., 1998). In line with above studies, C/EBPβ gene expression was predominantly localized to macrophages in intestine of SIV infected macaques (Mohan et al., 2008). Cell type specific regulation of LTR by Sp-1 (McAllister et al., 2000) and AP-1 has also been reported (Canonne-Hergaux et al., 1995). Another study demonstrates that selectively reduced tat mRNA expression with a concomitant decrease in Tat activity is responsible for the decline in productive HIV-1 infection in MDMs (Sonza et al., 2002).

Finally, the progeny virus is released from the plasma membrane in lymphocytes while in case of MDMs virions are accumulate in intracytoplasmic vesicles where they are accumulated (Orenstein et al., 1988). The release of virus is thought to occur through fusion between membrane of intracytoplasmic vesicles and plasma membrane (Raposo et al., 2002).

Virions assembled intracellularly retain infectivity for extended period of time (Sharova et al., 2005) and virus has developed strategy to avoid acidification of these compartments unlike virus free compartments in the same infected cell (Jouve et al., 2007). Thus understanding the replication of HIV in macrophages may reveal new therapeutic targets and may help to eliminate these viral reservoirs from AIDS patients.

5.4. Determinants of macrophage tropism

The permissiveness of macrophages to HIV-1 infection is attributed to cellular factors as well as viral determinants located in the glycoprotein gp120. Natural variation in third variable region of gp120, V3 loop, is associated with decreased fitness in primary macrophages (Lobritz et al., 2007). Another study linked X4 or R5 tropism with the charge of residues 11, 24, and 25 of the V3 loops from primary isolates and suggest that a positively charged amino acid at these positions defines X4; otherwise R5 tropism (Cardozo et al., 2007). Recently, role of Vpx to counteract antiviral block to lentivirus replication in macrophages was reported through targeting of VprBP-associated Cullin 4 ubiquitin ligase to enable efficient reverse transcription (Srivastava et al., 2008) and through damaged DNA binding protein 1 [DDB1] (Sharova et al., 2008).

Monocytes and macrophages are differentially permissive for HIV-1 replication. In fact different combinations of transcription factors present in different physiological or activation stage may regulate HIV LTR in a distinct manner. Replication of HIV-1 in monocytes is limited and efficient replication in MDMs requires full differentiation (Rich et al., 1992; Schuitemaker et al., 1992). A number of changes occur in cell during differentiation from monocytes to macrophages which might be linked with macrophage permissiveness to HIV-1. Expression of CCR5 increases during differentiation of monocyte to macrophages and is thought to mediate macrophage susceptibility to HIV-1 infection (Tuttle et al., 1998). However, viral entry may not be the only factor limiting viral replication in monocytes as postentry block in monocytes has also been reported. HIV-1 based vectors pseudotyped with VSV-G envelope, which enter the cells by endocytosis bypassing HIV-1 receptor/co-receptor, were unable to transduce freshly isolated monocytes (Neil et al., 2001). In addition, it was demonstrated that inability of X4-tropic viruses to infect macrophages correlate with bad conformation of the CXCR4 present on the macrophages which were unable to associate with CD4 for efficient viral entry (Lapham et al., 1999). APOBEC3 family has emerged as an important postentry restriction factor in the recent years and has been reported as critical factor in monocyte resistance to HIV infection. It

was shown that APOBEC3G and APOBEC3A expression are highly expressed in monocytes and diminishes during differentiation into macrophages. Consistently, APOBEC3A silencing reversed the monocyte resistance to HIV infection (Peng et al., 2007). Other cellular restriction factor against HIV-1 infection is TRIM5α which binds to incoming retroviruses via its C-terminal PRY/SPRY domain and recruits them to the proteasome resulting in their degradation before synthesis of viral DNA can occur (Huthoff and Towers, 2008).

In addition, expression pattern of cellular factors required for transcription from LTR changes during macrophage differentiation. NF-κB p50 homodimers is constitutively expressed in freshly isolated monocytes while their differentiation into MDMs and alveolar macrophages results in activation of transcriptionally active p50/p65 heterodimers while decreasing p50 homodimers (Lewin et al., 1997). Similarly, HIV infection resulted in activation of p50/p65 heterodimers in MDMs but not in monocytes (Lewin et al., 1997). In addition the ratio of Sp1 to Sp3 increases during differentiation of monocytes to macrophages (Kilareski et al., 2009). Another important factor which is require for HIV-1 replication in macrophages is Tat cofactor Cyclin T1 which is induced during monocyte differentiation (Yu et al., 2006). However, another study was unable to rescue Tat transactivation by transient expression Cyclin T1 in undifferentiated monocytes suggesting that CycT1 is not the only limiting factor of HIV-1 infection in monocytes (Dong et al., 2009). It was observed that the CDK9 expression remained constant during monocyte differentiation but phosphorylation of CDK9 was enhanced, which correlated with significantly increased HIV-1 infection in macrophages (Dong et al., 2009). Thus differentiation of monocytes to macrophages result in enhanced expression of positive regulators while decreased expression of negative regulators of HIV replication.

6. NF-κB signaling pathway and HIV-1

6.1. NF-κB regulated transcription from HIV-1 LTR

NF-κB is believed an important pathway for the regulation of LTR activity and is a potential candidate for antiviral therapy. NF-κB activates the LTR transcription through two NF-κB binding sites present in the enhancer of LTR and mutation of NF-κB sites abolishes the LTR activity (Nabel and Baltimore, 1987). IκBα super-repressor mutant which interferes with NF-κB activity has been reported to reduce HIV-1 replication in Jurkat cells (Kwon et al., 1998). Inhibition of NF-κB activity through dominant-negative IκBα reduces HIV transcription in macrophages as well (Asin et al., 2001). In addition, HIV-1 expressing a proteolysis-resistant IκBα (IkappaB-alphaS32/36A) reduced viral expression and was highly attenuated in both

Jurkat and peripheral blood mononuclear cells (Quinto et al., 1999). Accordingly, it was demonstrated that the replication of SIVmac239 virus expressing a proteolysis-resistant inhibitor of NF-κB, IκB-alphaS32/36A, was inhibited in cell cultures and in the SIV macaque model of AIDS (Quinto et al., 2004). In line with above findings, occupancy of HIV enhancer by p50/p65 heterodimer is required for LTR transcription in myeloid cells (Jacque et al., 1996).

However some previous studies demonstrated dispensable role of NF-κB in HIV-1 replication as deletion and mutation of NF-κB binding sites in HIV-1 promoter did not impair viral replication (Leonard et al., 1989; Ross et al., 1991). In fact the LTR sequences are optimized to suit a specific nuclear environment and requirement of NF-κB in the transcription of HIV-1 has been reported to be dependent upon amount of constitutive nuclear NF-κB in the cells. HIV-1 replication seems to be more dependent on NF-κB in cells with high basal level of NF-κB while independent of NF-κB in cells with low level of NF-κB activity. A panel of T-cell lines with different basal levels of NF-κB was infected with WT and NF-κB mutant viruses and it was demonstrated that mutation of NF-κB sites significantly decreased the viral transcription in one T-cell line with a constitutively high level of NF-κB, PM1, while another T-cell line with a low basal level of NF-κB, SupT1, did not show any effect on viral transcription or growth rate (Chen et al., 1997). It is likely that other transcription factors, probably present in more amounts in cells with low NF-κB activity, are compensating for absence of NF-κB on LTR of mutant viruses. Verhoef *et al.* isolated a fastly replicating mutant with one-nucleotide deletion in NF-κB binding site from long-term culture of Tat-mutant virus and found that the loss of NF-κB binding activity correlated with binding of another transcription factors GABP (Verhoef et al., 1999). Accordingly, introduction of GABP site in virus yielded a gain of fitness in SupT1 cells, known to contain low level of NF-κB activity (Verhoef et al., 1999).

In fact, deletion of all NF-κB and Sp-1 binding sites resulted in incompetent viral replication (Leonard et al., 1989) indicating that binding sites of different transcription factors in LTR possess functional redundancy which ensures virus replication in different cell types and is capable of changing depending on the particular combination of transcriptional factors present (Ross et al., 1991). Accordingly, cooperation between NF-κB and Sp-1 has been reported to result in activation of HIV-1 LTR (Perkins et al., 1993). Similarly, NF-κB and AP-1 synergistically transactivates the HIV-1 LTR through the NF-κB binding sites during signal-dependent activation of HIV-1 replication in latently infected U1 cells (Yang et al., 1999). Moreover IRF-1 form a functional complex with NF-κB at kappaB sites of LTR and is required

for full NF-κB-mediated LTR transcription (Sgarbanti et al., 2008). NF-κB occupancy at LTR has also been reported to be modulated by FoxP3 (Holmes et al., 2007).

As nuclear/cytoplasmic shuttling is required for efficient NF-κB activity, it was shown that inhibition of nuclear export by leptomycin B results in accumulation of IκB and p65 in nucleus resulting in inhibition of HIV-LTR dependent transcription and HIV replication in CD4+ T lymphocytes (Coiras et al., 2009). Another recent study reports a novel NF-κB independent control of HIV replication by IκB. It was observed that IκBα negatively regulates the HIV-1 expression and replication by directly binding to Tat, which results decreases transcriptional potential of Tat (Puca et al., 2007). Inhibition of NF-κB pathway by multiple approaches has been reported to inhibit HIV-1 replication. Inhibition of NF-κB by RelA-associated inhibitor (Takada et al., 2002) and IKK inhibitor noraristeromycin has been reported to inhibit HIV-1 replication (Asamitsu et al., 2008). Moreover, proteosome inhibitors known to inhibit NF-κB activation can block HIV transcription as well (Yu et al., 2009). In addition a number of viral proteins including Tat, Vpr, Nef and gp120 are associated with an increase in the binding of NF-κB to its consensus sequences in the viral promoter resulting in activation of LTR and viral replication (Bossis et al., 2002; Manna and Aggarwal, 2000; Varin et al., 2005; Varin et al., 2003).

6.2. NF-κB regulated transcription from LTR by coinfections

HIV-infection is related with severe immune suppression and HIV infected individuals are exposed to a number of opportunistic and non-opportunistic infections. As NF-κB is activated in response to different pathogens, interaction and synergism between HIV-1, other pathogens and their proteins may enhance HIV-1 replication through activation of NF-κB and may fuel the progression of HIV infection.

Activation of NF-κB and HIV-1 transcription from LTR has been reported in response to a number of viral infections. HSV infection (Gimble et al., 1988; Schafer et al., 1996; Vlach and Pitha, 1992) and its IE proteins ICP0 and ICP4 (Margolis et al., 1992) and StpC protein (Raymond et al., 2007) have been reported to activate HIV-1 LTR activity. Role of NF-κB in HSV-induced LTR activation was confirmed using an IKK inhibitor (Amici et al., 2004). Moreover HHV-6 infection (Ensoli et al., 1989), CMV E1/E2 proteins (Dal Monte et al., 1997) and EBV EBNA2 (Scala et al., 1993) can also activate LTR activity through NF-κB binding sites. In addition HBV (Gomez-Gonzalo et al., 2001; Siddiqui et al., 1989), and influenza virus (Flory et al., 2000; Sun et al., 2005a) have also been reported to activate HIV-1 LTR in T cells.

In addition HTLV-1 protein Tax (Cheng et al., 1998) and FLICE (Sun et al., 2005b) can activate HIV-1 replication in T cells in NF-κB dependent manner. Other viruses known to regulate HIV-1 transcription through NF-κB include human foamy virus (Marino et al., 1995), West Nile virus (Kesson and King, 2001), VZV (de Maisieres et al., 1998) and vaccinia virus (Chang et al., 1994). As HIV-1 and many of these viruses preferably infect distinct cell types, relevancy of the results in HIV-1 disease process is always questioned. However, the findings of transactivation of HIV-1 LTR by CMV IE1 protein in U937 cells seems relevant as both viruses replicate in macrophages (Kim et al., 1996).

An interesting study demonstrated that HSV virion contact with HIV-1 infected macrophage was able to activate HIV-1 expression in acutely infected MDMs. They show that infectious or heat-inactivated HSV type 1 or 2 virions induced HIV-1 expression in NF-κB dependent manner and neutralizing antibodies to the HSV glycoprotein gB or gD markedly attenuated these virion-mediated effects on HIV-1 expression in macrophages (Moriuchi et al., 2000). In fact the viral proteins found in sera of patients for example HIV Nef protein and HCV core protein (Fujii et al., 1996; Pivert et al., 2006) may have important implication in the progression of AIDS process through a bystander mechanism.

Other stimuli including bacteria *Mycobacterium tuberculosis* (Bernier et al., 1998a) and *Mycobacterium avium* (Ghassemi et al., 2000; Ghassemi et al., 2003), Mycoplasma and its lipid-associated membrane proteins [LAMPs] (Shimizu et al., 2004), and parasite Leshmania (Bernier et al., 1998b) have also be reported to activate transcription from HIV-1 LTR.

V. HIV/HCV coinfection

HIV-1 and Hepatitis C virus (HCV) are major health problems and establish persistent infection in the host. Owing to same route of transmission coinfection of HIV-1 and HCV is common. However prevalence of HIV-1/HCV coinfection varies in different HIV subpopulations ranging from <10% in homosexual men while >80% in injection drug users (Sulkowski and Thomas, 2003).

1. Virological interaction between HIV-1 and HCV

1.1. Impact of HIV-1 on pathogenesis of HCV disease

HIV-1/HCV coinfection is believed to accelerate HCV induced liver disease. Immune suppression resulting from HIV-1 infection can enhance HCV replication and impair HCV clearance. Higher HCV viral load has been reported in coinfected patients as compared with those infected with HCV alone (Bonacini et al., 1999; Sherman et al., 1993). Interestingly, the increase in viral load in coinfected patients correlated with decrease in intrahepatic CD4+ cell count (Canchis et al., 2004). Moreover, HCV has been detected in two major target cell types of HIV-1 replication, monocytes/macrophages and lymphoid cells, in co-infected patients (Laskus et al., 2000). Primary human macrophages have also been shown to be permissive for *in vitro* HCV replication and preceding as well as near-simultaneous HIV-1 infection make the macrophages more susceptible to HCV infection (Laskus et al., 2004).

In addition progression of fibrosis is more rapid in coinfected patients as compared to HCV mono-infected patients (Benhamou et al., 1999; Graham et al., 2001; Sulkowski et al., 2007). Similarly, onset of cirrhosis has been reported to be unusually rapid during HIV-1/HCV coinfection. It was observed that mean interval from estimated time of HCV infection to cirrhosis was 23.2 years in HIV-negative while 6.9 years in HIV-positive patients (Soto et al., 1997). Moreover, progression to hepatocellular carcinoma occurs at a younger age and after a shorter period of HCV infection in subjects coinfected with HIV (Garcia-Samaniego et al., 2001). The reasons for acceleration of liver disease in HIV-1/HCV coinfected patients are not clear. However, a number of mechanisms including direct viral effects, dysregulation of the immune system toward a profibrotic state, and increase in rate of apoptosis have been proposed (Kim and Chung, 2009). As several liver types including hepatocytes have been reported to be permissive for HIV-1 replication, direct interaction between both viruses in the liver is likely (Blackard and Sherman, 2008; Xiao et al., 2008). Moreover indirect interaction between

exogenous viral proteins of both viruses might contribute in the pathogenesis of HIV-1/HCV coinfection. The apoptosis of hepatocytes via surface binding of HIV-1 glycoprotein gp120 and HCV envelope protein E2 has been demonstrated via CXCR5 (Munshi et al., 2003).

1.2. Effect of HCV on pathogenesis of HIV-1 disease

Similar to the effects of HIV-1 on HCV infection, HCV has also been reported to increase the clinical progression of HIV-1 disease. A Swiss cohort study of 3111 HIV-infected patients out of which 1157 were coinfected with HCV showed that the probability of progression to clinical AIDS or to AIDS-related death are independently associated with HCV seropositivity. In contrast, smaller CD4-cell recovery was also correlated with HCV seropositivity indicating the possible role of lower CD4+ count in accelerated progression to AIDS (Greub et al., 2000). Other studies, although with less number of patients, show similar results (Daar et al., 2001; De Luca et al., 2002; Lesens et al., 1999; Piroth et al., 2000). Another study show that HIV disease progression differs by HCV genotype and is especially faster in individuals whose HCV infection involves more than one HCV genotype (van Asten and Prins, 2004). In addition HCV infection influences the tolerability of HAART negatively. Around 30% of patients discontinue antiretroviral therapy due to hepatotoxicity and HCV increases the risk of hepatotoxicity and discontinuation rates in coinfected patients (Roe and Hall, 2008). Incidence of HCV in patients with hepatotoxicity associated with antiretroviral therapy was 43% (Sulkowski et al., 2002). The effect of HCV infection on HIV-1 replication is not known.

2. Hepatitis C virus (HCV)

HCV infection is an infection of liver resulting in chronic hepatitis and many of which leads to cirrhosis and hepatocellular carcinoma. It infects 170 million people worldwide corresponding to 3% of world population. The virus is efficiently transmitted through large and repeated percutaneous exposures to blood e.g. transfusion or transplantation from infectious donors, illicit injection drug use. The transmission has been reported to be less efficient by single small-dose percutaneous exposures (e.g., accidental needle sticks) or by mucosal exposures to infected fluids (e.g., from mother to child, through sexual intercourse). Interestingly, coinfection with HIV increases the rate of transmission four- to fivefold (Lemon et al., 2007; Pawlotsky, 2004).

HCV is a RNA virus classified in the family *Flaviviridae* under the genus Hepacivirus. HCV virion is roughly a spherical particle of 55-65 nm in diameter with a icosahedral

nucleocapsid surrounded by lipid-containing envelope containing viral glycoproteins in the shape of spike-like projections (Kaito et al., 1994). Viral genome consists of a 9.6 kb single-stranded, positive-sense RNA genome with a single large ORF which encodes a large polyprotein with short 5' and 3' nontranlational RNA (NTR) segments. Replication of HCV occurs in the cytoplasm and the viral genome act as messenger RNA following its release in the cytoplasm. Translation of ORF is mediated by binding of the ribosomal 40S subunit with an internal ribosome entry site (IRES) present in the 5' NTR (Lemon et al., 2007).

The amino-terminal region of polyprotein comprise of structural proteins (Core, E1, E2) and central region include p7 and NS2 which are required for viral morphogenesis and release but are not essential for RNA replication. Carboxy-terminal that comprises the nonstructural proteins (NS3, NS4A, NS4B, NS5A, and NS5B) required for RNA replication. The large polyproteins is co- and post-translationally processed by cellular and viral proteases to produce individual proteins (Lemon et al., 2007).

2.1 HCV cellular tropism

The major site of viral replication is liver with the abundant presence of viral RNA. Presence of viral RNA and viral proteins (core, NS3) in the infected hepatocytes has been showed with highly sensitive in *situ* hybridization and immuohistochemistry techniques (Pal et al., 2006). Some studies have demonstrated the presence of viral antigen, viral RNA and negative-strand HCV RNA in PBMCs, biliary epithelial or sinusoidal lining cells, lymph nodes, spleen and brain indicating that HCV is not limited to hepatocytes (Laskus et al., 2000; Nouri-Aria et al., 1995; Okuda et al., 1999; Pal et al., 2006). However the replication of HCV in extrahepatic sites remains controversial and is not fully established yet. A number of studies demonstrated *in vitro* replication of virus in human hepatoma lines and primary cultured hepatocytes but the viral production was not robust (Bartenschlager and Lohmann, 2001). More recently, some robust culture systems have been developed (Lindenbach et al., 2005; Zhong et al., 2005). In addition HCV can replicate in lymphoid (Sung et al., 2003) and monocyte/macrophage cultures (Laskus et al., 2004). A recent study demonstrate the presence of HCV monocytic reservoirs in peripheral blood of HCV infected patients (Coquillard and Patterson, 2009).

2.2 HCV core protein

Core protein is a major structural protein of HCV which form the nucleocapsid. It is generated from the amino-terminal of precursor polyprotein after cleavage by a host signal

peptidase yielding the immature form of the protein which is further processed by a host signal peptide peptidase, yielding the mature form of the protein (Dubuisson, 2007). The proteins in found in sera of infected patients (Laperche et al., 2005; Leary et al., 2006; Pivert et al., 2006) and is released from tissue culture cell lines stably expressing the HCV core protein (Sabile et al., 1999).

Core protein is important for viral replication as it participate in viral RNA synthesis through its interaction with viral RNA polymerase (Kang et al., 2009), in encapsidation of the viral genome through interaction with 5'-UTR (Fan et al., 1999) and in virus assembly. In addition core protein has multiple effects on signal transduction, transcriptional activation, apoptosis and cell cycle regulation. HCV core protein activates a number of cell signaling pathways including NF-κB, AP-1, (Kato et al., 2000), JNK (Park et al., 2001), Raf1/MAPK (Aoki et al., 2000), STAT3 (Yoshida et al., 2002), Wnt/β-catenin pathway (Fukutomi et al., 2005), androgen-receptor signaling (Kanda et al., 2008), PI3K signaling (Alisi et al., 2008). It has been reported to modulate the immune system through inhibition of T cell responsiveness and IFN-α mediated antiviral activity (Irshad and Dhar, 2006). Importantly, core protein has transformation potential as shown in cell culture systems and this protein has been reported to alter several pathway potentially implicated in carcinogenesis (Levrero, 2006).

2.3. Activation of NF-κB during HCV infection

HCV core proteins can activate or inhibit NF-κB activation which seems dependent upon cell type and/or the genotype of the virus. HCV core protein has been reported to enhance lymphotoxin-β receptor and TNF-α triggered NF-κB activation. In addition HCV core protects cells from Fas and TNF-α induced apoptosis partially through activation of NF-κB (Marusawa et al., 1999; You et al., 1999). Another study showed that HCV core protein interacts with TNFR1 signaling complex and potentiates TNF-α-induced NF-κB activation through TRAF2-IKKβ-dependent pathway (Chung et al., 2001). It was shown that overexpression of HCV core proteins activates NF-κB through TRAF2/6 and IKKβ dependent pathway as dominant negative form of IKKβ and TRAF2/6 significantly blocked HCV core protein induced activation of the NF-κB pathway (Yoshida et al., 2001). In addition core protein may enhance NF-κB activation due to Hbx (Kanda et al., 2006) or alcohol (Kim et al., 2001). In contrast some studies have reported inhibition of NF-κB in response to HCV core proteins. HCV core protein interferes with IKK signalsome and IKKβ kinase activity resulting in NF-κB inhibition (Joo et al., 2005). Accordingly some other studies have also reported inhibition of NF-κB by HCV core (Lasarte et

al., 2003; Shrivastava et al., 1998). The difference between different studies is not fully known but genetic variation might be a reasonable explanation (Mann et al., 2006; Ray et al., 2002).

In recent years, constitutive activation of NF-κB has been reported to be implicated in hepatic oncogenesis through transcriptional regulation of genes involved in cellular transformation, proliferation, survival, invasion, and metastasis (Arsura and Cavin, 2005). HCV infection may also induce this process as higher frequency of NF-κB nuclear staining has been observed in HCV-infected liver tissues than in normal tissues (Tai et al., 2000). HCV core protein activates NF-κB and protects cells from TNF induced apoptosis in hepatocytes (Marusawa et al., 1999; Tai et al., 2000). Activation of NF-κB by core proteins may have important implications in HCV pathogenesis. HCV core induced NF-κB activation could confer resistance to TNF-α -induced apoptosis in HCV core-transfected cells (Tai et al., 2000). In addition it induces the expression of iNOS, pro- and anti-inflammatory cytokines through NF-κB (de Lucas et al., 2003; Dolganiuc et al., 2003). HCV core proteins may induce proliferation of hepatoma cells through enhancement of TFG-α expression in NF-κB dependent manner (Sato et al., 2006).

In addition to HCV core protein some other HCV proteins can also activate NF-κB but HCV core protein has been reported to have the strongest effect in reporter assay (Kato et al., 2000). Other proteins of HCV known to activate NF-κB include non-structural protein 2 [NS2] (Oem et al., 2008), NS3 (Hassan et al., 2007; Lu et al., 2008); NS4B (Li et al., 2009), and NS5A (Waris et al., 2003). NS5A has been reported to enhance HCV core protein induced NF-κB activation (Liao et al., 2005a). In line with above stated studies, *in vitro* HCV infection of primary hepatocytes has been reported to activate NF-κB and upregulated NF-κB-responsive genes (Guitart et al., 2005).

3

Materials and Methods

1. Reagents

Anti-p50, anti-p65, anti-RelB, anti-c-Rel, anti-p52, and anti-Bcl-3, and the single-stranded NF-κB oligonucleotide and mutated oligonucleotide were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-IκBα, anti-phospho-IκBα, anti-IKKα, anti-IKKβ, anti-IKKγ, anti-p- IKKγ, anti-p-IKKαβ, and anti-TRAF proteins were purchased from Cell Signaling Technologies (Beverly, MA); anti-pIKKα was purchased from Abcam (Cambridge, UK), anti-p-IKKβ was purchased from US Biological (Swampscatt, MA), and peroxidase-conjugated secondary anti-rabbit and anti-mouse immunoglobulin were obtained from Jackson ImmunoResearch (West Grove, PA). Antibodies against HIV-1 Nef and HCV core protein were provided by Chemicon/Millipore (Temecula, CA) and US biologicals, respectively. Recombinant myristoylated Nef protein derived from SF-2 HIV-1 strain was purchased from Jena Bioscience (Jena, Germany) and recombinant HCV core protein was purchased from US biologicals (Swampscott, MA). A scrambled control, p52, and Bcl-3 siRNA duplex were purchased from Santa Cruz Biotechnology. All other reagents were obtained from Sigma-Aldrich (St. Louis, MO) unless stated otherwise. Peripheral blood and human AB serum of a healthy donor was provided by Etablissement Français du Sang (EFS), Bourgogne Franche-Comté, France. Cell culture flasks and cell scrapers were purchased from Nunc (Roskilde, Denmark).

2. Cell culture

2.1. Isolation and culture of monocyte-derived macrophages

PBMCs were isolated by Ficoll gradient centrifugation, as previously reported (Herbein et al., 1998). Blood from a healthy donor was diluted with equal amounts of PBS (phosphate buffered saline), overlaid on Ficoll medium (Eurobio, Les Ulis, France), and centrifuged at 900×g for 30 min at 25°C. The PBMC band was removed and washed twice with PBS. Cell count was determined by Malassez cytometer (Poly Labo, Strasbourg, France) and the cells were resuspended in serum-free RPMI-1640 medium. The cells were plated in plastic cell culture flasks and incubated at 37°C. After 2 h, nonadherent cells were removed in order to enrich the culture for monocytes. Adherent cells were washed with sterile PBS and cultured in RPMI-1640 medium supplemented with 10% (v/v) human AB serum, penicillin (100 IU/ml), and streptomycin (100 µg/ml) for 7-10 days to allow the monocytes to differentiate into macrophages.

2.2. Cell lines

Promonocytic cells U937 were obtained from the American Tissue Cell Culture Collection (ATCC, Manassas, VA) and the promonocytic cell line U1 cells were a gift from Dr. C. Van Lint (Université Libre de Bruxelles, Belgium). The U1 cells are derived from cells surviving acute infection of the U937 cell line, contains two integrated HIV copies per cells (Folks et al., 1988). U937 and U1 cells were cultivated in RPMI-1640 supplemented with 10% fetal bovine serum, 1% L-glutamine and 1% penicillin/streptomycin. MRC5 human fibroblasts were obtained from BioMérieux (Marcy l'Étoile, France). MRC5 human fibroblasts were cultured in MEM supplemented with 10% FBS, penicillin (100 IU/ml), and streptomycin (100 µg/ml).

3. Preparation of viral stock

3.1. HCMV

Cell-free virus stock was prepared by propagating four strains of HCMV (high passage laboratory strain AD169; a clinical isolate HCMV-DB; TB40/E; and TB40/F) in MRC5 human fibroblasts as previously described (Coaquette et al., 2004). AD169 is a highly passaged laboratory strain of HCMV originally isolated from the adenoids of a child (Murphy et al., 2003). The clinical isolate HCMV-DB used in this study was isolated from a cervical swab specimen from a 30-year-old pregnant woman. Viral strains TB40/E and TB40/F were kindly provided by Dr C. Sinzger, University of Tuebingen, Tuebingen Germany. Strains TB40/E and TB40/F have been developed by 22 passages in endothelial cells and fibroblasts, respectively (Sinzger et al., 1999b). MRC5 cells were infected at 37°C with a viral isolate when the monolayer was confluent and virus was collected when cytopathic effects were >90%. Supernatants were clarified by centrifugation and stored at –80°C until use. Virus titers were determined by plaque-forming assay in MRC5 human fibroblasts as previously described (Arrode et al., 2002).

3.2. HIV

HIV-1 stock was prepared by propagation of HIV-1 89.6 strain in MDMs cultures as described previously (Crowe et al., 2004). HIV 89.6 is a laboratory-adapted macrophage-tropic strain of HIV-1 (Collman et al., 1992). MDMs cultures were infected on days 7-10 following isolation with viral stock overnight at 37°C in BSL-3 lab. Fresh medium was added to the culture the following day. Half of the supernatant was collected on day 7, 14, and 21

postinfection and the culture was supplemented with fresh medium. Supernatants were clarified by centrifugation, filtered through a 0.44 µM filter, aliquoted, and stored at –80°C until use. Concentration of capsid protein p24 in viral stocks was measured by an ELISA assay (Innogenetics, Gent Belgium).

4. Measurement of viral replication

4.1. Real-time PCR for quantification of HCMV titer

Quantification of HCMV titer in cell culture supernatants was performed by real-time PCR as previously described (Coaquette et al., 2004). DNA was extracted from 100 µl of supernatant using the KingFisher automatic instrument (Thermo Labsystems, Finland) and a QIAamp kit (Qiagen Inc., Valencia, CA) according to the recommendations of the manufacturer. After elution in 100 µl of buffer, 5 µl of the DNA was used for PCR. HCMV DNA in the samples was quantified by real-time PCR using Taqman technology on ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) according to the manufacturer's recommendations. The sequences of the primers used were 5' AACATAAGGACTTTTCACACTTTT and 5' GAATACAGACACTTAGAGCTCGGGT. The sequence of the TaqMan PCR probes was FAM 5' CTGGCCAGCACGTATCCCAACAGCA 3' TAMRA. Reaction samples had a final volume of 25 µl and contained 5µl of extracted DNA and 20µl of the cocktail containing 12.5µl of 2× TaqMan Universal PCR Master Mix (Applied Biosystems), 800 nM of each primer, 200 nM TaqMan probe. An internal positive control, TaqMan® Exogenous Internal Positive Control Reagents VIC™ Probe (Applied Biosystems), was included in each run to distinguish target negatives from PCR inhibition. A distilled water sample and a positive control with 3,000 copies of HCMV DNA were processed in parallel with samples. The amplification conditions were 50°C for 2 min and 95°C for 10 min followed by 45 cycles of 95°C for 15 s and 60°C for 1 min. To generate the HCMV external quantitative standard curve, PCR was performed on a plasmid containing one copy of the target sequence that had been serially diluted from 2 × 106 to 2 × 102 copies/ml. Final quantification was performed using comparative threshold value (CT) method using the ABI Prim 7000 SDS software.

4.2. Measurement of HIV-1 replication

The HIV-1 replication was measured by determining the concentration of capsid protein p24 in the supernatants using an ELISA assay (Innogenetics, Gent Belgium). The wells of

microtiter plates have been coated with human polyclonal antibodies to HIV. The test samples were incubated together with mixture of biotinylated anti-p24 monocolonal antibodies in microtiter plate. Next, well is washed and incubated with peroxidase-conjugated streptavidin and peroxidase substrate. Blue color produced by chromogen turn yellow when reaction is stopped with sulphuric acid. Absorbance was measured on an ELISA reader at 450 nm (PerkinElmer, Tarku, Finland).

5. HCMV entry assay

Viral entry into MDMs and MRC5 fibroblasts was assayed as described previously (Sainz et al., 2005). Cells were incubated at 37°C with HCMV-DB at MOIs of 1 and 10 for 2 hours and washed three times with PBS. Cells were treated with 0.25% trypsin for 10 minutes to release the virions that had adhered to the surface but had not entered the cell. The cells were pelleted and washed once with serum neutralization solution and three times with PBS. DNA was extracted from the cell pellet using the KingFisher automatic instrument (Thermo Labsystems, Finland) and a QIAamp kit (Qiagen Inc., Valencia, CA) according to the recommendations of the manufacturers. Samples of eluted DNA were analyzed by PCR using primers specific for the MIEP of HCMV (sense, 5’ -TGG GAC TTT CCT ACT TGG- 3’; antisense, 5’ -CCA GGC GAT CTG ACG GTT- 3’). The β-globin PCR gene was used as an internal control (sense, 5’ - TCC CCT CCT ACC CCT ACT TTC TA - 3’; antisense, 5’ - TGC CTG GAC TAA TCT GCA AGA G - 3’). The amplification products were resolved by 2% agarose gel electrophoresis and visualized by ethidium bromide staining.

