NF-κB activation can mediate inhibition of human cytomegalovirus replication

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The activation of NF-κB has long been considered a positive factor for human cytomegalovirus (HCMV) replication. The HCMV immediate-early promoter, the initial transcriptional element in the HCMV replication cycle, is activated by the transcription factor NF-κB, and several HCMV gene products have been demonstrated to activate this transcription factor. However, the role of NF-κB in the full replication cycle of the virus has not been carefully examined. A series of experiments that demonstrate an important inhibitory role of NF-κB for HCMV replication in fibroblasts is presented here. Using both genetic and pharmaceutical methods, it was shown that blocking NF-κB activation in cell culture does not inhibit HCMV replication, but rather leads to a modest increase in replication. Two cytokines inhibitory for HCMV, tumour necrosis factor-α and interferon-γ, no longer inhibit HCMV when NF-κB activation is blocked. Furthermore, forced expression of the NF-κB activating IκB kinase β (IKKβ), but not a kinase inactive mutant, also inhibits HCMV replication. In addition, it was shown that NF-κB signalling is essential for the production of an anti-viral factor in the supernatant of HCMV-infected fibroblasts, and identified interferon-β as this factor. Thus, the role of NF-κB in fibroblasts is to activate a host defence against HCMV.

INTRODUCTION

The NF-κB transcription factor family is abundant, potent, broadly expressed and rapidly activated by a large variety of cellular events involving cellular or environmental stress, DNA damage, cytokines, or the presence of pathogen components that activate either the Toll-like receptors or the Nod pathway. Because many of these cellular events occur during viral infections, it is not surprising that many viruses have evolved to include NF-κB binding sites in their transcriptional control elements. One interpretation of this phenomenon is that these pathogens in fact exploit the NF-κB pathway to drive their own transcriptional events, because it is strongly activated during infection.

The HCMV immediate-early (IE) gene promoter encodes several functional NF-κB binding sites and the promoter function is increased by NF-κB activation (Sambucetti et al., 1989; Prosch et al., 1995). Furthermore, HCMV encodes and expresses proteins that can themselves activate the NF-κB pathway (Yurochko et al., 1994, 1997a, b). For these reasons, one could conclude that NF-κB activation plays a positive role for HCMV replication and that inhibiting NF-κB activation would be a useful strategy for blocking virus replication. Under some conditions and in some cell types, NF-κB activation is important for anti-apoptotic, cellular repair and survival pathways, and perhaps the viral activation of NF-κB is a viral mechanism to avoid cellular apoptosis that would limit virus replication and spread. However, NF-κB is also an important transcription factor for a number of cellular anti-viral genes including inflammatory and anti-viral cytokines and genes whose products are part of the host innate immune response. Because of the evolutionary potential of viruses, it is quite possible that NF-κB is primarily a transcription factor activated in response to pathogen entry and important for driving anti-pathogen responses, but viruses have evolved to take advantage of the strongly and rapidly induced transcription factors available during infection.

Recently the requirement for NF-κB in cytomegalovirus replication has been examined using specific methods that assess the role of the transcription factor in the entire replication process (Benedict et al., 2004). These investigators provided evidence that viral transcription and replication proceed normally when either the NF-κB pathway is blocked or when established NF-κB binding sites in the viral genome are eliminated.

We are interested in defining the host signal transduction pathways used by HCMV for replication. This is an important study, both for understanding how HCMV replicates

Supplementary material available in JGV Online.
as well as for identifying new targets for pharmaceutical intervention of this replication. Accordingly, we have examined the role of NF-κB activation during the entire replication cycle of HCMV to distinguish if this activation is a pro-viral event (which would be consistent with a number of transcriptional studies of HCMV which, however, fail to examine the full HCMV life cycle) or if NF-κB activation drives an anti-viral response in the HCMV-infected cell.

