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Measles Virus Activates NF-κB and STAT Transcription Factors and Production of IFN-α/β and IL-6 in the Human Lung Epithelial Cell Line A549

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Epithelial cells of the respiratory tract are the primary targets of measles virus (MV) infection. In this work we have studied the effect of MV infection on the activation of transcription factors nuclear factor (NF)-κB and signal transducer and activator of transcription (STAT) and the production of cytokines in the lung epithelial A549 cell line. NF-κB and STAT activation were induced by MV in A549 cells as analyzed by electrophoretic mobility shift assay. NF-κB activation was rapid and it was not inhibited by the protein synthesis inhibitor cycloheximide, suggesting that MV directly activates NF-κB. In contrast, Stat1, Stat3, and interferon-stimulated gene factor 3 (ISGF3) DNA binding was induced by MV infection with delayed kinetics compared to NF-κB activation. MV infection also resulted in an efficient interferon (IFN)-α/β and interleukin-6 production. Cycloheximide and neutralizing anti-IFN-α/β antibodies inhibited MV-induced activation of Stat1, Stat3, and ISGF3 DNA binding in A549 cells. In conclusion, the results suggest that MV infection activates transcription factors involved in the initiation of innate immune responses in epithelial cells by two different mechanisms: directly by leading to NF-κB activation and indirectly via IFN-α/β leading to STAT activation.

Key Words: measles virus; STAT; NF-κB; IRF-1; ISGF3; ISRE; SIE; GAS; A549; IFN-α; IFN-β; IL-6; immunosuppression.

INTRODUCTION

Tracheal and bronchial epithelial cells are the primary targets of measles virus (MV) infection and together with endothelial cells and monocytes they play an important role in spreading of the virus in the body. MV infection results in a lifelong protective immunity (Griffin, 1995) although certain immunological abnormalities may be found during and after the infection. Because of immunosuppression measles continues to be a leading cause of death in children in developing countries, where vaccination coverage is insufficient. MV infection exposes mainly malnourished infants to opportunistic infections, resulting in annual mortality of 1 to 2 million people worldwide.

MV-induced immunosuppression is caused by abnormalities in cell-mediated immunity during and after the infection. Many functions of lymphocytes, such as lymphoproliferative response to mitogenic and recall antigen stimulation, are impaired in MV infection (Yanagi *et al.,* 1992; Fujinami *et al.,* 1998; Sun *et al.,* 1998). The lytic activity of natural killer (NK) cells may also be decreased (Casali *et al.,* 1984; Griffin *et al.,* 1990). MV infection increases the production of T helper type 2 (Th2) cytokine interleukin (IL)-4 *in vivo* and *in vitro,* whereas the production of Th1 cytokine interferon (IFN)-γ is not induced. The production of lipopolysaccharide-induced IL-12 in macrophages is also suppressed by MV infection (Karp *et al.,* 1996), which may impair the activation of lymphocytes (Schmitt *et al.,* 1994; Trinchieri, 1997) and lead to secondary bacterial infections. On the whole, the predominant humoral immune response and the suppression of cellular immunity in MV infection lead to the preferential Th2 polarization (Griffin and Ward, 1993; Ward and Griffin, 1993).

Activation of innate immunity is essential for the control of viral infections since it slows down the spread of the infection before the activation of adaptive immune responses. IFN-α/β plays an important role in the initiation of innate immunity by a number of different mechanisms. IFN-α/β enhances the killing of virus-infected cells by NK cells and activates the production of IFN-γ in NK cells, which leads to further activation of macrophages. IFN-α/β also directly inhibits the replication of viruses by stimulating the expression of several antiviral proteins, like (2′, 5′)oligoadenylate synthetase, ds-RNA-dependent protein kinase and Mx proteins (De Maeyer and De Maeyer-Guignard, 1998; Stark *et al.,* 1998). Gene knockout studies have also shown that the IFN-α/β system is essential for the clearance of viral infections (Durbin *et al.,* 1996; Meraz *et al.,* 1996).

IFN-α/β signals through the Janus tyrosine kinase–signal transducer and activator of transcription (JAK–

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STAT) pathway (Darnell *et al.,* 1994; Ihle, 1996). IFN-α/β induces tyrosine phosphorylation of Stat1 and Stat2 by the IFN-α/β receptor-associated JAK1 and TYK-2. The activated Stat1 and Stat2 heterodimerize and together with p48 (IRF9) form the IFN-stimulated gene factor 3 (ISGF3) complex. In the nucleus ISGF3 complex binds to the conserved IFN-stimulated response elements (ISRE) and initiates the transcription of IFN-α/β target genes. IFN-α/β and IFN-γ have some overlapping functions which are due to IFN-α/β- and IFN-γ-induced STAT DNA binding to the γ-activating sequence (GAS) elements in the IFN target genes. The activated Stat1 and Stat3 are known to interact also with the c-sis-inducible element (SIE) of the c-fos gene (Gronowski *et al.,* 1995).

Since the respiratory epithelium is the target of MV infection, we wanted to investigate whether innate immune mechanisms are fully and efficiently exploited by the lung epithelial cells during the infection. The A549 cell line used in this study has retained many features of the type II alveolar epithelial cells, including the synthesis of surfactant and cytoplasmic multilamellar inclusion bodies (Lieber *et al.,* 1976). In A549 cells MV infection induced a rapid activation of nuclear factor (NF)-κB DNA binding and activation of the JAK–STAT pathway with a slower kinetics, indicating a relatively strong antiviral response in lung epithelial cells.