6. Preparation of cellular extracts

Preparation of nuclear and cytoplasmic extracts was performed as previously described (Varin et al., 2003). Cells were scraped from the plastic surface of the culture dishes and washed with wash buffer (10 mM HEPES, pH 7.6, 10 mM KCl, 2 mM $MgCl_2$, 1 mM EDTA). Cell pellets were then incubated on ice with cytoplasmic isolation buffer (10 mM HEPES, pH 7.6, 10 mM KCl, 2 mM $MgCl_2$, 1 mM EDTA, 0.02% NP-40). Cytoplasmic extracts were collected by centrifugation and the nuclear pellets were washed twice in wash buffer, spun, and incubated for 15 min on ice with nuclear isolation buffer (20 mM HEPES pH 7.6, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 25% glycerol). Supernatants containing nuclear extracts were collected by centrifugation and stored at –80°C. Total cellular extract for western blot and co-IP experiments was prepared by incubation of cell pallet with RIPA buffer (150 mM NaCl, 1% NP-10, 0.5% Sodium deoxycholate, 0.1% SDS, 50 mM Tris pH 8) for 15 minutes on ice.

Supernatants containing total cellular extracts were collected by centrifugation and stored at –80°C. Protease inhibitors (1 mM DTT, 1 mM PMSF, 1 µg/ml aprotinin, 1 µg/ml leupeptin, 1 µg/ml pepstatin) and phosphatase inhibitors were added to all solutions. Protein concentration in cellular extracts was determined by the Bradford method using a BioPhotometer (Eppendorf, Hamburg, Germany).

7. Electrophoretic Mobility Shift Assay

To measure NF-κB activation, electrophoretic mobility shift assay (EMSA) was carried out as previously described (Davis et al., 2004). Briefly, nuclear extracts were incubated with 20 fmol of biotin-end-labeled 45-mer double-stranded NF-κB oligonucleotide,

5-TTGTTACAAGGGACTTTCCGCTGGGGACTTTCCAGGGAGGCGTGG-3 (bolded letters indicate NF-κB binding sites in the MIEP of HCMV) in the presence of binding buffer [10 mM Tris, 50 mM KCl, 1 mM DTT at pH 7.5 and 50 ng/µl Poly (dI•dC)]. NF-κB oligonucleotide was labeled with biotin using the Biotin 3' End DNA Labeling kit (Pierce, Rockford, IL) and complementary pairs were annealed by heating in boiling water for 5 min and then cooling slowly to room temperature. DNA-protein complexes were resolved from free oligonucleotide on a 6% native polyacrylamide gel in 1× Tris-borate-EDTA buffer using a Mini-PROTEAN 3 Cell (Bio-Rad, Hercules, CA) and were transferred to a Biodyne precut nylon membrane (Pierce) using the Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad). Biotin-end-labeled DNA was detected using the LightShift Chemiluminescent EMSA kit (Pierce). Control Epstein-Barr Nuclear Ag (EBNA) System (Pierce) containing biotin-EBNA control DNA and EBNA extract were assayed in parallel with the sample to ensure that the kit components and the overall procedure was working properly.

8. Microwell colorimetric NF-κB assay

Microwell colorimetric NF-κB assay was performed as described previously (Renard et al., 2001) using the Trans-AM NF-κB family Transcription factor assay kit (Active Motif, Carlsbad, CA). Briefly, cell extracts were incubated in a 96-well plate coated with an oligonucleotide containing the NF-κB consensus binding site (5´-GGGACTTTCC-3´). Activated transcription factors from extracts that bound specifically to the respective immobilized oligonucleotide were detected using Abs to NF-κB p50, p52, p65, RelB, and c-Rel subunits followed by a secondary Ab conjugated to horseradish peroxidase (HRP) in an ELISA-like assay. Absorbance was read within 5 min on an ELISA reader (PerkinElmer, Tarku, Finland) at

450 nm. The specificity of the assay was validated by including both the WT and mutated oligonucleotides in the reaction. Raji nuclear extract was used a positive control.

9. Western blot

Expression of different proteins in total, cytoplasmic, and nuclear extract was examined by western blot according to previously described procedures (Varin et al., 2003). Cellular extracts were resolved by 10% SDS-PAGE using a Mini-PROTEAN 3 Cell (Bio-Rad). The proteins were electrotransferred onto a PVDF membrane (Amersham Biosciences, Buckinghamshire, UK) using Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad). The membranes were probed with primary Abs followed by HRP-conjugated secondary immunoglobulin raised against the appropriate species; bands were detected using the ECL Plus kit (Amersham). The band density was quantified using ImageJ 1.40 software (NIH) and the results are shown as relative intensities.

10. Co-immunoprecipitation test

Total cellular extracts were precleared with 50 µl Protein A Sepharose 50% suspension (Amersham) for 1 h at 4°C. Immunoprecipitating and control Abs were added to the cleared supernatant and the mixture was incubated overnight at 4°C. Immune complexes were precipitated with 50 µl Protein A Sepharose suspension, washed with PBS, and bound proteins were eluted by incubating with 30 µl Laemmli sample buffer at 100°C for 5 minutes. SDS-PAGE and western blot were performed as described above using primary Ab against interacting protein.

11. Chromatin immunoprecipitation assay

Chromatin immunoprecipitation assay (ChIP) assay was performed on HCMV-infected MDMs using EZ-Magna ChIP A (Upstate, Temecula, CA) according to a previously described procedure (Mahlknecht et al., 2004). Briefly, MDMs (0.5×10^6 cells /reaction) were cross linked with 1% formaldehyde, lysed, and nuclear extracts were sonicated to obtain DNA fragments approximately 200-1000-bp long. After centrifugation, the nuclear extracts were diluted 10-fold with ChIP dilution buffer and 1% of the material was saved as input. Nuclear extracts were incubated with 5 µg control IgG, anti-Bcl-3 or anti-p52 Ab, and 20 µl of Protein A magnetic beads slurry overnight at 4°C. Magnetic beads were separated and washed using a magnetic separator (Upstate). Immune complexes were eluted and cross-linking was reversed by adding Proteinase K and incubating at 62°C for 2 h. Immunoprecipitated DNA and input were analyzed by PCR using primers specific for the MIEP of HCMV as described above. The amplification

product was run in a 2% agarose gel and visualized by ethidium bromide staining. Enrichment of MIEP was measured by real-time PCR using the ABI Prism 7000 Sequence Detection System (Applied Biosystems) according to the manufacturer's recommendations.

12. Plasmid constructs and transfections

Plasmid constructs p52 (pUNO-hNFkBp52a), p50 (pUNO-hNFkBp50), and p65 (pUNO-hNFkBp65) were purchased from Invivogen (San Diego, CA). Bcl-3-expressing plasmid (pCMV6-XL4-Bcl-3) was obtained from Origene (Rockville, MD). Luciferase gene reporter plasmid pLTR(1-789)-luc, and pLTR(1-789)mut-NF-κB-luc were kindly provided by the Dr C. Van Lint, Université Libre de Bruxelles, Belgium (Van Lint et al., 1996) and pCMV-Luc was purchased from PlasmidFactory (Bielefeld, Germany). U937 cells (2×10^6) were transfected with plasmids using the GenePulser XL electroporation system (Bio-Rad) according to manufacturer's instructions. MRC5 fibroblasts were transfected with p52, Bcl-3 and p52+Bcl-3-expression plasmid using lipofectamine RNAiMAX according to manufacturer's instructions (Invitrogen, Carlsbad, CA).

13. Quantification of luciferase activity

Luciferase activity in cell extracts was measured using luciferase assay system (Promega Madison, WI) and a 20/20n Luminometer (Turner Biosystems, Sunnyvale, CA). Firefly luciferase catalyzes luciferin oxidation using ATP.Mg^{2+} as a cosubstrate forming the product oxyluciferin and converts the chemical energy of luciferin oxidation into light. Cells were rinsed with PBS and lysed with 75 µl of 1X cell lysis reagent (Promega Madison, WI). Luciferase activity is measured after adding 20 µl of extract in 100 µl of luciferase assay reagent and was normalized to the total protein concentration and expressed as RLU/ µg of protein.

14. RNA interference

MDMs cultures (0.5×10^6 cells) were transfected with a scrambled control or p52+Bcl-3 or p50+p65 siRNA duplex (Santa Cruz Biotechnology) using lipofectamine RNAiMAX (Invitrogen). MDMs were infected with the clinical isolate HCMV-DB at 24 h post transfection and viral titers in culture supernatants were determined at the indicated times post infection by real-time PCR, as described above. For monitoring knockdown, total cellular extracts were prepared daily for three days post transfection. Expression of p52, Bcl-3, p50, and p65 protein was analyzed by western blot as described above. Transfection efficiency was monitored using a fluorescein-conjugated scrambled control duplex and exceeded 50% in MDMs and 90% in MRC5 fibroblasts.

15. Statistical analysis

Figures show the means and SDs of independent experiments. Statistical analysis was performed by student's *t* test and differences were considered significant at $p < 0.05$. Microsoft Excel was used to construct the plots.

4

Results

Publication 1

Bcl-3-Regulated Transcription from Major Immediate-Early Promoter of Human Cytomegalovirus in Monocyte-Derived Macrophages

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The Journal of Immunology, 2009, 182: 7784–7794.

The results of these studies were presented in part at the following conferences;

- **Khan KA,** Coaquette A, and Herbein G. (2007) Restricted growth of Human Cytomegalovirus (HCMV) in monocyte-derived macrophages is dependent on cell-type-specific binding of NF-kappaB p52 homodimers. 11th International CMV and Beta Herpesvirus Workshop, May 13-17, 2007. INSERM Toulouse, France.
- **Khan KA,** Coaquette A, and Herbein G. (2007) Signal transduction in macrophages in response to human cytomegalovirus (HCMV) infection. 13e Forum des Jeunes Chercheurs, June 14-15, 2007. Université de Bourgogne, Dijon, France.
- **Khan KA,** Coaquette A, and Herbein G. (2008) Human cytomegalovirus (HCMV) gene expression in primary macrophages. 14e Forum des Jeunes Chercheurs, June 12-13, 2008. Université de Franche-Comté, Besançon, France.
- **Khan KA,** and Herbein G. (2009) Transcription du promoteur très précore IE (MIEP) du cytomégalovirus humain (HCMV) par le facteur proto-oncogenique Bcl-3. Journee Scientific de l'IFR 133, Novembre 6, 2009, Chambre de commerce et d'industrie, Besançon, France.

Human cytomegalovirus (HCMV) is an opportunistic, species-specific herpesvirus that infects a large part of the population worldwide and causes asymptomatic latent infection in healthy people. However, it can cause severe disease in the absence of an effective immune response, especially in patients with AIDS and in immunocompromised solid-organ and bone marrow allograft recipients. Furthermore, HCMV infection during pregnancy can cause permanent birth defects. Breast feeding is the most common route of transmission from mother to child, however HCMV can be transmitted via the placenta and during delivery. An association between HCMV infection and the development of atherosclerosis and malignancy has also been described. The presence of HCMV infected cells has been demonstrated in virtually all organs and the virus targets a variety of cell types *in vivo* and *in vitro*, including macrophages. HCMV DNA and antigens have been detected in monocytes during acute HCMV infection and the macrophage is the predominant infiltrating cell type found in infected organs. The monocyte-derived macrophages (MDMs) are permissive for HCMV replication *in vitro* but the rate of viral production in these cells is considerably lower than in fibroblasts, the standard cell type, which produces high titers of infectious virus after *in vitro* infection. The cellular and/or viral factors responsible for the low production of HCMV in primary human MDMs remain unknown.

HCMV infection has been reported to activate the NF-κB signaling pathway and like many other viruses, HCMV contains NF-κB binding sites in its transcriptional response element to exploit cellular NF-κB to drive its own transcription. NF-κB and upstream IKKβ have been demonstrated as requirements for efficient transactivation of the major immediate-early promoter (MIEP), late gene expression, and viral replication of HCMV. Because distinct NF-κB complexes can be activated in different cell types in response to similar stimuli, we hypothesized that this mechanistic versatility might be involved in the varied transcription of HCMV in different cell types. NF-κB is a family of DNA-binding proteins with five members in mammals: Rel (c-Rel), RelA (p65), RelB, NF-κB1 (p50 and its precursor p105), and NF-κB2 (p52 and its precursor p100), and these proteins exist in various homo- and heterodimeric complexes. Binding of different dimers to NF-κB binding sites at comparable levels of recruitment can support different levels of transcription. Thus, the presence of distinct NF-κB dimers in different cell types might contribute to varied levels of viral replication in the different cell types.

Preliminary studies showed that HCMV could grow in primary MDMs culture but that the viral titer in culture supernatants was lower than that observed in the supernatants of more permissive MRC5 fibroblasts. Electrophoretic mobility shift assay and microwell colorimetric

NF-κB assay demonstrated that HCMV infection of MDMs increased p52 binding activity without activating the canonical p50/p65 complex. Moreover, Bcl-3 was upregulated and was demonstrated to associate with p52, indicating p52/ Bcl-3 complexes as the major component of the NF-κB complex in MDMs. In addition, we assayed the composition of NF-κB early after infection and found that the canonical p50/p65 NF-κB complex was activated during early phases of MDMs infection by HCMV, with a shift to the p52/ Bcl-3 complex occurring later during infection. Luciferase assays in promonocytic U937 cells transfected with an MIEP-luciferase reporter construct demonstrated MIEP activation in response to p52 and Bcl-3 overexpression. Chromatin immunoprecipitation assay demonstrated that p52 and Bcl-3 bind the MIEP in acutely HCMV-infected MDMs. In contrast, HCMV infection of MRC5 fibroblasts resulted in activation of p50/p65 heterodimers. Finally, MDMs and MRC5 fibroblast cultures were transfected with p52+Bcl-3 and p50+p65-specific siRNA and infected with HCMV. Knockdown of p50/p65 resulted in decreased growth of HCMV-DB in MDM cultures on day 1, while p52/Bcl-3 ablation resulted in decreased viral growth on days 4-6 post infection. In addition, p52/Bcl-3 knockdown did not block viral replication in MRC5 fibroblasts while p50/p65 ablation resulted in decreased growth of HCMV-DB in fibroblasts. Altogether, our results indicate that in HCMV infected MDMs, HCMV MIEP is preferentially activated by a p52/ Bcl-3 complex.

Our findings, presented herewith, suggest a model of HCMV MIEP transcription by NF-κB in macrophages and in fibroblasts. HCMV infection of fibroblasts leads to activation of classical p50/p65 heterodimers, which are strong activators of MIEP transcription. HCMV infection of MDMs leads to activation of classical p50/p65 heterodimers early after infection but later during infection there is a dimer change to p52/Bcl-3 complexes resulting from Bcl-3 activation and processing of p100 into p52. The association of Bcl-3 with p52 homodimers activates MIEP transcription at low levels resulting in persistent low-level growth in macrophages.

Publication 2

HCV Core and HIV-1 Nef Proteins Activate NF-kappaB and Synergistically Enhance HIV-1 Replication in Primary Macrophages

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Manuscript under preparation

The results of this study were presented in part at the following conference;

- **Khan KA**, Dichamp I, and Herbein G (2009) Effects of HIV-1 Nef and HCV core proteins on transcription of HIV in macrophages. 15^{e} Forum des Jeunes Chercheurs, June 25-26, 2009. Université de Bourgogne, Dijon, France.

Transcription of the HIV-1 provirus integrated in the host genome is regulated by a number of cellular transcription factors including NF-κB, Sp-1, AP-1, NFAT, C/EBP, CREB, YY1 etc. NF-κB is activated in response to a number of stimuli including viral infections and viral proteins and translocates to the nucleus to bind with the NF-κB binding sites present in LTR to activate HIV-1 transcription. HIV Nef protein plays a critical role in AIDS pathogenesis by activating NF-κB and enhancing viral replication in infected cells, especially macrophages. In addition, NF-κB activation has been reported during hepatitis C virus (HCV) infection and in response to HCV core protein but the role of NF-κB activation in HIV replication during HIV-1/HCV co-infection is unknown. Hepatitis C virus (HCV) infection is common in HIV-1 infected patients and each of these infections may affect the other. Several reports found that HIV infection accelerates the development of severe liver disease, and HIV facilitates infection and replication of HCV in human macrophages Thus both viruses can infect macrophages and the intracellular interaction between them can favor HIV-1 replication in macrophages of HIV/HCV coinfected subjects. In addition viral proteins HIV-1 Nef and HCV core proteins, which are detected in the serum from HIV-1/HCV coinfected subjects, can also activate NF-κB and in turn HIV-1 replication in macrophages.

We observed, *ex vivo*, highest levels of HIV-1 replication and NF-κB activation in MDMs isolated from the peripheral blood of HIV-1/HCV coinfected subjects in comparison with HIV-1 and HCV mononinfected patients. To assess the potential role of HIV-1 Nef and HCV core proteins in this phenomenon, we studied their respective role with regard to NF-κB activation and HIV-1 replication in MDMs. We demonstrated a direct interaction of HIV-1 Nef and HCV core proteins *in vitro* and *ex vivo* within promonocytic U937 cells as measured by coimmunoprecipitation assays.

Our results indicate a synergistic and sustained effect of HIV-1 Nef and HCV core proteins on NF-κB activation in primary macrophages. The composition of NF-κB assayed by EMSA and microwell colorimetric NF-κB assay showed that NF-κB complexes in response to HIV-1 Nef and HCV core proteins consisted of canonical p50/p65 complexes. Consistently, degradation of IκBα, and phosphorylation of IKKα, IKKβ was observed in response to both HIV-1 Nef and HCV core protein indicating the activation of canonical NF-κB pathway. In addition, HIV-1 Nef and HCV core proteins stimulated synergistically the HIV-1 LTR in luciferase assay. Subsequently both proteins synergistically enhanced HIV-1 replication in both chronically infected promonocytic U1 cells and acutely HIV-1 infected MDMs.

Therefore, our results indicate that HIV-1 Nef and HCV core proteins activate NF-κB in MDMs through activation of IKK complex and synergize to activate LTR activation resulting in enhanced HIV-1 replication in chronically infected cells U1 and in the acutely HIV-1-infected MDMs. Thus HCV infection of macrophages and core protein present in blood can stimulate the effects of Nef on HIV-1 replication in HIV-1/HCV coinfected patients. Our findings underline the key role of both HIV-1 Nef and HCV core proteins in the formation of HIV-1 reservoir in mononuclear phagocytes in HIV/HCV coinfected patients.

Publication 3

Is HIV infection a TNF receptor signalling-driven disease?

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Trends in Immunology, 2008, 29 (2): 61-67.

The progressive depletion of both CD4+ and CD8+ T cells and the formation of viral reservoirs are two of the hallmarks of human immunodeficiency virus (HIV) infection. Recent studies indicate that TNF receptor signalling is a key player in HIV pathogenesis as several HIV encoded proteins target multiple components of this signalling pathway. This article proposes a new model that highlights the role of HIV proteins in the modulation of TNF receptor signalling and could explain both the formation of viral reservoirs and immune suppression during HIV infection and could thereby further enhance our understanding of the pathogenesis of HIV-mediated disease.

At early stages of HIV infection levels of proinflammatory proteins and C-C chemokines are low and viral encoded proteins, particularly Nef, Vpr and Tat, mimic the TNFR signalling fuelling the formation of viral reservoirs through sustained viral growth and interference with apoptotic machinery. These viral proteins activate NF-κB in monocytic cells and primary macrophages resulting in the positive regulation of the HIV LTR. In the meantime, Vpr protein blocks the production of proinflammatory cytokines and chemokines, and soluble Nef and Tat proteins favor the recruitment of both T cells and monocytes/macrophages, further indicating a critical role for viral proteins in taking control of HIV-1 replication. At a later stage of the disease, at the onset of AIDS, proinflammatory cytokines such as TNF and C-C chemokines are produced abundantly due to chronic immune stimulation and viral proteins, rather than mimicking TNFR signalling, will in fact enhance TNF-mediated T cell apoptosis. At that point late produced HIV-1 proteins such as gp120 and Vpu are detected. The T cell proapoptotic effect results from both increased cell surface expression of TNF and TNFR molecules, especially TNFR1, in the context of high level of proinflammatory cytokines triggered by gp120 and from impaired anti-apoptotic effect mediated via TNFR/TRAF1 pathway triggered by Vpu. This will result in accelerated T cell apoptosis and in increased release of mature infectious virions from the infected cells, thus favoring the immune failure.

Therefore, targeting TNFR signalling pathway might confer a critical advantage to HIV via increased viral replication in the context of immune suppression.

Publication 4

Macrophage signalling in HIV-1 infection

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Retrovirology, 2010, In press

The human immunodeficiency virus-1 (HIV-1) infection is characterized by sustained activation of the immune system. As macrophages, along with other cell types, are permissive to HIV-1 infection, the virus can activate the immune system through modulation of the cell signaling pathways. HIV-1 does not rely exclusively on host machinery, but rather on codes for several accessory, regulatory and structural proteins, acting as molecular switches during the viral life cycle, and playing significant functions in viral pathogenesis, notably by modulating cell signalling. Even signalling pathways can be modulated in uninfected macrophages by the soluble gp120 protein, virion gp120, or by soluble HIV-1 proteins such as Nef, Tat, and Vpr which are detected in serum of HIV-1 infected patients, possibly released by infected/apoptotic cells. Soluble exogenous HIV-1 proteins are able to enter macrophages, and modulate both cellular machinery and viral transcription.

Both accessory regulatory and structural HIV-1 proteins interact with cell signalling pathways in infected macrophages. Regarding intracellular signalling induced by Nef treatment of MDMs, it has been reported that Nef modulates the expression of a significant number of genes, as early as 2 hours after treatment. Nef results in rapid activation of IKK/NF-κB, MAPK, and IRF-3 signalling pathways and induces prompt phosphorylation of the three MAPK, i.e., ERK1/2, JNK, and p38. NF-κB activation induced by Nef is responsible for activation of HIV-1 replication. In addition, production of cytokines, such as macrophage inflammatory protein-1 alpha (MIP1α), MIP1β, TNFα, IL-1β, and IL-6 involved in the inflammatory response. Similarly, exogenous Tat activates NF-κB, JNK, and AP-1. Thus exogenous Nef and Tat may modulate intracellular signalling pathways downstream of TNFRs, and thus mimic the effects of TNFα on primary macrophages. High concentrations of rVpr, as well as the carboxy-terminal Vpr peptide, are cytotoxic to macrophages but at low concentrations, rVpr was shown to enhance the activity of several transcription factors, including AP-1, c-Jun, and, NF-κB resulting in activation of HIV-1 LTR and viral replication in acutely and latently infected cells. In addition Vpr has the ability to interfere with cytokine production in macrophages and PBLs. Furthermore, HIV-1 proteins, e.g., gp120, may exert their effects by interacting with cell surface membrane receptors, especially chemokine co-receptors. By activating the signalling pathways, such as NF-κB, MAPK, and JAK/STAT, HIV-1 proteins promote viral replication by stimulating transcription from the LTR in infected macrophages; they are also involved in macrophage-mediated bystander T cell apoptosis.

The macrophages play critical role in HIV-1 disease progression through loss of T lymphocytes and formation of viral reservoirs. The role of HIV-1 proteins in the modulation of

macrophage signalling is discussed in the paper with regard to the formation of viral reservoirs and macrophage-mediated T cell apoptosis during HIV-1 infection. A better understanding of the manner by which HIV-1 modulates signalling in macrophages may be instrumental in the development of new therapeutic approaches, ultimately restricting or decreasing the size of cellular virion reservoirs in HIV-1-infected patients.

Publication 5

Exogenous HIV-1 proteins, macrophages, and AIDS pathogenesis

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In: HIV and the Macrophage, Herbein G. ed.,
Transworld Research Network, 2007. p. 55-77.

The primary goal of any virus is to replicate its genome in an appropriate host cell in order to produce progeny virions for the infection of new target cells. In this ongoing battle, the viruses redirect numerous cellular mechanisms including the defense tools of the infected cell, to their own benefit. Human immunodeficiency virus type 1 (HIV-1), is a member of lentivirus genus that, unlike simpler oncoviruses, do not rely exclusively on host cell machinery but code for additional accessory and regulatory proteins that act as molecular switches during viral life cycle and play important functions for viral pathogenesis. In addition to structural proteins (Gag, Pol, and Env), HIV-1 encodes a group of six accessory regulatory proteins. Among these, the Tat and Rev are considered essential for viral replication while defects in other accessory genes (Nef, Vif, Vpr, and Vpu) are known to impair virus replication but do still allow the production of the new virion. However, it becomes increasingly clear that these proteins exert important functions in their relevant target cells *in vivo*, and most of the HIV accessory proteins seem to exert multiple independent functions, including modulation of viral replication events and cell apoptosis. Among these exogenous Tat, Nef, and Vpr proteins have been detected in the serum of HIV-1 infected patients possibly after release from infected/apoptotic cells. Hence, they have the potential to enter the cells to modulate cellular machinery as well as viral transcription. In addition, they may exert their effects by interaction with cell membrane surface receptors. The biologic effects of exogenous proteins may be exerted in neighboring or distant tissues, not infected by the virus, and may have significant consequences for the pathogenesis of HIV-1 infection. The role of these proteins in viral replication, T cell apoptosis and formation of viral reservoirs during HIV-1 infection is discussed in this chapter.

The role of exogenous HIV-1 proteins (Tat, Nef, and Vpr) seems to be, in part, similar in HIV-1 pathogenesis. They may stimulate transcription from LTR and HIV-1 replication after entering infected cells while enhancing expression of coreceptors for HIV-1 in order to favor the entry of virus into uninfected cells. In addition to direct cytotoxic effect on a variety of cells, they can potentiate bystander cell killing by expression of apoptosis-inducing ligands while they are responsible for antiapoptotic signals in infected macrophages and latently infected T cells resulting in formation of viral reservoirs at the same time. In addition they may contribute to HIV-1 pathogenesis by enhancing secretion of important cytokines from macrophages. Moreover, exogenously added proteins might modulate the intracellular signaling pathways downstream of TNF receptors that seem to mimic the effect of TNF-α. Interaction and synergism between different exogenous proteins may enhance further their pathogenic effects.

So extracellular proteins present in sera of AIDS patients may have important impact on HIV-1 pathogenesis and therapeutic targeting of these proteins might help to limit the depletion of bystander T cells and the formation of viral reservoirs in HIV-infected patients.

Publication 6

Macrophages-mediated T cell death in HIV-1 infection

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In: HIV and the Macrophage, Herbein G. ed.,
Transworld Research Network, 2007. p. 183-195.

Macrophages constitute an important cellular component of the immune responses against viruses. They serve as antigen presenting cells and also secrete inflammatory mediators to activate innate and adaptive immune cells. Following HIV-1 infection, effector functions of macrophages such as phagocytosis, chemotaxis, intracellular killing, inflammatory responses and antigen presentation are impaired. Furthermore, macrophages serve as reservoirs of HIV in chronically infected patients and contribute to approximately one percent of the plasma viral load. In addition, the clearance of apoptotic cells by HIV-1 infected macrophages contributes to persistent viremia in patients infected with HIV-1. Multiple contributing factors may favor the macrophage as a resilient host of HIV-1 supporting viral infection and promoting viral replication and persistence. In addition, monocyte differentiation into macrophages leads to an apoptosis resistant phenotype characterized by upregulation of antiapoptotic molecules and lower levels of proapoptotic molecules. The development of these stable antiretroviral resistant cells represents the major obstacle in achieving a complete sterile cure.

Progressive depletion of both CD4+ and CD8+ populations of T cells is a characteristic feature of HIV-1 infection and plays an important role in AIDS pathogenesis. Apoptosis or programmed cell death has been proposed as the underlying mechanism of this T cell loss in HIV-1 patients. In addition to spontaneous apoptosis of circulating CD4+ and CD8+ T cells, activation induced apoptosis have been reported in lymph nodes of HIV-1 patients. The accelerated apoptosis may be explained by CD4 cross-linking by glycoprotein 120 leading to aberrant T cell signaling, cytokines, Fas/FasL interaction, superantigen activity encoded by HIV-1 products, or the involvement of accessory cells including macrophages. Soluble factors, especially those produced by macrophages play an important role in the induction of cell death. Macrophages are cellular reservoirs for HIV-1, and are particularly implicated in the death of CD4+ and CD8+ T cells during HIV-1 infection. We will review here the different mechanisms that can explain the macrophage-mediated T cell death in HIV-l infection and the role of the CD4+ and CD8+ T cell death in HIV pathogenesis which include the escape to the immune response and the increase of the spread of the infection.

Both direct and indirect mechanisms are involved in the CD4+ T cell depletion. HIV-1 infected macrophages in lymph nodes confer a death signal to neighboring primed uninfected T lymphocytes leading to their apoptosis. *In vitro* culture models demonstrate that uninfected CD4+ T cells undergo apoptosis upon contact with HIV-infected cells. These observations support the hypothesis that a direct cell contact between a susceptible CD4+ T cell and antigen-presenting cells like macrophages that potentially expresses apoptosis-inducing ligands is

required. The lymphocyte apoptosis during HIV-1 infection is dependent upon the expression of a family of ligands and receptors (FasL, TNF, Tumor necrosis factor-related apoptosis-inducing ligand [TRAIL/APO2L], Fas, TNFR). FasL is expressed on the cell surface of uninfected macrophages, and that its expression is upregulated following HIV infection, resulting in the selective killing of uninfected CD4+ T lymphocytes. In addition to enhanced apoptosis of CD4+ cells, CD8+ T cell apoptosis has also been observed in HIV-infected individuals. Increased apoptosis could contribute to HIV pathogenesis *via* the premature elimination of effector CD8+ T cells. Mechanisms of CD8+ T cell apoptosis in HIV involve cellular interactions between macrophages and T cells and is mediated by interaction of membrane bound TNF on macrophages and TNFR2 receptor on CD8+ T cells.

A better understanding of the mechanisms underlying T cell apoptosis during the HIV-1 infection could allow the development of new therapeutical approaches to block the disease progression in HIV-infected persons.

5

Discussion and Perspectives

NF-κB is activated in response to a number of viral infections. On one hand, NF-κB activation during viral infection can be exploited by the viruses for its own replication while on the other hand NF-κB activation can modulate the cellular genes of macrophage resulting in modulation of its function or change in its phenotype. The overall goal of this thesis was to assess the role of NF-κB in the transcription of HCMV and HIV-1 in primary macrophages.

In fact NF-κB is a master regulator of transcription and it can be exploited by the virus in multiple ways. On one hand, we show that multiple NF-κB subunits are activated during different phase of HCMV infection, which may result in viral replication at multiple paces during different phases of viral infection. HCMV infection of macrophages leads to activation of classical p50/p65 heterodimers early after infection but later during infection there is a dimer change to p52/Bcl-3 complexes and transcriptional regulation of MIEP of HCMV in macrophages. Low-level transcription of MIEP by this novel complex results in persistent low-level viral growth in macrophages. On the other hand, we show that NF-κB is activated by multiple stimuli which in turn activate transcription of HIV-1. Our results demonstrate activation of p50/p65 complexes in response to treatment of macrophages with HIV-1 Nef and HCV core proteins and in turn transcriptional regulation of HIV-1 LTR in macrophages.

I. Pathogenesis of HCMV infection: Role of NF-κB signaling

HCMV gene expression occurs in a cascade which consists of IE, DE, and L genes. The IE gene products are necessary for viral replication through expression of subsequent viral genes. Expression of IE genes is regulated by MIEP, activity of which is the outcome of interplay between a number of cellular transcription factors and viral proteins which regulate MIEP either in a positive or negative way. MIEP contain binding sites for a number of cellular transcription factors including NF-κB, AP-1, CREB/ATF, SP1, serum response factor, ELK-1, and retinoic acid/ retinoid X receptor. Transcription factor NF-κB is an important transcription factor which is activated in response to HCMV infection and in response to different viral proteins. NF-κB is required for IE gene expression and successful viral replication in fibroblasts (DeMeritt and Yurochko, 2006). Inhibition of NF-κB activity through IKK inhibitors and transfection of dominant negative phenotype of IκB has been reported to inhibit MIEP transactivation and HCMV replication (Caposio et al., 2007b; DeMeritt et al., 2004). Role of NF-κB in MIEP transcription and HCMV replication in macrophages remain unknown.