METHODS

Cell culture. Primary human foreskin fibroblast (HFF) cell cultures were grown in DMEM containing 10 % FCS, 2 mM glutamine and 100 μg penicillin and streptomycin ml⁻¹ (DMEM 10 % FCS). A NIH3T3 cell clone expressing an NF-κB-dependent luciferase cDNA (3T3KLuc) was grown in DMEM 10 % FCS. For NF-κB luciferase assays, 3 × 10⁴ NIH3T3 cells were seeded per well of 96-well plates on day 1. After 24 h, the cells were treated with the indicated reagents or infected with recombinant adenovirus (see below) and cultured for an additional 24 h. Luciferase activity was then detected using standardized extracts and a commercial luciferase detection system.

Recombinant adenovirus vector construction and purification. The recombinant adenoviruses used in this study were E1, E3 defective derivatives of adenovirus type 5 (reviewed by Russell, 2000). The cDNAs of desired proteins were cloned into a transfer vector: 5'-GACATCCCTGAGG-AGATTAAGCA-3' and 5'-GGGACATCTCATAGTGTGCAAATG-3'; hybridization probe: 5'-FAM-GGTCCCCTCTTGGAACTGCTG-CAG-TAMRA-3'. GAPDH was quantified using an established TaqMan assay kit (ABI Prism TaqMan assay reagent for human GAPDH; Applied Biosystems).

RESULTS

Establishing methods of blocking the tumour necrosis factor (TNF)-α and IFN-γ activation of NF-κB

The standard routes for NF-κB activation have been defined, which has facilitated the development of inhibitors of this pathway. Exposure of cells to a variety of stimuli leads to activation of an IKK complex comprising IKKα, IKKβ, and IKKγ, resulting in the phosphorylation followed by ubiquitination and degradation of the inhibitor of NF-κB, IκB (reviewed by Ghosh & Karin, 2002). These events produce transcriptionally active p50/p65 NF-κB subunits, which either alone or in cooperation with additional transcription factors promote the expression of a panoply of genes involved in cell signalling, growth control and apoptosis. To examine the role of NF-κB in HCMV replication, we used an IκBα Ser 32, 36 Ala mutant (Brown et al., 1995, 1996) that lacks the serine residues which either alone or in cooperation with additional transcription factors promote the expression of a panoply of genes involved in cell signalling, growth control and apoptosis. To examine the role of NF-κB in HCMV replication, we used an IκBα Ser 32, 36 Ala mutant (Brown et al., 1995, 1996) that lacks the serine residues phosphorylated by IκB kinases during NF-κB activation. These phosphorylation events must occur to promote IκB ubiquitination, degradation and the ensuing NF-κB activation. IκBα (S32,36A; IκBα) is a potent inhibitor of NF-κB activation mechanisms. These reagents were tested in either cells permissive to HCMV replication (U373, Fig. 1a, b left panels; and HFF, Fig. 1c) or in murine NIH3T3 cells (Fig. 1a, b right panels). Cells infected with HCMV or stimulated with TNF-α or IFN-γ, show a clear activation of NF-κB. The expression of IκBα directed by recombinant adenovirus transduction (Fig. 1a insert) suppresses the induction of NF-κB from all cell types (Fig. 1a, white bars
**Fig. 1.** Both non-degradable IkBα (IkBΔ) and Dex block the induction of NF-κB. For NF-κB luciferase assays in NIH3T3 cells, 3 x 10^4 3T3 NF-κB luc cells were seeded in 96-well plates. For NF-κB luciferase assays in U373 and HFF cells, 5 x 10^4 cells were seeded in 24-well plates. (a) U373 cells were transfected with 275 ng reporter plasmid p3K, 75 ng pGFP for normalization or 90 ng non-degradable IkB (pIkBΔ white bars) or control plasmid (pPM7, black bars) using Lipofectamine (Invitrogen). After 4 h, the medium was replaced and cells were cultured for another 24 h. Before lysis, cells were stimulated for 2 h with 10 ng TNF-α ml⁻¹ (lanes 2), 4 h with 1 U IFN-γ ml⁻¹ (lanes 3) or with a purified HCMV strain AD169 with an m.o.i. of 0:03 (lanes 4). NIH3T3 cells were infected with 1000 viral particles per cell (p/c) of adenoviruses expressing a non-degradable IkB (AdIkBΔ white bars) or a control adenovirus AdΔ5 (black bars). After 4 h, the medium was replaced with fresh DMEM without FCS and the cells cultured for another 24 h. Four hours before lysis, cells were stimulated with 1 ng TNF-α ml⁻¹ (lanes 2), 100 U IFN-γ ml⁻¹ (lanes 3) or an HCMV strain AD169 with an m.o.i. of 0:3 (lanes 4). For expression controls, cells were lysed with RIPA-buffer, lysates were separated by SDS-PAGE (10% gel), transferred to nitrocellulose and probed with an anti-IkB antibody (Santa Cruz Biotechnology). (b) U373 cells were transfected with 275 ng reporter plasmid p3K and 75 ng pGFP for normalization. After transfection (24 h), cells were treated with 1 μM Dex and 1 h later stimulated with 10 ng TNF-α ml⁻¹ (2 h, lanes 2), 1 U IFN-γ ml⁻¹ (4 h, lanes 3) or a purified HCMV strain AD169 with an m.o.i. of 0:03 (4 h, lanes 4). After (24 h) seeding, NIH3T3 cells were treated with 1 μM Dex (white bars) or solvent control (black bars). After 1 h, NIH3T3 cells were stimulated for 4 h with 2 ng TNF-α ml⁻¹ (lanes 2), 20 U IFN-γ ml⁻¹ (lanes 3) or HCMV strain AD169 with an m.o.i. of 0:3 (lanes 4), and lysed for analysis of luciferase activity. Luciferase activity was normalized with GFP expression. (c) HFF cells were transfected with 170 ng reporter plasmid p3K, 30 ng pGFP for normalization and the indicated amounts of non-degradable IkB or control plasmid using FuGene (Roche). After 8 h, the medium was replaced and cells were cultured for another 16 h. After (24 h) transfection, cells were treated with the indicated amounts of Dex (right panel) or left untreated (left panel). After 1 h, cells were stimulated with TNF-α or HCMV as described above.
and Fig. 1c, left panel). Transduction with control adenoviruses of the same genotype (E1, E3 defective) with either no expression cassette or with irrelevant gene expression cassettes did not impair the NF-κB activation (results not shown).