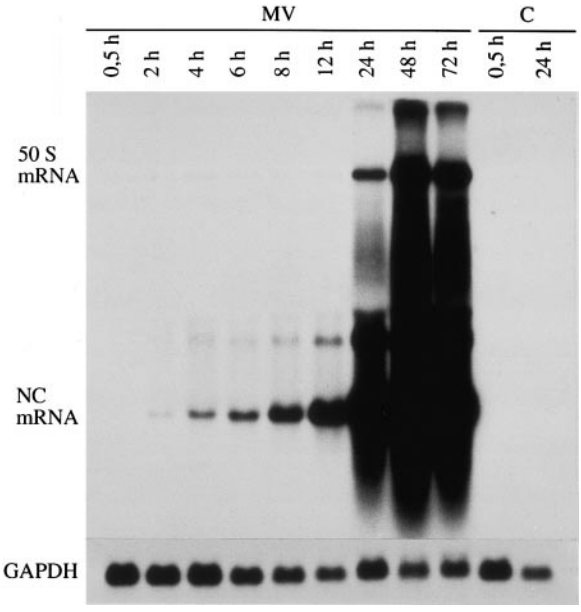

FIG. 1. Kinetics of viral NC mRNA and viral full-length 50 S genomic mRNA expression in MV-infected A549 cells. Cells were infected with MV and harvested at times indicated, and total cellular RNA was isolated. Equal amounts of RNA (20 μg) were size-fractionated in agarose gels, transferred to a nylon membrane, and hybridized with ^{32}P-labeled cDNA probes as indicated.

RESULTS

Kinetics of expression of viral RNA and proteins in MV-infected lung epithelial A549 cells

To study the infection kinetics in detail, lung epithelial A549 cells were infected with MV at a multiplicity of infection (m.o.i.) of 5. The cells were harvested at various time points, and the expression of viral mRNA was analyzed by Northern blotting (Fig. 1). MV nucleocapsid (NC) mRNA expression was detected already at 4 h p.i., whereas the expression of full-length 50 S mRNA was observed at 24 h p.i., corresponding to a complete viral replication cycle of MV.

Kinetics of MV-induced IFN-α/β and IL-6 production in A549 cells

Most cell types respond rapidly to viral infections by secreting IFN-α/β, which is the most important antiviral host factor (Goodbourn *et al.,* 2000; Guidotti and Chisari, 2000). Further evidence to support an important role of type I IFNs in antiviral defense has been obtained from IFN-α/β receptor or IFN-β deficient mice which are unable to clear viral infections (Deonarain *et al.,* 2000; McClary *et al.,* 2000).

To study MV-induced cytokine production in A549 cells, we analyzed the kinetics and magnitude of IFN-α/β, tumor necrosis factor (TNF)-α, IL-1β, and IL-6 production in response to MV infection. MV-infected A549 cell culture supernatants were collected at times indicated in Fig. 2 and IFN-α/β activity was measured. During the first 24 h of infection IFN-α/β levels were undetectable (<3 IU/ml) but after that a high level of IFN-α/β activity was found in the cell culture medium (up to 300 IU/ml at 72 h p.i.; Fig. 2A). Also the production of IL-6 was strongly induced in MV-infected A549 cells (Fig. 2B). Compared to control cells, some IL-6 was produced already at the first time point measured (3 h p.i.; 10 pg/ml in control and 160 pg/ml in MV-infected cell culture supernatants). Later a strong increase in IL-6 production occurred from 48 h p.i. onward (maximum at 96 h p.i. 2750 pg/ml). No significant TNF-α or IL-1β production was detected in MV-infected A549 cells (data not shown).

Analysis of NF-κB DNA-binding activity in MV-infected A549 cells

IL-6 and IFN-β genes are under the transcriptional control of NF-κB (Lenardo *et al.,* 1989; Libermann and Baltimore, 1990; Shimizu *et al.,* 1990). As the production of these cytokines was up-regulated in MV-infected A549 cells, we studied whether NF-κB DNA-binding activity was induced in response to MV infection. Nuclear extracts were prepared from MV-infected and control cells and analyzed by electrophoretic mobility shift assay (EMSA). Interestingly, NF-κB DNA-binding activity was seen starting from 1 h p.i. and it remained clearly detectable for 24 h after the infection (Fig. 3A). The NF-κB protein–DNA complexes were supershifted with p50-