We observed that infection of MDMs with the clinical isolate HCMV-DB and the laboratory strain AD169 resulted in low-level sustained growth and concluded that HCMV was

able to infect MDM cultures but that the viral titers in the culture supernatants were much lower than the viral titers of infected fibroblasts. Most importantly, data presented in this study show activation of p52/ Bcl-3 complexes in MDMs following HCMV infection and activation of MIEP. Although this is the first report of regulation of the MIEP of HCMV by p52/ Bcl-3 complexes, others have reported activation of a number of human genes by Bcl-3, including P-selectin (Pan and McEver, 1995), cyclin D1 (Westerheide et al., 2001), Bcl-2 (Viatour et al., 2003), inducible NO synthase (Dai et al., 2007), and epidermal growth factor receptor (Thornburg and Raab-Traub, 2007) through NF-κB sites.

In fact NF-κB activation in response to HCMV infection could consist of two steps: the early phase represents release of preformed stores of NF-κB in response to the binding of viral glycoproteins gB and gH with cell membrane receptors, while the second phase represents *de novo* synthesis of NF-κB proteins (DeMeritt and Yurochko, 2006). Our results demonstrate the activation of p50/p65, expression of p65, and binding of p50 and p65 to MIEP early after infection but not later in infection, underscoring a scenario in which the first phase of NF-κB activation in MDMs involves canonical p50/p65 complexes — leading to initiation of HCMV gene expression, as reported by others studies (DeMeritt et al., 2004; DeMeritt et al., 2006). Later during infection, there is a shift toward the involvement of p52 homodimer/ Bcl-3 complexes — leading to sustained low-level growth of HCMV in MDMs. So Bcl-3 regulated low-level transcription might lead to persistent low-level growth of HCMV in macrophages and can influence cellular genes expression pattern as well. Persistent infection of macrophages has strong implications *in vivo* as high endothelial tropism is associated with better viral growth in macrophages (Sinzger et al., 2006). So persistently infected macrophages may act as a viral reservoir and may transmit the virus to other permissive cell types including endothelial cells, epithelial cells, and fibroblasts present in the vicinity of infected macrophages.

The concept of low-level persistent infection has broader implications beyond the infection of macrophages. Presence and replication of HCMV in cancerous and chronically inflamed tissues has been reported in recent years. Highly sensitive techniques (immunohistochemistry, *in situ* hybridization and PCR techniques) have detected the HCMV proteins and DNA in tumor cells of a number of cancers including glioma, colon, prostate, breast, and some skin cancers but not in the adjacent healthy tissues (Michaelis et al., 2009b). In addition, the presence of HCMV has also been reported in several chronic inflammatory disease tissues using the same sensitive detection techniques (Soderberg-Naucler, 2008). The replication appears to be a low-level, persistent infection releasing low titers of virus, which have been

suggested as "microinfection" (Soderberg-Naucler, 2008). As low-level transcription from MIEP of HCMV by Bcl-3 results into low-level growth of virus in macrophages (Khan et al., 2009), it is possible that the same phenomenon might be involved in transcription of HCMV in cancer cells under the control of Bcl-3, which is highly expressed in many type of cancers. Thus, instead of a lytic infection, HCMV microinfection is potentially a valuable source of multi-faceted pro-tumoral proteins and micro RNAs.

It is well established that HCMV infection result in depression of immune system and thus avoid recognition by the immune system. Macrophage being an essential tool of immune system is an ideal target of the virus. As HCMV infection of macrophages is productive and expresses all stages of viral proteins, it is logical that virus and viral protein may affect macrophage functions. Macrophage contributes to host defense and targeting of the immune cells by HCMV infection may lead to generalized immunologic hyporesponsiveness associated with HCMV disease. HCMV infected macrophages had an altered adhesion and decreased phagocytosis of yeast/fungus through a bystander mechanism (Gafa et al., 2005; Gredmark et al., 2004b). In addition, it was observed that HCMV reduces migratory properties of monocyte (Frascaroli et al., 2006) and macrophages (Frascaroli et al., 2009). Effects on macrophages were also observed in uninfected neighboring macrophages through release of macrophage migration inhibitory factor (MIF) and reorganization of the cytoskeleton (Frascaroli et al., 2009).

In addition HCMV infection of macrophage has been reported to negatively affect macrophage differentiation which in turn can lead to immune suppression. HCMV infection of monocyte was demonstrated to inhibit macrophage differentiation through IFN-α (Noraz et al., 1997). Subsequent studies done by Gredmark et al. show that HCMV inhibits cytokine-mediated differentiation of monocytes to macrophage. Cells lacked macrophage morphology and showed impairment in migration in response to chemoattractants (Gredmark et al., 2004b). This effect was transient as restimulation of HCMV treated monocytes with cytokines induced differentiation. This effect of HCMV did not require virus replication and was linked with binding of virus particles and gB protein to the cell surface molecule CD13/aminopeptidase N (Gredmark et al., 2004a; Gredmark et al., 2004b). Subsequently the same group shows that HCMV inhibits human neural precursor cells differentiation into astrocytes (Odeberg et al., 2007). As it is well established that permissiveness to viral replication is correlated with differentiation state of the cell, shut down of differentiation pathway may favor the latency of virus in monocytes. These studies suggest an efficient strategy of cytomegalovirus for immune evasion and could be associated with generalized immune suppression observed in HCMV

patients. In direct contrast, another study report that HCMV induce differentiation of monocytes into macrophages as a strategy for viral dissemination and persistence (Smith et al., 2004a). They propose the model in which HCMV infects monocytes and activates NF-κB and PI3K which induces transendothelial migration of monocytes followed by its differentiation to macrophages permissive for replication of the original input virus (Yurochko, 2008). The reason for discrepancy might be based on differences in experimental conditions but it is common in all studies that viral replication is not necessary for effects of HCMV as replication deficient virus can induce the same effects. Another explanation for this controversy might be the heterogeneity of macrophages. It can be hypothesized that HCMV suppresses the differentiation of macrophages resulting in immune suppression while using a subset of cells for dissemination strategy.

The switch in NF-κB family members after HCMV infection of MDMs could result in modulation of monocyte/macrophage gene expression resulting in functional and phenotypical changes for example an M2 phenotype. Bcl-3 has been reported to inhibit LPS-induced inflammatory response from macrophages through IL-10 (Kuwata et al., 2003; Wessells et al., 2004). Another recent study reported that resolution of inflammation was associated with expression of Bcl-3 in macrophages and change in the phenotype of macrophage (de Kozak et al., 2007). M2 phenotype of macrophage, unlike M1 proinflammatory phenotype, produces higher levels of anti-inflammatory cytokines e.g. IL-10, expresses lower levels of HLA class II, and produce less IL-12 and NO. M2 phenotype is less efficient in antigen presentation and is related with resolution of inflammation (Gordon, 2003).

Data on the effects of HCMV on macrophage activation is limited but some studies show a possible link between HCMV infection and M2 macrophage phenotype. HCMV infection of primary macrophages (Khan et al., 2009) and monocytic cell lines (Nordoy et al., 2003) result in induction of IL-10. The finding that HCMV encodes a homologue of IL-10 (Kotenko et al., 2000) is another argument in this regard. Moreover HCMV infection of MDMs results in impairment of surface expression of HLA class II molecules and T lymphocyte immune surveillance. HCMV infection of macrophages resulted in reduced expression of HLA Class II molecules and macrophages were unable to stimulate a specific CD4+ T-cell response (Fish et al., 1996; Odeberg and Soderberg-Naucler, 2001). Similarly, MCMV specifically downregulates MHC class II surface expression through transient activation of host IL-10 very early in the course of infection (Redpath et al., 1999). Virally encoded IL-10 (cmvIL-10) and latency-

associated cmvIL-10 transcript (LAcmvIL-10) has also been reported to downregulate HLA class I and II molecules (Jenkins et al., 2008; Spencer et al., 2002).

Transcriptome analysis of HCMV infected monocytes shows that HCMV results a unique M1/M2 polarization signature in which macrophage exhibits characteristics of a proinflammatory M1 as well as anti-inflammatory M2 macrophage (Chan et al., 2008a; Chan et al., 2009). It was found that 65% of genes strictly associated with M1 polarization were up-regulated, while 4% of genes associated with M2 polarization were up-regulated including anti-inflammatory cytokines IL-10 and IL-1Ra (Chan et al., 2008a). The same group revealed that HCMV induced differentiation of monocytes to macrophages is dependent upon NF-κB and PI(3)K. Transcriptome analysis demonstrated that 48%, 7% and 31% of HCMV-induced M1-associated genes were dependent on NF-κB, PI(3)K or both activities, respectively; while 100% of HCMV-induced M2-associated genes required both NF-κB and PI(3)K activities (Chan et al., 2009). Thus the macrophage phenotype in response to HCMV might be the result of the differential activation of NF-κB dimmers during different phases of HCMV infection of macrophages (Khan et al., 2009). Similarly M2 phenotype has also been observed in solid organ tumors. These macrophages are called tumor-associated macrophages (TAM) and they are correlated negatively with progression of cancer unlike lymphocytes (Sica et al., 2008). A macrophage phenotype has also been reported in mammary tumor of mice which was neither M1 nor M2 (Torroella-Kouri et al., 2009). NF-κB is believed as a central regulator of TAM polarization. TAM produces less level of proinflammatory cytokines and show inhibition of p65 nuclear translocation (Biswas et al., 2006). As macrophages and HCMV are present in the tumor microenvironment which may lead to their possible interaction, it would be interesting to study if HCMV play any role in signals required for M2 phenotype of TAM present in tumors.

Altogether, the activation of multiple NF-κB dimers after HCMV infection of MDMs might influence the outcome of HCMV infection by modulating the expression of both viral and cellular genes.

II. Role of NF-κB signaling in pathogenesis of HIV-1/HCV co-infection

Transcription of HIV-1 provirus integrated in the host genome is regulated by a number of cellular transcription factors especially at the early phase and results in the production of early viral gene products. HIV-1 transcription is driven by LTR which is responsive to a number of transcription factors including NF-κB, Sp-1, AP-1, NFAT, C/EBP, CREB, YY1 etc. Transcription factor NF-κB is an important player of immunity and inflammation. It is activated

in response to a number of stimuli including viral infections and viral proteins. Activated NF-κB translocates to the nucleus and binds with the NF-κB binding sites present in LTR to activate HIV-1 transcription. Involvement of NF-κB in successful viral replication has been reported in different cell types including T cells, thymocytes, microglial cells, and macrophages (Rohr et al., 2003). Expression of IκBα repressor mutants interferes with NF-κB activity and reduces HIV-1 replication in T cells cultures *in vitro* (Kwon et al., 1998). Inhibition of HIV replication has also been reported by inhibition of NF-κB activity through IKK inhibitors (Victoriano et al., 2006). HIV Nef protein plays a critical role in AIDS pathogenesis by activating NF-κB and enhancing viral replication in infected cells, especially macrophages. In addition, NF-κB activation has been reported during hepatitis C virus (HCV) infection and in response to HCV core protein but the role of NF-κB activation in HIV replication during HIV-1/HCV co-infection is unknown.

Our results indicate a synergistic and sustained effect of HIV-1 Nef and HCV core proteins on HIV-1 replication in primary macrophages. We observed that both proteins activated canonical NF-κB pathway as demonstrated by activation of p50/p65 complexes, degradation of IκBα, and phosphorylation of IKKα, IKKβ. In addition, HIV-1 Nef and HCV core proteins stimulated synergistically the HIV-1 LTR in luciferase assay. Subsequently both proteins synergistically enhanced HIV-1 replication in both chronically infected promonocytic U1 cells and acutely HIV-1 infected MDMs.

Previous studies have shown that TNF-α stimulates HIV-1 replication in chronically infected promonocytic U1 cell line through activation of NF-κB and transactivation of the proviral LTR. Recent studies demonstrated that the features observed in promonocytic cells U937 and macrophages in response to exogenous HIV-1 proteins are similar to those observed following TNF-α treatment (Khan et al., 2007). Different HIV-1 proteins have been reported to activate NF-κB and in turn activate LTR driven transcription. Exogenous SIV and HIV-1 Nef proteins activates the transcription factors NF-κB in promonocytic U937 cells and stimulates HIV-1 LTR via NF-κB activation resulting in enhanced viral replication in the chronically infected promonocytic cells U1 (Varin et al., 2003). Subsequent studies from our laboratory showed that Nef and TNF-α synergistically activate NF-κB and AP-1 in U937 cells and MDMs resulting in enhanced stimulation of LTR and in turn viral replication (Herbein et al., 2008). In addition, synthetic Vpr protein activates NF-κB in the promonocytic cell line U937, in primary macrophages and stimulated transcription from pLTR-Luc in U937 cells. Moreover, synthetic Vpr stimulated HIV-1 replication in chronically infected U1 promonocytic cells and acutely infected primary macrophages (Varin et al., 2005). Similarly, exogenous Tat protein has been

shown to amplify the activity of TNF-α (Westendorp et al., 1995) but the mechanism of action is not the same. HIV-1 Tat induced activation of NF-κB, AP-1, JNK required $p56^{lck}$ while TNF-induced activation was found to be $p56^{lck}$-independent (Manna and Aggarwal, 2000).

HIV-infection is related with severe immune suppression and HIV infected individuals are exposed to a number of opportunistic and non-opportunistic infections. As NF-κB is activated in response to different viral infections, interaction and synergism between viruses and their viral proteins may enhance HIV-1 replication through activation of NF-κB and may fuel the progression of HIV infection. A number of viral infections have been reported to activate HIV-1 LTR through activation of NF-κB. Although activation of HIV-1 transcription has been reported by influenza virus infection (Flory et al., 2000), HHV-6 infection (Ensoli et al., 1989), HSV infection and its IE proteins ICP0 and ICP4 (Margolis et al., 1992), HVS StpC protein (Raymond et al., 2007), and HTLV-1 encoded proteins tax (Cheng et al., 1998) and FLICE (Sun et al., 2005b), HBV HBx protein (Siddiqui et al., 1989) and CMV E1/E2 (Dal Monte et al., 1997) proteins but relevancy of the results in HIV-1 disease process is always questioned as many of these viruses preferably infect distinct cell types.

Our results have important implications in the progression of AIDS process as HIV-1 Nef and HCV core proteins are found in blood of infected patients (Fujii et al., 1996; Pivert et al., 2006). HIV-1 Nef and HCV core proteins present in blood may contribute in the replication of enhanced HIV-1 in macrophages. In addition to exogenous viral proteins, the interaction of HCV and HIV-1 is possible in macrophages as both viruses have been reported to replicate in macrophages (Laskus et al., 2000). An interesting study demonstrated that HSV virion's contact with HIV-1 infected macrophage was able to activate HIV-1 expression in acutely infected MDMs. It was demonstrated that infectious or heat-inactivated HSV type 1 or 2 virions induced HIV-1 expression in NF-κB dependent manner and neutralizing antibodies to the HSV glycoprotein gB or gD markedly attenuated these virion-mediated effects on HIV-1 expression in macrophages (Moriuchi et al., 2000). Similarly, the findings of transactivation of HIV-1 LTR by CMV IE1 protein in U937 cells seems relevant as both viruses replicate in macrophages (Kim et al., 1996).

On one hand, activation of NF-κB by HIV Nef and HCV core protein result in enhanced HIV-1 replication while on the other hand it may result in activation of cellular genes as well. HIV-1 infection of macrophages induces cytokine and chemokine through NF-κB (Choe et al., 2002). Treatment of MDMs with rNef increases the transcription of genes for several inflammatory factors including macrophage inflammatory proteins 1alpha and 1beta, IL-1beta,

IL-6, and TNF-alpha through NF-κB (Olivetta et al., 2003). NF-κB is also responsible for production of IL-8 from macrophages by Vpr protein (Roux et al., 2000). Another important function of NF-κB in the macrophages is the survival signals which results in enhanced formation of viral reservoir. NF-κB is activated during HIV-1 infection of myeloid cells which protects them from TNF and cycloheximide induced apoptosis (DeLuca et al., 1998). Absence of apoptosis in macrophages by HIV was correlated with upregulation of Bcl-x(L) through NF-κB dependent mechanism (Choi and Smithgall, 2004; Guillemard et al., 2004). Similarly, HCV core protein has also been reported to suppress apoptosis through activation of NF-κB (Marusawa et al., 1999; Tai et al., 2000). Thus both proteins may favor the formation of viral reservoir through activation of viral transcription and inhibition of apoptosis.

Altogether, our results indicate that HIV-1 Nef and HCV core proteins activate NF-κB in MDMs and synergize to activate LTR activation resulting in enhanced HIV-1 replication in chronically infected U1 cells and in the acutely HIV-1-infected MDMs. Thus HCV infection of macrophages and core protein present in blood can stimulate the effects of Nef on HIV-1 replication in HIV-1/HCV coinfected patients. Our findings underline the key role of both HIV-1 Nef and HCV core proteins in the formation of HIV-1 reservoir in mononuclear phagocytes in HIV/HCV coinfected patients.

III. Conclusion

NF-κB is an important tool used by both ends in the war between the host and the virus. Host uses NF-κB to mount antiviral immune response while viruses hijack this for its own benefit. It is worth noting that some viruses targets NF-κB signaling at multiple steps through multiple viral products to ensure effective modulation of this important pathway which is then exploited by the viruses. In other words host senses different viral proteins by different signaling molecules for mounting defense mechanism in an efficient manner.

In conclusion, our findings show that NF-κB is activated during HCMV infection and HIV-1/HCV coinfection of macrophages which is exploited by viruses in different manners for persistent viral replication in macrophages. These results have important implication in terms of viral persistence and formation of viral reservoirs in macrophages during HCMV infection and HIV-1/HCV coinfection. Moreover, NF-κB activation during viral infections might influence the outcome of disease by modulating the expression of cellular genes as well.

References

Abbink, T.E., and Berkhout, B. (2008). HIV-1 reverse transcription initiation: a potential target for novel antivirals? Virus Res *134*, 4-18.

Acheampong, E.A., Parveen, Z., Muthoga, L.W., Kalayeh, M., Mukhtar, M., and Pomerantz, R.J. (2005). Human Immunodeficiency virus type 1 Nef potently induces apoptosis in primary human brain microvascular endothelial cells via the activation of caspases. J Virol *79*, 4257-4269.

Ahmad, N., and Venkatesan, S. (1988). Nef protein of HIV-1 is a transcriptional repressor of HIV-1 LTR. Science *241*, 1481-1485.

Akari, H., Bour, S., Kao, S., Adachi, A., and Strebel, K. (2001). The human immunodeficiency virus type 1 accessory protein Vpu induces apoptosis by suppressing the nuclear factor kappaB-dependent expression of antiapoptotic factors. J Exp Med *194*, 1299-1311.

Alisi, A., Giannini, C., Spaziani, A., Caini, P., Zignego, A.L., and Balsano, C. (2008). Involvement of PI3K in HCV-related lymphoproliferative disorders. J Cell Physiol *214*, 396-404.

Alvarez, S., Serramia, M.J., Fresno, M., and Munoz-Fernandez, M. (2005). Human immunodeficiency virus type 1 envelope glycoprotein 120 induces cyclooxygenase-2 expression in neuroblastoma cells through a nuclear factor-kappaB and activating protein-1 mediated mechanism. J Neurochem *94*, 850-861.

Amici, C., Belardo, G., Rozera, C., Bernasconi, D., and Santoro, M.G. (2004). Inhibition of herpesvirus-induced HIV-1 replication by cyclopentenone prostaglandins: role of IkappaB kinase (IKK). AIDS *18*, 1271-1280.

Amici, C., Rossi, A., Costanzo, A., Ciafre, S., Marinari, B., Balsamo, M., Levrero, M., and Santoro, M.G. (2006). Herpes simplex virus disrupts NF-kappaB regulation by blocking its recruitment on the IkappaBalpha promoter and directing the factor on viral genes. J Biol Chem *281*, 7110-7117.

Ammon, C., Mondal, K., Andreesen, R., and Krause, S.W. (2000). Differential expression of the transcription factor NF-kappaB during human mononuclear phagocyte differentiation to macrophages and dendritic cells. Biochem Biophys Res Commun *268*, 99-105.

Aoki, H., Hayashi, J., Moriyama, M., Arakawa, Y., and Hino, O. (2000). Hepatitis C virus core protein interacts with 14-3-3 protein and activates the kinase Raf-1. J Virol *74*, 1736-1741.

Arrode, G., Boccaccio, C., Abastado, J.P., and Davrinche, C. (2002). Cross-presentation of human cytomegalovirus pp65 (UL83) to CD8+ T cells is regulated by virus-induced, soluble-mediator-dependent maturation of dendritic cells. J Virol *76*, 142-150.

Arsura, M., and Cavin, L.G. (2005). Nuclear factor-kappaB and liver carcinogenesis. Cancer Lett *229*, 157-169.

Asamitsu, K., Yamaguchi, T., Nakata, K., Hibi, Y., Victoriano, A.F., Imai, K., Onozaki, K., Kitade, Y., and Okamoto, T. (2008). Inhibition of human immunodeficiency virus type 1 replication by blocking IkappaB kinase with noraristeromycin. J Biochem *144*, 581-589.

Asante-Appiah, E., and Skalka, A.M. (1997). Molecular mechanisms in retrovirus DNA integration. Antiviral Res *36*, 139-156.

Ashburner, B.P., Westerheide, S.D., and Baldwin, A.S., Jr. (2001). The p65 (RelA) subunit of NF-kappaB interacts with the histone deacetylase (HDAC) corepressors HDAC1 and HDAC2 to negatively regulate gene expression. Mol Cell Biol *21*, 7065-7077.

Asin, S., Bren, G.D., Carmona, E.M., Solan, N.J., and Paya, C.V. (2001). NF-kappaB cis-acting motifs of the human immunodeficiency virus (HIV) long terminal repeat regulate HIV transcription in human macrophages. J Virol *75*, 11408-11416.

Assogba, B.D., Choi, B.H., and Rho, H.M. (2002). Transcriptional activation of the promoter of human cytomegalovirus immediate early gene (CMV-IE) by the hepatitis B viral X protein (HBx) through the NF-kappaB site. Virus Res *84*, 171-179.

Azad, A.A. (2000). Could Nef and Vpr proteins contribute to disease progression by promoting depletion of bystander cells and prolonged survival of HIV-infected cells? Biochem Biophys Res Commun *267*, 677-685.

Bachelerie, F., Alcami, J., Arenzana-Seisdedos, F., and Virelizier, J.L. (1991). HIV enhancer activity perpetuated by NF-kappa B induction on infection of monocytes. Nature *350*, 709-712.

Bae, J.S., Jang, M.K., Hong, S., An, W.G., Choi, Y.H., Kim, H.D., and Cheong, J. (2003). Phosphorylation of NF-kappa B by calmodulin-dependent kinase IV activates anti-apoptotic gene expression. Biochem Biophys Res Commun *305*, 1094-1098.

Bagnarelli, P., Valenza, A., Menzo, S., Sampaolesi, R., Varaldo, P.E., Butini, L., Montroni, M., Perno, C.F., Aquaro, S., Mathez, D., *et al.* (1996). Dynamics and modulation of human immunodeficiency virus type 1 transcripts in vitro and in vivo. J Virol *70*, 7603-7613.

Bakri, Y., Amzazi, S., Mannioui, A., and Benjouad, A. (2001). The susceptibility of macrophages to human immunodeficiency virus type 1 X4 isolates depends on their activation state. Biomed Pharmacother *55*, 32-38.

Ballard, D.W., Bohnlein, E., Lowenthal, J.W., Wano, Y., Franza, B.R., and Greene, W.C. (1988). HTLV-I tax induces cellular proteins that activate the kappa B element in the IL-2 receptor alpha gene. Science *241*, 1652-1655.

Bao, X., Liu, T., Spetch, L., Kolli, D., Garofalo, R.P., and Casola, A. (2007). Airway epithelial cell response to human metapneumovirus infection. Virology *368*, 91-101.

Barre-Sinoussi, F., Chermann, J.C., Rey, F., Nugeyre, M.T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., *et al.* (1983). Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science *220*, 868-871.

Bartenschlager, R., and Lohmann, V. (2001). Novel cell culture systems for the hepatitis C virus. Antiviral Res *52*, 1-17.

Basak, S., Kim, H., Kearns, J.D., Tergaonkar, V., O'Dea, E., Werner, S.L., Benedict, C.A., Ware, C.F., Ghosh, G., Verma, I.M., *et al.* (2007). A fourth IkappaB protein within the NF-kappaB signaling module. Cell *128*, 369-381.

Basak, S., Shih, V.F., and Hoffmann, A. (2008). Generation and activation of multiple dimeric transcription factors within the NF-kappaB signaling system. Mol Cell Biol *28*, 3139-3150.

Beg, A.A., and Baldwin, A.S., Jr. (1994). Activation of multiple NF-kappa B/Rel DNA-binding complexes by tumor necrosis factor. Oncogene *9*, 1487-1492.

Bellas, R.E., Hopkins, N., and Li, Y. (1993). The NF-kappa B binding site is necessary for efficient replication of simian immunodeficiency virus of macaques in primary macrophages but not in T cells in vitro. J Virol *67*, 2908-2913.

Benedict, C.A., Angulo, A., Patterson, G., Ha, S., Huang, H., Messerle, M., Ware, C.F., and Ghazal, P. (2004). Neutrality of the canonical NF-kappaB-dependent pathway for human and murine cytomegalovirus transcription and replication in vitro. J Virol *78*, 741-750.

Benhamou, Y., Bochet, M., Di Martino, V., Charlotte, F., Azria, F., Coutellier, A., Vidaud, M., Bricaire, F., Opolon, P., Katlama, C., *et al.* (1999). Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfected patients. The Multivirc Group. Hepatology *30*, 1054-1058.

Berger, E.A., Doms, R.W., Fenyo, E.M., Korber, B.T., Littman, D.R., Moore, J.P., Sattentau, Q.J., Schuitemaker, H., Sodroski, J., and Weiss, R.A. (1998). A new classification for HIV-1. Nature *391*, 240.

Bernier, R., Barbeau, B., Olivier, M., and Tremblay, M.J. (1998a). Mycobacterium tuberculosis mannose-capped lipoarabinomannan can induce NF-kappaB-dependent activation of human immunodeficiency virus type 1 long terminal repeat in T cells. J Gen Virol *79 (Pt 6)*, 1353-1361.

Bernier, R., Barbeau, B., Tremblay, M.J., and Olivier, M. (1998b). The lipophosphoglycan of Leishmania donovani up-regulates HIV-1 transcription in T cells through the nuclear factor-kappaB elements. J Immunol *160*, 2881-2888.

Biggs, T.E., Cooke, S.J., Barton, C.H., Harris, M.P., Saksela, K., and Mann, D.A. (1999). Induction of activator protein 1 (AP-1) in macrophages by human immunodeficiency virus type-1 NEF is a cell-type-specific response that requires both hck and MAPK signaling events. J Mol Biol *290*, 21-35.

Bissinger, A.L., Sinzger, C., Kaiserling, E., and Jahn, G. (2002). Human cytomegalovirus as a direct pathogen: correlation of multiorgan involvement and cell distribution with clinical and pathological findings in a case of congenital inclusion disease. J Med Virol *67*, 200-206.

Biswas, S.K., Gangi, L., Paul, S., Schioppa, T., Saccani, A., Sironi, M., Bottazzi, B., Doni, A., Vincenzo, B., Pasqualini, F., *et al.* (2006). A distinct and unique transcriptional program expressed by tumor-associated macrophages (defective NF-kappaB and enhanced IRF-3/STAT1 activation). Blood *107*, 2112-2122.

Blackard, J.T., and Sherman, K.E. (2008). HCV/ HIV co-infection: time to re-evaluate the role of HIV in the liver? J Viral Hepat *15*, 323-330.

Bleul, C.C., Wu, L., Hoxie, J.A., Springer, T.A., and Mackay, C.R. (1997). The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes. Proc Natl Acad Sci U S A *94*, 1925-1930.

Bohuslav, J., Chen, L.F., Kwon, H., Mu, Y., and Greene, W.C. (2004). p53 induces NF-kappaB activation by an IkappaB kinase-independent mechanism involving phosphorylation of p65 by ribosomal S6 kinase 1. J Biol Chem *279*, 26115-26125.

Bonacini, M., Govindarajan, S., Blatt, L.M., Schmid, P., Conrad, A., and Lindsay, K.L. (1999). Patients co-infected with human immunodeficiency virus and hepatitis C virus demonstrate higher levels of hepatic HCV RNA. J Viral Hepat *6*, 203-208.

Borst, E.M., and Messerle, M. (2005). Analysis of human cytomegalovirus oriLyt sequence requirements in the context of the viral genome. J Virol *79*, 3615-3626.

Bossis, G., Salinas, S., Cartier, C., Devaux, C., and Briant, L. (2002). NF-kappaB activation upon interaction of HIV-1 envelope glycoproteins with cell surface CD4 involves IkappaB kinases. FEBS Lett *516*, 257-264.

Bouazzaoui, A., Kreutz, M., Eisert, V., Dinauer, N., Heinzelmann, A., Hallenberger, S., Strayle, J., Walker, R., Rubsamen-Waigmann, H., Andreesen, R., *et al.* (2006). Stimulated trans-acting factor of 50 kDa (Staf50) inhibits HIV-1 replication in human monocyte-derived macrophages. Virology *356*, 79-94.

Boulares, A.H., Ferran, M.C., and Lucas-Lenard, J. (1996). NF-kappaB activation Is delayed in mouse L929 cells infected with interferon suppressing, but not inducing, vesicular stomatitis virus strains. Virology *218*, 71-80.

Bour, S., Geleziunas, R., and Wainberg, M.A. (1995). The human immunodeficiency virus type 1 (HIV-1) CD4 receptor and its central role in promotion of HIV-1 infection. Microbiol Rev *59*, 63-93.

Bour, S., Perrin, C., Akari, H., and Strebel, K. (2001). The human immunodeficiency virus type 1 Vpu protein inhibits NF-kappa B activation by interfering with beta TrCP-mediated degradation of Ikappa B. J Biol Chem *276*, 15920-15928.

Bours, V., Franzoso, G., Azarenko, V., Park, S., Kanno, T., Brown, K., and Siebenlist, U. (1993). The oncoprotein Bcl-3 directly transactivates through kappa B motifs via association with DNA-binding p50B homodimers. Cell *72*, 729-739.

Bowers, W.J., Baglia, L.A., and Ruddel, A. (1996). Regulation of avian leukosis virus long terminal repeat-enhanced transcription by C/EBP-Rel interactions. J Virol *70*, 3051-3059.

Bowie, A., Kiss-Toth, E., Symons, J.A., Smith, G.L., Dower, S.K., and O'Neill, L.A. (2000). A46R and A52R from vaccinia virus are antagonists of host IL-1 and toll-like receptor signaling. Proc Natl Acad Sci U S A *97*, 10162-10167.

Bren, G.D., Trushin, S.A., Whitman, J., Shepard, B., and Badley, A.D. (2009). HIV gp120 induces, NF-kappaB dependent, HIV replication that requires procaspase 8. PLoS One *4*, e4875.

Briggs, S.D., Scholtz, B., Jacque, J.M., Swingler, S., Stevenson, M., and Smithgall, T.E. (2001). HIV-1 Nef promotes survival of myeloid cells by a Stat3-dependent pathway. J Biol Chem *276*, 25605-25611.

Brinkmann, M.M., Glenn, M., Rainbow, L., Kieser, A., Henke-Gendo, C., and Schulz, T.F. (2003). Activation of mitogen-activated protein kinase and NF-kappaB pathways by a Kaposi's sarcoma-associated herpesvirus K15 membrane protein. J Virol *77*, 9346-9358.

Brinkmann, M.M., and Schulz, T.F. (2006). Regulation of intracellular signalling by the terminal membrane proteins of members of the Gammaherpesvirinae. J Gen Virol *87*, 1047-1074.

Britt, W. (2008). Manifestations of human cytomegalovirus infection: proposed mechanisms of acute and chronic disease. Curr Top Microbiol Immunol *325*, 417-470.

Britt, W.J., and Boppana, S. (2004). Human cytomegalovirus virion proteins. Hum Immunol *65*, 395-402.

Brooks, P.A., Cockerell, G.L., and Nyborg, J.K. (1998). Activation of BLV transcription by NF-kappa B and Tax. Virology *243*, 94-98.

Browne, E.P., and Shenk, T. (2003). Human cytomegalovirus UL83-coded pp65 virion protein inhibits antiviral gene expression in infected cells. Proc Natl Acad Sci U S A *100*, 11439-11444.