To increase the reliability of our conclusions, an alternative method of modulating NF-κB activation was sought. Glucocorticoids, such as Dex, are potent inhibitors of NF-κB signalling with at least three mechanisms functional in this activity (reviewed by McKay & Cidlowski 1999; Almawi & Melemedjian, 2002). The activated glucocorticoid receptor can promote transcription of the inhibitor of NF-κB (IκB), can form complexes with p65, recruiting transcriptional inhibitory histone deacetylase to the promoters of NF-κB target genes, and can sequester limiting components of the transcriptional machinery to impair NF-κB activated gene expression.

To demonstrate that Dex could block NF-κB signalling, cells were exposed to Dex and infected with HCMV or treated with NF-κB activating levels of TNF-α or IFN-γ. Similar to the results obtained with the expression of IκBΔ, Dex treatment of U373, NIH3T3 or HFF cells clearly blocks the activation of NF-κB (Fig. 1b and 1c, right panel). Thus, two distinct methods of blocking the NF-κB activation are available: the expression of a IκBΔ directed by recombinant adenovirus and the pharmacological inhibition of NF-κB signalling by Dex.

That HCMV infection itself activates NF-κB has been known for some time (Kowalik et al., 1993). Several distinct mechanisms have been described for this activation, including upregulation of both p105/p50 and p65 and relB expression at the transcriptional level by HCMV early gene expression (Yurochko et al., 1995, 1997b; Jiang et al., 2002). The activation of NF-κB by HCMV can occur independently of virus replication and the inhibition of this function by pertussis toxin suggests a role for G-protein activation (Carlquist et al., 1999). The activation of NF-κB by HCMV infection is demonstrated in Fig. 1(a) and (b), four sample sets, and Fig. 1(c). Importantly, both IκBΔ expression (Fig. 1a and c) and Dex treatment (Fig. 1b and c) block the HCMV-stimulated NF-κB activation. Interestingly, UV inactivation of the virus (which should eliminate viral gene expression) leads to slight increase in the NF-κB activation (see supplementary material Fig. S1), consistent with the published report that HCMV encodes gene products which counteract NF-κB signalling and block the induction of an innate immune response (Brownie & Shenk, 2003).