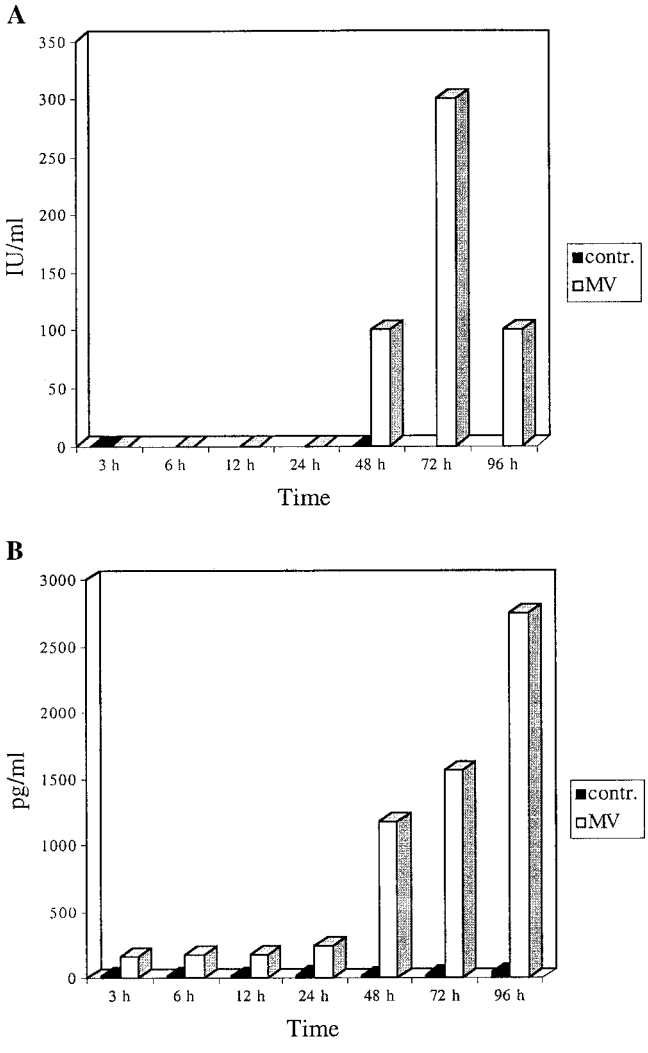

FIG. 2. Kinetics of IFN-α/β (A) and IL-6 (B) production in MV-infected A549 cells. Cells were infected with MV (5 m.o.i.) and cell culture supernatants were collected at times indicated. IFN-α/β and IL-6 levels were determined as described under Materials and Methods. A representative example of three independent experiments is shown.

and p65-specific antibodies, suggesting that the activated NF-κB complex in MV-infected cells consisted predominantly of p50 and p65 proteins (Fig. 3B). Protein synthesis inhibitor cycloheximide (CHX) treatment had no effect on the formation and quality of NF-κB DNA-binding complex (compare Figs. 3A and 8A), suggesting that virus-induced NF-κB activation was not dependent on ongoing protein synthesis.

Activation of Stat1, Stat3, and ISGF3 DNA binding in MV-infected A549 cells

Both NF-κB and STAT are involved in cytokine signaling during innate immune responses. We therefore studied the possible activation of STAT DNA binding during MV infection in A549 cells. STAT activation was analyzed by EMSA with interferon regulatory factor (IRF)-1 GAS, SIE, and ISRE15 oligonucleotide probes using nuclear extracts prepared from MV-infected A549 cells. STAT binding to IRF-1 GAS in response to MV infection was detected at 12 h p.i. and the intensity of this complex increased up to 24 h p.i. (Fig. 4A). Supershift experiments with anti-Stat1, -Stat3, and -Stat5b antibodies showed that both Stat1 and Stat3 DNA binding was activated in MV infection (Fig. 4B). MV infection also activated the STAT DNA binding beginning at 12 h p.i. as analyzed by the SIE element (Fig. 5A). The intensity of the complexes increased up to 24 h p.i. Supershift experiments with anti-Stat1, -Stat3, and -Stat5b antibodies showed that the MV-induced SIE-binding complexes consisted of Stat1 and Stat3 homodimers as well as Stat1/3 heterodimers

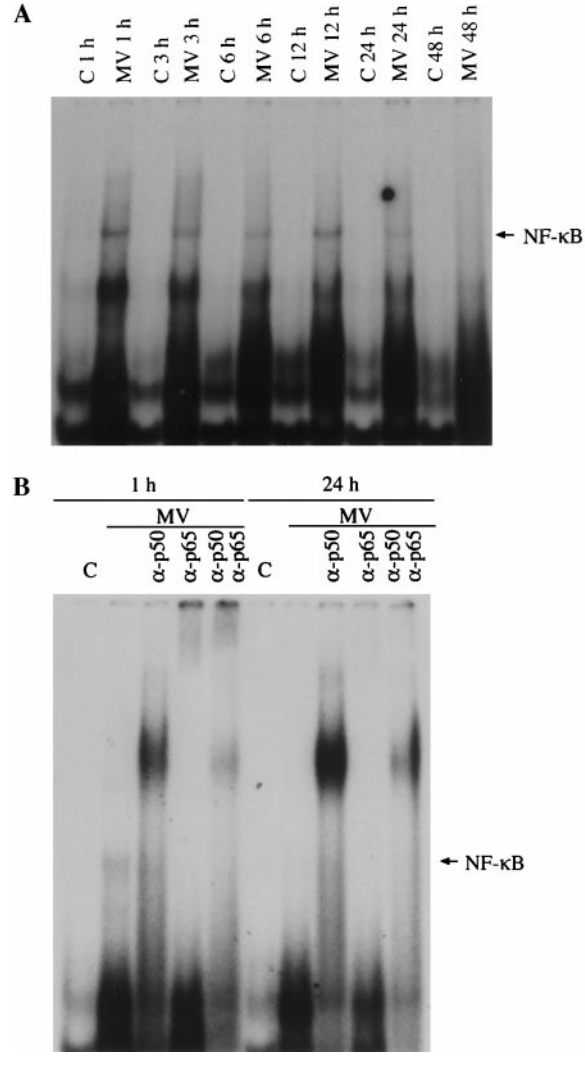

FIG. 3. MV-induced NF-κB DNA binding in A549 cells. Cells were infected with MV (5 m.o.i.) or treated with the uninfected control supernatant for the times indicated (A). Nuclear extracts were incubated with ^{32}P-labeled NF-κB consensus probe and analyzed by EMSA. In supershift experiments (B) nuclear extracts were incubated for 1 h on ice with anti-p50 and anti-p65 antibodies followed by EMSA with NF-κB consensus probe. A representative example of three independent experiments is shown.