Brune, W., Menard, C., Heesemann, J., and Koszinowski, U.H. (2001). A ribonucleotide reductase homolog of cytomegalovirus and endothelial cell tropism. Science *291*, 303-305.

Buerki, C., Rothgiesser, K.M., Valovka, T., Owen, H.R., Rehrauer, H., Fey, M., Lane, W.S., and Hottiger, M.O. (2008). Functional relevance of novel p300-mediated lysine 314 and 315 acetylation of RelA/p65. Nucleic Acids Res *36*, 1665-1680.

Bukrinsky, M.I., Sharova, N., Dempsey, M.P., Stanwick, T.L., Bukrinskaya, A.G., Haggerty, S., and Stevenson, M. (1992). Active nuclear import of human immunodeficiency virus type 1 preintegration complexes. Proc Natl Acad Sci U S A *89*, 6580-6584.

Bukrinsky, M.I., Sharova, N., McDonald, T.L., Pushkarskaya, T., Tarpley, W.G., and Stevenson, M. (1993). Association of integrase, matrix, and reverse transcriptase antigens of human immunodeficiency virus type 1 with viral nucleic acids following acute infection. Proc Natl Acad Sci U S A *90*, 6125-6129.

Burdo, T.H., Nonnemacher, M., Irish, B.P., Choi, C.H., Krebs, F.C., Gartner, S., and Wigdahl, B. (2004). High-affinity interaction between HIV-1 Vpr and specific sequences that span the C/EBP and adjacent NF-kappaB sites within the HIV-1 LTR correlate with HIV-1-associated dementia. DNA Cell Biol *23*, 261-269.

Buss, H., Dorrie, A., Schmitz, M.L., Frank, R., Livingstone, M., Resch, K., and Kracht, M. (2004a). Phosphorylation of serine 468 by GSK-3beta negatively regulates basal p65 NF-kappaB activity. J Biol Chem *279*, 49571-49574.

Buss, H., Dorrie, A., Schmitz, M.L., Hoffmann, E., Resch, K., and Kracht, M. (2004b). Constitutive and interleukin-1-inducible phosphorylation of p65 NF-{kappa}B at serine 536 is mediated by multiple protein kinases including I{kappa}B kinase (IKK)-{alpha}, IKK{beta}, IKK{epsilon}, TRAF family member-associated (TANK)-binding kinase 1 (TBK1), and an unknown kinase and couples p65 to TATA-binding protein-associated factor II31-mediated interleukin-8 transcription. J Biol Chem *279*, 55633-55643.

Calao, M., Burny, A., Quivy, V., Dekoninck, A., and Van Lint, C. (2008). A pervasive role of histone acetyltransferases and deacetylases in an NF-kappaB-signaling code. Trends Biochem Sci *33*, 339-349.

Camus-Bouclainville, C., Fiette, L., Bouchiha, S., Pignolet, B., Counor, D., Filipe, C., Gelfi, J., and Messud-Petit, F. (2004). A virulence factor of myxoma virus colocalizes with NF-kappaB in the nucleus and interferes with inflammation. J Virol *78*, 2510-2516.

Canchis, P.W., Yee, H.T., Fiel, M.I., Dieterich, D.T., Liu, R.C., Chiriboga, L., Jacobson, I.M., Edlin, B.R., and Talal, A.H. (2004). Intrahepatic CD4+ cell depletion in hepatitis C virus/HIV-coinfected patients. J Acquir Immune Defic Syndr *37*, 1125-1131.

Canonne-Hergaux, F., Aunis, D., and Schaeffer, E. (1995). Interactions of the transcription factor AP-1 with the long terminal repeat of different human immunodeficiency virus type 1 strains in Jurkat, glial, and neuronal cells. J Virol *69*, 6634-6642.

Caposio, P., Dreano, M., Garotta, G., Gribaudo, G., and Landolfo, S. (2004). Human cytomegalovirus stimulates cellular IKK2 activity and requires the enzyme for productive replication. J Virol *78*, 3190-3195.

Caposio, P., Luganini, A., Bronzini, M., Landolfo, S., and Gribaudo, G. (2010). The Elk-1 and Serum Response Factor Binding Sites in the Major Immediate-Early Promoter of the Human Cytomegalovirus are Required for Efficient Viral Replication in Quiescent Cells and Compensate for Inactivation of the NF-{kappa}B Sites in Proliferating Cells. J Virol.

Caposio, P., Luganini, A., Hahn, G., Landolfo, S., and Gribaudo, G. (2007a). Activation of the virus-induced IKK/NF-kappaB signalling axis is critical for the replication of human cytomegalovirus in quiescent cells. Cell Microbiol *9*, 2040-2054.

Caposio, P., Musso, T., Luganini, A., Inoue, H., Gariglio, M., Landolfo, S., and Gribaudo, G. (2007b). Targeting the NF-kappaB pathway through pharmacological inhibition of IKK2 prevents human cytomegalovirus replication and virus-induced inflammatory response in infected endothelial cells. Antiviral Res *73*, 175-184.

Cara, A., and Klotman, M.E. (2006). Retroviral E-DNA: persistence and gene expression in nondividing immune cells. J Leukoc Biol *80*, 1013-1017.

Cardozo, T., Kimura, T., Philpott, S., Weiser, B., Burger, H., and Zolla-Pazner, S. (2007). Structural basis for coreceptor selectivity by the HIV type 1 V3 loop. AIDS Res Hum Retroviruses *23*, 415-426.

Carlquist, J.F., Edelman, L., Bennion, D.W., and Anderson, J.L. (1999). Cytomegalovirus induction of interleukin-6 in lung fibroblasts occurs independently of active infection and involves a G protein and the transcription factor, NF-kappaB. J Infect Dis *179*, 1094-1100.

Carmody, R.J., Ruan, Q., Palmer, S., Hilliard, B., and Chen, Y.H. (2007). Negative regulation of toll-like receptor signaling by NF-kappaB p50 ubiquitination blockade. Science *317*, 675-678.

Cassol, E., Alfano, M., Biswas, P., and Poli, G. (2006). Monocyte-derived macrophages and myeloid cell lines as targets of HIV-1 replication and persistence. J Leukoc Biol *80*, 1018-1030.

Cavrois, M., Neidleman, J., Kreisberg, J.F., Fenard, D., Callebaut, C., and Greene, W.C. (2006). Human immunodeficiency virus fusion to dendritic cells declines as cells mature. J Virol *80*, 1992-1999.

Chan, G., Bivins-Smith, E.R., Smith, M.S., Smith, P.M., and Yurochko, A.D. (2008a). Transcriptome analysis reveals human cytomegalovirus reprograms monocyte differentiation toward an M1 macrophage. J Immunol *181*, 698-711.

Chan, G., Bivins-Smith, E.R., Smith, M.S., and Yurochko, A.D. (2008b). Transcriptome analysis of NF-kappaB- and phosphatidylinositol 3-kinase-regulated genes in human cytomegalovirus-infected monocytes. J Virol *82*, 1040-1046.

Chan, G., Bivins-Smith, E.R., Smith, M.S., and Yurochko, A.D. (2009). NF-kappaB and phosphatidylinositol 3-kinase activity mediates the HCMV-induced atypical M1/M2 polarization of monocytes. Virus Res *144*, 329-333.

Chang, P.Y., Stellrecht, K., Melana, S., and Pogo, B.G. (1994). Elements in the long terminal repeat of HIV-1 that interact with nuclear extracts from Jurkat cells persistently infected with vaccinia virus. Virus Res *34*, 127-138.

Chang, T.H., Liao, C.L., and Lin, Y.L. (2006). Flavivirus induces interferon-beta gene expression through a pathway involving RIG-I-dependent IRF-3 and PI3K-dependent NF-kappaB activation. Microbes Infect *8*, 157-171.

Chen, B.K., Feinberg, M.B., and Baltimore, D. (1997). The kappaB sites in the human immunodeficiency virus type 1 long terminal repeat enhance virus replication yet are not absolutely required for viral growth. J Virol *71*, 5495-5504.

Chen, L.F., and Greene, W.C. (2004). Shaping the nuclear action of NF-kappaB. Nat Rev Mol Cell Biol *5*, 392-401.

Chen, L.F., Mu, Y., and Greene, W.C. (2002). Acetylation of RelA at discrete sites regulates distinct nuclear functions of NF-kappaB. EMBO J *21*, 6539-6548.

Chen, L.F., Williams, S.A., Mu, Y., Nakano, H., Duerr, J.M., Buckbinder, L., and Greene, W.C. (2005). NF-kappaB RelA phosphorylation regulates RelA acetylation. Mol Cell Biol *25*, 7966-7975.

Chen, Z.J. (2005). Ubiquitin signalling in the NF-kappaB pathway. Nat Cell Biol *7*, 758-765.

Cheng, H., Tarnok, J., and Parks, W.P. (1998). Human immunodeficiency virus type 1 genome activation induced by human T-cell leukemia virus type 1 Tax protein is through cooperation of NF-kappaB and Tat. J Virol *72*, 6911-6916.

Chirmule, N., Kalyanaraman, V.S., and Pahwa, S. (1994). Signals transduced through the CD4 molecule on T lymphocytes activate NF-kappa B. Biochem Biophys Res Commun *203*, 498-505.

Cho, I.R., Jeong, S., Jhun, B.H., An, W.G., Lee, B., Kwak, Y.T., Lee, S.H., Jung, J.U., and Chung, Y.H. (2007). Activation of non-canonical NF-kappaB pathway mediated by STP-A11, an oncoprotein of Herpesvirus saimiri. Virology *359*, 37-45.

Choe, W., Volsky, D.J., and Potash, M.J. (2002). Activation of NF-kappaB by R5 and X4 human immunodeficiency virus type 1 induces macrophage inflammatory protein 1alpha and tumor necrosis factor alpha in macrophages. J Virol *76*, 5274-5277.

Choi, H.J., and Smithgall, T.E. (2004). HIV-1 Nef promotes survival of TF-1 macrophages by inducing Bcl-XL expression in an extracellular signal-regulated kinase-dependent manner. J Biol Chem *279*, 51688-51696.

Chu, Z.L., Shin, Y.A., Yang, J.M., DiDonato, J.A., and Ballard, D.W. (1999). IKKgamma mediates the interaction of cellular IkappaB kinases with the tax transforming protein of human T cell leukemia virus type 1. J Biol Chem *274*, 15297-15300.

Chung, Y.H., Jhun, B.H., Ryu, S.C., Kim, H.S., Kim, C.M., Kim, B.S., Kim, Y.O., and Lee, S.J. (2007). STP-C, an oncoprotein of herpesvirus saimiri augments the activation of NF-kappaB through ubiquitination of TRAF6. J Biochem Mol Biol *40*, 341-348.

Chung, Y.M., Park, K.J., Choi, S.Y., Hwang, S.B., and Lee, S.Y. (2001). Hepatitis C virus core protein potentiates TNF-alpha-induced NF-kappaB activation through TRAF2-IKKbeta-dependent pathway. Biochem Biophys Res Commun *284*, 15-19.

Clement, J.F., Meloche, S., and Servant, M.J. (2008). The IKK-related kinases: from innate immunity to oncogenesis. Cell Res *18*, 889-899.

Coaquette, A., Bourgeois, A., Dirand, C., Varin, A., Chen, W., and Herbein, G. (2004). Mixed cytomegalovirus glycoprotein B genotypes in immunocompromised patients. Clin Infect Dis *39*, 155-161.

Coffin, J.M., Hughes, S.H., and Varmus, H.E. (1997). Retroviruses (Plainview NY, Cold Spring Harbor Laboratory Press).

Cohen, E.A., Dehni, G., Sodroski, J.G., and Haseltine, W.A. (1990). Human immunodeficiency virus vpr product is a virion-associated regulatory protein. J Virol *64*, 3097-3099.

Coiras, M., Lopez-Huertas, M.R., Perez-Olmeda, M., and Alcami, J. (2009). Understanding HIV-1 latency provides clues for the eradication of long-term reservoirs. Nat Rev Microbiol *7*, 798-812.

Collman, R., Balliet, J.W., Gregory, S.A., Friedman, H., Kolson, D.L., Nathanson, N., and Srinivasan, A. (1992). An infectious molecular clone of an unusual macrophage-tropic and highly cytopathic strain of human immunodeficiency virus type 1. J Virol *66*, 7517-7521.

Compton, T., Nowlin, D.M., and Cooper, N.R. (1993). Initiation of human cytomegalovirus infection requires initial interaction with cell surface heparan sulfate. Virology *193*, 834-841.

Connolly, J.L., Rodgers, S.E., Clarke, P., Ballard, D.W., Kerr, L.D., Tyler, K.L., and Dermody, T.S. (2000). Reovirus-induced apoptosis requires activation of transcription factor NF-kappaB. J Virol *74*, 2981-2989.

Conti, L., Hiscott, J., Papacchini, M., Roulston, A., Wainberg, M.A., Belardelli, F., and Gessani, S. (1997). Induction of relA(p65) and I kappa B alpha subunit expression during differentiation of human peripheral blood monocytes to macrophages. Cell Growth Differ *8*, 435-442.

Coquillard, G., and Patterson, B.K. (2009). Determination of hepatitis C virus-infected, monocyte lineage reservoirs in individuals with or without HIV coinfection. J Infect Dis *200*, 947-954.

Crabtree, G.R. (1999). Generic signals and specific outcomes: signaling through Ca2+, calcineurin, and NF-AT. Cell *96*, 611-614.

Crotti, A., Lusic, M., Lupo, R., Lievens, P.M., Liboi, E., Della Chiara, G., Tinelli, M., Lazzarin, A., Patterson, B.K., Giacca, M., *et al.* (2007). Naturally occurring C-terminally truncated STAT5 is a negative regulator of HIV-1 expression. Blood *109*, 5380-5389.

Crowe, S., Maslin, C., Ellery, P., and Kedzierska, K. (2004). Culture of HIV in monocytes and macrophages. Curr Protoc Immunol *Chapter 12*, Unit 12 14.

Cuevas-Bennett, C., and Shenk, T. (2008). Dynamic histone H3 acetylation and methylation at human cytomegalovirus promoters during replication in fibroblasts. J Virol *82*, 9525-9536.

D'Addario, M., Ahmad, A., Xu, J.W., and Menezes, J. (1999). Epstein-Barr virus envelope glycoprotein gp350 induces NF-kappaB activation and IL-1beta synthesis in human monocytes-macrophages involving PKC and PI3-K. FASEB J *13*, 2203-2213.

Daar, E.S., Lynn, H., Donfield, S., Gomperts, E., O'Brien, S.J., Hilgartner, M.W., Hoots, W.K., Chernoff, D., Arkin, S., Wong, W.Y., *et al.* (2001). Hepatitis C virus load is associated with human immunodeficiency virus type 1 disease progression in hemophiliacs. J Infect Dis *183*, 589-595.

Dai, R., Phillips, R.A., and Ahmed, S.A. (2007). Despite inhibition of nuclear localization of NF-kappa B p65, c-Rel, and RelB, 17-beta estradiol up-regulates NF-kappa B signaling in mouse splenocytes: the potential role of Bcl-3. J Immunol *179*, 1776-1783.

Dal Monte, P., Landini, M.P., Sinclair, J., Virelizier, J.L., and Michelson, S. (1997). TAR and Sp1-independent transactivation of HIV long terminal repeat by the Tat protein in the presence of human cytomegalovirus IE1/IE2. AIDS *11*, 297-303.

Darnell, G.A., Schroder, W.A., Gardner, J., Harrich, D., Yu, H., Medcalf, R.L., Warrilow, D., Antalis, T.M., Sonza, S., and Suhrbier, A. (2006). SerpinB2 is an inducible host factor involved in enhancing HIV-1 transcription and replication. J Biol Chem *281*, 31348-31358.

Davis, M.E., Grumbach, I.M., Fukai, T., Cutchins, A., and Harrison, D.G. (2004). Shear stress regulates endothelial nitric-oxide synthase promoter activity through nuclear factor kappaB binding. J Biol Chem *279*, 163-168.

de Kozak, Y., Omri, B., Smith, J.R., Naud, M.C., Thillaye-Goldenberg, B., and Crisanti, P. (2007). Protein kinase Czeta (PKCzeta) regulates ocular inflammation and apoptosis in endotoxin-induced uveitis (EIU): signaling molecules involved in EIU resolution by PKCzeta inhibitor and interleukin-13. Am J Pathol *170*, 1241-1257.

de Los Santos, T., Diaz-San Segundo, F., and Grubman, M.J. (2007). Degradation of nuclear factor kappa B during foot-and-mouth disease virus infection. J Virol *81*, 12803-12815.

De Luca, A., Bugarini, R., Lepri, A.C., Puoti, M., Girardi, E., Antinori, A., Poggio, A., Pagano, G., Tositti, G., Cadeo, G., *et al.* (2002). Coinfection with hepatitis viruses and outcome of initial antiretroviral regimens in previously naive HIV-infected subjects. Arch Intern Med *162*, 2125-2132.

de Lucas, S., Bartolome, J., Amaro, M.J., and Carreno, V. (2003). Hepatitis C virus core protein transactivates the inducible nitric oxide synthase promoter via NF-kappaB activation. Antiviral Res *60*, 117-124.

de Maisieres, P.D., Baudoux-Tebache, L., Merville, M.P., Rentier, B., Bours, V., and Piette, J. (1998). Activation of the human immunodeficiency virus long terminal repeat by varicella-zoster virus IE4 protein requires nuclear factor-kappaB and involves both the amino-terminal and the carboxyl-terminal cysteine-rich region. J Biol Chem *273*, 13636-13644.

Deacon, N.J., Tsykin, A., Solomon, A., Smith, K., Ludford-Menting, M., Hooker, D.J., McPhee, D.A., Greenway, A.L., Ellett, A., Chatfield, C., *et al.* (1995). Genomic structure of an attenuated quasi species of HIV-1 from a blood transfusion donor and recipients. Science *270*, 988-991.

Dechend, R., Hirano, F., Lehmann, K., Heissmeyer, V., Ansieau, S., Wulczyn, F.G., Scheidereit, C., and Leutz, A. (1999). The Bcl-3 oncoprotein acts as a bridging factor between NF-kappaB/Rel and nuclear co-regulators. Oncogene *18*, 3316-3323.

Dejardin, E., Droin, N.M., Delhase, M., Haas, E., Cao, Y., Makris, C., Li, Z.W., Karin, M., Ware, C.F., and Green, D.R. (2002). The lymphotoxin-beta receptor induces different patterns of gene expression via two NF-kappaB pathways. Immunity *17*, 525-535.

Delelis, O., Carayon, K., Saib, A., Deprez, E., and Mouscadet, J.F. (2008). Integrase and integration: biochemical activities of HIV-1 integrase. Retrovirology *5*, 114.

DeLuca, C., Kwon, H., Pelletier, N., Wainberg, M.A., and Hiscott, J. (1998). NF-kappaB protects HIV-1-infected myeloid cells from apoptosis. Virology *244*, 27-38.

DeLuca, C., Petropoulos, L., Zmeureanu, D., and Hiscott, J. (1999). Nuclear IkappaBbeta maintains persistent NF-kappaB activation in HIV-1-infected myeloid cells. J Biol Chem *274*, 13010-13016.

DeMeritt, I.B., Milford, L.E., and Yurochko, A.D. (2004). Activation of the NF-kappaB pathway in human cytomegalovirus-infected cells is necessary for efficient transactivation of the major immediate-early promoter. J Virol *78*, 4498-4507.

DeMeritt, I.B., Podduturi, J.P., Tilley, A.M., Nogalski, M.T., and Yurochko, A.D. (2006). Prolonged activation of NF-kappaB by human cytomegalovirus promotes efficient viral replication and late gene expression. Virology *346*, 15-31.

DeMeritt, I.B., and Yurochko, A.D. (2006). The role of cellular transcription factors in the immediate-early stages of β-herpesvirus replication. In Recent Research Developments in Virology (Kerala, India, Transworld Research Network), pp. 55-107.

Deneka, M., Pelchen-Matthews, A., Byland, R., Ruiz-Mateos, E., and Marsh, M. (2007). In macrophages, HIV-1 assembles into an intracellular plasma membrane domain containing the tetraspanins CD81, CD9, and CD53. J Cell Biol *177*, 329-341.

Deng, H., Liu, R., Ellmeier, W., Choe, S., Unutmaz, D., Burkhart, M., Di Marzio, P., Marmon, S., Sutton, R.E., Hill, C.M., *et al.* (1996). Identification of a major co-receptor for primary isolates of HIV-1. Nature *381*, 661-666.

Devergne, O., Cahir McFarland, E.D., Mosialos, G., Izumi, K.M., Ware, C.F., and Kieff, E. (1998). Role of the TRAF binding site and NF-kappaB activation in Epstein-Barr virus latent membrane protein 1-induced cell gene expression. J Virol *72*, 7900-7908.

Devergne, O., Hatzivassiliou, E., Izumi, K.M., Kaye, K.M., Kleijnen, M.F., Kieff, E., and Mosialos, G. (1996). Association of TRAF1, TRAF2, and TRAF3 with an Epstein-Barr virus LMP1 domain important for B-lymphocyte transformation: role in NF-kappaB activation. Mol Cell Biol *16*, 7098-7108.

Dhib-Jalbut, S., Xia, J., Rangaviggula, H., Fang, Y.Y., and Lee, T. (1999). Failure of measles virus to activate nuclear factor-kappa B in neuronal cells: implications on the immune response to viral infections in the central nervous system. J Immunol *162*, 4024-4029.

Diamond, T.L., Roshal, M., Jamburuthugoda, V.K., Reynolds, H.M., Merriam, A.R., Lee, K.Y., Balakrishnan, M., Bambara, R.A., Planelles, V., Dewhurst, S., *et al.* (2004). Macrophage tropism of HIV-1 depends on efficient cellular dNTP utilization by reverse transcriptase. J Biol Chem *279*, 51545-51553.

Diel, D.G., Delhon, G., Luo, S., Flores, E.F., and Rock, D.L. (2010). A novel inhibitor of the NF-{kappa}B signaling pathway encoded by the parapoxvirus orf virus. J Virol.

Digel, M., and Sinzger, C. (2006). Regulation of Human Cytomegalovirus Gene Expression by Chromatin Remodeling. In Cytomegalovirus: Molecular Biology and Immunology, M.J. Reddehase, ed. (Caister Academic Press, Norfolk UK), pp. 445-466.

DiPerna, G., Stack, J., Bowie, A.G., Boyd, A., Kotwal, G., Zhang, Z., Arvikar, S., Latz, E., Fitzgerald, K.A., and Marshall, W.L. (2004). Poxvirus protein N1L targets the I-kappaB kinase complex, inhibits signaling to NF-kappaB by the tumor necrosis factor superfamily

of receptors, and inhibits NF-kappaB and IRF3 signaling by toll-like receptors. J Biol Chem *279*, 36570-36578.

Dolganiuc, A., Kodys, K., Kopasz, A., Marshall, C., Do, T., Romics, L., Jr., Mandrekar, P., Zapp, M., and Szabo, G. (2003). Hepatitis C virus core and nonstructural protein 3 proteins induce pro- and anti-inflammatory cytokines and inhibit dendritic cell differentiation. J Immunol *170*, 5615-5624.

Dong, C., Kwas, C., and Wu, L. (2009). Transcriptional restriction of human immunodeficiency virus type 1 gene expression in undifferentiated primary monocytes. J Virol *83*, 3518-3527.

Dong, J., Jimi, E., Zhong, H., Hayden, M.S., and Ghosh, S. (2008). Repression of gene expression by unphosphorylated NF-kappaB p65 through epigenetic mechanisms. Genes Dev *22*, 1159-1173.

Dorfleutner, A., Talbott, S.J., Bryan, N.B., Funya, K.N., Rellick, S.L., Reed, J.C., Shi, X., Rojanasakul, Y., Flynn, D.C., and Stehlik, C. (2007). A Shope Fibroma virus PYRIN-only protein modulates the host immune response. Virus Genes *35*, 685-694.

Dreyfus, D.H., Nagasawa, M., Pratt, J.C., Kelleher, C.A., and Gelfand, E.W. (1999). Inactivation of NF-kappaB by EBV BZLF-1-encoded ZEBRA protein in human T cells. J Immunol *163*, 6261-6268.

Dubuisson, J. (2007). Hepatitis C virus proteins. World J Gastroenterol *13*, 2406-2415.

Duran, A., Diaz-Meco, M.T., and Moscat, J. (2003). Essential role of RelA Ser311 phosphorylation by zetaPKC in NF-kappaB transcriptional activation. EMBO J *22*, 3910-3918.

Edelstein, L.C., Pan, A., and Collins, T. (2005). Chromatin modification and the endothelial-specific activation of the E-selectin gene. J Biol Chem *280*, 11192-11202.

Eickhoff, J., Hanke, M., Stein-Gerlach, M., Kiang, T.P., Herzberger, K., Habenberger, P., Muller, S., Klebl, B., Marschall, M., Stamminger, T., *et al.* (2004). RICK activates a NF-kappaB-dependent anti-human cytomegalovirus response. J Biol Chem *279*, 9642-9652.

Eickhoff, J.E., and Cotten, M. (2005). NF-kappaB activation can mediate inhibition of human cytomegalovirus replication. J Gen Virol *86*, 285-295.

Endres, M.J., Clapham, P.R., Marsh, M., Ahuja, M., Turner, J.D., McKnight, A., Thomas, J.F., Stoebenau-Haggarty, B., Choe, S., Vance, P.J., *et al.* (1996). CD4-independent infection by HIV-2 is mediated by fusin/CXCR4. Cell *87*, 745-756.

Ensoli, B., Lusso, P., Schachter, F., Josephs, S.F., Rappaport, J., Negro, F., Gallo, R.C., and Wong-Staal, F. (1989). Human herpes virus-6 increases HIV-1 expression in co-infected T cells via nuclear factors binding to the HIV-1 enhancer. EMBO J *8*, 3019-3027.

Esfandiarei, M., Boroomand, S., Suarez, A., Si, X., Rahmani, M., and McManus, B. (2007). Coxsackievirus B3 activates nuclear factor kappa B transcription factor via a phosphatidylinositol-3 kinase/protein kinase B-dependent pathway to improve host cell viability. Cell Microbiol *9*, 2358-2371.

Fan, Z., Yang, Q.R., Twu, J.S., and Sherker, A.H. (1999). Specific in vitro association between the hepatitis C viral genome and core protein. J Med Virol *59*, 131-134.

Fang, X., Gao, J., Zheng, H., Li, B., Kong, L., Zhang, Y., Wang, W., Zeng, Y., and Ye, L. (2007). The membrane protein of SARS-CoV suppresses NF-kappaB activation. J Med Virol *79*, 1431-1439.

Federico, M., Percario, Z., Olivetta, E., Fiorucci, G., Muratori, C., Micheli, A., Romeo, G., and Affabris, E. (2001). HIV-1 Nef activates STAT1 in human monocytes/macrophages through the release of soluble factors. Blood *98*, 2752-2761.

Feng, Y., Broder, C.C., Kennedy, P.E., and Berger, E.A. (1996). HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science *272*, 872-877.

Field, N., Low, W., Daniels, M., Howell, S., Daviet, L., Boshoff, C., and Collins, M. (2003). KSHV vFLIP binds to IKK-gamma to activate IKK. J Cell Sci *116*, 3721-3728.

Fish, K.N., Britt, W., and Nelson, J.A. (1996). A novel mechanism for persistence of human cytomegalovirus in macrophages. J Virol *70*, 1855-1862.

Flory, E., Kunz, M., Scheller, C., Jassoy, C., Stauber, R., Rapp, U.R., and Ludwig, S. (2000). Influenza virus-induced NF-kappaB-dependent gene expression is mediated by overexpression of viral proteins and involves oxidative radicals and activation of IkappaB kinase. J Biol Chem *275*, 8307-8314.

Folks, T.M., Justement, J., Kinter, A., Schnittman, S., Orenstein, J., Poli, G., and Fauci, A.S. (1988). Characterization of a promonocyte clone chronically infected with HIV and inducible by 13-phorbol-12-myristate acetate. J Immunol *140*, 1117-1122.

Fontaine, V., van der Meijden, E., de Graaf, J., ter Schegget, J., and Struyk, L. (2000). A functional NF-kappaB binding site in the human papillomavirus type 16 long control region. Virology *272*, 40-49.

Foster, J.L., and Garcia, J.V. (2007). Role of Nef in HIV-1 replication and pathogenesis. Adv Pharmacol *55*, 389-409.

Frascaroli, G., Varani, S., Blankenhorn, N., Pretsch, R., Bacher, M., Leng, L., Bucala, R., Landini, M.P., and Mertens, T. (2009). Human cytomegalovirus paralyzes macrophage motility through down-regulation of chemokine receptors, reorganization of the cytoskeleton, and release of macrophage migration inhibitory factor. J Immunol *182*, 477-488.

Frascaroli, G., Varani, S., Moepps, B., Sinzger, C., Landini, M.P., and Mertens, T. (2006). Human cytomegalovirus subverts the functions of monocytes, impairing chemokine-mediated migration and leukocyte recruitment. J Virol *80*, 7578-7589.

Freed, E.O. (2004). HIV-1 and the host cell: an intimate association. Trends Microbiol *12*, 170-177.

Freed, E.O., and Martin, M.A. (2007). HIVs and Their Replication. In Fields Virology, D.M. Knipe, and P.M. Howley, eds. (Philadelphia, Lippincott Williams & Wilkins), pp. 2107-2185.

Friess, M., Engelhardt, P., Dobbelaere, D., Zurbriggen, A., and Grone, A. (2005). Reduced nuclear translocation of nuclear factor (NF)-kappaB p65 in the footpad epidermis of dogs infected with distemper virus. J Comp Pathol *132*, 82-89.

Fujii, Y., Otake, K., Tashiro, M., and Adachi, A. (1996). Soluble Nef antigen of HIV-1 is cytotoxic for human CD4+ T cells. FEBS Lett *393*, 93-96.

Fujita, M., Otsuka, M., Miyoshi, M., Khamsri, B., Nomaguchi, M., and Adachi, A. (2008). Vpx is critical for reverse transcription of the human immunodeficiency virus type 2 genome in macrophages. J Virol *82*, 7752-7756.

Fujita, T., Nolan, G.P., Liou, H.C., Scott, M.L., and Baltimore, D. (1993). The candidate proto-oncogene bcl-3 encodes a transcriptional coactivator that activates through NF-kappa B p50 homodimers. Genes Dev *7*, 1354-1363.

Fukutomi, T., Zhou, Y., Kawai, S., Eguchi, H., Wands, J.R., and Li, J. (2005). Hepatitis C virus core protein stimulates hepatocyte growth: correlation with upregulation of wnt-1 expression. Hepatology *41*, 1096-1105.

Furia, B., Deng, L., Wu, K., Baylor, S., Kehn, K., Li, H., Donnelly, R., Coleman, T., and Kashanchi, F. (2002). Enhancement of nuclear factor-kappa B acetylation by coactivator p300 and HIV-1 Tat proteins. J Biol Chem *277*, 4973-4980.

Furukawa, T., Fioretti, A., and Plotkin, S. (1973). Growth characteristics of cytomegalovirus in human fibroblasts with demonstration of protein synthesis early in viral replication. J Virol *11*, 991-997.

Gafa, V., Manches, O., Pastor, A., Drouet, E., Ambroise-Thomas, P., Grillot, R., and Aldebert, D. (2005). Human cytomegalovirus downregulates complement receptors (CR3, CR4) and decreases phagocytosis by macrophages. J Med Virol *76*, 361-366.

Garcia-Samaniego, J., Rodriguez, M., Berenguer, J., Rodriguez-Rosado, R., Carbo, J., Asensi, V., and Soriano, V. (2001). Hepatocellular carcinoma in HIV-infected patients with chronic hepatitis C. Am J Gastroenterol *96*, 179-183.

Garofalo, R., Sabry, M., Jamaluddin, M., Yu, R.K., Casola, A., Ogra, P.L., and Brasier, A.R. (1996). Transcriptional activation of the interleukin-8 gene by respiratory syncytial virus infection in alveolar epithelial cells: nuclear translocation of the RelA transcription factor as a mechanism producing airway mucosal inflammation. J Virol *70*, 8773-8781.

Gealy, C., Humphreys, C., Dickinson, V., Stinski, M., and Caswell, R. (2007). An activation-defective mutant of the human cytomegalovirus IE2p86 protein inhibits NF-kappaB-mediated stimulation of the human interleukin-6 promoter. J Gen Virol *88*, 2435-2440.