The replication of HCMV in cell culture can be inhibited by treating cells with either TNF-α (Ito & O’Malley, 1987; Manor & Sarov, 1988; Paya et al., 1988) or IFN-γ (Yamamoto et al., 1987; Torigoe et al., 1993; Bodaghi et al., 1999). Both cytokines activate NF-κB (see Fig. 1); however, in the case of HCMV replication, the exact role of NF-κB in the cellular anti-viral responses activated by TNF-α and IFN-γ has not been examined directly. We first demonstrated that under the conditions used here, treatment with either agent inhibited HCMV replication (Fig. 2). TNF-α, and to a lesser extent IFN-γ, inhibited HCMV replication comparably with high level ganciclovir (GCV) treatment (Fig. 2b), which inhibits the virus by targeting a viral kinase and DNA polymerase. To investigate the requirement for NF-κB activation by the cytokines in the HCMV inhibition, IκBΔ or Dex were tested for their ability to modulate the HCMV inhibitory pathway activated by TNF-α and IFN-γ. Both IκBΔ and Dex reduced the anti-viral effects of TNF-α and IFN-γ on HCMV replication (Fig. 2). Control transduction with adenovirus Ad5 bearing an empty expression cassette does not influence the HCMV replication assay (see Eickhoff et al., 2004 and Fig. 2c, d). However, as expected, Dex (Fig. 2b) did not reverse the GCV inhibition of HCMV, which is not predicted to require NF-κB for function. Thus, two different anti-viral cytokines, TNF-α and IFN-γ, both activate NF-κB and inhibit HCMV replication. Inhibition of NF-κB activation by either expression of IκBΔ or treatment of cells with Dex prevented the inhibitory activity of the two cytokines. Very similar results were obtained when virus replication was assayed by traditional plaque titration (Fig. 2c, 2d) confirming the validity of the GFP assay results. (For a direct comparison between the plaque titration- and the GFP-replication assay, see supplementary Fig. S2.) Thus, the activation and function of the transcription factor NF-κB is essential for the inhibition of HCMV replication by TNF-α and IFN-γ.

**Blocking NF-κB activation does not impair HCMV gene expression**

Activation of NF-κB is clearly not required for HCMV replication in this system. Examination of the promoter structure of several HCMV genes has revealed NF-κB binding sites, and indeed, where examined, some of these promoters can be activated by NF-κB (see Benedict et al., 2004 for a detailed analysis of this point). The most thoroughly characterized of these promoters is the IE enhancer promoter; thus we monitored the consequences of blocking NF-κB activation on the activity of this promoter during a productive infection (Fig. 3). We found that neither IκBΔ expression nor Dex changed the timing or level of expression of IEp72, a gene product driven by the IE promoter (Fig. 3). Furthermore, expression of the early-late transcript glycoprotein B, monitored at 24 and 72 h, was also not changed (Fig. 3). Indeed, since the total replication of HCMV, as measured by GFP expression, was not inhibited by blocking NF-κB, these results were not unexpected. A detailed analysis of the importance of NF-κB for both HCMV and murine CMV was recently published (Benedict et al., 2004) and provided similar conclusions: the NF-κB sites in the IE promoter were not required for productive infection, and a similar dominant negative IκB system did not impair HCMV replication.
NF-κB activation mediates inhibition of HCMV replication