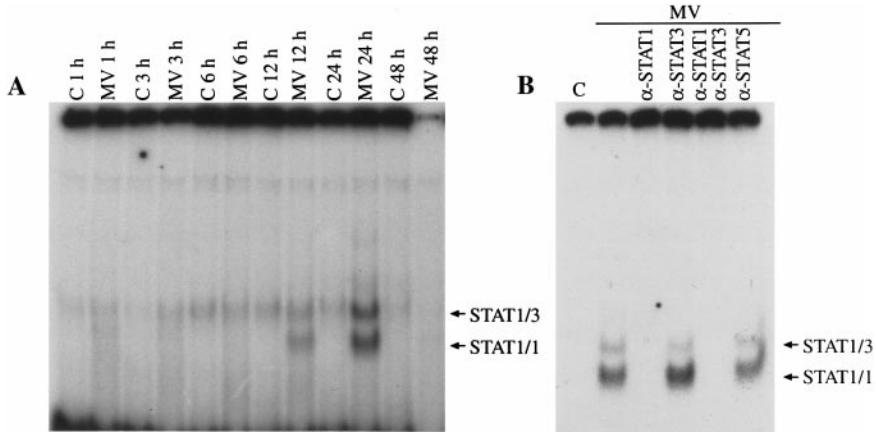

FIG. 4. Kinetics of MV-induced STAT DNA binding to IRF-1 GAS in A549 cells. Cells were infected with MV (5 m.o.i.) or treated with the uninfected control supernatant for the times indicated (A). Nuclear extracts were prepared and analyzed by EMSA with IRF-1 GAS probe. In supershift experiments (B) nuclear extracts were incubated for 1 h on ice with anti-Stat1, anti-Stat3, and anti-Stat5b antibodies followed by EMSA with IRF-1 GAS probe. A representative example of three independent experiments is shown.

(Fig. 5B). ISGF3 complex, binding to the ISRE15 element, was detectable at 12 h p.i. and the complex started to disappear after 24 h p.i. (Fig. 6A). The presence of p48 and Stat1 in ISGF3 complex was confirmed with anti-Stat1- and anti-p48-specific antibodies (Fig. 6B).

Activation of NF-κB and STAT DNA binding in Sendai virus-infected A549 cells

In addition to MV, we used Sendai virus to study innate immune response in A549 cells. Sendai (murine parainfluenza type 1) virus infects upper respiratory tract epithelial cells and has widely been used to study virus-induced gene activation. Nuclear extracts were prepared from Sendai virus-infected cells and control cells and analyzed by EMSA with NF-κB and SIE probes. NF-κB DNA binding was detected at 3 h after Sendai virus infection, and NF-κB activation peaked at 6 and 12 h p.i. (Fig. 7A). Similar to NF-κB DNA binding, Sendai virus-induced Stat1 and Stat3 DNA binding to SIE probe was seen at 3 h p.i. The Sendai virus-induced STAT complexes were detectable up to 48 h p.i. (Fig. 7B).

Effect of protein synthesis inhibition on the activation of STAT by MV

To analyze the requirement of ongoing protein synthesis for STAT activation, A549 cells were infected with MV for 1 or 12 h in the presence or absence of CHX. Unlike NF-κB, MV-induced Stat1 and Stat3 DNA binding to IRF-1 GAS and SIE elements was inhibited by CHX (Figs. 8B and 8C). Similarly, MV-induced ISGF3 complex formation was abolished by CHX (Fig. 8D). The results suggest that MV-induced cytokines are involved in STAT activation.

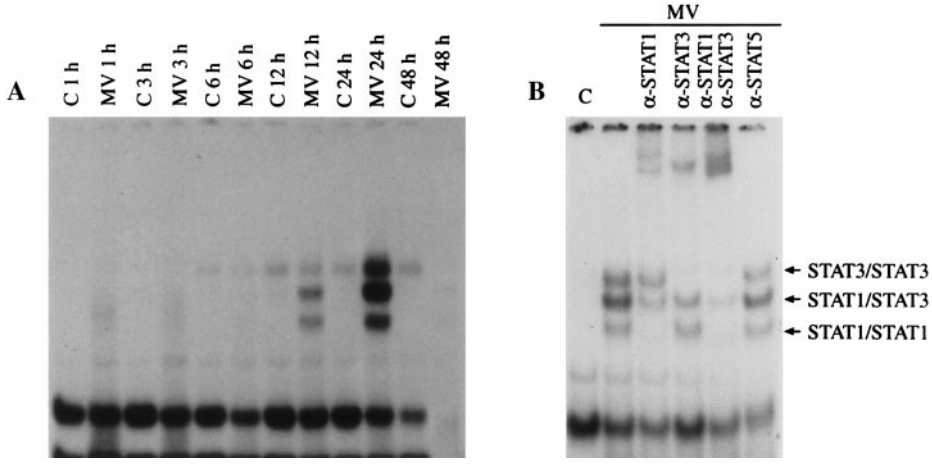

FIG. 5. Kinetics of MV-induced STAT DNA binding to SIE in A549 cells. Cells were infected with MV (5 m.o.i.) or treated with the uninfected control supernatant for the times indicated (A) and nuclear extracts were prepared and analyzed by EMSA with SIE probe. In supershift experiments (B) nuclear extracts were incubated for 1 h on ice with anti-Stat1, anti-Stat3, and anti-Stat5b antibodies. A representative example of three independent experiments is shown.