Gedey, R., Jin, X.L., Hinthong, O., and Shisler, J.L. (2006). Poxviral regulation of the host NF-kappaB response: the vaccinia virus M2L protein inhibits induction of NF-kappaB activation via an ERK2 pathway in virus-infected human embryonic kidney cells. J Virol *80*, 8676-8685.

Geijtenbeek, T.B., Kwon, D.S., Torensma, R., van Vliet, S.J., van Duijnhoven, G.C., Middel, J., Cornelissen, I.L., Nottet, H.S., KewalRamani, V.N., Littman, D.R., *et al.* (2000). DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. Cell *100*, 587-597.

Geleziunas, R., Xu, W., Takeda, K., Ichijo, H., and Greene, W.C. (2001). HIV-1 Nef inhibits ASK1-dependent death signalling providing a potential mechanism for protecting the infected host cell. Nature *410*, 834-838.

Geng, H., Wittwer, T., Dittrich-Breiholz, O., Kracht, M., and Schmitz, M.L. (2009). Phosphorylation of NF-kappaB p65 at Ser468 controls its COMMD1-dependent ubiquitination and target gene-specific proteasomal elimination. EMBO Rep *10*, 381-386.

Ghaffari, G., Tuttle, D.L., Briggs, D., Burkhardt, B.R., Bhatt, D., Andiman, W.A., Sleasman, J.W., and Goodenow, M.M. (2005). Complex determinants in human immunodeficiency virus type 1 envelope gp120 mediate CXCR4-dependent infection of macrophages. J Virol *79*, 13250-13261.

Ghassemi, M., Asadi, F.K., Andersen, B.R., and Novak, R.M. (2000). Mycobacterium avium induces HIV upregulation through mechanisms independent of cytokine induction. AIDS Res Hum Retroviruses *16*, 435-440.

Ghassemi, M., Novak, R.M., Khalili, M.F., and Zhou, J. (2003). Viable Mycobacterium avium is required for the majority of human immunodeficiency virus-induced upregulation in monocytoid cells. J Med Microbiol *52*, 877-882.

Ghosh, S., and Hayden, M.S. (2008). New regulators of NF-kappaB in inflammation. Nat Rev Immunol *8*, 837-848.

Ghosh, S., and Karin, M. (2002). Missing pieces in the NF-kappaB puzzle. Cell *109 Suppl*, S81-96.

Gibson, W. (2006). Assembly and Maturation of the Capsid. In Cytomegalovirus: Molecular Biology and Immunology, M.J. Reddehase, ed. (Caister Academic Press, Norfolk UK), pp. 231-244.

Gilmore, T.D. (2010). Inducers of NF-kB activity. In Rel/NF-kB Transcription factors available at www nf-kb org (Boston, MA).

Gilmore, T.D., and Mosialos, G. (2003). Viuses as Inducers in the Rel/NF-kB Siganling Pathway. In Nucear Factor-kB: Regulation and Role in Disease, R. Beyaert, ed. (Dordrecht, The Netherlands, Kluwer Academic Publishers), pp. 91-115.

Gimble, J.M., Duh, E., Ostrove, J.M., Gendelman, H.E., Max, E.E., and Rabson, A.B. (1988). Activation of the human immunodeficiency virus long terminal repeat by herpes simplex virus type 1 is associated with induction of a nuclear factor that binds to the NF-kappa B/core enhancer sequence. J Virol *62*, 4104-4112.

Goila-Gaur, R., and Strebel, K. (2008). HIV-1 Vif, APOBEC, and intrinsic immunity. Retrovirology *5*, 51.

Gomez-Gonzalo, M., Carretero, M., Rullas, J., Lara-Pezzi, E., Aramburu, J., Berkhout, B., Alcami, J., and Lopez-Cabrera, M. (2001). The hepatitis B virus X protein induces HIV-1 replication and transcription in synergy with T-cell activation signals: functional roles of NF-kappaB/NF-AT and SP1-binding sites in the HIV-1 long terminal repeat promoter. J Biol Chem *276*, 35435-35443.

Gordon, S. (2003). Alternative activation of macrophages. Nat Rev Immunol *3*, 23-35.

Graff, J.W., Ettayebi, K., and Hardy, M.E. (2009). Rotavirus NSP1 inhibits NFkappaB activation by inducing proteasome-dependent degradation of beta-TrCP: a novel mechanism of IFN antagonism. PLoS Pathog *5*, e1000280.

Graham, C.S., Baden, L.R., Yu, E., Mrus, J.M., Carnie, J., Heeren, T., and Koziel, M.J. (2001). Influence of human immunodeficiency virus infection on the course of hepatitis C virus infection: a meta-analysis. Clin Infect Dis *33*, 562-569.

Granelli-Piperno, A., Delgado, E., Finkel, V., Paxton, W., and Steinman, R.M. (1998). Immature dendritic cells selectively replicate macrophagetropic (M-tropic) human immunodeficiency virus type 1, while mature cells efficiently transmit both M- and T-tropic virus to T cells. J Virol *72*, 2733-2737.

Granja, A.G., Sabina, P., Salas, M.L., Fresno, M., and Revilla, Y. (2006). Regulation of inducible nitric oxide synthase expression by viral A238L-mediated inhibition of p65/RelA acetylation and p300 transactivation. J Virol *80*, 10487-10496.

Gredmark, S., Britt, W.B., Xie, X., Lindbom, L., and Soderberg-Naucler, C. (2004a). Human cytomegalovirus induces inhibition of macrophage differentiation by binding to human aminopeptidase N/CD13. J Immunol *173*, 4897-4907.

Gredmark, S., Tilburgs, T., and Soderberg-Naucler, C. (2004b). Human cytomegalovirus inhibits cytokine-induced macrophage differentiation. J Virol *78*, 10378-10389.

Greenway, A.L., McPhee, D.A., Allen, K., Johnstone, R., Holloway, G., Mills, J., Azad, A., Sankovich, S., and Lambert, P. (2002). Human immunodeficiency virus type 1 Nef binds to tumor suppressor p53 and protects cells against p53-mediated apoptosis. J Virol *76*, 2692-2702.

Greub, G., Ledergerber, B., Battegay, M., Grob, P., Perrin, L., Furrer, H., Burgisser, P., Erb, P., Boggian, K., Piffaretti, J.C., *et al.* (2000). Clinical progression, survival, and immune recovery during antiretroviral therapy in patients with HIV-1 and hepatitis C virus coinfection: the Swiss HIV Cohort Study. Lancet *356*, 1800-1805.

Gribaudo, G., Ravaglia, S., Gaboli, M., Gariglio, M., Cavallo, R., and Landolfo, S. (1995). Interferon-alpha inhibits the murine cytomegalovirus immediate-early gene expression by down-regulating NF-kappa B activity. Virology *211*, 251-260.

Gribaudo, G., Ravaglia, S., Guandalini, L., Cavallo, R., Gariglio, M., and Landolfo, S. (1996). The murine cytomegalovirus immediate-early 1 protein stimulates NF-kappa B activity by transactivating the NF-kappa B p105/p50 promoter. Virus Res *45*, 15-27.

Griffin, G.E., Leung, K., Folks, T.M., Kunkel, S., and Nabel, G.J. (1989). Activation of HIV gene expression during monocyte differentiation by induction of NF-kappa B. Nature *339*, 70-73.

Groves, I.J., Reeves, M.B., and Sinclair, J.H. (2009). Lytic infection of permissive cells with human cytomegalovirus is regulated by an intrinsic 'pre-immediate-early' repression of viral gene expression mediated by histone post-translational modification. J Gen Virol *90*, 2364-2374.

Guan, H., Hou, S., and Ricciardi, R.P. (2005). DNA binding of repressor nuclear factor-kappaB p50/p50 depends on phosphorylation of Ser337 by the protein kinase A catalytic subunit. J Biol Chem *280*, 9957-9962.

Guasparri, I., Bubman, D., and Cesarman, E. (2008). EBV LMP2A affects LMP1-mediated NF-kappaB signaling and survival of lymphoma cells by regulating TRAF2 expression. Blood *111*, 3813-3820.

Guasparri, I., Wu, H., and Cesarman, E. (2006). The KSHV oncoprotein vFLIP contains a TRAF-interacting motif and requires TRAF2 and TRAF3 for signalling. EMBO Rep *7*, 114-119.

Guerra, S., Lopez-Fernandez, L.A., Conde, R., Pascual-Montano, A., Harshman, K., and Esteban, M. (2004). Microarray analysis reveals characteristic changes of host cell gene expression in response to attenuated modified vaccinia virus Ankara infection of human HeLa cells. J Virol *78*, 5820-5834.

Guillemard, E., Jacquemot, C., Aillet, F., Schmitt, N., Barre-Sinoussi, F., and Israel, N. (2004). Human immunodeficiency virus 1 favors the persistence of infection by activating macrophages through TNF. Virology *329*, 371-380.

Guitart, A., Riezu-Boj, J.I., Elizalde, E., Larrea, E., Berasain, C., Aldabe, R., Civeira, M.P., and Prieto, J. (2005). Hepatitis C virus infection of primary tupaia hepatocytes leads to selection of quasispecies variants, induction of interferon-stimulated genes and NF-kappaB nuclear translocation. J Gen Virol *86*, 3065-3074.

Gustems, M., Borst, E., Benedict, C.A., Perez, C., Messerle, M., Ghazal, P., and Angulo, A. (2006). Regulation of the transcription and replication cycle of human cytomegalovirus is insensitive to genetic elimination of the cognate NF-kappaB binding sites in the enhancer. J Virol *80*, 9899-9904.

Hacker, H., and Karin, M. (2006). Regulation and function of IKK and IKK-related kinases. Sci STKE *2006*, re13.

Halwachs-Baumann, G., Wilders-Truschnig, M., Desoye, G., Hahn, T., Kiesel, L., Klingel, K., Rieger, P., Jahn, G., and Sinzger, C. (1998). Human trophoblast cells are permissive to the complete replicative cycle of human cytomegalovirus. J Virol *72*, 7598-7602.

Hammarskjold, M.L., and Simurda, M.C. (1992). Epstein-Barr virus latent membrane protein transactivates the human immunodeficiency virus type 1 long terminal repeat through induction of NF-kappa B activity. J Virol *66*, 6496-6501.

Hanson, L.K., and Campbell, A.N. (2006). Determinants of macrophage tropism. In Cytomegaloviruses: Molecular Biology and Immunology, M.J. Reddehase, ed. (Caister Academic Press, Norfolk UK), pp. 419-443.

Hanson, L.K., Slater, J.S., Cavanaugh, V.J., Newcomb, W.W., Bolin, L.L., Nelson, C.N., Fetters, L.D., Tang, Q., Brown, J.C., Maul, G.G., *et al.* (2009). Murine cytomegalovirus capsid assembly is dependent on US22 family gene M140 in infected macrophages. J Virol *83*, 7449-7456.

Hanson, L.K., Slater, J.S., Karabekian, Z., Ciocco-Schmitt, G., and Campbell, A.E. (2001). Products of US22 genes M140 and M141 confer efficient replication of murine cytomegalovirus in macrophages and spleen. J Virol *75*, 6292-6302.

Hanson, L.K., Slater, J.S., Karabekian, Z., Virgin, H.W.t., Biron, C.A., Ruzek, M.C., van Rooijen, N., Ciavarra, R.P., Stenberg, R.M., and Campbell, A.E. (1999). Replication of murine cytomegalovirus in differentiated macrophages as a determinant of viral pathogenesis. J Virol *73*, 5970-5980.

Harcourt, B.H., Rota, P.A., Hummel, K.B., Bellini, W.J., and Offermann, M.K. (1999). Induction of intercellular adhesion molecule 1 gene expression by measles virus in human umbilical vein endothelial cells. J Med Virol *57*, 9-16.

Hargett, D., Rice, S., and Bachenheimer, S.L. (2006). Herpes simplex virus type 1 ICP27-dependent activation of NF-kappaB. J Virol *80*, 10565-10578.

Harrich, D., Garcia, J., Wu, F., Mitsuyasu, R., Gonazalez, J., and Gaynor, R. (1989). Role of SP1-binding domains in in vivo transcriptional regulation of the human immunodeficiency virus type 1 long terminal repeat. J Virol *63*, 2585-2591.

Hassan, M., Selimovic, D., Ghozlan, H., and Abdel-Kader, O. (2007). Induction of high-molecular-weight (HMW) tumor necrosis factor(TNF) alpha by hepatitis C virus (HCV) non-structural protein 3 (NS3) in liver cells is AP-1 and NF-kappaB-dependent activation. Cell Signal *19*, 301-311.

Hayden, M.S., and Ghosh, S. (2004). Signaling to NF-kappaB. Genes Dev *18*, 2195-2224.

Hayden, M.S., and Ghosh, S. (2008). Shared principles in NF-kappaB signaling. Cell *132*, 344-362.

He, G., and Margolis, D.M. (2002). Counterregulation of chromatin deacetylation and histone deacetylase occupancy at the integrated promoter of human immunodeficiency virus type 1 (HIV-1) by the HIV-1 repressor YY1 and HIV-1 activator Tat. Mol Cell Biol *22*, 2965-2973.

Heieren, M.H., Kim, Y.K., and Balfour, H.H., Jr. (1988). Human cytomegalovirus infection of kidney glomerular visceral epithelial and tubular epithelial cells in culture. Transplantation *46*, 426-432.

Henderson, A.J., and Calame, K.L. (1997). CCAAT/enhancer binding protein (C/EBP) sites are required for HIV-1 replication in primary macrophages but not CD4(+) T cells. Proc Natl Acad Sci U S A *94*, 8714-8719.

Henriet, S., Mercenne, G., Bernacchi, S., Paillart, J.C., and Marquet, R. (2009). Tumultuous relationship between the human immunodeficiency virus type 1 viral infectivity factor (Vif) and the human APOBEC-3G and APOBEC-3F restriction factors. Microbiol Mol Biol Rev *73*, 211-232.

Herbein, G. (2006). Autologous Cell-Mediated Killing. In Cell Death During HIV Infection, A.D. Badley, ed. (Boca Raton, FL, CRC Press), pp. 253-270.

Herbein, G., and Khan, K.A. (2008). Is HIV infection a TNF receptor signalling-driven disease? Trends Immunol *29*, 61-67.

Herbein, G., Mahlknecht, U., Batliwalla, F., Gregersen, P., Pappas, T., Butler, J., O'Brien, W.A., and Verdin, E. (1998). Apoptosis of CD8+ T cells is mediated by macrophages through interaction of HIV gp120 with chemokine receptor CXCR4. Nature *395*, 189-194.

Herbein, G., Varin, A., Larbi, A., Fortin, C., Mahlknecht, U., Fulop, T., and Aggarwal, B.B. (2008). Nef and TNFalpha are coplayers that favor HIV-1 replication in monocytic cells and primary macrophages. Curr HIV Res *6*, 117-129.

Hildt, E., Saher, G., Bruss, V., and Hofschneider, P.H. (1996). The hepatitis B virus large surface protein (LHBs) is a transcriptional activator. Virology *225*, 235-239.

Hiscott, J., Alper, D., Cohen, L., Leblanc, J.F., Sportza, L., Wong, A., and Xanthoudakis, S. (1989). Induction of human interferon gene expression is associated with a nuclear factor that interacts with the NF-kappa B site of the human immunodeficiency virus enhancer. J Virol *63*, 2557-2566.

Hiscott, J., Nguyen, T.L., Arguello, M., Nakhaei, P., and Paz, S. (2006). Manipulation of the nuclear factor-kappaB pathway and the innate immune response by viruses. Oncogene *25*, 6844-6867.

Hishiki, T., Ohshima, T., Ego, T., and Shimotohno, K. (2007). BCL3 acts as a negative regulator of transcription from the human T-cell leukemia virus type 1 long terminal repeat through interactions with TORC3. J Biol Chem *282*, 28335-28343.

Ho, D.D., Rota, T.R., Andrews, C.A., and Hirsch, M.S. (1984). Replication of human cytomegalovirus in endothelial cells. J Infect Dis *150*, 956-957.

Hoberg, J.E., Popko, A.E., Ramsey, C.S., and Mayo, M.W. (2006). IkappaB kinase alpha-mediated derepression of SMRT potentiates acetylation of RelA/p65 by p300. Mol Cell Biol *26*, 457-471.

Hoffmann, A., Levchenko, A., Scott, M.L., and Baltimore, D. (2002). The IkappaB-NF-kappaB signaling module: temporal control and selective gene activation. Science *298*, 1241-1245.

Holmes, D., Knudsen, G., Mackey-Cushman, S., and Su, L. (2007). FoxP3 enhances HIV-1 gene expression by modulating NFkappaB occupancy at the long terminal repeat in human T cells. J Biol Chem *282*, 15973-15980.

Honda, Y., Rogers, L., Nakata, K., Zhao, B.Y., Pine, R., Nakai, Y., Kurosu, K., Rom, W.N., and Weiden, M. (1998). Type I interferon induces inhibitory 16-kD CCAAT/ enhancer binding protein (C/EBP)beta, repressing the HIV-1 long terminal repeat in macrophages: pulmonary tuberculosis alters C/EBP expression, enhancing HIV-1 replication. J Exp Med *188*, 1255-1265.

Hrimech, M., Yao, X.J., Bachand, F., Rougeau, N., and Cohen, E.A. (1999). Human immunodeficiency virus type 1 (HIV-1) Vpr functions as an immediate-early protein during HIV-1 infection. J Virol *73*, 4101-4109.

Hu, J., Gardner, M.B., and Miller, C.J. (2000). Simian immunodeficiency virus rapidly penetrates the cervicovaginal mucosa after intravaginal inoculation and infects intraepithelial dendritic cells. J Virol *74*, 6087-6095.

Huang, W.C., Ju, T.K., Hung, M.C., and Chen, C.C. (2007). Phosphorylation of CBP by IKKalpha promotes cell growth by switching the binding preference of CBP from p53 to NF-kappaB. Mol Cell *26*, 75-87.

Hummel, M., Zhang, Z., Yan, S., DePlaen, I., Golia, P., Varghese, T., Thomas, G., and Abecassis, M.I. (2001). Allogeneic transplantation induces expression of cytomegalovirus immediate-early genes in vivo: a model for reactivation from latency. J Virol *75*, 4814-4822.

Huthoff, H., and Towers, G.J. (2008). Restriction of retroviral replication by APOBEC3G/F and TRIM5alpha. Trends Microbiol *16*, 612-619.

Ibanez, C.E., Schrier, R., Ghazal, P., Wiley, C., and Nelson, J.A. (1991). Human cytomegalovirus productively infects primary differentiated macrophages. J Virol *65*, 6581-6588.

Imai, K., and Okamoto, T. (2006). Transcriptional repression of human immunodeficiency virus type 1 by AP-4. J Biol Chem *281*, 12495-12505.

Irshad, M., and Dhar, I. (2006). Hepatitis C virus core protein: an update on its molecular biology, cellular functions and clinical implications. Med Princ Pract *15*, 405-416.

Isomura, H., Stinski, M.F., Kudoh, A., Nakayama, S., Murata, T., Sato, Y., Iwahori, S., and Tsurumi, T. (2008). A cis element between the TATA Box and the transcription start site of the major immediate-early promoter of human cytomegalovirus determines efficiency of viral replication. J Virol *82*, 849-858.

Isomura, H., Tsurumi, T., and Stinski, M.F. (2004). Role of the proximal enhancer of the major immediate-early promoter in human cytomegalovirus replication. J Virol *78*, 12788-12799.

Iwamoto, G.K., and Konicek, S.A. (1997). Cytomegalovirus immediate early genes upregulate interleukin-6 gene expression. J Investig Med *45*, 175-182.

Jacque, J.M., Fernandez, B., Arenzana-Seisdedos, F., Thomas, D., Baleux, F., Virelizier, J.L., and Bachelerie, F. (1996). Permanent occupancy of the human immunodeficiency virus type 1 enhancer by NF-kappa B is needed for persistent viral replication in monocytes. J Virol *70*, 2930-2938.

Jacque, J.M., and Stevenson, M. (2006). The inner-nuclear-envelope protein emerin regulates HIV-1 infectivity. Nature *441*, 641-645.

Jamaluddin, M., Choudhary, S., Wang, S., Casola, A., Huda, R., Garofalo, R.P., Ray, S., and Brasier, A.R. (2005). Respiratory syncytial virus-inducible BCL-3 expression antagonizes the STAT/IRF and NF-kappaB signaling pathways by inducing histone deacetylase 1 recruitment to the interleukin-8 promoter. J Virol *79*, 15302-15313.

Jamaluddin, M., Tian, B., Boldogh, I., Garofalo, R.P., and Brasier, A.R. (2009). Respiratory syncytial virus infection induces a reactive oxygen species-MSK1-phospho-Ser-276 RelA pathway required for cytokine expression. J Virol *83*, 10605-10615.

James, C.O., Huang, M.B., Khan, M., Garcia-Barrio, M., Powell, M.D., and Bond, V.C. (2004). Extracellular Nef protein targets CD4+ T cells for apoptosis by interacting with CXCR4 surface receptors. J Virol *78*, 3099-3109.

James, M.A., Lee, J.H., and Klingelhutz, A.J. (2006). Human papillomavirus type 16 E6 activates NF-kappaB, induces cIAP-2 expression, and protects against apoptosis in a PDZ binding motif-dependent manner. J Virol *80*, 5301-5307.

Jarvis, M.A., Borton, J.A., Keech, A.M., Wong, J., Britt, W.J., Magun, B.E., and Nelson, J.A. (2006). Human cytomegalovirus attenuates interleukin-1beta and tumor necrosis factor alpha proinflammatory signaling by inhibition of NF-kappaB activation. J Virol *80*, 5588-5598.

Jarvis, M.A., and Nelson, J.A. (2002). Human cytomegalovirus persistence and latency in endothelial cells and macrophages. Curr Opin Microbiol *5*, 403-407.

Jayakumar, P., Berger, I., Autschbach, F., Weinstein, M., Funke, B., Verdin, E., Goldsmith, M.A., and Keppler, O.T. (2005). Tissue-resident macrophages are productively infected ex vivo by primary X4 isolates of human immunodeficiency virus type 1. J Virol *79*, 5220-5226.

Jeang, K.T., Xiao, H., and Rich, E.A. (1999). Multifaceted activities of the HIV-1 transactivator of transcription, Tat. J Biol Chem *274*, 28837-28840.

Jenkins, C., Garcia, W., Godwin, M.J., Spencer, J.V., Stern, J.L., Abendroth, A., and Slobedman, B. (2008). Immunomodulatory properties of a viral homolog of human interleukin-10 expressed by human cytomegalovirus during the latent phase of infection. J Virol *82*, 3736-3750.

Jeong, S., Cho, I.R., An, W.G., Jhun, B.H., Lee, B., Park, K., and Chung, Y.H. (2007). STP-A11, an oncoprotein of Herpesvirus saimiri augments both NF-kappaB and AP-1 transcription activity through TRAF6. Exp Mol Med *39*, 56-64.

Jiang, G., Espeseth, A., Hazuda, D.J., and Margolis, D.M. (2007). c-Myc and Sp1 contribute to proviral latency by recruiting histone deacetylase 1 to the human immunodeficiency virus type 1 promoter. J Virol *81*, 10914-10923.

Johannessen, M., Olsen, P.A., Johansen, B., Seternes, O.M., and Moens, U. (2003). Activation of the coactivator four-and-a-half-LIM-only protein FHL2 and the c-fos promoter through inhibition of protein phosphatase 2A. Biochem Pharmacol *65*, 1317-1328.

Jones, J.O., and Arvin, A.M. (2006). Inhibition of the NF-kappaB pathway by varicella-zoster virus in vitro and in human epidermal cells in vivo. J Virol *80*, 5113-5124.

Jones, K.A., Kadonaga, J.T., Luciw, P.A., and Tjian, R. (1986). Activation of the AIDS retrovirus promoter by the cellular transcription factor, Sp1. Science *232*, 755-759.

Joo, M., Hahn, Y.S., Kwon, M., Sadikot, R.T., Blackwell, T.S., and Christman, J.W. (2005). Hepatitis C virus core protein suppresses NF-kappaB activation and cyclooxygenase-2 expression by direct interaction with IkappaB kinase beta. J Virol *79*, 7648-7657.

Joseph, A.M., Kumar, M., and Mitra, D. (2005). Nef: "necessary and enforcing factor" in HIV infection. Curr HIV Res *3*, 87-94.

Jouve, M., Sol-Foulon, N., Watson, S., Schwartz, O., and Benaroch, P. (2007). HIV-1 buds and accumulates in "nonacidic" endosomes of macrophages. Cell Host Microbe *2*, 85-95.

Kaito, M., Watanabe, S., Tsukiyama-Kohara, K., Yamaguchi, K., Kobayashi, Y., Konishi, M., Yokoi, M., Ishida, S., Suzuki, S., and Kohara, M. (1994). Hepatitis C virus particle detected by immunoelectron microscopic study. J Gen Virol *75 (Pt 7)*, 1755-1760.

Kalejta, R.F. (2008). Tegument proteins of human cytomegalovirus. Microbiol Mol Biol Rev *72*, 249-265.

Kanda, T., Steele, R., Ray, R., and Ray, R.B. (2008). Hepatitis C virus core protein augments androgen receptor-mediated signaling. J Virol *82*, 11066-11072.

Kanda, T., Yokosuka, O., Nagao, K., and Saisho, H. (2006). State of hepatitis C viral replication enhances activation of NF-kB- and AP-1-signaling induced by hepatitis B virus X. Cancer Lett *234*, 143-148.

Kang, M.H., So, E.Y., Park, H., and Kim, B.S. (2008). Replication of Theiler's virus requires NF-kappa B-activation: higher viral replication and spreading in astrocytes from susceptible mice. Glia *56*, 942-953.

Kang, S.M., Choi, J.K., Kim, S.J., Kim, J.H., Ahn, D.G., and Oh, J.W. (2009). Regulation of hepatitis C virus replication by the core protein through its interaction with viral RNA polymerase. Biochem Biophys Res Commun *386*, 55-59.

Kanno, M., Fromental, C., Staub, A., Ruffenach, F., Davidson, I., and Chambon, P. (1989). The SV40 TC-II(kappa B) and the related H-2Kb enhansons exhibit different cell type specific and inducible proto-enhancer activities, but the SV40 core sequence and the AP-2 binding site have no enhanson properties. EMBO J *8*, 4205-4214.

Karayiannis, P. (2005). The hepatitis C virus NS3/4A protease complex interferes with pathways of the innate immune response. J Hepatol *43*, 743-745.

Karin, M. (2006). Nuclear factor-kappaB in cancer development and progression. Nature *441*, 431-436.

Kaspari, M., Tavalai, N., Stamminger, T., Zimmermann, A., Schilf, R., and Bogner, E. (2008). Proteasome inhibitor MG132 blocks viral DNA replication and assembly of human cytomegalovirus. FEBS Lett *582*, 666-672.

Kato, N., Yoshida, H., Ono-Nita, S.K., Kato, J., Goto, T., Otsuka, M., Lan, K., Matsushima, K., Shiratori, Y., and Omata, M. (2000). Activation of intracellular signaling by hepatitis B and C viruses: C-viral core is the most potent signal inducer. Hepatology *32*, 405-412.

Kaufman, P.A., Weinberg, J.B., and Greene, W.C. (1992). Nuclear expression of the 50- and 65-kD Rel-related subunits of nuclear factor-kappa B is differentially regulated in human monocytic cells. J Clin Invest *90*, 121-129.

Keller, M.J., Wheeler, D.G., Cooper, E., and Meier, J.L. (2003). Role of the human cytomegalovirus major immediate-early promoter's 19-base-pair-repeat cyclic AMP-response element in acutely infected cells. J Virol *77*, 6666-6675.

Kelly, J., Beddall, M.H., Yu, D., Iyer, S.R., Marsh, J.W., and Wu, Y. (2008). Human macrophages support persistent transcription from unintegrated HIV-1 DNA. Virology *372*, 300-312.

Kesson, A.M., and King, N.J. (2001). Transcriptional regulation of major histocompatibility complex class I by flavivirus West Nile is dependent on NF-kappaB activation. J Infect Dis *184*, 947-954.

Kestler, H.W., 3rd, Ringler, D.J., Mori, K., Panicali, D.L., Sehgal, P.K., Daniel, M.D., and Desrosiers, R.C. (1991). Importance of the nef gene for maintenance of high virus loads and for development of AIDS. Cell *65*, 651-662.

Kfoury, Y., Nasr, R., Hermine, O., de The, H., and Bazarbachi, A. (2005). Proapoptotic regimes for HTLV-I-transformed cells: targeting Tax and the NF-kappaB pathway. Cell Death Differ *12 Suppl 1*, 871-877.

Khan, K.A., Coaquette, A., Davrinche, C., and Herbein, G. (2009). Bcl-3-regulated transcription from major immediate-early promoter of human cytomegalovirus in monocyte-derived macrophages. J Immunol *182*, 7784-7794.

Khan, K.A., Varin, A., and Herbein, G. (2007). Exogenous HIV-1 proteins, macrophages, and AIDS pathogenesis. In HIV and the Macrophage, H. G, ed. (Kerala, India, Transworld Research Network), pp. 55-77.

Kiernan, R., Bres, V., Ng, R.W., Coudart, M.P., El Messaoudi, S., Sardet, C., Jin, D.Y., Emiliani, S., and Benkirane, M. (2003). Post-activation turn-off of NF-kappa B-dependent transcription is regulated by acetylation of p65. J Biol Chem *278*, 2758-2766.

Kilareski, E.M., Shah, S., Nonnemacher, M.R., and Wigdahl, B. (2009). Regulation of HIV-1 transcription in cells of the monocyte-macrophage lineage. Retrovirology *6*, 118.

Kim, A.Y., and Chung, R.T. (2009). Coinfection with HIV-1 and HCV--a one-two punch. Gastroenterology *137*, 795-814.

Kim, S., Yu, S.S., and Kim, V.N. (1996). Essential role of NF-kappa B in transactivation of the human immunodeficiency virus long terminal repeat by the human cytomegalovirus 1E1 protein. J Gen Virol *77 (Pt 1)*, 83-91.

Kim, W.H., Hong, F., Jaruga, B., Hu, Z., Fan, S., Liang, T.J., and Gao, B. (2001). Additive activation of hepatic NF-kappaB by ethanol and hepatitis B protein X (HBX) or HCV core

protein: involvement of TNF-alpha receptor 1-independent and -dependent mechanisms. FASEB J *15*, 2551-2553.

Kim, Y.M., Sharma, N., and Nyborg, J.K. (2008). The proto-oncogene Bcl3, induced by Tax, represses Tax-mediated transcription via p300 displacement from the human T-cell leukemia virus type 1 promoter. J Virol *82*, 11939-11947.

Kinoshita, S., Su, L., Amano, M., Timmerman, L.A., Kaneshima, H., and Nolan, G.P. (1997). The T cell activation factor NF-ATc positively regulates HIV-1 replication and gene expression in T cells. Immunity *6*, 235-244.

Kirchhoff, F., Greenough, T.C., Brettler, D.B., Sullivan, J.L., and Desrosiers, R.C. (1995). Brief report: absence of intact nef sequences in a long-term survivor with nonprogressive HIV-1 infection. N Engl J Med *332*, 228-232.

Kitagawa, R., Takahashi, Y., Takahashi, M., Imazu, H., Yasuda, M., Sadanari, H., and Tanaka, J. (2009). Hexamethylene bisacetamide can convert nonpermissive human cells to a permissive state for expressing the major immediate-early genes of human cytomegalovirus by up-regulating NF-kappaB activity. Virology *383*, 195-206.

Klatzmann, D., Barre-Sinoussi, F., Nugeyre, M.T., Danquet, C., Vilmer, E., Griscelli, C., Brun-Veziret, F., Rouzioux, C., Gluckman, J.C., Chermann, J.C., *et al.* (1984). Selective tropism of lymphadenopathy associated virus (LAV) for helper-inducer T lymphocytes. Science *225*, 59-63.

Komatsu, T., Takeuchi, K., Yokoo, J., and Gotoh, B. (2004). C and V proteins of Sendai virus target signaling pathways leading to IRF-3 activation for the negative regulation of interferon-beta production. Virology *325*, 137-148.

Kondo, K., Xu, J., and Mocarski, E.S. (1996). Human cytomegalovirus latent gene expression in granulocyte-macrophage progenitors in culture and in seropositive individuals. Proc Natl Acad Sci U S A *93*, 11137-11142.

Korin, Y.D., and Zack, J.A. (1998). Progression to the G1b phase of the cell cycle is required for completion of human immunodeficiency virus type 1 reverse transcription in T cells. J Virol *72*, 3161-3168.