Fig. 2. Both IκBΔ and Dex block the inhibitory effect of IFN-γ and TNF-α on HCMV replication. (a) Subconfluent monolayers of HFFs were transduced with 1000 p/c of adenoviruses expressing IκBΔ (white bars) or a control adenovirus AdJ5 (black bars). At 24 h post-adenoviral transduction, cells were infected with an HCMV strain AD169-GFP; where indicated, 2 h after HCMV infection the culture medium was replaced with fresh medium containing 10 ng TNF-α ml⁻¹ (lanes 3), 10 U IFN-γ ml⁻¹ (lanes 4) or solvent control (lanes 2) and cells cultured for an additional 7 days. For analysis, cells were lysed in 25 mM Tris pH 7.5, 2 mM DTT, 1% Triton and 10% glycerol and analysed for EGFP content using a Wallac Victor fluorescence detector. All reported values are derived from duplicate infections with mean and SD values shown. (b) Cells were infected with HCMV AD169-GFP. At 1 h after HCMV infection, the medium was replaced with fresh medium containing 10 nM Dex (white bars) or solvent control (black bars). After 1 h addition of Dex, cells were treated with 10 μM GCV (lanes 2), 2 ng TNF-α ml⁻¹ (lanes 3) or 0-005U IFN-γ (lanes 4) and cultured for an additional 7 days. Analysis for EGFP content was performed as described above. (c) Subconfluent monolayers of HFF cells were transduced with 1000 p/c of adenovirus expressing IκBΔ or a control adenovirus AdJ5 and cultured for 24 h (lanes 6–9), or treated with Dex for 1 h (lanes 3 and 4) before infection with an HCMV strain AD169-GFP. After HCMV infection (1 h), the culture medium was replaced with fresh medium containing 0.3 ng TNF-α ml⁻¹ (lanes 3–5) or 0.03 ng TNF-α ml⁻¹ (lanes 8 and 9). After cultivation for 7 days, supernatants were tested for virus particle load in a plaque assay. For this, subconfluent HFF monolayers were infected with dilutions of the supernatants. After cultivation for additional 8 days, cells were stained with crystal violet and the numbers of viral plaques per well counted. All measurements were based on duplicate infections; mean and SD values are shown. (d) HFF cells were transduced with 3000 p/c of adenovirus expressing IκBΔ or a control adenovirus (lanes 6–9), or treated with Dex for 1 h (lanes 3 and 4) before infection with HCMV as described above. After HCMV infection (1 h), the culture medium was replaced with fresh medium containing 0.01 U ml⁻¹ (lanes 3–5) or 0.1 U ml⁻¹ (lanes 8 and 9) IFN-γ. After 7 days, supernatants were taken off and analysed for virus particle load in a plaque assay as described above.
Deliberate activation of the NF-κB pathway by overexpression of the kinase IKKβ inhibits HCMV replication

Both TNF-α and IFN-γ have a variety of effects on the cell and can activate a number of events in addition to NF-κB signalling. The above experiments demonstrate that NF-κB is essential for the anti-viral effects of TNF-α and IFN-γ, but the data do not demonstrate that it is sufficient. We thus used an alternative, and perhaps more specific, method of activating the NF-κB pathway to determine if NF-κB activation alone is sufficient to inhibit HCMV.

The activation pathway of NF-κB is primarily driven by stimuli that activate the IKK complex comprising the kinases IKKα and IKKβ and the non-catalytic subunit IKKγ/NEMO (reviewed by Ghosh & Karin, 2002). Overexpression of IKKβ with no additional stimulus was shown to be sufficient to activate the NF-κB pathway (Woronicz et al., 1997). This results in phosphorylation of both IκB as well as p105 with ensuing processing, and p65 or p50 release and NF-κB activation (Lang et al., 2003). Consistent with the literature, overexpression of IKKβ alone activates NF-κB dependent gene expression and will inhibit HCMV replication (Eickhoff et al., 2004), although in this published report, the control demonstrating that kinase activity was indeed required, was lacking. We examined this phenomenon in more detail here, including the appropriate control: a similar adenovirus expressing a kinase inactive point mutant of the kinase (Fig. 4). Expression of wild-type IKKβ provides a dose-dependent increase in NF-κB dependent gene expression (Fig. 4a, lanes 2–5) while expression of a kinase inactive version of IKKβ no longer activates NF-κB (Fig. 4a, lanes 6–9). When tested on HCMV replication, adenovirus-directed expression of IKKβ results in the expected inhibition of replication (Fig. 4b, lanes 3–5), similar to the inhibition observed with the more pleiotropic NF-κB activators TNF-α and IFN-γ (Fig. 2).