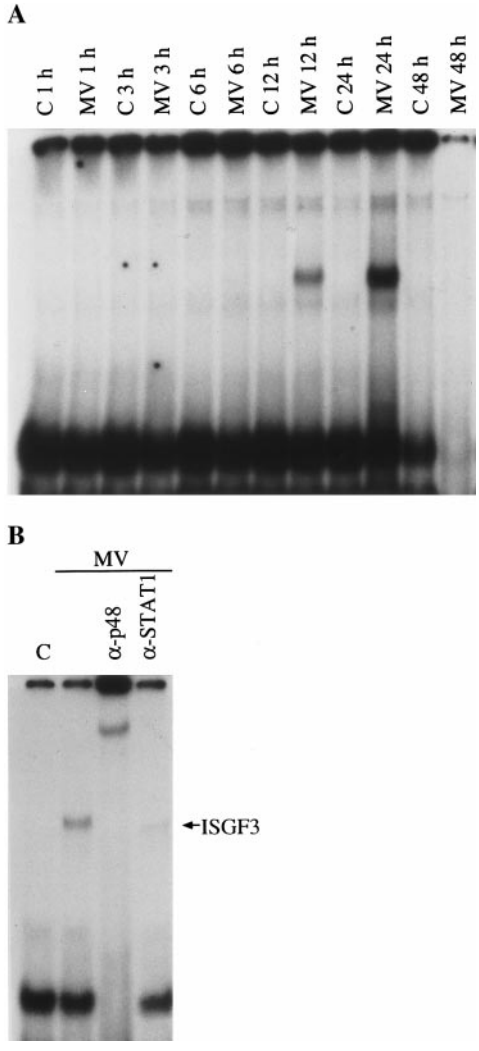

FIG. 6. Activation of ISGF3 DNA binding to ISRE15 in MV-infected A549 cells. Nuclear extracts prepared from MV-infected or control supernatant-treated cells (A) were analyzed by EMSA with ISRE15 probe. In supershift experiments (B) nuclear extracts were incubated for 1 h on ice with anti-Stat1 and anti-p48 antibodies followed by EMSA with ISRE15 probe. A representative example of three independent experiments is shown.

MV-induced STAT DNA binding is dependent on IFN-α/β production

To study the role of IFN-α/β and IL-6 in virus-induced STAT activation, we used neutralizing antibodies. A549 cells were infected with MV in the presence or absence of neutralizing antibodies against IFN-α/β or IL-6. Stat1 binding to IRF-1 GAS by MV was completely abolished by IFN-α/β antibodies and also Stat3 binding was markedly reduced (Fig. 9A). IFN-α/β antibodies also clearly reduced the MV-induced Stat1 and Stat3 binding to SIE or ISGF3 binding to ISRE15 (Figs. 9B and 9C). Anti-IL-6 antibodies had no detectable effect on Stat1 or Stat3 DNA binding to IRF-1 GAS or SIE elements, nor had it any effect on ISGF3 DNA binding (Figs. 9A, 9B, and 9C).

DISCUSSION

In this work we have analyzed the activation of immediate immune responses during MV infection in the lung epithelial A549 cell line. A549 cells are known to be susceptible to different viruses (Smith *et al.,* 1986; Coffin and Hodinka, 1995; Huang *et al.,* 1995; Ronni *et al.,* 1997) including MV (Helin *et al.,* 1999). Analysis of the kinetics of MV infection revealed that viral RNA levels increased up to 48 h p.i. followed by a decrease thereafter probably due to direct cell destruction caused by MV (Fig. 1).

MV infection of A549 cells resulted in marked IFN-α/β and IL-6 production, whereas IL-1β and TNF-α production remained at very low levels. IFN-α/β has antiviral activity, inhibits cell growth and proliferation, modulates the immune system by enhancing the expression of MHC classes I and II, and stimulates macrophage activation (Stark *et al.,* 1998; Goodbourn *et al.,* 2000; Guidotti and Chisari, 2000). IFNs directly activate antiviral pathways, while IL-6 contributes to the antiviral response indirectly by activating host inflammatory responses. In A549 cells an efficient IFN-α/β and IL-6 production was

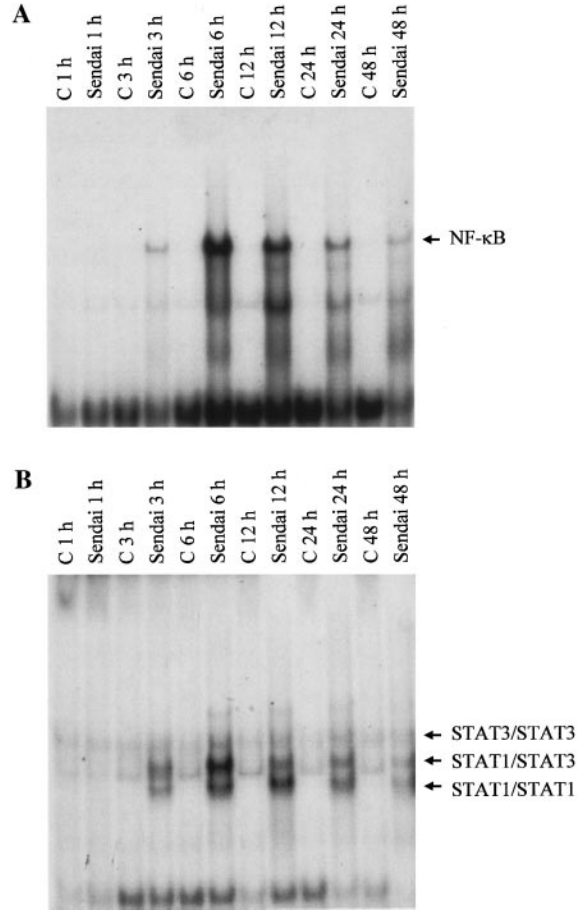

FIG. 7. Kinetics of Sendai virus-induced NF-κB and STAT DNA binding in A549 cells. Cells were infected with Sendai virus for the indicated times. Nuclear extracts were prepared, and EMSA with NF-κB (A) and SIE (B) probes followed.