Kotenko, S.V., Saccani, S., Izotova, L.S., Mirochnitchenko, O.V., and Pestka, S. (2000). Human cytomegalovirus harbors its own unique IL-10 homolog (cmvIL-10). Proc Natl Acad Sci U S A *97*, 1695-1700.

Kowalik, T.F., Wing, B., Haskill, J.S., Azizkhan, J.C., Baldwin, A.S., Jr., and Huang, E.S. (1993). Multiple mechanisms are implicated in the regulation of NF-kappa B activity during human cytomegalovirus infection. Proc Natl Acad Sci U S A *90*, 1107-1111.

Kumar, N., Xin, Z.T., Liang, Y., and Ly, H. (2008). NF-kappaB signaling differentially regulates influenza virus RNA synthesis. J Virol *82*, 9880-9889.

Kung, C.P., and Raab-Traub, N. (2008). Epstein-Barr virus latent membrane protein 1 induces expression of the epidermal growth factor receptor through effects on Bcl-3 and STAT3. J Virol *82*, 5486-5493.

Kuritzkes, D.R., and Walker, B.D. (2007). HIV-1: Pathogenesis, Clinical Manifestations, and Treatment. In Fields Virology, D.M. Knipe, and P.M. Howley, eds. (Philadelphia, Lippincott Williams & Wilkins), pp. 2187-2214.

Kuwata, H., Watanabe, Y., Miyoshi, H., Yamamoto, M., Kaisho, T., Takeda, K., and Akira, S. (2003). IL-10-inducible Bcl-3 negatively regulates LPS-induced TNF-alpha production in macrophages. Blood *102*, 4123-4129.

Kwon, H., Pelletier, N., DeLuca, C., Genin, P., Cisternas, S., Lin, R., Wainberg, M.A., and Hiscott, J. (1998). Inducible expression of IkappaBalpha repressor mutants interferes with NF-kappaB activity and HIV-1 replication in Jurkat T cells. J Biol Chem *273*, 7431-7440.

Kwon, J.A., and Rho, H.M. (2002). Hepatitis B viral core protein activates the hepatitis B viral enhancer II/pregenomic promoter through the nuclear factor kappaB binding site. Biochem Cell Biol *80*, 445-455.

LaMonica, R., Kocer, S.S., Nazarova, J., Dowling, W., Geimonen, E., Shaw, R.D., and Mackow, E.R. (2001). VP4 differentially regulates TRAF2 signaling, disengaging JNK activation while directing NF-kappa B to effect rotavirus-specific cellular responses. J Biol Chem *276*, 19889-19896.

Landolfo, S., Gariglio, M., Gribaudo, G., and Lembo, D. (2003). The human cytomegalovirus. Pharmacol Ther *98*, 269-297.

Laperche, S., Le Marrec, N., Girault, A., Bouchardeau, F., Servant-Delmas, A., Maniez-Montreuil, M., Gallian, P., Levayer, T., Morel, P., and Simon, N. (2005). Simultaneous detection of hepatitis C virus (HCV) core antigen and anti-HCV antibodies improves the early detection of HCV infection. J Clin Microbiol *43*, 3877-3883.

Lapham, C.K., Zaitseva, M.B., Lee, S., Romanstseva, T., and Golding, H. (1999). Fusion of monocytes and macrophages with HIV-1 correlates with biochemical properties of CXCR4 and CCR5. Nat Med *5*, 303-308.

Lasarte, J.J., Sarobe, P., Boya, P., Casares, N., Arribillaga, L., de Cerio, A.L., Gorraiz, M., Borras-Cuesta, F., and Prieto, J. (2003). A recombinant adenovirus encoding hepatitis C virus core and E1 proteins protects mice against cytokine-induced liver damage. Hepatology *37*, 461-470.

Lashmit, P., Wang, S., Li, H., Isomura, H., and Stinski, M.F. (2009). The CREB site in the proximal enhancer is critical for cooperative interaction with the other transcription factor binding sites to enhance transcription of the major intermediate-early genes in human cytomegalovirus-infected cells. J Virol *83*, 8893-8904.

Laskus, T., Radkowski, M., Jablonska, J., Kibler, K., Wilkinson, J., Adair, D., and Rakela, J. (2004). Human immunodeficiency virus facilitates infection/replication of hepatitis C virus in native human macrophages. Blood *103*, 3854-3859.

Laskus, T., Radkowski, M., Piasek, A., Nowicki, M., Horban, A., Cianciara, J., and Rakela, J. (2000). Hepatitis C virus in lymphoid cells of patients coinfected with human immunodeficiency virus type 1: evidence of active replication in monocytes/macrophages and lymphocytes. J Infect Dis *181*, 442-448.

Lathey, J.L., and Spector, S.A. (1991). Unrestricted replication of human cytomegalovirus in hydrocortisone-treated macrophages. J Virol *65*, 6371-6375.

Law, A.H., Lee, D.C., Cheung, B.K., Yim, H.C., and Lau, A.S. (2007). Role for nonstructural protein 1 of severe acute respiratory syndrome coronavirus in chemokine dysregulation. J Virol *81*, 416-422.

Lawrence, T., Bebien, M., Liu, G.Y., Nizet, V., and Karin, M. (2005). IKKalpha limits macrophage NF-kappaB activation and contributes to the resolution of inflammation. Nature *434*, 1138-1143.

Leary, T.P., Gutierrez, R.A., Muerhoff, A.S., Birkenmeyer, L.G., Desai, S.M., and Dawson, G.J. (2006). A chemiluminescent, magnetic particle-based immunoassay for the detection of hepatitis C virus core antigen in human serum or plasma. J Med Virol *78*, 1436-1440.

Lee, C.W., Lin, W.N., Lin, C.C., Luo, S.F., Wang, J.S., Pouyssegur, J., and Yang, C.M. (2006a). Transcriptional regulation of VCAM-1 expression by tumor necrosis factor-alpha in

human tracheal smooth muscle cells: involvement of MAPKs, NF-kappaB, p300, and histone acetylation. J Cell Physiol *207*, 174-186.

Lee, D.C., Cheung, C.Y., Law, A.H., Mok, C.K., Peiris, M., and Lau, A.S. (2005). p38 mitogen-activated protein kinase-dependent hyperinduction of tumor necrosis factor alpha expression in response to avian influenza virus H5N1. J Virol *79*, 10147-10154.

Lee, E.G., Boone, D.L., Chai, S., Libby, S.L., Chien, M., Lodolce, J.P., and Ma, A. (2000). Failure to regulate TNF-induced NF-kappaB and cell death responses in A20-deficient mice. Science *289*, 2350-2354.

Lee, E.S., Sarma, D., Zhou, H., and Henderson, A.J. (2002). CCAAT/enhancer binding proteins are not required for HIV-1 entry but regulate proviral transcription by recruiting coactivators to the long-terminal repeat in monocytic cells. Virology *299*, 20-31.

Lee, G.C., Yi, H.A., and Lee, C.H. (2006b). Stimulation of interferon-beta gene expression by human cytomegalovirus via nuclear factor kappa B and phosphatidylinositol 3-kinase pathway. Virus Res *117*, 209-214.

Lee, S.M., and Kleiboeker, S.B. (2005). Porcine arterivirus activates the NF-kappaB pathway through IkappaB degradation. Virology *342*, 47-59.

Lee, Y., Sohn, W.J., Kim, D.S., and Kwon, H.J. (2004). NF-kappaB- and c-Jun-dependent regulation of human cytomegalovirus immediate-early gene enhancer/promoter in response to lipopolysaccharide and bacterial CpG-oligodeoxynucleotides in macrophage cell line RAW 264.7. Eur J Biochem *271*, 1094-1105.

Legler, D.F., Micheau, O., Doucey, M.A., Tschopp, J., and Bron, C. (2003). Recruitment of TNF receptor 1 to lipid rafts is essential for TNFalpha-mediated NF-kappaB activation. Immunity *18*, 655-664.

Lemmers, B., Salmena, L., Bidere, N., Su, H., Matysiak-Zablocki, E., Murakami, K., Ohashi, P.S., Jurisicova, A., Lenardo, M., Hakem, R., *et al.* (2007). Essential role for caspase-8 in Toll-like receptors and NFkappaB signaling. J Biol Chem *282*, 7416-7423.

Lemon, S.M., Walker, C., Alter, M.J., and Yi, M. (2007). Hepatitis C Virus. In Fields Virology, D.M. Knipe, and P.M. Howley, eds. (Philadelphia, Lippincott Williams & Wilkins), pp. 1253-1304.

Leonard, J., Parrott, C., Buckler-White, A.J., Turner, W., Ross, E.K., Martin, M.A., and Rabson, A.B. (1989). The NF-kappa B binding sites in the human immunodeficiency virus type 1 long terminal repeat are not required for virus infectivity. J Virol *63*, 4919-4924.

Lernbecher, T., Muller, U., and Wirth, T. (1993). Distinct NF-kappa B/Rel transcription factors are responsible for tissue-specific and inducible gene activation. Nature *365*, 767-770.

Lesens, O., Deschenes, M., Steben, M., Belanger, G., and Tsoukas, C.M. (1999). Hepatitis C virus is related to progressive liver disease in human immunodeficiency virus-positive hemophiliacs and should be treated as an opportunistic infection. J Infect Dis *179*, 1254-1258.

Levrero, M. (2006). Viral hepatitis and liver cancer: the case of hepatitis C. Oncogene *25*, 3834-3847.

Lewin, S.R., Lambert, P., Deacon, N.J., Mills, J., and Crowe, S.M. (1997). Constitutive expression of p50 homodimer in freshly isolated human monocytes decreases with in vitro and in vivo differentiation: a possible mechanism influencing human immunodeficiency virus replication in monocytes and mature macrophages. J Virol *71*, 2114-2119.

Ley, J.A. (2007). HIV and the pathogenesis of AIDS, 3rd edn (Washington DC ASM Press).

Li, L., Li, H.S., Pauza, C.D., Bukrinsky, M., and Zhao, R.Y. (2005a). Roles of HIV-1 auxiliary proteins in viral pathogenesis and host-pathogen interactions. Cell Res *15*, 923-934.

Li, Q., Lu, Q., Bottero, V., Estepa, G., Morrison, L., Mercurio, F., and Verma, I.M. (2005b). Enhanced NF-kappaB activation and cellular function in macrophages lacking IkappaB kinase 1 (IKK1). Proc Natl Acad Sci U S A *102*, 12425-12430.

Li, S., Ye, L., Yu, X., Xu, B., Li, K., Zhu, X., Liu, H., Wu, X., and Kong, L. (2009). Hepatitis C virus NS4B induces unfolded protein response and endoplasmic reticulum overload response-dependent NF-kappaB activation. Virology *391*, 257-264.

Liao, G., Zhang, M., Harhaj, E.W., and Sun, S.C. (2004). Regulation of the NF-kappaB-inducing kinase by tumor necrosis factor receptor-associated factor 3-induced degradation. J Biol Chem *279*, 26243-26250.

Liao, Q.J., Ye, L.B., Timani, K.A., She, Y.L., Yang, X.J., Ye, L., and Wu, Z.H. (2005a). Hepatitis C virus non-structural 5A protein can enhance full-length core protein-induced nuclear factor-kappaB activation. World J Gastroenterol *11*, 6433-6439.

Liao, Q.J., Ye, L.B., Timani, K.A., Zeng, Y.C., She, Y.L., Ye, L., and Wu, Z.H. (2005b). Activation of NF-kappaB by the full-length nucleocapsid protein of the SARS coronavirus. Acta Biochim Biophys Sin (Shanghai) *37*, 607-612.

Lin, K.I., Lee, S.H., Narayanan, R., Baraban, J.M., Hardwick, J.M., and Ratan, R.R. (1995a). Thiol agents and Bcl-2 identify an alphavirus-induced apoptotic pathway that requires activation of the transcription factor NF-kappa B. J Cell Biol *131*, 1149-1161.

Lin, R., Gewert, D., and Hiscott, J. (1995b). Differential transcriptional activation in vitro by NF-kappa B/Rel proteins. J Biol Chem *270*, 3123-3131.

Lin, S., Wu, M., Xu, Y., Xiong, W., Yi, Z., Zhang, X., and Zhenghong, Y. (2007). Inhibition of hepatitis B virus replication by MyD88 is mediated by nuclear factor-kappaB activation. Biochim Biophys Acta *1772*, 1150-1157.

Lin, S.C., Chung, J.Y., Lamothe, B., Rajashankar, K., Lu, M., Lo, Y.C., Lam, A.Y., Darnay, B.G., and Wu, H. (2008). Molecular basis for the unique deubiquitinating activity of the NF-kappaB inhibitor A20. J Mol Biol *376*, 526-540.

Lindenbach, B.D., Evans, M.J., Syder, A.J., Wolk, B., Tellinghuisen, T.L., Liu, C.C., Maruyama, T., Hynes, R.O., Burton, D.R., McKeating, J.A., *et al.* (2005). Complete replication of hepatitis C virus in cell culture. Science *309*, 623-626.

Liu, L., Eby, M.T., Rathore, N., Sinha, S.K., Kumar, A., and Chaudhary, P.M. (2002). The human herpes virus 8-encoded viral FLICE inhibitory protein physically associates with and persistently activates the Ikappa B kinase complex. J Biol Chem *277*, 13745-13751.

Liu, X., Fitzgerald, K., Kurt-Jones, E., Finberg, R., and Knipe, D.M. (2008). Herpesvirus tegument protein activates NF-kappaB signaling through the TRAF6 adaptor protein. Proc Natl Acad Sci U S A *105*, 11335-11339.

Liu, Y., Denlinger, C.E., Rundall, B.K., Smith, P.W., and Jones, D.R. (2006). Suberoylanilide hydroxamic acid induces Akt-mediated phosphorylation of p300, which promotes acetylation and transcriptional activation of RelA/p65. J Biol Chem *281*, 31359-31368.

Lobritz, M.A., Marozsan, A.J., Troyer, R.M., and Arts, E.J. (2007). Natural variation in the V3 crown of human immunodeficiency virus type 1 affects replicative fitness and entry inhibitor sensitivity. J Virol *81*, 8258-8269.

Lombardi, L., Ciana, P., Cappellini, C., Trecca, D., Guerrini, L., Migliazza, A., Maiolo, A.T., and Neri, A. (1995). Structural and functional characterization of the promoter regions of the NFKB2 gene. Nucleic Acids Res *23*, 2328-2336.

Lu, L., Wei, L., Peng, G., Mu, Y., Wu, K., Kang, L., Yan, X., Zhu, Y., and Wu, J. (2008). NS3 protein of hepatitis C virus regulates cyclooxygenase-2 expression through multiple signaling pathways. Virology *371*, 61-70.

Ludwig, S., and Planz, O. (2008). Influenza viruses and the NF-kappaB signaling pathway - towards a novel concept of antiviral therapy. Biol Chem *389*, 1307-1312.

Luftig, M., Prinarakis, E., Yasui, T., Tsichritzis, T., Cahir-McFarland, E., Inoue, J., Nakano, H., Mak, T.W., Yeh, W.C., Li, X., *et al.* (2003). Epstein-Barr virus latent membrane protein 1 activation of NF-kappaB through IRAK1 and TRAF6. Proc Natl Acad Sci U S A *100*, 15595-15600.

Lum, J.J., and Badley, A.D. (2003). Resistance to apoptosis: mechanism for the development of HIV reservoirs. Curr HIV Res *1*, 261-274.

Lusic, M., Marcello, A., Cereseto, A., and Giacca, M. (2003). Regulation of HIV-1 gene expression by histone acetylation and factor recruitment at the LTR promoter. EMBO J *22*, 6550-6561.

Mabb, A.M., and Miyamoto, S. (2007). SUMO and NF-kappaB ties. Cell Mol Life Sci *64*, 1979-1996.

Mahlknecht, U., Will, J., Varin, A., Hoelzer, D., and Herbein, G. (2004). Histone deacetylase 3, a class I histone deacetylase, suppresses MAPK11-mediated activating transcription factor-2 activation and represses TNF gene expression. J Immunol *173*, 3979-3990.

Maier, H.J., Marienfeld, R., Wirth, T., and Baumann, B. (2003). Critical role of RelB serine 368 for dimerization and p100 stabilization. J Biol Chem *278*, 39242-39250.

Maine, G.N., Mao, X., Komarck, C.M., and Burstein, E. (2007). COMMD1 promotes the ubiquitination of NF-kappaB subunits through a cullin-containing ubiquitin ligase. EMBO J *26*, 436-447.

Mallardo, M., Dragonetti, E., Baldassarre, F., Ambrosino, C., Scala, G., and Quinto, I. (1996). An NF-kappaB site in the 5'-untranslated leader region of the human immunodeficiency virus type 1 enhances the viral expression in response to NF-kappaB-activating stimuli. J Biol Chem *271*, 20820-20827.

Mangino, G., Percario, Z.A., Fiorucci, G., Vaccari, G., Manrique, S., Romeo, G., Federico, M., Geyer, M., and Affabris, E. (2007). In vitro treatment of human monocytes/macrophages with myristoylated recombinant Nef of human immunodeficiency virus type 1 leads to the activation of mitogen-activated protein kinases, IkappaB kinases, and interferon regulatory factor 3 and to the release of beta interferon. J Virol *81*, 2777-2791.

Mankan, A.K., Lawless, M.W., Gray, S.G., Kelleher, D., and McManus, R. (2009). NF-kappaB regulation: the nuclear response. J Cell Mol Med *13*, 631-643.

Mann, E.A., Stanford, S., and Sherman, K.E. (2006). Prevalence of mutations in hepatitis C virus core protein associated with alteration of NF-kappaB activation. Virus Res *121*, 51-57.

Manna, S.K., and Aggarwal, B.B. (2000). Differential requirement for p56lck in HIV-tat versus TNF-induced cellular responses: effects on NF-kappa B, activator protein-1, c-Jun N-terminal kinase, and apoptosis. J Immunol *164*, 5156-5166.

Manninen, A., Renkema, G.H., and Saksela, K. (2000). Synergistic activation of NFAT by HIV-1 nef and the Ras/MAPK pathway. J Biol Chem *275*, 16513-16517.

Marban, C., Suzanne, S., Dequiedt, F., de Walque, S., Redel, L., Van Lint, C., Aunis, D., and Rohr, O. (2007). Recruitment of chromatin-modifying enzymes by CTIP2 promotes HIV-1 transcriptional silencing. EMBO J *26*, 412-423.

Marchant, D., Neil, S.J., and McKnight, A. (2006). Human immunodeficiency virus types 1 and 2 have different replication kinetics in human primary macrophage culture. J Gen Virol *87*, 411-418.

Margolis, D.M., Rabson, A.B., Straus, S.E., and Ostrove, J.M. (1992). Transactivation of the HIV-1 LTR by HSV-1 immediate-early genes. Virology *186*, 788-791.

Marienfeld, R., Berberich-Siebelt, F., Berberich, I., Denk, A., Serfling, E., and Neumann, M. (2001). Signal-specific and phosphorylation-dependent RelB degradation: a potential mechanism of NF-kappaB control. Oncogene *20*, 8142-8147.

Marino, S., Kretschmer, C., Brandner, S., Cavard, C., Zider, A., Briand, P., Isenmann, S., Wagner, E.F., and Aguzzi, A. (1995). Activation of HIV transcription by human foamy virus in transgenic mice. Lab Invest *73*, 103-110.

Marshall, H.E., Hess, D.T., and Stamler, J.S. (2004). S-nitrosylation: physiological regulation of NF-kappaB. Proc Natl Acad Sci U S A *101*, 8841-8842.

Martin, K.A., Wyatt, R., Farzan, M., Choe, H., Marcon, L., Desjardins, E., Robinson, J., Sodroski, J., Gerard, C., and Gerard, N.P. (1997). CD4-independent binding of SIV gp120 to rhesus CCR5. Science *278*, 1470-1473.

Martinez, O., Valmas, C., and Basler, C.F. (2007). Ebola virus-like particle-induced activation of NF-kappaB and Erk signaling in human dendritic cells requires the glycoprotein mucin domain. Virology *364*, 342-354.

Marusawa, H., Hijikata, M., Chiba, T., and Shimotohno, K. (1999). Hepatitis C virus core protein inhibits Fas- and tumor necrosis factor alpha-mediated apoptosis via NF-kappaB activation. J Virol *73*, 4713-4720.

Massoumi, R., Chmielarska, K., Hennecke, K., Pfeifer, A., and Fassler, R. (2006). Cyld inhibits tumor cell proliferation by blocking Bcl-3-dependent NF-kappaB signaling. Cell *125*, 665-677.

Mattioli, I., Geng, H., Sebald, A., Hodel, M., Bucher, C., Kracht, M., and Schmitz, M.L. (2006). Inducible phosphorylation of NF-kappa B p65 at serine 468 by T cell costimulation is mediated by IKK epsilon. J Biol Chem *281*, 6175-6183.

Maussang, D., Langemeijer, E., Fitzsimons, C.P., Stigter-van Walsum, M., Dijkman, R., Borg, M.K., Slinger, E., Schreiber, A., Michel, D., Tensen, C.P., *et al.* (2009). The human cytomegalovirus-encoded chemokine receptor US28 promotes angiogenesis and tumor formation via cyclooxygenase-2. Cancer Res *69*, 2861-2869.

McAllister, J.J., Phillips, D., Millhouse, S., Conner, J., Hogan, T., Ross, H.L., and Wigdahl, B. (2000). Analysis of the HIV-1 LTR NF-kappaB-proximal Sp site III: evidence for cell type-specific gene regulation and viral replication. Virology *274*, 262-277.

McElhinny, J.A., MacMorran, W.S., Bren, G.D., Ten, R.M., Israel, A., and Paya, C.V. (1995). Regulation of I kappa B alpha and p105 in monocytes and macrophages persistently infected with human immunodeficiency virus. J Virol *69*, 1500-1509.

Medin, C.L., Fitzgerald, K.A., and Rothman, A.L. (2005). Dengue virus nonstructural protein NS5 induces interleukin-8 transcription and secretion. J Virol *79*, 11053-11061.

Meier, J.L., and Pruessner, J.A. (2000). The human cytomegalovirus major immediate-early distal enhancer region is required for efficient viral replication and immediate-early gene expression. J Virol *74*, 1602-1613.

Meier, J.L., and Stinski, M.F. (2006). Major Immediate-early Enhancer and its Gene Products. In Cytomegalovirus: Molecular Biology and Immunology, M.J. Reddehase, ed. (Caister Academic Press, Norfolk UK), pp. 151-166.

Menard, C., Wagner, M., Ruzsics, Z., Holak, K., Brune, W., Campbell, A.E., and Koszinowski, U.H. (2003). Role of murine cytomegalovirus US22 gene family members in replication in macrophages. J Virol *77*, 5557-5570.

Meyer, M., Caselmann, W.H., Schluter, V., Schreck, R., Hofschneider, P.H., and Baeuerle, P.A. (1992). Hepatitis B virus transactivator MHBst: activation of NF-kappa B, selective inhibition by antioxidants and integral membrane localization. EMBO J *11*, 2991-3001.

Michaelis, M., Doerr, H.W., and Cinatl, J. (2009a). The story of human cytomegalovirus and cancer: increasing evidence and open questions. Neoplasia *11*, 1-9.

Michaelis, M., Doerr, H.W., and Cinatl, J., Jr. (2009b). Oncomodulation by human cytomegalovirus: evidence becomes stronger. Med Microbiol Immunol *198*, 79-81.

Michaelis, M., Kohler, N., Reinisch, A., Eikel, D., Gravemann, U., Doerr, H.W., Nau, H., and Cinatl, J., Jr. (2004). Increased human cytomegalovirus replication in fibroblasts after treatment with therapeutical plasma concentrations of valproic acid. Biochem Pharmacol *68*, 531-538.

Michelson, S. (1997). Interaction of human cytomegalovirus with monocytes/macrophages: a love-hate relationship. Pathol Biol (Paris) *45*, 146-158.

Mishra, A., Bharti, A.C., Varghese, P., Saluja, D., and Das, B.C. (2006). Differential expression and activation of NF-kappaB family proteins during oral carcinogenesis: Role of high risk human papillomavirus infection. Int J Cancer *119*, 2840-2850.

Mocarski, E.S., Shenk, T., and Pass, R.F. (2007). Cytomegaloviruses. In Fields Virology, D.M. Knipe, and P.M. Howley, eds. (Lippincott Williams & Wilkins), pp. 2701–2772.

Moffatt, S., Tanaka, N., Tada, K., Nose, M., Nakamura, M., Muraoka, O., Hirano, T., and Sugamura, K. (1996). A cytotoxic nonstructural protein, NS1, of human parvovirus B19 induces activation of interleukin-6 gene expression. J Virol *70*, 8485-8491.

Mohamed, M.R., Rahman, M.M., Rice, A., Moyer, R.W., Werden, S.J., and McFadden, G. (2009). Cowpox virus expresses a novel ankyrin repeat NF-kappaB inhibitor that controls inflammatory cell influx into virus-infected tissues and is critical for virus pathogenesis. J Virol *83*, 9223-9236.

Mohan, M., Aye, P.P., Borda, J.T., Alvarez, X., and Lackner, A.A. (2008). CCAAT/enhancer binding protein beta is a major mediator of inflammation and viral replication in the gastrointestinal tract of simian immunodeficiency virus-infected rhesus macaques. Am J Pathol *173*, 106-118.

Montag, C., Wagner, J., Gruska, I., and Hagemeier, C. (2006). Human cytomegalovirus blocks tumor necrosis factor alpha- and interleukin-1beta-mediated NF-kappaB signaling. J Virol *80*, 11686-11698.

Moran, J.M., Moxley, M.A., Buller, R.M., and Corbett, J.A. (2005). Encephalomyocarditis virus induces PKR-independent mitogen-activated protein kinase activation in macrophages. J Virol *79*, 10226-10236.

Mordmuller, B., Krappmann, D., Esen, M., Wegener, E., and Scheidereit, C. (2003). Lymphotoxin and lipopolysaccharide induce NF-kappaB-p52 generation by a co-translational mechanism. EMBO Rep *4*, 82-87.

Mori, N., and Prager, D. (1996). Transactivation of the interleukin-1alpha promoter by human T-cell leukemia virus type I and type II Tax proteins. Blood *87*, 3410-3417.

Moriuchi, M., Moriuchi, H., Williams, R., and Straus, S.E. (2000). Herpes simplex virus infection induces replication of human immunodeficiency virus type 1. Virology *278*, 534-540.

Mortola, E., and Larsen, A. (2009). [Bluetongue virus infection: signaling pathway activated during apoptosis]. Rev Argent Microbiol *41*, 134-140.

Mosoian, A., Teixeira, A., High, A.A., Christian, R.E., Hunt, D.F., Shabanowitz, J., Liu, X., and Klotman, M. (2006). Novel function of prothymosin alpha as a potent inhibitor of human

immunodeficiency virus type 1 gene expression in primary macrophages. J Virol *80*, 9200-9206.

Muller, J.R., and Siebenlist, U. (2003). Lymphotoxin beta receptor induces sequential activation of distinct NF-kappa B factors via separate signaling pathways. J Biol Chem *278*, 12006-12012.

Munshi, N., Balasubramanian, A., Koziel, M., Ganju, R.K., and Groopman, J.E. (2003). Hepatitis C and human immunodeficiency virus envelope proteins cooperatively induce hepatocytic apoptosis via an innocent bystander mechanism. J Infect Dis *188*, 1192-1204.

Murao, L.E., and Shisler, J.L. (2005). The MCV MC159 protein inhibits late, but not early, events of TNF-alpha-induced NF-kappaB activation. Virology *340*, 255-264.

Murayama, T., Ohara, Y., Obuchi, M., Khabar, K.S., Higashi, H., Mukaida, N., and Matsushima, K. (1997). Human cytomegalovirus induces interleukin-8 production by a human monocytic cell line, THP-1, through acting concurrently on AP-1- and NF-kappaB-binding sites of the interleukin-8 gene. J Virol *71*, 5692-5695.

Murphy, E., and Shenk, T. (2008). Human cytomegalovirus genome. Curr Top Microbiol Immunol *325*, 1-19.

Murphy, E., Yu, D., Grimwood, J., Schmutz, J., Dickson, M., Jarvis, M.A., Hahn, G., Nelson, J.A., Myers, R.M., and Shenk, T.E. (2003). Coding potential of laboratory and clinical strains of human cytomegalovirus. Proc Natl Acad Sci U S A *100*, 14976-14981.

Murphy, J.C., Fischle, W., Verdin, E., and Sinclair, J.H. (2002). Control of cytomegalovirus lytic gene expression by histone acetylation. EMBO J *21*, 1112-1120.

Nabel, G., and Baltimore, D. (1987). An inducible transcription factor activates expression of human immunodeficiency virus in T cells. Nature *326*, 711-713.

Nachtwey, J., and Spencer, J.V. (2008). HCMV IL-10 suppresses cytokine expression in monocytes through inhibition of nuclear factor-kappaB. Viral Immunol *21*, 477-482.

Nadella, M.V., Dirksen, W.P., Nadella, K.S., Shu, S., Cheng, A.S., Morgenstern, J.A., Richard, V., Fernandez, S.A., Huang, T.H., Guttridge, D., *et al.* (2007). Transcriptional regulation of parathyroid hormone-related protein promoter P2 by NF-kappaB in adult T-cell leukemia/lymphoma. Leukemia *21*, 1752-1762.

Nakamichi, K., Saiki, M., Sawada, M., Takayama-Ito, M., Yamamuro, Y., Morimoto, K., and Kurane, I. (2005). Rabies virus-induced activation of mitogen-activated protein kinase and NF-kappaB signaling pathways regulates expression of CXC and CC chemokine ligands in microglia. J Virol *79*, 11801-11812.

Nakamura, H., Ishii, C., Suehiro, M., Iguchi, A., Kuroda, K., Shimizu, K., Shimizu, N., Imadome, K., Yajima, M., and Fujiwara, S. (2008). The latent membrane protein 1 (LMP1) encoded by Epstein-Barr virus induces expression of the putative oncogene Bcl-3 through activation of the nuclear factor-kappaB. Virus Res *131*, 170-179.

Neil, S., Martin, F., Ikeda, Y., and Collins, M. (2001). Postentry restriction to human immunodeficiency virus-based vector transduction in human monocytes. J Virol *75*, 5448-5456.

Neumann, M., and Naumann, M. (2007). Beyond IkappaBs: alternative regulation of NF-kappaB activity. FASEB J *21*, 2642-2654.

Nevels, M., Paulus, C., and Shenk, T. (2004). Human cytomegalovirus immediate-early 1 protein facilitates viral replication by antagonizing histone deacetylation. Proc Natl Acad Sci U S A *101*, 17234-17239.

Neznanov, N., Chumakov, K.M., Neznanova, L., Almasan, A., Banerjee, A.K., and Gudkov, A.V. (2005). Proteolytic cleavage of the p65-RelA subunit of NF-kappaB during poliovirus infection. J Biol Chem *280*, 24153-24158.

Nichols, D.B., and Shisler, J.L. (2006). The MC160 protein expressed by the dermatotropic poxvirus molluscum contagiosum virus prevents tumor necrosis factor alpha-induced NF-kappaB activation via inhibition of I kappa kinase complex formation. J Virol *80*, 578-586.

Nitahara-Kasahara, Y., Kamata, M., Yamamoto, T., Zhang, X., Miyamoto, Y., Muneta, K., Iijima, S., Yoneda, Y., Tsunetsugu-Yokota, Y., and Aida, Y. (2007). Novel nuclear import of Vpr promoted by importin alpha is crucial for human immunodeficiency virus type 1 replication in macrophages. J Virol *81*, 5284-5293.

Nogalski, M.T., Podduturi, J.P., DeMeritt, I.B., Milford, L.E., and Yurochko, A.D. (2007). The human cytomegalovirus virion possesses an activated casein kinase II that allows for the rapid phosphorylation of the inhibitor of NF-kappaB, IkappaBalpha. J Virol *81*, 5305-5314.

Nomaguchi, M., Fujita, M., and Adachi, A. (2008). Role of HIV-1 Vpu protein for virus spread and pathogenesis. Microbes Infect *10*, 960-967.

Noraz, N., Lathey, J.L., and Spector, S.A. (1997). Human cytomegalovirus-associated immunosuppression is mediated through interferon-alpha. Blood *89*, 2443-2452.

Nordoy, I., Rollag, H., Lien, E., Sindre, H., Degre, M., Aukrust, P., Froland, S.S., and Muller, F. (2003). Cytomegalovirus infection induces production of human interleukin-10 in macrophages. Eur J Clin Microbiol Infect Dis *22*, 737-741.