We tested the IKKβ K44R mutant for its capacity to inhibit NF-κB signalling, as this mutant has been proposed to inhibit HCMV-induced NF-κB activation in fibroblast cells (Caposio et al., 2004), yet its effect on a full HCMV replication cycle has not yet been examined. Importantly, expression of comparable levels of kinase inactive IKKβ K44R mutant does not impair HCMV replication (Fig. 4b, lanes 6–8), consistent with the demonstration that the mutant does not activate NF-κB (Fig. 4a). Transduction with an empty adenovirus of the same genotype, but lacking an expression cassette (AdJ5) is also not inhibitory to HCMV replication under these conditions (Fig. 4b, lane 2).

NF-κB activation is required to mount the cellular anti-viral response

One could define two types of NF-κB driven events in the infected cell in terms of virus replication: the transcription of negative factors such as cytokines and the transcription of positive factors, such as the HCMV genes themselves. We had previously demonstrated that activation of NF-κB, by overexpression of RIP-like interacting CLARP kinase (RICK), can lead to the secretion of anti-viral levels of IFN-β (Eickhoff et al., 2004, Fig. 7a). We had previously observed a modest increase in IFN-β expression in cells infected with a low m.o.i. of HCMV (Eickhoff et al., 2004, Fig. 7b). We hypothesized that infection with higher m.o.i. of HCMV could lead to inhibitory levels of the cytokine appearing in the cell supernatant. To distinguish the effects of NF-κB activation on the cellular anti-viral response from any requirement that HCMV may have for NF-κB for its own replication needs, we used a supernatant transfer method. With this method, NF-κB activation is manipulated in the initial HCMV-infected cell, and supernatants from these cells were tested for their ability to inhibit HCMV replication in fresh cells.

Either control fibroblasts or fibroblasts in which the NF-κB pathway was blocked using IκBα, were infected with HCMV. The supernatants from these cells were then collected and tested for their ability to inhibit HCMV
However, if the original cells are transduced with increasing amounts of HCMV, this inhibition was not significantly altered by transduction of the original cells with a control adenovirus (AdJ5, Fig. 5, lanes 3, 4).

The addition of an IFN-κB neutralizing antibody to the supernatants abolished the inhibition of virus replication as shown in Fig. 6(b), revealing IFN-κB as the responsible inhibitory cytokine. A modest increase of basal replication of HCMV in the presence of anti-IFN-κB occurs, which can be explained by an IFN-κB secretion in the course of the replication assay.

**DISCUSSION**

Two distinct methods of blocking NF-κB activation, the expression of a mutant IκB or the treatment of cells with the glucocorticoid Dex were used to examine the requirement for NF-κB activation on HCMV replication. We observed no inhibitory effects of blocking NF-κB on HCMV IE gene expression, consistent with published data from Benedict et al. (2004). Surprisingly, when full HCMV replication was examined, two different methods of blocking NF-κB activation resulted in modest increases in replication in a GFP-replication assay. In addition it was shown that two
cytokines that are established inhibitors of HCMV replication, TNF-α and IFN-γ, and which are relevant to control of virus growth in vivo, lose their inhibitory function when the activation of NF-κB is prevented, demonstrating that although the cytokines may have other transcriptional targets, the activation of NF-κB is essential for the HCMV inhibition. Moreover, it was demonstrated that cells over-expressing IKKβ, a more specific activator of NF-κB than either of the cytokines, are also refractory to HCMV replication, whereas a kinase inactive IKKβ K44R does not inhibit virus replication. Finally, it was revealed that NF-κB signalling is essential for the production of an anti-viral factor in the supernatant of HCMV-infected fibroblasts, and this factor was identified as IFN-β. Thus, NF-κB activation does not promote HCMV replication in fibroblasts, but rather drives a cellular inhibitory response against the virus.