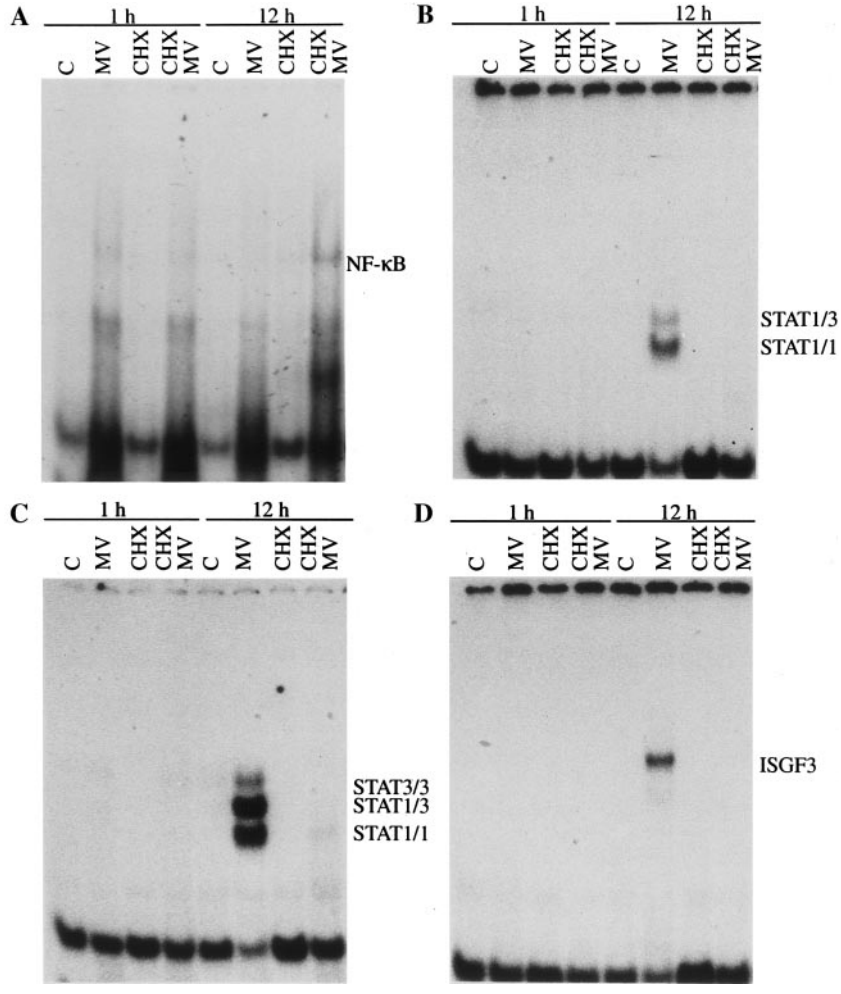

FIG. 8. Effect of protein synthesis inhibition on NF-κB, STAT, and ISGF3 transcription factor complex formation in MV-infected A549 cells. Cells were infected with MV in the presence or absence of the protein synthesis inhibitor CHX (10 μg/ml). Cells were collected, and nuclear extracts were prepared and analyzed in EMSA with NF-κB consensus (A), IRF-1 GAS (B), SIE (C), or ISRE15 (D) probe. A representative example of three independent experiments is shown.

induced during MV infection (Fig. 2). This finding is in agreement with previous results by others in several different cell types both *in vivo* and *in vitro* (Karp *et al.,* 1996; Auwaerter *et al.,* 1999; Manchester *et al.,* 1999). MV infection induces IFN-α production in PBMC and IFN-β production in glial cells and primary human umbilical vein endothelial cells (HUVEC) (Dhib-Jalbut and Cowan, 1993; Feldman *et al.,* 1994). Also mouse macrophages expressing human CD46, a receptor for MV, produce IFN-α/β in response to MV infection. The production is greatly attenuated by a deletion in the cytoplasmic domain of CD46 (Katayama *et al.,* 2000). Neurons, instead, are incapable of producing IFN-β in MV infection (Dhib-Jalbut *et al.,* 1995) and the difference in IFN-β production between neuronal and glial cells has been explained by the failure of MV to activate NF-κB in neuronal cells (Dhib-Jalbut *et al.,* 1999). In addition to glial cells, MV induces the activation of NF-κB in HUVEC and human B cells (Harcourt *et al.,* 1999; Imani *et al.,* 1999). Human brain cells have been shown to express MxA protein during MV infection (Schneider-Schaulies *et al.,* 1994). This provides indirect evidence of IFN-α/β production, since the human MxA gene is known to be controlled strictly by IFN-α/β (Ronni *et al.,* 1997, 1998).