Nouri-Aria, K.T., Sallie, R., Mizokami, M., Portmann, B.C., and Williams, R. (1995). Intrahepatic expression of hepatitis C virus antigens in chronic liver disease. J Pathol *175*, 77-83.

Odeberg, J., and Soderberg-Naucler, C. (2001). Reduced expression of HLA class II molecules and Iinterleukin-10- and transforming growth factor beta1-independent suppression of T-cell proliferation in human cytomegalovirus-infected macrophage cultures. J Virol *75*, 5174-5181.

Odeberg, J., Wolmer, N., Falci, S., Westgren, M., Sundtrom, E., Seiger, A., and Soderberg-Naucler, C. (2007). Late human cytomegalovirus (HCMV) proteins inhibit differentiation of human neural precursor cells into astrocytes. J Neurosci Res *85*, 583-593.

Oem, J.K., Jackel-Cram, C., Li, Y.P., Kang, H.N., Zhou, Y., Babiuk, L.A., and Liu, Q. (2008). Hepatitis C virus non-structural protein-2 activates CXCL-8 transcription through NF-kappaB. Arch Virol *153*, 293-301.

Oie, K.L., and Pickup, D.J. (2001). Cowpox virus and other members of the orthopoxvirus genus interfere with the regulation of NF-kappaB activation. Virology *288*, 175-187.

Okada, H., Takei, R., and Tashiro, M. (1997). HIV-1 Nef protein-induced apoptotic cytolysis of a broad spectrum of uninfected human blood cells independently of CD95(Fas). FEBS Lett *414*, 603-606.

Okuda, M., Hino, K., Korenaga, M., Yamaguchi, Y., Katoh, Y., and Okita, K. (1999). Differences in hypervariable region 1 quasispecies of hepatitis C virus in human serum, peripheral blood mononuclear cells, and liver. Hepatology *29*, 217-222.

Olivetta, E., and Federico, M. (2006). HIV-1 Nef protects human-monocyte-derived macrophages from HIV-1-induced apoptosis. Exp Cell Res *312*, 890-900.

Olivetta, E., Percario, Z., Fiorucci, G., Mattia, G., Schiavoni, I., Dennis, C., Jager, J., Harris, M., Romeo, G., Affabris, E., *et al.* (2003). HIV-1 Nef induces the release of inflammatory

factors from human monocyte/macrophages: involvement of Nef endocytotic signals and NF-kappa B activation. J Immunol *170*, 1716-1727.

Omoto, S., and Fujii, Y.R. (2005). Regulation of human immunodeficiency virus 1 transcription by nef microRNA. J Gen Virol *86*, 751-755.

Orenstein, J.M., Meltzer, M.S., Phipps, T., and Gendelman, H.E. (1988). Cytoplasmic assembly and accumulation of human immunodeficiency virus types 1 and 2 in recombinant human colony-stimulating factor-1-treated human monocytes: an ultrastructural study. J Virol *62*, 2578-2586.

Pahl, H.L., and Baeuerle, P.A. (1995). Expression of influenza virus hemagglutinin activates transcription factor NF-kappa B. J Virol *69*, 1480-1484.

Pahl, H.L., Sester, M., Burgert, H.G., and Baeuerle, P.A. (1996). Activation of transcription factor NF-kappaB by the adenovirus E3/19K protein requires its ER retention. J Cell Biol *132*, 511-522.

Pak, J., and Faller, D.V. (1996). Moloney murine leukemia virus activates NF-kappa B. J Virol *70*, 4167-4172.

Pal, S., Shuhart, M.C., Thomassen, L., Emerson, S.S., Su, T., Feuerborn, N., Kae, J., and Gretch, D.R. (2006). Intrahepatic hepatitis C virus replication correlates with chronic hepatitis C disease severity in vivo. J Virol *80*, 2280-2290.

Palmer, S., and Chen, Y.H. (2008). Bcl-3, a multifaceted modulator of NF-kappaB-mediated gene transcription. Immunol Res *42*, 210-218.

Pan, J., and McEver, R.P. (1995). Regulation of the human P-selectin promoter by Bcl-3 and specific homodimeric members of the NF-kappa B/Rel family. J Biol Chem *270*, 23077-23083.

Park, C.Y., Oh, S.H., Kang, S.M., Lim, Y.S., and Hwang, S.B. (2009). Hepatitis delta virus large antigen sensitizes to TNF-alpha-induced NF-kappaB signaling. Mol Cells *28*, 49-55.

Park, K.J., Choi, S.H., Koh, M.S., Kim, D.J., Yie, S.W., Lee, S.Y., and Hwang, S.B. (2001). Hepatitis C virus core protein potentiates c-Jun N-terminal kinase activation through a signaling complex involving TRADD and TRAF2. Virus Res *74*, 89-98.

Park, K.J., Choi, S.H., Lee, S.Y., Hwang, S.B., and Lai, M.M. (2002). Nonstructural 5A protein of hepatitis C virus modulates tumor necrosis factor alpha-stimulated nuclear factor kappa B activation. J Biol Chem *277*, 13122-13128.

Park, S.G., Chung, C., Kang, H., Kim, J.Y., and Jung, G. (2006). Up-regulation of cyclin D1 by HBx is mediated by NF-kappaB2/BCL3 complex through kappaB site of cyclin D1 promoter. J Biol Chem *281*, 31770-31777.

Patel, A., Hanson, J., McLean, T.I., Olgiate, J., Hilton, M., Miller, W.E., and Bachenheimer, S.L. (1998). Herpes simplex type 1 induction of persistent NF-kappa B nuclear translocation increases the efficiency of virus replication. Virology *247*, 212-222.

Pawlotsky, J.M. (2004). Pathophysiology of hepatitis C virus infection and related liver disease. Trends Microbiol *12*, 96-102.

Peng, G., Greenwell-Wild, T., Nares, S., Jin, W., Lei, K.J., Rangel, Z.G., Munson, P.J., and Wahl, S.M. (2007). Myeloid differentiation and susceptibility to HIV-1 are linked to APOBEC3 expression. Blood *110*, 393-400.

Percario, Z., Olivetta, E., Fiorucci, G., Mangino, G., Peretti, S., Romeo, G., Affabris, E., and Federico, M. (2003). Human immunodeficiency virus type 1 (HIV-1) Nef activates STAT3 in primary human monocyte/macrophages through the release of soluble factors: involvement of Nef domains interacting with the cell endocytotic machinery. J Leukoc Biol *74*, 821-832.

Pereira, L.A., Bentley, K., Peeters, A., Churchill, M.J., and Deacon, N.J. (2000). A compilation of cellular transcription factor interactions with the HIV-1 LTR promoter. Nucleic Acids Res *28*, 663-668.

Perkins, N.D. (2006). Post-translational modifications regulating the activity and function of the nuclear factor kappa B pathway. Oncogene *25*, 6717-6730.

Perkins, N.D., Edwards, N.L., Duckett, C.S., Agranoff, A.B., Schmid, R.M., and Nabel, G.J. (1993). A cooperative interaction between NF-kappa B and Sp1 is required for HIV-1 enhancer activation. EMBO J *12*, 3551-3558.

Piguet, V., and Steinman, R.M. (2007). The interaction of HIV with dendritic cells: outcomes and pathways. Trends Immunol *28*, 503-510.

Pion, M., Granelli-Piperno, A., Mangeat, B., Stalder, R., Correa, R., Steinman, R.M., and Piguet, V. (2006). APOBEC3G/3F mediates intrinsic resistance of monocyte-derived dendritic cells to HIV-1 infection. J Exp Med *203*, 2887-2893.

Piroth, L., Grappin, M., Cuzin, L., Mouton, Y., Bouchard, O., Raffi, F., Rey, D., Peyramond, D., Gourdon, F., Drobacheff, C., *et al.* (2000). Hepatitis C virus co-infection is a negative prognostic factor for clinical evolution in human immunodeficiency virus-positive patients. J Viral Hepat *7*, 302-308.

Pivert, A., Payan, C., Morand, P., Fafi-Kremer, S., Deshayes, J., Carrat, F., Pol, S., Cacoub, P., Perronne, C., and Lunel, F. (2006). Comparison of serum hepatitis C virus (HCV) RNA and core antigen levels in patients coinfected with human immunodeficiency virus and HCV and treated with interferon plus ribavirin. J Clin Microbiol *44*, 417-422.

Poland, S.D., Costello, P., Dekaban, G.A., and Rice, G.P. (1990). Cytomegalovirus in the brain: in vitro infection of human brain-derived cells. J Infect Dis *162*, 1252-1262.

Poole, E., Atkins, E., Nakayama, T., Yoshie, O., Groves, I., Alcami, A., and Sinclair, J. (2008). NF-kappaB-mediated activation of the chemokine CCL22 by the product of the human cytomegalovirus gene UL144 escapes regulation by viral IE86. J Virol *82*, 4250-4256.

Poole, E., Groves, I., MacDonald, A., Pang, Y., Alcami, A., and Sinclair, J. (2009). Identification of TRIM23 as a cofactor involved in the regulation of NF-kappaB by human cytomegalovirus. J Virol *83*, 3581-3590.

Poole, E., King, C.A., Sinclair, J.H., and Alcami, A. (2006). The UL144 gene product of human cytomegalovirus activates NFkappaB via a TRAF6-dependent mechanism. EMBO J *25*, 4390-4399.

Prakash, O., Swamy, O.R., Peng, X., Tang, Z.Y., Li, L., Larson, J.E., Cohen, J.C., Gill, J., Farr, G., Wang, S., *et al.* (2005). Activation of Src kinase Lyn by the Kaposi sarcoma-associated herpesvirus K1 protein: implications for lymphomagenesis. Blood *105*, 3987-3994.

Prosch, S., Priemer, C., Hoflich, C., Liebenthaf, C., Babel, N., Kruger, D.H., and Volk, H.D. (2003). Proteasome inhibitors: a novel tool to suppress human cytomegalovirus replication and virus-induced immune modulation. Antivir Ther *8*, 555-567.

Prosch, S., Staak, K., Stein, J., Liebenthal, C., Stamminger, T., Volk, H.D., and Kruger, D.H. (1995). Stimulation of the human cytomegalovirus IE enhancer/promoter in HL-60 cells by TNFalpha is mediated via induction of NF-kappaB. Virology *208*, 197-206.

Prosch, S., Wuttke, R., Kruger, D.H., and Volk, H.D. (2002). NF-kappaB--a potential therapeutic target for inhibition of human cytomegalovirus (re)activation? Biol Chem *383*, 1601-1609.

Puca, A., Fiume, G., Palmieri, C., Trimboli, F., Olimpico, F., Scala, G., and Quinto, I. (2007). IkappaB-alpha represses the transcriptional activity of the HIV-1 Tat transactivator by promoting its nuclear export. J Biol Chem *282*, 37146-37157.

Qing, G., and Xiao, G. (2005). Essential role of IkappaB kinase alpha in the constitutive processing of NF-kappaB2 p100. J Biol Chem *280*, 9765-9768.

Quaranta, M.G., Mattioli, B., Giordani, L., and Viora, M. (2004). HIV-1 Nef equips dendritic cells to reduce survival and function of CD8+ T cells: a mechanism of immune evasion. Faseb J *18*, 1459-1461.

Quinto, I., Mallardo, M., Baldassarre, F., Scala, G., Englund, G., and Jeang, K.T. (1999). Potent and stable attenuation of live-HIV-1 by gain of a proteolysis-resistant inhibitor of NF-kappaB (IkappaB-alphaS32/36A) and the implications for vaccine development. J Biol Chem *274*, 17567-17572.

Quinto, I., Puca, A., Greenhouse, J., Silvera, P., Yalley-Ogunro, J., Lewis, M.G., Palmieri, C., Trimboli, F., Byrum, R., Adelsberger, J., *et al.* (2004). High attenuation and immunogenicity of a simian immunodeficiency virus expressing a proteolysis-resistant inhibitor of NF-kappaB. J Biol Chem *279*, 1720-1728.

Quivy, V., De Walque, S., and Van Lint, C. (2007). Chromatin-associated regulation of HIV-1 transcription: implications for the development of therapeutic strategies. Subcell Biochem *41*, 371-396.

Quivy, V., and Van Lint, C. (2002). Diversity of acetylation targets and roles in transcriptional regulation: the human immunodeficiency virus type 1 promoter as a model system. Biochem Pharmacol *64*, 925-934.

Rahman, M.M., Mohamed, M.R., Kim, M., Smallwood, S., and McFadden, G. (2009). Co-regulation of NF-kappaB and inflammasome-mediated inflammatory responses by myxoma virus pyrin domain-containing protein M013. PLoS Pathog *5*, e1000635.

Ranganathan, P.N., and Khalili, K. (1993). The transcriptional enhancer element, kappa B, regulates promoter activity of the human neurotropic virus, JCV, in cells derived from the CNS. Nucleic Acids Res *21*, 1959-1964.

Ranjbar, S., Tsytsykova, A.V., Lee, S.K., Rajsbaum, R., Falvo, J.V., Lieberman, J., Shankar, P., and Goldfeld, A.E. (2006). NFAT5 regulates HIV-1 in primary monocytes via a highly conserved long terminal repeat site. PLoS Pathog *2*, e130.

Raposo, G., Moore, M., Innes, D., Leijendekker, R., Leigh-Brown, A., Benaroch, P., and Geuze, H. (2002). Human macrophages accumulate HIV-1 particles in MHC II compartments. Traffic *3*, 718-729.

Ray, R.B., Steele, R., Basu, A., Meyer, K., Majumder, M., Ghosh, A.K., and Ray, R. (2002). Distinct functional role of Hepatitis C virus core protein on NF-kappaB regulation is linked to genomic variation. Virus Res *87*, 21-29.

Raymond, A.D., Hasham, M., Tsygankov, A.Y., and Henderson, E.E. (2007). Herpesvirus saimiri terminal membrane proteins modulate HIV-1 replication by altering Nef and Tat functions. Curr HIV Res *5*, 79-86.

Redpath, S., Angulo, A., Gascoigne, N.R., and Ghazal, P. (1999). Murine cytomegalovirus infection down-regulates MHC class II expression on macrophages by induction of IL-10. J Immunol *162*, 6701-6707.

Reeves, M.B., MacAry, P.A., Lehner, P.J., Sissons, J.G., and Sinclair, J.H. (2005). Latency, chromatin remodeling, and reactivation of human cytomegalovirus in the dendritic cells of healthy carriers. Proc Natl Acad Sci U S A *102*, 4140-4145.

Reimers, K., Buchholz, K., and Werchau, H. (2005). Respiratory syncytial virus M2-1 protein induces the activation of nuclear factor kappa B. Virology *331*, 260-268.

Renard, P., Ernest, I., Houbion, A., Art, M., Le Calvez, H., Raes, M., and Remacle, J. (2001). Development of a sensitive multi-well colorimetric assay for active NFkappaB. Nucleic Acids Res *29*, E21.

Renkema, G.H., Manninen, A., and Saksela, K. (2001). Human immunodeficiency virus type 1 Nef selectively associates with a catalytically active subpopulation of p21-activated kinase 2 (PAK2) independently of PAK2 binding to Nck or beta-PIX. J Virol *75*, 2154-2160.

Renner, F., and Schmitz, M.L. (2009). Autoregulatory feedback loops terminating the NF-kappaB response. Trends Biochem Sci *34*, 128-135.

Reuse, S., Calao, M., Kabeya, K., Guiguen, A., Gatot, J.S., Quivy, V., Vanhulle, C., Lamine, A., Vaira, D., Demonte, D., *et al.* (2009). Synergistic activation of HIV-1 expression by deacetylase inhibitors and prostratin: implications for treatment of latent infection. PLoS One *4*, e6093.

Revilla, Y., Callejo, M., Rodriguez, J.M., Culebras, E., Nogal, M.L., Salas, M.L., Vinuela, E., and Fresno, M. (1998). Inhibition of nuclear factor kappaB activation by a virus-encoded IkappaB-like protein. J Biol Chem *273*, 5405-5411.

Rich, E.A., Chen, I.S., Zack, J.A., Leonard, M.L., and O'Brien, W.A. (1992). Increased susceptibility of differentiated mononuclear phagocytes to productive infection with human immunodeficiency virus-1 (HIV-1). J Clin Invest *89*, 176-183.

Riegler, S., Hebart, H., Einsele, H., Brossart, P., Jahn, G., and Sinzger, C. (2000). Monocyte-derived dendritic cells are permissive to the complete replicative cycle of human cytomegalovirus. J Gen Virol *81*, 393-399.

Rocha, S., Garrett, M.D., Campbell, K.J., Schumm, K., and Perkins, N.D. (2005). Regulation of NF-kappaB and p53 through activation of ATR and Chk1 by the ARF tumour suppressor. EMBO J *24*, 1157-1169.

Rodrigues, L., Filipe, J., Seldon, M.P., Fonseca, L., Anrather, J., Soares, M.P., and Simas, J.P. (2009). Termination of NF-kappaB activity through a gammaherpesvirus protein that assembles an EC5S ubiquitin-ligase. EMBO J *28*, 1283-1295.

Rodriguez, C.I., Nogal, M.L., Carrascosa, A.L., Salas, M.L., Fresno, M., and Revilla, Y. (2002). African swine fever virus IAP-like protein induces the activation of nuclear factor kappa B. J Virol *76*, 3936-3942.

Roe, B., and Hall, W.W. (2008). Cellular and molecular interactions in coinfection with hepatitis C virus and human immunodeficiency virus. Expert Rev Mol Med *10*, e30.

Roebuck, K.A., and Saifuddin, M. (1999). Regulation of HIV-1 transcription. Gene Expr *8*, 67-84.

Rohr, O., Aunis, D., and Schaeffer, E. (1997). COUP-TF and Sp1 interact and cooperate in the transcriptional activation of the human immunodeficiency virus type 1 long terminal repeat in human microglial cells. J Biol Chem *272*, 31149-31155.

Rohr, O., Marban, C., Aunis, D., and Schaeffer, E. (2003). Regulation of HIV-1 gene transcription: from lymphocytes to microglial cells. J Leukoc Biol *74*, 736-749.

Romani, B., and Engelbrecht, S. (2009). Human immunodeficiency virus type 1 Vpr: functions and molecular interactions. J Gen Virol *90*, 1795-1805.

Romani, B., Engelbrecht, S., and Glashoff, R.H. (2010). Functions of Tat: the versatile protein of human immunodeficiency virus type 1. J Gen Virol *91*, 1-12.

Romieu-Mourez, R., Kim, D.W., Shin, S.M., Demicco, E.G., Landesman-Bollag, E., Seldin, D.C., Cardiff, R.D., and Sonenshein, G.E. (2003). Mouse mammary tumor virus c-rel transgenic mice develop mammary tumors. Mol Cell Biol *23*, 5738-5754.

Ross, E.K., Buckler-White, A.J., Rabson, A.B., Englund, G., and Martin, M.A. (1991). Contribution of NF-kappa B and Sp1 binding motifs to the replicative capacity of human immunodeficiency virus type 1: distinct patterns of viral growth are determined by T-cell types. J Virol *65*, 4350-4358.

Ross, J.A., and Auger, M.J. (2002). The biology of the macrophage. In The Macrophage, B. Burke, Lewis, C.E., ed. (Oxford, Oxford University Press), pp. 1-72.

Roux, P., Alfieri, C., Hrimech, M., Cohen, E.A., and Tanner, J.E. (2000). Activation of transcription factors NF-kappaB and NF-IL-6 by human immunodeficiency virus type 1 protein R (Vpr) induces interleukin-8 expression. J Virol *74*, 4658-4665.

Ryo, A., Suizu, F., Yoshida, Y., Perrem, K., Liou, Y.C., Wulf, G., Rottapel, R., Yamaoka, S., and Lu, K.P. (2003). Regulation of NF-kappaB signaling by Pin1-dependent prolyl isomerization and ubiquitin-mediated proteolysis of p65/RelA. Mol Cell *12*, 1413-1426.

Sabbah, A., and Bose, S. (2009). Retinoic acid inducible gene I activates innate antiviral response against human parainfluenza virus type 3. Virol J *6*, 200.

Sabile, A., Perlemuter, G., Bono, F., Kohara, K., Demaugre, F., Kohara, M., Matsuura, Y., Miyamura, T., Brechot, C., and Barba, G. (1999). Hepatitis C virus core protein binds to apolipoprotein AII and its secretion is modulated by fibrates. Hepatology *30*, 1064-1076.

Saccani, S., Marazzi, I., Beg, A.A., and Natoli, G. (2004). Degradation of promoter-bound p65/RelA is essential for the prompt termination of the nuclear factor kappaB response. J Exp Med *200*, 107-113.

Saccani, S., Pantano, S., and Natoli, G. (2003). Modulation of NF-kappaB activity by exchange of dimers. Mol Cell *11*, 1563-1574.

Saffert, R.T., and Kalejta, R.F. (2006). Inactivating a cellular intrinsic immune defense mediated by Daxx is the mechanism through which the human cytomegalovirus pp71 protein stimulates viral immediate-early gene expression. J Virol *80*, 3863-3871.

Sainz, B., Jr., LaMarca, H.L., Garry, R.F., and Morris, C.A. (2005). Synergistic inhibition of human cytomegalovirus replication by interferon-alpha/beta and interferon-gamma. Virol J *2*, 14.

Salim, A., and Ratner, L. (2008). Modulation of beta-catenin and E-cadherin interaction by Vpu increases human immunodeficiency virus type 1 particle release. J Virol *82*, 3932-3938.

Samanta, M., Iwakiri, D., Kanda, T., Imaizumi, T., and Takada, K. (2006). EB virus-encoded RNAs are recognized by RIG-I and activate signaling to induce type I IFN. EMBO J *25*, 4207-4214.

Sambucetti, L.C., Cherrington, J.M., Wilkinson, G.W., and Mocarski, E.S. (1989). NF-kappa B activation of the cytomegalovirus enhancer is mediated by a viral transactivator and by T cell stimulation. EMBO J *8*, 4251-4258.

Sato, Y., Kato, J., Takimoto, R., Takada, K., Kawano, Y., Miyanishi, K., Kobune, M., Takayama, T., Matunaga, T., and Niitsu, Y. (2006). Hepatitis C virus core protein promotes proliferation of human hepatoma cells through enhancement of transforming growth factor alpha expression via activation of nuclear factor-kappaB. Gut *55*, 1801-1808.

Scala, G., Quinto, I., Ruocco, M.R., Mallardo, M., Ambrosino, C., Squitieri, B., Tassone, P., and Venuta, S. (1993). Epstein-Barr virus nuclear antigen 2 transactivates the long terminal repeat of human immunodeficiency virus type 1. J Virol *67*, 2853-2861.

Schafer, S.L., Hiscott, J., and Pitha, P.M. (1996). Differential regulation of the HIV-1 LTR by specific NF-kappa B subunits in HSV-1-infected cells. Virology *224*, 214-223.

Schierling, K., Stamminger, T., Mertens, T., and Winkler, M. (2004). Human cytomegalovirus tegument proteins ppUL82 (pp71) and ppUL35 interact and cooperatively activate the major immediate-early enhancer. J Virol *78*, 9512-9523.

Schmidtmayerova, H., Ancuta, P., and Pancino, G. (2007). Monocyte and macrophage susceptibility to HIV infection: Pathophysiological implications. In HIV and the Macrophage, H. G, ed. (Kerala, India, Transworld Research Network), pp. 1-34.

Schuitemaker, H., Kootstra, N.A., Koppelman, M.H., Bruisten, S.M., Huisman, H.G., Tersmette, M., and Miedema, F. (1992). Proliferation-dependent HIV-1 infection of monocytes occurs during differentiation into macrophages. J Clin Invest *89*, 1154-1160.

Schwabe, R.F., and Sakurai, H. (2005). IKKbeta phosphorylates p65 at S468 in transactivaton domain 2. FASEB J *19*, 1758-1760.

Schwarz, E.M., Badorff, C., Hiura, T.S., Wessely, R., Badorff, A., Verma, I.M., and Knowlton, K.U. (1998). NF-kappaB-mediated inhibition of apoptosis is required for encephalomyocarditis virus virulence: a mechanism of resistance in p50 knockout mice. J Virol *72*, 5654-5660.

Sciortino, M.T., Medici, M.A., Marino-Merlo, F., Zaccaria, D., Giuffre-Cuculletto, M., Venuti, A., Grelli, S., Bramanti, P., and Mastino, A. (2008). Involvement of gD/HVEM interaction in NF-kB-dependent inhibition of apoptosis by HSV-1 gD. Biochem Pharmacol *76*, 1522-1532.

Secchiero, P., Zella, D., Curreli, S., Mirandola, P., Capitani, S., Gallo, R.C., and Zauli, G. (2000). Engagement of CD28 modulates CXC chemokine receptor 4 surface expression in both resting and CD3-stimulated CD4+ T cells. J Immunol *164*, 4018-4024.

Senftleben, U., Cao, Y., Xiao, G., Greten, F.R., Krahn, G., Bonizzi, G., Chen, Y., Hu, Y., Fong, A., Sun, S.C., *et al.* (2001). Activation by IKKalpha of a second, evolutionary conserved, NF-kappa B signaling pathway. Science *293*, 1495-1499.

Seo, T., Park, J., Lim, C., and Choe, J. (2004). Inhibition of nuclear factor kappaB activity by viral interferon regulatory factor 3 of Kaposi's sarcoma-associated herpesvirus. Oncogene *23*, 6146-6155.

Sgarbanti, M., Remoli, A.L., Marsili, G., Ridolfi, B., Borsetti, A., Perrotti, E., Orsatti, R., Ilari, R., Sernicola, L., Stellacci, E., *et al.* (2008). IRF-1 is required for full NF-kappaB transcriptional activity at the human immunodeficiency virus type 1 long terminal repeat enhancer. J Virol *82*, 3632-3641.

Sharova, N., Swingler, C., Sharkey, M., and Stevenson, M. (2005). Macrophages archive HIV-1 virions for dissemination in trans. EMBO J *24*, 2481-2489.

Sharova, N., Wu, Y., Zhu, X., Stranska, R., Kaushik, R., Sharkey, M., and Stevenson, M. (2008). Primate lentiviral Vpx commandeers DDB1 to counteract a macrophage restriction. PLoS Pathog *4*, e1000057.

Sherman, K.E., O'Brien, J., Gutierrez, A.G., Harrison, S., Urdea, M., Neuwald, P., and Wilber, J. (1993). Quantitative evaluation of hepatitis C virus RNA in patients with concurrent human immunodeficiency virus infections. J Clin Microbiol *31*, 2679-2682.

Sherry, B. (2009). Rotavirus and reovirus modulation of the interferon response. J Interferon Cytokine Res *29*, 559-567.

Shimizu, T., Kida, Y., and Kuwano, K. (2004). Lipid-associated membrane proteins of Mycoplasma fermentans and M. penetrans activate human immunodeficiency virus long-terminal repeats through Toll-like receptors. Immunology *113*, 121-129.

Shisler, J.L., and Jin, X.L. (2004). The vaccinia virus K1L gene product inhibits host NF-kappaB activation by preventing IkappaBalpha degradation. J Virol *78*, 3553-3560.

Shrivastava, A., Manna, S.K., Ray, R., and Aggarwal, B.B. (1998). Ectopic expression of hepatitis C virus core protein differentially regulates nuclear transcription factors. J Virol *72*, 9722-9728.

Shun, M.C., Daigle, J.E., Vandegraaff, N., and Engelman, A. (2007). Wild-type levels of human immunodeficiency virus type 1 infectivity in the absence of cellular emerin protein. J Virol *81*, 166-172.

Shurman, L., Sen, R., and Bergman, Y. (1989). Adenovirus E1A products activate the Ig k-chain enhancer in fibroblasts. A possible involvement of the NF-kB binding site. J Immunol *143*, 3806-3812.

Sica, A., Allavena, P., and Mantovani, A. (2008). Cancer related inflammation: the macrophage connection. Cancer Lett *267*, 204-215.

Siddiqui, A., Gaynor, R., Srinivasan, A., Mapoles, J., and Farr, R.W. (1989). trans-activation of viral enhancers including long terminal repeat of the human immunodeficiency virus by the hepatitis B virus X protein. Virology *169*, 479-484.

Simmons, A., Aluvihare, V., and McMichael, A. (2001). Nef triggers a transcriptional program in T cells imitating single-signal T cell activation and inducing HIV virulence mediators. Immunity *14*, 763-777.

Simon, C.O., Seckert, C.K., Dreis, D., Reddehase, M.J., and Grzimek, N.K. (2005). Role for tumor necrosis factor alpha in murine cytomegalovirus transcriptional reactivation in latently infected lungs. J Virol *79*, 326-340.

Sinclair, J. (2009). Chromatin structure regulates human cytomegalovirus gene expression during latency, reactivation and lytic infection. Biochim Biophys Acta.

Sinclair, J., and Sissons, P. (1996). Latent and persistent infections of monocytes and macrophages. Intervirology *39*, 293-301.

Sinclair, J., and Sissons, P. (2006). Latency and reactivation of human cytomegalovirus. J Gen Virol *87*, 1763-1779.

Sinzger, C., Bissinger, A.L., Viebahn, R., Oettle, H., Radke, C., Schmidt, C.A., and Jahn, G. (1999a). Hepatocytes are permissive for human cytomegalovirus infection in human liver cell culture and In vivo. J Infect Dis *180*, 976-986.

Sinzger, C., Digel, M., and Jahn, G. (2008). Cytomegalovirus cell tropism. Curr Top Microbiol Immunol *325*, 63-83.

Sinzger, C., Eberhardt, K., Cavignac, Y., Weinstock, C., Kessler, T., Jahn, G., and Davignon, J.L. (2006). Macrophage cultures are susceptible to lytic productive infection by endothelial-cell-propagated human cytomegalovirus strains and present viral IE1 protein to CD4+ T cells despite late downregulation of MHC class II molecules. J Gen Virol *87*, 1853-1862.

Sinzger, C., Grefte, A., Plachter, B., Gouw, A.S., The, T.H., and Jahn, G. (1995). Fibroblasts, epithelial cells, endothelial cells and smooth muscle cells are major targets of human cytomegalovirus infection in lung and gastrointestinal tissues. J Gen Virol *76 (Pt 4)*, 741-750.

Sinzger, C., Muntefering, H., Loning, T., Stoss, H., Plachter, B., and Jahn, G. (1993). Cell types infected in human cytomegalovirus placentitis identified by immunohistochemical double staining. Virchows Arch A Pathol Anat Histopathol *423*, 249-256.

Sinzger, C., Plachter, B., Grefte, A., The, T.H., and Jahn, G. (1996). Tissue macrophages are infected by human cytomegalovirus in vivo. J Infect Dis *173*, 240-245.

Sinzger, C., Schmidt, K., Knapp, J., Kahl, M., Beck, R., Waldman, J., Hebart, H., Einsele, H., and Jahn, G. (1999b). Modification of human cytomegalovirus tropism through

propagation in vitro is associated with changes in the viral genome. J Gen Virol *80 (Pt 11)*, 2867-2877.

Smith, M.S., Bentz, G.L., Alexander, J.S., and Yurochko, A.D. (2004a). Human cytomegalovirus induces monocyte differentiation and migration as a strategy for dissemination and persistence. J Virol *78*, 4444-4453.

Smith, M.S., Bentz, G.L., Smith, P.M., Bivins, E.R., and Yurochko, A.D. (2004b). HCMV activates PI(3)K in monocytes and promotes monocyte motility and transendothelial migration in a PI(3)K-dependent manner. J Leukoc Biol *76*, 65-76.

Smith, M.S., Bivins-Smith, E.R., Tilley, A.M., Bentz, G.L., Chan, G., Minard, J., and Yurochko, A.D. (2007). Roles of phosphatidylinositol 3-kinase and NF-kappaB in human cytomegalovirus-mediated monocyte diapedesis and adhesion: strategy for viral persistence. J Virol *81*, 7683-7694.

Soderberg-Naucler, C. (2008). HCMV microinfections in inflammatory diseases and cancer. J Clin Virol *41*, 218-223.

Soderberg-Naucler, C., Fish, K.N., and Nelson, J.A. (1997a). Interferon-gamma and tumor necrosis factor-alpha specifically induce formation of cytomegalovirus-permissive monocyte-derived macrophages that are refractory to the antiviral activity of these cytokines. J Clin Invest *100*, 3154-3163.

Soderberg-Naucler, C., Fish, K.N., and Nelson, J.A. (1997b). Reactivation of latent human cytomegalovirus by allogeneic stimulation of blood cells from healthy donors. Cell *91*, 119-126.

Soderberg-Naucler, C., Streblow, D.N., Fish, K.N., Allan-Yorke, J., Smith, P.P., and Nelson, J.A. (2001). Reactivation of latent human cytomegalovirus in CD14(+) monocytes is differentiation dependent. J Virol *75*, 7543-7554.