There are a series of relevant observations on the ability of glucocorticoids, potent NF-κB inhibitory agents, to promote HCMV or murine CMV replication in cell culture, as well as in patients and in mice (Jordan et al., 1977; Velasco et al., 1984; Tanaka et al., 1984; Koment, 1985, 1989; Forbes et al., 1990; Lathey & Spector, 1991). We demonstrate here that two different methods of blocking NF-κB activation reverse the inhibitory effects of IFN-γ and TNF-α. These results provide a molecular mechanism to explain a number of older literature reports on the stimulatory effects of glucocorticoids on HCMV replication and pathology.

Although these results are clearly surprising in light of earlier work examining HCMV IE promoter function and NF-κB activation, they are consistent with more recent publications on this phenomenon. Benedict et al. (2001) had previously found that HCMV replication was not impaired in cells expressing a non-degradable IkB mutant; this was the first demonstration that inhibition of NF-κB did not produce a barrier to HCMV. Furthermore, Eickhoff et al. (2004) showed that the kinase RICK induces IFN-β via NF-κB signalling, which leads to inhibition of HCMV growth. In addition, Benedict et al. (2004) demonstrated...
that the NF-κB sites in the IE promoter are not essential for either murine CMV or HCMV replication, and that both viruses grow in the presence of a non-degradable IκB or in p65−/− fibroblasts. Using an alternative approach, we show in the current work that three different methods of manipulating NF-κB support the same conclusions: functional NF-κB activation is not only dispensable for productive HCMV replication in fibroblasts, but rather an essential part of an anti-viral host defence mechanism, which is mediated by IFN-β production. Blocking NF-κB by glucocorticoid or IκB does not impair replication, but blocks the inhibitory effect of IFN-γ or TNF, which has in vivo relevance for the control of virus growth, on HCMV replication and hampers host cell anti-viral response.

The response of a particular gene to NF-κB activation is influenced by the collection of other transcription factors that can be recruited to the promoter, the tissue expression of these transcription factors, local chromatin structure, as well as the strength, duration and nature of the NF-κB stimulus. It is clear that there are different kinetics of induction, strengths of expression, tissue expressions governed by different forms of NF-κB subunits expressed in the target cells as clearly demonstrated by Hoffmann et al. (2003). Furthermore, the NF-κB-dependent induction of the three isoforms of IκB can also strongly influence the resulting gene expression (Hoffmann et al., 2002). Thus, it is an oversimplification to consider all NF-κB activation events as equal. It is possible that some low-level NF-κB activation, promoting a cell survival, might be beneficial to HCMV replication, and these subtleties would not appear in our studies. Alternatively, it is possible that HCMV replication in other cell types might have a different response to NF-κB activation. Certainly the survival role of the NF-κB pathway can have profoundly different importance in, for example, lymphocytes compared with fibroblasts. Thus, the NF-κB activation driven by HCMV capsid protein binding (Boyle et al., 1999; Compton et al., 2003) or by US28 (Casarosa et al., 2001; Waldhoer et al., 2002) may serve an important role in the survival of infected lymphocytes. In the evolution of the virus, this cell survival must be balanced against whatever negative consequences NF-κB activation may generate. It is now becoming clear that viruses encode mechanisms to block the inhibitory consequences of NF-κB activation, such as the ability of HCMV pp65 to impair NF-κB and IRF1 signalling (Browne & Shenk, 2003). These strategies may allow the virus to take advantage of the positive features of activating NF-κB while preventing the negative consequences.

Finally, it should be noted that in vivo HCMV infection is not limited to fibroblasts. A full understanding of NF-κB in HCMV function in vivo requires an analysis in other, clinically relevant, cell types. There is evidence that HCMV can reside latently in monocytic cell lineages (Soderberg-Naucler et al., 1997; Pollock et al., 1997), and it is possible that NF-κB activation may contribute to reactivation of virus replication. Indeed, Soderberg-Naucler and colleagues (1997) have shown that IFN-γ and TNF-α can promote HCMV reactivation in monocytes and this facet of the HCMV replication cycle must be considered. However, for the development of anti-HCMV strategies, it is becoming clear that blocking NF-κB function is not an effective method of impairing the replication of this virus.

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