NF-κB regulates the transcription of a large number of genes involved in immune responses, and it plays an important role in the antiviral response by regulating IFN gene expression. NF-κB also enhances the transcription of TNF-α and IL-6 genes. In this work we show that MV infection activates NF-κB DNA-binding activity with fast kinetics in the lung epithelial A549 cell line (Fig. 3). MV-induced NF-κB consisted of p50 and p65 proteins, which are known to be the most abundant heterodimeric form of NF-κB in human cells. The strongest NF-κB activity was seen already at the early stages of MV infection. Similar rapid activation of NF-κB was detected also in Sendai virus-infected A549 cells (Fig. 7A). Many viral gene products, like the hemagglutinin of influenza A virus, the Tax protein of type 1 human T cell leukemia virus, or the X protein of hepatitis B virus, are known to

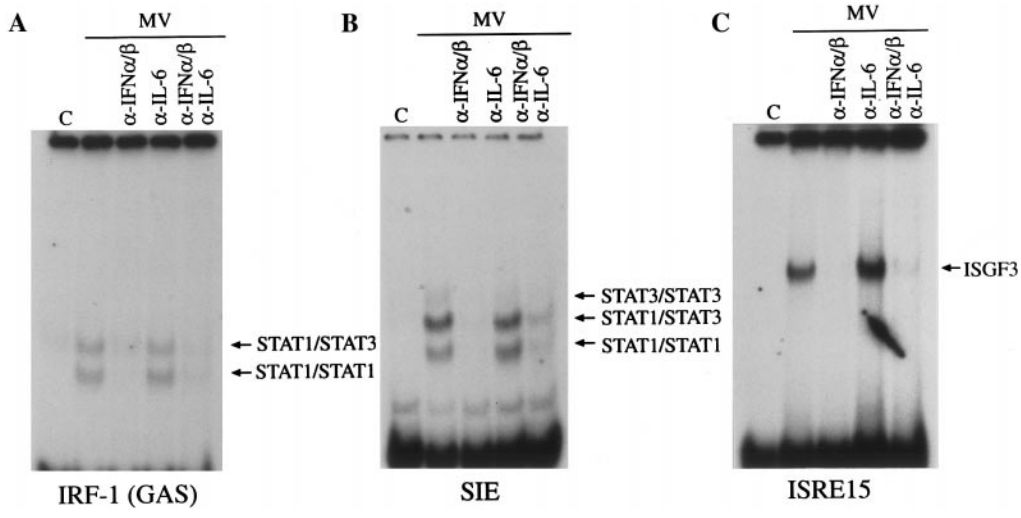

FIG. 9. The effects of neutralizing anti-IFN-α/β and anti-IL-6 antibodies on MV-induced STAT and ISGF3 DNA binding in A549 cells. Cells were infected with MV in the presence or absence of neutralizing anti-IFN-α/β and anti-IL-6 antibodies. Nuclear extracts were prepared and analyzed by EMSA with IRF-1 GAS (A), SIE (B), or ISRE15 (C) probe. A representative example of three independent experiments is shown.

directly activate NF-κB (Pahl and Baeuerle, 1999; Sun and Ballard, 1999; Weil *et al.,* 1999). The rapid activation without significant cytokine production at the early phase of infection (Fig. 2) suggests that MV is also able to directly activate NF-κB. Neutralizing anti-IFN-α/β or anti-IL-6 antibodies had no effect on MV-induced NF-κB activation (Fig. 9) and NF-κB DNA binding was activated by MV in the presence of protein synthesis inhibitor CHX (Fig. 8), further suggesting that MV directly activated NF-κB. Toll-like receptors (TLR) are innate immune receptors that have been shown to be involved in bacteria- and respiratory syncytial virus (RSV)-induced activation of NF-κB and production of cytokines (Brightbill and Modlin, 2000; Kurt-Jones *et al.,* 2000). Whether RSV-like TLR-mediated NF-κB activation mechanisms take place during MV infection is an open question.

The activation of STAT transcription factors, namely Stat1 and Stat3 DNA binding to IRF-1 GAS (Fig. 4) and SIE elements (Fig. 5), was induced by MV at 12 h p.i. in A549 cells. In contrast, Sendai virus activated Stat1 and Stat3 DNA binding to SIE probe already at 3 h p.i. (Fig. 7B). MV-induced STAT DNA binding appeared to be dependent on *de novo* protein synthesis (Fig. 8), suggesting that STAT activation was cytokine mediated. Neutralizing anti-IFN-α/β antibodies strongly reduced MV-induced Stat1 and Stat3 DNA binding (Fig. 9), although we were unable to detect any IFN-α/β production in MV-infected 24-h samples (Fig. 2). However, IFN-α/β production was strongly induced at 48 p.i. and it is possible that the low amounts of IFN-α/β produced in the earlier phases of infection were consumed by the cells. As in the case of MV infection, Sendai virus-activated Stat1 and Stat3 DNA binding was abolished by neutralizing anti-IFN-α/β antibodies (data not shown), suggesting that IFN-α/β has an important role in innate response to both of these viruses. Neutralization of IL-6 had no effect on MV-induced (Fig. 9) or Sendai virus-induced (data not shown) activation of STATs.

Formation of ISGF3 complex is specifically induced by IFN-α (Schindler *et al.,* 1992) and it has an essential role in the host defense against viral infections. Some viruses can interfere with the JAK–STAT pathway. For instance, hepatitis B virus has specific ISGF3-binding ISRE-like promoter-sequences in its genome (Nakao *et al.,* 1999) and human papillomavirus can physically interact with TYK-2 and prevent the phosphorylation of Stat1 and Stat2 (Li *et al.,* 1999). In the present study MV was found to induce the formation of ISGF3 complex in an IFN-α/β-dependent manner (Figs. 6 and 9).