Solan, N.J., Miyoshi, H., Carmona, E.M., Bren, G.D., and Paya, C.V. (2002). RelB cellular regulation and transcriptional activity are regulated by p100. J Biol Chem *277*, 1405-1418.

Song, H.Y., Rothe, M., and Goeddel, D.V. (1996). The tumor necrosis factor-inducible zinc finger protein A20 interacts with TRAF1/TRAF2 and inhibits NF-kappaB activation. Proc Natl Acad Sci U S A *93*, 6721-6725.

Sonza, S., Mutimer, H.P., O'Brien, K., Ellery, P., Howard, J.L., Axelrod, J.H., Deacon, N.J., Crowe, S.M., and Purcell, D.F. (2002). Selectively reduced tat mRNA heralds the decline in productive human immunodeficiency virus type 1 infection in monocyte-derived macrophages. J Virol *76*, 12611-12621.

Sorokina, E.M., Merlo, J.J., Jr., and Tsygankov, A.Y. (2004). Molecular mechanisms of the effect of herpesvirus saimiri protein StpC on the signaling pathway leading to NF-kappaB activation. J Biol Chem *279*, 13469-13477.

Soto, B., Sanchez-Quijano, A., Rodrigo, L., del Olmo, J.A., Garcia-Bengoechea, M., Hernandez-Quero, J., Rey, C., Abad, M.A., Rodriguez, M., Sales Gilabert, M., *et al.* (1997). Human immunodeficiency virus infection modifies the natural history of chronic parenterally-acquired hepatitis C with an unusually rapid progression to cirrhosis. J Hepatol *26*, 1-5.

Speir, E., Shibutani, T., Yu, Z.X., Ferrans, V., and Epstein, S.E. (1996). Role of reactive oxygen intermediates in cytomegalovirus gene expression and in the response of human smooth muscle cells to viral infection. Circ Res *79*, 1143-1152.

Spencer, J.V., Lockridge, K.M., Barry, P.A., Lin, G., Tsang, M., Penfold, M.E., and Schall, T.J. (2002). Potent immunosuppressive activities of cytomegalovirus-encoded interleukin-10. J Virol *76*, 1285-1292.

Spitkovsky, D., Hehner, S.P., Hofmann, T.G., Moller, A., and Schmitz, M.L. (2002). The human papillomavirus oncoprotein E7 attenuates NF-kappa B activation by targeting the Ikappa B kinase complex. J Biol Chem *277*, 25576-25582.

Srivastava, S., Swanson, S.K., Manel, N., Florens, L., Washburn, M.P., and Skowronski, J. (2008). Lentiviral Vpx accessory factor targets VprBP/DCAF1 substrate adaptor for cullin 4 E3 ubiquitin ligase to enable macrophage infection. PLoS Pathog *4*, e1000059.

Stinski, M.F., and Petrik, D.T. (2008). Functional roles of the human cytomegalovirus essential IE86 protein. Curr Top Microbiol Immunol *325*, 133-152.

Strahl, B.D., and Allis, C.D. (2000). The language of covalent histone modifications. Nature *403*, 41-45.

Streblow, D.N., Dumortier, J., Moses, A.V., Orloff, S.L., and Nelson, J.A. (2008). Mechanisms of cytomegalovirus-accelerated vascular disease: induction of paracrine factors that promote angiogenesis and wound healing. Curr Top Microbiol Immunol *325*, 397-415.

Streblow, D.N., Varnum, S.M., Smith, R.D., and Nelson, J.A. (2006). A Proteomics Analysis of Human Cytomegalovirus Particle. In Cytomegaloviruses: Molecular Biology and Immunology, M.J. Reddehase, ed. (Caister Academic Press, Norfolk UK), pp. 91-110.

Su, H., Bidere, N., Zheng, L., Cubre, A., Sakai, K., Dale, J., Salmena, L., Hakem, R., Straus, S., and Lenardo, M. (2005). Requirement for caspase-8 in NF-kappaB activation by antigen receptor. Science *307*, 1465-1468.

Sugano, N., Chen, W., Roberts, M.L., and Cooper, N.R. (1997). Epstein-Barr virus binding to CD21 activates the initial viral promoter via NF-kappaB induction. J Exp Med *186*, 731-737.

Sulkowski, M.S., Mehta, S.H., Torbenson, M.S., Higgins, Y., Brinkley, S.C., de Oca, R.M., Moore, R.D., Afdhal, N.H., and Thomas, D.L. (2007). Rapid fibrosis progression among HIV/hepatitis C virus-co-infected adults. AIDS *21*, 2209-2216.

Sulkowski, M.S., and Thomas, D.L. (2003). Hepatitis C in the HIV-Infected Person. Ann Intern Med *138*, 197-207.

Sulkowski, M.S., Thomas, D.L., Mehta, S.H., Chaisson, R.E., and Moore, R.D. (2002). Hepatotoxicity associated with nevirapine or efavirenz-containing antiretroviral therapy: role of hepatitis C and B infections. Hepatology *35*, 182-189.

Sun, J., Bergeron, M., Barbeau, B., Boivin, G., and Tremblay, M.J. (2005a). Influenza virus activates human immunodeficiency virus type-1 gene expression in human CD4-expressing T cells through an NF-kappaB-dependent pathway. Clin Immunol *114*, 190-198.

Sun, Q., Matta, H., and Chaudhary, P.M. (2005b). Kaposi's sarcoma associated herpes virus-encoded viral FLICE inhibitory protein activates transcription from HIV-1 Long Terminal Repeat via the classical NF-kappaB pathway and functionally cooperates with Tat. Retrovirology *2*, 9.

Sun, S.C., Ganchi, P.A., Ballard, D.W., and Greene, W.C. (1993). NF-kappa B controls expression of inhibitor I kappa B alpha: evidence for an inducible autoregulatory pathway. Science *259*, 1912-1915.

Sung, V.M., Shimodaira, S., Doughty, A.L., Picchio, G.R., Can, H., Yen, T.S., Lindsay, K.L., Levine, A.M., and Lai, M.M. (2003). Establishment of B-cell lymphoma cell lines persistently infected with hepatitis C virus in vivo and in vitro: the apoptotic effects of virus infection. J Virol *77*, 2134-2146.

Svarovskaia, E.S., Cheslock, S.R., Zhang, W.H., Hu, W.S., and Pathak, V.K. (2003). Retroviral mutation rates and reverse transcriptase fidelity. Front Biosci *8*, d117-134.

Tada, K., Okazaki, T., Sakon, S., Kobarai, T., Kurosawa, K., Yamaoka, S., Hashimoto, H., Mak, T.W., Yagita, H., Okumura, K., *et al.* (2001). Critical roles of TRAF2 and TRAF5 in tumor necrosis factor-induced NF-kappa B activation and protection from cell death. J Biol Chem *276*, 36530-36534.

Tai, D.I., Tsai, S.L., Chen, Y.M., Chuang, Y.L., Peng, C.Y., Sheen, I.S., Yeh, C.T., Chang, K.S., Huang, S.N., Kuo, G.C., *et al.* (2000). Activation of nuclear factor kappaB in hepatitis C virus infection: implications for pathogenesis and hepatocarcinogenesis. Hepatology *31*, 656-664.

Takada, N., Sanda, T., Okamoto, H., Yang, J.P., Asamitsu, K., Sarol, L., Kimura, G., Uranishi, H., Tetsuka, T., and Okamoto, T. (2002). RelA-associated inhibitor blocks transcription of human immunodeficiency virus type 1 by inhibiting NF-kappaB and Sp1 actions. J Virol *76*, 8019-8030.

Tamanini, A., Nicolis, E., Bonizzato, A., Bezzerri, V., Melotti, P., Assael, B.M., and Cabrini, G. (2006). Interaction of adenovirus type 5 fiber with the coxsackievirus and adenovirus receptor activates inflammatory response in human respiratory cells. J Virol *80*, 11241-11254.

Tanaka, J., Ogura, T., Kamiya, S., Yoshie, T., Yabuki, Y., and Hatano, M. (1984). Dexamethasone enhances human cytomegalovirus replication in human epithelial cell cultures. Virology *136*, 448-452.

Tanaka, T., Grusby, M.J., and Kaisho, T. (2007). PDLIM2-mediated termination of transcription factor NF-kappaB activation by intranuclear sequestration and degradation of the p65 subunit. Nat Immunol *8*, 584-591.

Tang, Q., and Maul, G.G. (2003). Mouse cytomegalovirus immediate-early protein 1 binds with host cell repressors to relieve suppressive effects on viral transcription and replication during lytic infection. J Virol *77*, 1357-1367.

Taylor-Wiedeman, J., Sissons, P., and Sinclair, J. (1994). Induction of endogenous human cytomegalovirus gene expression after differentiation of monocytes from healthy carriers. J Virol *68*, 1597-1604.

Taylor, R.T., and Bresnahan, W.A. (2006). Human cytomegalovirus IE86 attenuates virus- and tumor necrosis factor alpha-induced NFkappaB-dependent gene expression. J Virol *80*, 10763-10771.

Ten, R.M., Blank, V., Le Bail, O., Kourilsky, P., and Israel, A. (1993). Two factors, IRF1 and KBF1/NF-kappa B, cooperate during induction of MHC class I gene expression by interferon alpha beta or Newcastle disease virus. C R Acad Sci III *316*, 496-501.

Thierry, S., Marechal, V., Rosenzwajg, M., Sabbah, M., Redeuilh, G., Nicolas, J.C., and Gozlan, J. (2004). Cell cycle arrest in G2 induces human immunodeficiency virus type 1 transcriptional activation through histone acetylation and recruitment of CBP, NF-kappaB, and c-Jun to the long terminal repeat promoter. J Virol *78*, 12198-12206.

Thoetkiattikul, H., Beck, M.H., and Strand, M.R. (2005). Inhibitor kappaB-like proteins from a polydnavirus inhibit NF-kappaB activation and suppress the insect immune response. Proc Natl Acad Sci U S A *102*, 11426-11431.

Thornburg, N.J., Pathmanathan, R., and Raab-Traub, N. (2003). Activation of nuclear factor-kappaB p50 homodimer/Bcl-3 complexes in nasopharyngeal carcinoma. Cancer Res *63*, 8293-8301.

Thornburg, N.J., and Raab-Traub, N. (2007). Induction of epidermal growth factor receptor expression by Epstein-Barr virus latent membrane protein 1 C-terminal-activating region 1 is mediated by NF-kappaB p50 homodimer/Bcl-3 complexes. J Virol *81*, 12954-12961.

Tobiume, M., Fujinaga, K., Suzuki, S., Komoto, S., Mukai, T., and Ikuta, K. (2002). Extracellular Nef protein activates signal transduction pathway from Ras to mitogen-activated protein kinase cascades that leads to activation of human immunodeficiency virus from latency. AIDS Res Hum Retroviruses *18*, 461-467.

Torroella-Kouri, M., Silvera, R., Rodriguez, D., Caso, R., Shatry, A., Opiela, S., Ilkovitch, D., Schwendener, R.A., Iragavarapu-Charyulu, V., Cardentey, Y., *et al.* (2009). Identification of a subpopulation of macrophages in mammary tumor-bearing mice that are neither M1 nor M2 and are less differentiated. Cancer Res *69*, 4800-4809.

Tsavachidou, D., Podrzucki, W., Seykora, J., and Berger, S.L. (2001). Gene array analysis reveals changes in peripheral nervous system gene expression following stimuli that result in reactivation of latent herpes simplex virus type 1: induction of transcription factor Bcl-3. J Virol *75*, 9909-9917.

Tugizov, S., Maidji, E., and Pereira, L. (1996). Role of apical and basolateral membranes in replication of human cytomegalovirus in polarized retinal pigment epithelial cells. J Gen Virol *77 (Pt 1)*, 61-74.

Tumilowicz, J.J., Gawlik, M.E., Powell, B.B., and Trentin, J.J. (1985). Replication of cytomegalovirus in human arterial smooth muscle cells. J Virol *56*, 839-845.

Tung, W.H., Hsieh, H.L., and Yang, C.M. (2010). Enterovirus 71 induces COX-2 expression via MAPKs, NF-kappaB, and AP-1 in SK-N-SH cells: Role of PGE(2) in viral replication. Cell Signal *22*, 234-246.

Turner, B.G., and Summers, M.F. (1999). Structural biology of HIV. J Mol Biol *285*, 1-32.

Tuttle, D.L., Harrison, J.K., Anders, C., Sleasman, J.W., and Goodenow, M.M. (1998). Expression of CCR5 increases during monocyte differentiation and directly mediates macrophage susceptibility to infection by human immunodeficiency virus type 1. J Virol *72*, 4962-4969.

Twu, J.S., Chu, K., and Robinson, W.S. (1989). Hepatitis B virus X gene activates kappa B-like enhancer sequences in the long terminal repeat of human immunodeficiency virus 1. Proc Natl Acad Sci U S A *86*, 5168-5172.

Tyagi, M., and Karn, J. (2007). CBF-1 promotes transcriptional silencing during the establishment of HIV-1 latency. EMBO J *26*, 4985-4995.

UNAIDS (2010). HIV Data (Geneva, Switzerland, UNAIDS Secretariat).

Unutmaz, D., KewalRamani, V.N., Marmon, S., and Littman, D.R. (1999). Cytokine signals are sufficient for HIV-1 infection of resting human T lymphocytes. J Exp Med *189*, 1735-1746.

Valentine, R., Dawson, C.W., Hu, C., Shah, K.M., Owen, T.J., Date, K.L., Maia, S.P., Shao, J., Arrand, J.R., Young, L.S., *et al.* (2010). Epstein-Barr virus-encoded EBNA1 inhibits the canonical NF-kappaB pathway in carcinoma cells by inhibiting IKK phosphorylation. Mol Cancer *9*, 1.

van Asten, L., and Prins, M. (2004). Infection with concurrent multiple hepatitis C virus genotypes is associated with faster HIV disease progression. AIDS *18*, 2319-2324.

Van Lint, C., Emiliani, S., Ott, M., and Verdin, E. (1996). Transcriptional activation and chromatin remodeling of the HIV-1 promoter in response to histone acetylation. EMBO J *15*, 1112-1120.

Varin, A., Decrion, A.Z., Sabbah, E., Quivy, V., Sire, J., Van Lint, C., Roques, B.P., Aggarwal, B.B., and Herbein, G. (2005). Synthetic Vpr protein activates activator protein-1, c-Jun N-terminal kinase, and NF-kappaB and stimulates HIV-1 transcription in promonocytic cells and primary macrophages. J Biol Chem *280*, 42557-42567.

Varin, A., Manna, S.K., Quivy, V., Decrion, A.Z., Van Lint, C., Herbein, G., and Aggarwal, B.B. (2003). Exogenous Nef protein activates NF-kappa B, AP-1, and c-Jun N-terminal kinase and stimulates HIV transcription in promonocytic cells. Role in AIDS pathogenesis. J Biol Chem *278*, 2219-2227.

Varnum, S.M., Streblow, D.N., Monroe, M.E., Smith, P., Auberry, K.J., Pasa-Tolic, L., Wang, D., Camp, D.G., 2nd, Rodland, K., Wiley, S., *et al.* (2004). Identification of proteins in human cytomegalovirus (HCMV) particles: the HCMV proteome. J Virol *78*, 10960-10966.

Verani, A., Gras, G., and Pancino, G. (2005). Macrophages and HIV-1: dangerous liaisons. Mol Immunol *42*, 195-212.

Verdin, E., Paras, P., Jr., and Van Lint, C. (1993). Chromatin disruption in the promoter of human immunodeficiency virus type 1 during transcriptional activation. EMBO J *12*, 3249-3259.

Verhoef, K., Sanders, R.W., Fontaine, V., Kitajima, S., and Berkhout, B. (1999). Evolution of the human immunodeficiency virus type 1 long terminal repeat promoter by conversion of an NF-kappaB enhancer element into a GABP binding site. J Virol *73*, 1331-1340.

Vermeulen, L., De Wilde, G., Van Damme, P., Vanden Berghe, W., and Haegeman, G. (2003). Transcriptional activation of the NF-kappaB p65 subunit by mitogen- and stress-activated protein kinase-1 (MSK1). EMBO J *22*, 1313-1324.

Viatour, P., Bentires-Alj, M., Chariot, A., Deregowski, V., de Leval, L., Merville, M.P., and Bours, V. (2003). NF- kappa B2/p100 induces Bcl-2 expression. Leukemia *17*, 1349-1356.

Viatour, P., Dejardin, E., Warnier, M., Lair, F., Claudio, E., Bureau, F., Marine, J.C., Merville, M.P., Maurer, U., Green, D., *et al.* (2004a). GSK3-mediated BCL-3 phosphorylation modulates its degradation and its oncogenicity. Mol Cell *16*, 35-45.

Viatour, P., Merville, M.P., Bours, V., and Chariot, A. (2004b). Protein phosphorylation as a key mechanism for the regulation of BCL-3 activity. Cell Cycle *3*, 1498-1501.

Viatour, P., Merville, M.P., Bours, V., and Chariot, A. (2005). Phosphorylation of NF-kappaB and IkappaB proteins: implications in cancer and inflammation. Trends Biochem Sci *30*, 43-52.

Victoriano, A.F., Asamitsu, K., Hibi, Y., Imai, K., Barzaga, N.G., and Okamoto, T. (2006). Inhibition of human immunodeficiency virus type 1 replication in latently infected cells by a novel IkappaB kinase inhibitor. Antimicrob Agents Chemother *50*, 547-555.

Vlach, J., and Pitha, P.M. (1992). Herpes simplex virus type 1-mediated induction of human immunodeficiency virus type 1 provirus correlates with binding of nuclear proteins to the NF-kappa B enhancer and leader sequence. J Virol *66*, 3616-3623.

Waldhoer, M., Kledal, T.N., Farrell, H., and Schwartz, T.W. (2002). Murine cytomegalovirus (CMV) M33 and human CMV US28 receptors exhibit similar constitutive signaling activities. J Virol *76*, 8161-8168.

Wang, D., Westerheide, S.D., Hanson, J.L., and Baldwin, A.S., Jr. (2000). Tumor necrosis factor alpha-induced phosphorylation of RelA/p65 on Ser529 is controlled by casein kinase II. J Biol Chem *275*, 32592-32597.

Wang, W., Ye, L., Li, B., Gao, B., Zeng, Y., Kong, L., Fang, X., Zheng, H., Wu, Z., and She, Y. (2007). Up-regulation of IL-6 and TNF-alpha induced by SARS-coronavirus spike protein in murine macrophages via NF-kappaB pathway. Virus Res *128*, 1-8.

Wang, W., Yu, P., Zhang, P., Shi, Y., Bu, H., and Zhang, L. (2008). The infection of human primary cells and cell lines by human cytomegalovirus: new tropism and new reservoirs for HCMV. Virus Res *131*, 160-169.

Wang, X., Huang, D.Y., Huong, S.M., and Huang, E.S. (2005). Integrin alphavbeta3 is a coreceptor for human cytomegalovirus. Nat Med *11*, 515-521.

Wang, X., Huong, S.M., Chiu, M.L., Raab-Traub, N., and Huang, E.S. (2003). Epidermal growth factor receptor is a cellular receptor for human cytomegalovirus. Nature *424*, 456-461.

Waris, G., Livolsi, A., Imbert, V., Peyron, J.F., and Siddiqui, A. (2003). Hepatitis C virus NS5A and subgenomic replicon activate NF-kappaB via tyrosine phosphorylation of IkappaBalpha and its degradation by calpain protease. J Biol Chem *278*, 40778-40787.

Weil, R., Sirma, H., Giannini, C., Kremsdorf, D., Bessia, C., Dargemont, C., Brechot, C., and Israel, A. (1999). Direct association and nuclear import of the hepatitis B virus X protein with the NF-kappaB inhibitor IkappaBalpha. Mol Cell Biol *19*, 6345-6354.

Wessells, J., Baer, M., Young, H.A., Claudio, E., Brown, K., Siebenlist, U., and Johnson, P.F. (2004). BCL-3 and NF-kappaB p50 attenuate lipopolysaccharide-induced inflammatory responses in macrophages. J Biol Chem *279*, 49995-50003.

Westendorp, M.O., Li-Weber, M., Frank, R.W., and Krammer, P.H. (1994). Human immunodeficiency virus type 1 Tat upregulates interleukin-2 secretion in activated T cells. J Virol *68*, 4177-4185.

Westendorp, M.O., Shatrov, V.A., Schulze-Osthoff, K., Frank, R., Kraft, M., Los, M., Krammer, P.H., Droge, W., and Lehmann, V. (1995). HIV-1 Tat potentiates TNF-induced NF-kappa B activation and cytotoxicity by altering the cellular redox state. Embo J *14*, 546-554.

Westerheide, S.D., Mayo, M.W., Anest, V., Hanson, J.L., and Baldwin, A.S., Jr. (2001). The putative oncoprotein Bcl-3 induces cyclin D1 to stimulate G(1) transition. Mol Cell Biol *21*, 8428-8436.

Whittaker, G.R., Kann, M., and Helenius, A. (2000). Viral entry into the nucleus. Annu Rev Cell Dev Biol *16*, 627-651.

Williams, J.L., Garcia, J., Harrich, D., Pearson, L., Wu, F., and Gaynor, R. (1990). Lymphoid specific gene expression of the adenovirus early region 3 promoter is mediated by NF-kappa B binding motifs. EMBO J *9*, 4435-4442.

Williams, S.A., Chen, L.F., Kwon, H., Ruiz-Jarabo, C.M., Verdin, E., and Greene, W.C. (2006). NF-kappaB p50 promotes HIV latency through HDAC recruitment and repression of transcriptional initiation. EMBO J *25*, 139-149.

Wilson, R.L., Fuentes, S.M., Wang, P., Taddeo, E.C., Klatt, A., Henderson, A.J., and He, B. (2006). Function of small hydrophobic proteins of paramyxovirus. J Virol *80*, 1700-1709.

Wolf, D., Witte, V., Clark, P., Blume, K., Lichtenheld, M.G., and Baur, A.S. (2008). HIV Nef enhances Tat-mediated viral transcription through a hnRNP-K-nucleated signaling complex. Cell Host Microbe *4*, 398-408.

Wolf, D., Witte, V., Laffert, B., Blume, K., Stromer, E., Trapp, S., d'Aloja, P., Schurmann, A., and Baur, A.S. (2001). HIV-1 Nef associated PAK and PI3-kinases stimulate Akt-independent Bad-phosphorylation to induce anti-apoptotic signals. Nat Med *7*, 1217-1224.

Woodhall, D.L., Groves, I.J., Reeves, M.B., Wilkinson, G., and Sinclair, J.H. (2006). Human Daxx-mediated repression of human cytomegalovirus gene expression correlates with a repressive chromatin structure around the major immediate early promoter. J Biol Chem *281*, 37652-37660.

Wright, E., Bain, M., Teague, L., Murphy, J., and Sinclair, J. (2005). Ets-2 repressor factor recruits histone deacetylase to silence human cytomegalovirus immediate-early gene expression in non-permissive cells. J Gen Virol *86*, 535-544.

Xiao, G., Cvijic, M.E., Fong, A., Harhaj, E.W., Uhlik, M.T., Waterfield, M., and Sun, S.C. (2001a). Retroviral oncoprotein Tax induces processing of NF-kappaB2/p100 in T cells: evidence for the involvement of IKKalpha. EMBO J *20*, 6805-6815.

Xiao, G., Fong, A., and Sun, S.C. (2004). Induction of p100 processing by NF-kappaB-inducing kinase involves docking IkappaB kinase alpha (IKKalpha) to p100 and IKKalpha-mediated phosphorylation. J Biol Chem *279*, 30099-30105.

Xiao, G., Harhaj, E.W., and Sun, S.C. (2001b). NF-kappaB-inducing kinase regulates the processing of NF-kappaB2 p100. Mol Cell *7*, 401-409.

Xiao, G., Rabson, A.B., Young, W., Qing, G., and Qu, Z. (2006). Alternative pathways of NF-kappaB activation: a double-edged sword in health and disease. Cytokine Growth Factor Rev *17*, 281-293.

Xiao, G., and Sun, S.C. (2000). Activation of IKKalpha and IKKbeta through their fusion with HTLV-I tax protein. Oncogene *19*, 5198-5203.

Xiao, P., Usami, O., Suzuki, Y., Ling, H., Shimizu, N., Hoshino, H., Zhuang, M., Ashino, Y., Gu, H., and Hattori, T. (2008). Characterization of a CD4-independent clinical HIV-1 that can efficiently infect human hepatocytes through chemokine (C-X-C motif) receptor 4. AIDS *22*, 1749-1757.

Xie, P., Hostager, B.S., and Bishop, G.A. (2004). Requirement for TRAF3 in signaling by LMP1 but not CD40 in B lymphocytes. J Exp Med *199*, 661-671.

Xu, X.N., Laffert, B., Screaton, G.R., Kraft, M., Wolf, D., Kolanus, W., Mongkolsapay, J., McMichael, A.J., and Baur, A.S. (1999). Induction of Fas ligand expression by HIV involves the interaction of Nef with the T cell receptor zeta chain. J Exp Med *189*, 1489-1496.

Yan, M., Peng, J., Jabbar, I.A., Liu, X., Filgueira, L., Frazer, I.H., and Thomas, R. (2005). Activation of dendritic cells by human papillomavirus-like particles through TLR4 and NF-kappaB-mediated signalling, moderated by TGF-beta. Immunol Cell Biol *83*, 83-91.

Yang, X., Chen, Y., and Gabuzda, D. (1999). ERK MAP kinase links cytokine signals to activation of latent HIV-1 infection by stimulating a cooperative interaction of AP-1 and NF-kappaB. J Biol Chem *274*, 27981-27988.

Yao, Z., Fanslow, W.C., Seldin, M.F., Rousseau, A.M., Painter, S.L., Comeau, M.R., Cohen, J.I., and Spriggs, M.K. (1995). Herpesvirus Saimiri encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. Immunity *3*, 811-821.

Yedowitz, J.C., and Blaho, J.A. (2005). Herpes simplex virus 2 modulates apoptosis and stimulates NF-kappaB nuclear translocation during infection in human epithelial HEp-2 cells. Virology *342*, 297-310.

Yeh, P.Y., Yeh, K.H., Chuang, S.E., Song, Y.C., and Cheng, A.L. (2004). Suppression of MEK/ERK signaling pathway enhances cisplatin-induced NF-kappaB activation by protein phosphatase 4-mediated NF-kappaB p65 Thr dephosphorylation. J Biol Chem *279*, 26143-26148.

Yeung, F., Hoberg, J.E., Ramsey, C.S., Keller, M.D., Jones, D.R., Frye, R.A., and Mayo, M.W. (2004). Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. EMBO J *23*, 2369-2380.

Yoshida, H., Kato, N., Shiratori, Y., Otsuka, M., Maeda, S., Kato, J., and Omata, M. (2001). Hepatitis C virus core protein activates nuclear factor kappa B-dependent signaling through tumor necrosis factor receptor-associated factor. J Biol Chem *276*, 16399-16405.

Yoshida, T., Hanada, T., Tokuhisa, T., Kosai, K., Sata, M., Kohara, M., and Yoshimura, A. (2002). Activation of STAT3 by the hepatitis C virus core protein leads to cellular transformation. J Exp Med *196*, 641-653.

You, L.R., Chen, C.M., and Lee, Y.H. (1999). Hepatitis C virus core protein enhances NF-kappaB signal pathway triggering by lymphotoxin-beta receptor ligand and tumor necrosis factor alpha. J Virol *73*, 1672-1681.

Yu, L., Mohanram, V., Simonson, O.E., Smith, C.I., Spetz, A.L., and Mohamed, A.J. (2009). Proteasome inhibitors block HIV-1 replication by affecting both cellular and viral targets. Biochem Biophys Res Commun *385*, 100-105.

Yu, W., Wang, Y., Shaw, C.A., Qin, X.F., and Rice, A.P. (2006). Induction of the HIV-1 Tat co-factor cyclin T1 during monocyte differentiation is required for the regulated expression of a large portion of cellular mRNAs. Retrovirology *3*, 32.

Yurochko, A.D. (2008). Human cytomegalovirus modulation of signal transduction. Curr Top Microbiol Immunol *325*, 205-220.

Yurochko, A.D., and Huang, E.S. (1999). Human cytomegalovirus binding to human monocytes induces immunoregulatory gene expression. J Immunol *162*, 4806-4816.

Yurochko, A.D., Hwang, E.S., Rasmussen, L., Keay, S., Pereira, L., and Huang, E.S. (1997a). The human cytomegalovirus UL55 (gB) and UL75 (gH) glycoprotein ligands initiate the rapid activation of Sp1 and NF-kappaB during infection. J Virol *71*, 5051-5059.

Yurochko, A.D., Kowalik, T.F., Huong, S.M., and Huang, E.S. (1995). Human cytomegalovirus upregulates NF-kappa B activity by transactivating the NF-kappa B p105/p50 and p65 promoters. J Virol *69*, 5391-5400.

Yurochko, A.D., Mayo, M.W., Poma, E.E., Baldwin, A.S., Jr., and Huang, E.S. (1997b). Induction of the transcription factor Sp1 during human cytomegalovirus infection mediates upregulation of the p65 and p105/p50 NF-kappaB promoters. J Virol *71*, 4638-4648.

Zaitseva, M., Romantseva, T., Manischewitz, J., Wang, J., Goucher, D., and Golding, H. (2005). Increased CXCR4-dependent HIV-1 fusion in activated T cells: role of CD4/CXCR4 association. J Leukoc Biol *78*, 1306-1317.

Zaragoza, C., Saura, M., Padalko, E.Y., Lopez-Rivera, E., Lizarbe, T.R., Lamas, S., and Lowenstein, C.J. (2006). Viral protease cleavage of inhibitor of kappaBalpha triggers host cell apoptosis. Proc Natl Acad Sci U S A *103*, 19051-19056.

Zarnegar, B., Yamazaki, S., He, J.Q., and Cheng, G. (2008). Control of canonical NF-kappaB activation through the NIK-IKK complex pathway. Proc Natl Acad Sci U S A *105*, 3503-3508.

Zhang, S.Q., Kovalenko, A., Cantarella, G., and Wallach, D. (2000). Recruitment of the IKK signalosome to the p55 TNF receptor: RIP and A20 bind to NEMO (IKKgamma) upon receptor stimulation. Immunity *12*, 301-311.

Zheng, H., Ye, L., Fang, X., Li, B., Wang, Y., Xiang, X., Kong, L., Wang, W., Zeng, Y., Wu, Z., *et al.* (2007). Torque teno virus (SANBAN isolate) ORF2 protein suppresses NF-kappaB pathways via interaction with IkappaB kinases. J Virol *81*, 11917-11924.

Zhong, H., May, M.J., Jimi, E., and Ghosh, S. (2002). The phosphorylation status of nuclear NF-kappa B determines its association with CBP/p300 or HDAC-1. Mol Cell *9*, 625-636.

Zhong, H., Voll, R.E., and Ghosh, S. (1998). Phosphorylation of NF-kappa B p65 by PKA stimulates transcriptional activity by promoting a novel bivalent interaction with the coactivator CBP/p300. Mol Cell *1*, 661-671.

Zhong, J., Gastaminza, P., Cheng, G., Kapadia, S., Kato, T., Burton, D.R., Wieland, S.F., Uprichard, S.L., Wakita, T., and Chisari, F.V. (2005). Robust hepatitis C virus infection in vitro. Proc Natl Acad Sci U S A *102*, 9294-9299.

Zhu, T., Mo, H., Wang, N., Nam, D.S., Cao, Y., Koup, R.A., and Ho, D.D. (1993). Genotypic and phenotypic characterization of HIV-1 patients with primary infection. Science *261*, 1179-1181.

Zhu, Z., Tang, W., Ray, A., Wu, Y., Einarsson, O., Landry, M.L., Gwaltney, J., Jr., and Elias, J.A. (1996). Rhinovirus stimulation of interleukin-6 in vivo and in vitro. Evidence for nuclear factor kappa B-dependent transcriptional activation. J Clin Invest *97*, 421-430.

Zink, W., Ryan, L., and Gendelman, H.E. (2002). Macrophage–virus interactions In The Macrophage, B. Burke, Lewis, C.E., ed. (Oxford, Oxford University Press), pp. 138-211.

Zipris, D., Lien, E., Nair, A., Xie, J.X., Greiner, D.L., Mordes, J.P., and Rossini, A.A. (2007). TLR9-signaling pathways are involved in Kilham rat virus-induced autoimmune diabetes in the biobreeding diabetes-resistant rat. J Immunol *178*, 693-701.