Young and colleagues (2000) demonstrated that Paramyxoviridae were able to interfere with the IFN signaling system with different molecular mechanisms. MV, as the only member of the Morbillivirus genus, was not, however, included in this study. Our results suggested that in lung epithelial A549 cells the IFN-α/β signaling system functioned normally during MV infection, although A549 cells seemed to be highly susceptible to MV infection and were incapable of restricting the infection *in vitro* despite strong IFN production. STAT activation in MV infection of A549 cells seemed to occur relatively slowly (12 h p.i.), allowing the virus to replicate prior the activation of the antiviral defense system. Recently, Naniche and coworkers described that the wild-type isolates of MV induce lower levels of IFN-α/β compared to Vero cell-grown strains of MV (Naniche *et al.,* 2000). We used the Vero cell-adapted Halonen strain of MV in our studies and it may be that this strain induces higher levels of IFN-α/β compared to virulent strains of MV. Therefore, it would be interesting to compare the ability of wild-type isolates of MV and Vero cell-grown

strains of MV to activate STAT signaling in lung epithelial cells.

In this study we have shown that MV infection readily enhanced the production of IFN-α/β and IL-6. We also demonstrated that MV infection directly activated NF-κB, whereas the JAK–STAT pathway was indirectly activated via the production of IFN-α/β. This suggests that a relatively strong antiviral response is induced by MV in lung epithelial cells. This is an interesting observation because it is known that *in vivo* MV replicates during the first 2 to 4 days locally in bronchial epithelial cells before it spreads to local lymph nodes and other tissues. Despite relatively strong innate immune response, MV-infected A549 cells were incapable of restricting the infection. In addition to epithelial cells endothelial cells and monocytes/macrophages are also the target cells for MV infection *in vivo.* In monocytes MV infection is, however, restricted and it would be of great interest to elucidate the role of IFNs and IFN-inducible transcription factors in monocytes and their possible contribution to the pathogenesis of MV infection.

MATERIALS AND METHODS

Viruses and cell line

A wild-type MV (Halonen strain; Vainionpää *et al.,* 1978) with a high infectivity titer ($>5 \times 10^7$ PFU/ml) was used throughout the study. The inoculum virus was propagated in Vero cells. In all experiments a m.o.i. of 5 was used. Control cells were treated with an equal volume of the supernatant of uninfected Vero cells. The murine Sendai virus (strain Cantell) was grown as previously described (Pirhonen *et al.,* 1999). The hemagglutination titer of Sendai virus used was 150 U/ml.

The human lung adenocarcinoma cell line A549 (ATCC CCC-185) was cultured in Ham's F-12 medium supplemented with 1% FBS.

Cytokine ELISA

The levels of IL-1β, IL-6, and TNF-α in cell culture supernatants were determined by enzyme-linked immunosorbent assay as previously described by Miettinen *et al.* (1998).

Biological assay for IFN-α/β

Cell culture supernatants were treated at pH 2 and assayed for the presence of IFN-α/β in Hep2 cells by vesicular stomatitis virus plaque reduction (Cantell *et al.,* 1991). The results are expressed as IU/ml, using an international control IFN-α preparation as a standard.

Electrophoretic mobility shift assay

Nuclear extracts were prepared according to Andrews *et al.* (1991) and nuclear protein/DNA-binding reactions were performed as previously described by Matikainen *et al.* (1996). IRF-1 GAS (5′-AGCTTCAGCCTGATTTCCCCGAAATGACGGA-3′), SIE (5′-GATCTAGGGATTTCCGGGAAATGAAGCT-3′), and ISRE15 (5′-AGCTTGATCGGGAAAGGGAAACCGAAACTGAAGCCA-3′) (Raz *et al.,* 1994) oligonucleotides were synthesized with an Applied Biotechnology, Inc., oligonucleotide synthesizer and purified on PAGE gels in the presence of 8 M urea. NF-κB binding oligonucleotide (5′-AGTTGAGGGGACTTTCCCAGCC-3′) was purchased from Promega (Madison, WI). The probes were end-labeled with [γ-^{32}P]dATP (3000 Ci/mol; Amersham) by T4 polynucleotide kinase. Samples were analyzed by electrophoresis on 4–6% nondenaturing low-ionic-strength polyacrylamide gels in 0.25× Tris–borate–EDTA. Gels were dried and visualized by autoradiography.

For supershift assays, nuclear protein extracts were incubated with antibodies (1/20 dilution) for 1 h on ice before the addition of the radiolabeled probe. Antibodies used in supershift experiments were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The following antibodies were used: anti-Stat1 (sc-345X), anti-Stat3 (sc-482X), anti-Stat5b (sc-835X), anti-p50 (sc-1190X), and anti-p65 (sc-372X).

CHX treatment and neutralization of cytokine activity

For protein synthesis inhibition the A549 cells were incubated in the presence of 10 μg/ml CHX (Sigma, St. Louis, MO). To neutralize extracellular IFN-α/β or IL-6 activity, MV-infected cells were treated with neutralizing anti-IFN-α/β (Mogensen *et al.,* 1975) or anti-IL-6 (R&D Systems, Abingdon, UK) antibodies.

RNA isolation and Northern blot analysis

For Northern blotting total cellular RNA was extracted using the thiocyanate–CsCl method (Chirgwin *et al.,* 1979). Twenty micrograms of RNA was separated in formaldehyde-containing 0.8% agarose gel, transferred to a nylon membrane (ZETA-Probe; Bio-Rad), and hybridized with ^{32}P-labeled (Amersham, Inc.) MV NC-specific cDNA (a kind gift from Dr. Roberto Cattaneo, University of Zurich, Switzerland; Cattaneo *et al.,* 1987) probe. A rat glyceraldehyde-3-phosphate dehydrogenase probe (Fort *et al.,* 1985) was used to control the proportional quantities of RNA.

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