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Thogoto virus ML protein suppresses IRF3 function

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Abstract

The *Thogoto virus* (THOV) is a member of the family *Orthomyxoviridae*. It prevents induction of alpha/beta interferons (IFN) in cell culture and in vivo via the action of the viral ML protein. Phenotypically, the effect of THOV ML resembles that of the NS1 protein of *influenza A virus* (FLUAV) in that it blocks the expression of IFN genes. IFN expression depends on IFN regulatory factor 3 (IRF3). Upon activation, IRF3 forms homodimers and accumulates in the nucleus where it binds the transcriptional coactivator CREB-binding protein (CBP). Here, we show that expression of ML blocked the transcriptional activity of IRF3 after stimulation by virus infection. Further biochemical analysis revealed that ML acts by blocking IRF3 dimerization and association with CBP. Surprisingly, however, ML did not interfere with the nuclear transport of IRF3. Thus, the action of ML differs strikingly from that of FLUAV NS1 that prevents IFN induction by retaining IRF3 in the cytoplasm.

Keywords: Orthomyxoviruses; *Thogoto virus*; ML protein; *Influenza virus*; NS1 protein; Interferon antagonist; Interferon-regulatory factor 3; IRF3

Introduction

The mammalian interferon (IFN) system represents the basis of an early host response against viral infections (Samuel, 2001). Mice lacking a functional type I IFN receptor (Fiette et al., 1995; Müller et al., 1994) or humans with defects in IFN receptor signaling (Dupuis et al., 2003) are highly susceptible to infections with viral pathogens. Upon viral infection, recognition of virus particles (Collins et al., 2004; Servant et al., 2002) or virus-specific molecular patterns like single-stranded and double-stranded RNA (dsRNA) triggers the expression of type I IFNs, mainly IFNα and β (Alexopoulou et al., 2001; Diebold et al., 2004; Stark et al., 1998). IFNβ, known as the immediate early IFN, leads via a positive feedback mechanism to the upregulation of the IFNα genes (Erlandsson et al., 1998; Marie et al., 1998). Both IFNα and β induce the expression of IFN-stimulated genes, thus establishing an antiviral state in cells (Der et al., 1998; Staeheli, 1990).

Production of IFNβ is one of the earliest host cell responses to infection with viral pathogens. The transcriptional induction of the IFNβ promoter involves activation of IFN regulatory factor-3 (IRF3), activating protein-1 (AP1), and nuclear factor kappa B (NF-κB) (Algarte et al., 1999; Schafer et al., 1998; Wathelet et al., 1998; Yoneyama et al., 1998). Among these transcription factors, IRF3 is the most essential for the immediate early induction of IFNβ expression (Sato et al., 2000). IRF3 is constitutively expressed and in its inactive, unphosphorylated form

Abbreviations: dsRNA, double-stranded RNA; FF-Luc, firefly luciferase; FLUAV, *influenza A virus*; IFN, interferon; M, matrix protein; ML, matrix protein long; NDV, newcastle disease virus; NP, nucleoprotein; PFU, plaque forming units; REN-Luc, Renilla luciferase; THOV, *Thogoto virus*; VLP, virus-like particle; vRNP, viral ribonucleoprotein complex.

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predominantly resides in the cytoplasm. Viral infection triggers activation of IRF3 through phosphorylation on serine and threonine residues in its C-terminal part (between residues 385 and 405) (Lin et al., 1998; Mori et al., 2004; Yoneyama et al., 1998). The responsible kinases for this essential activation step were recently characterized as two unconventional IκB kinases, IKKε and TBK1 (Fitzgerald et al., 2003; Sharma et al., 2003). Activation of IRF3 induces a sequence of events including nuclear accumulation (Kumar et al., 2000; Lin et al., 1998), homodimerization, and association with transcriptional coactivators like the CREB-binding protein (CBP) in order to gain full transcriptional activity (Suhara et al., 2000; Weaver et al., 1998; Yang et al., 2002). The IRF3/CBP holocomplex then binds and activates target gene promoters containing IRF3 binding sites such as the IFNβ and ISG56 promoter (Grandvaux et al., 2002; Peters et al., 2002; Wathelet et al., 1998).

Viruses have evolved different strategies to circumvent the induction of type I IFNs (Goodbourn et al., 2000; Levy and Garcia-Sastre, 2001; Weber et al., 2003). IRF3 as a key factor for the induction of the early antiviral host response is targeted by many viruses (Baigent et al., 2002; Basler et al., 2003; Bossert et al., 2003; Dauber et al., 2004; Foy et al., 2003; Graff et al., 2002; Lin et al., 2001; Poole et al., 2002; Ronco et al., 1998; Xiang et al., 2002). One of the best studied examples is the NS1 protein of FLUAV that was shown to prevent activation of the IFNβ promoter by suppressing activation of IRF3 by dsRNA (Talon et al., 2000). Similar to FLUAV, infection with *Thogoto virus* (THOV) attenuates activation of the IFNβ promoter (Hagmaier et al., 2003).

Together with the influenza viruses, THOV belongs to the family of *Orthomyxoviridae* (Van Regenmortel et al., 2000). In contrast to other orthomyxoviruses, THOV is an arbovirus that replicates both in mammalian and in tick cells (Jones and Nuttall, 1989). Ticks are the reservoir of THOV (Nuttall et al., 1995). The virus persistently infects these animals but is not transmitted horizontally or transovarially between ticks (Davies et al., 1986), raising the question how the virus persists in this reservoir. THOV is transmitted to vertebrates through tick bites. After a viremic phase, the virus infects other ticks feeding on the same animal, favoring the spread of the virus back to its arthropod reservoir. To successfully perform this replication cycle, THOV has evolved a strategy to inhibit the induction of the innate immune response in the vertebrate host. The virus has been shown to effectively suppress the induction of type I IFN in infected cells and in animals (Hagmaier et al., 2003; Pichlmair et al., 2004), and therefore prevents induction of the early antiviral host response that is mainly based on IFN-stimulated *Mx* genes (Haller et al., 1995; Pavlovic et al., 1995).

The genome of THOV consists of six single-stranded RNA segments of negative polarity that have a coding capacity for seven proteins: six essential structural proteins such as the three subunits of the viral RNA-dependent RNA polymerase, the nucleoprotein (NP), the transmembrane glycoprotein, the matrix protein (M), and one non-essential accessory protein, the ML protein (Hagmaier et al., 2003; Haller and Kochs, 2002). The proteins M and ML are both encoded by the smallest viral RNA segment. The M protein is translated from a spliced transcript in which the stop codon terminating the M reading frame is created by a splicing event (Kochs et al., 2000), whereas the ML protein is translated from the full-length, unspliced transcript. Thus the 304 amino acid long ML represents an elongated version of M (266 amino acids) containing 38 additional amino acids at the C terminus. We recently found that ML functions as an IFN antagonist that prevents activation of the IFNβ promoter (Hagmaier et al., 2003). Accordingly, a recombinant THOV lacking the ML gene showed enhanced induction of IFN in infected cells and, in contrast to the highly virulent wild-type virus, was strongly attenuated in mice (Hagmaier et al., 2003; Pichlmair et al., 2004).

Here, we demonstrate that ML targets a distinct step in IRF3 activation. ML does not interfere with the initial IRF3 activation and nuclear translocation. Instead, it blocks IRF3 dimerization and its interaction with the coactivator CBP. Therefore, unlike the NS1 protein of FLUAV that prevents IRF3 activation in the cytoplasm, THOV ML antagonizes the IFN system by specifically inhibiting IRF3 transcriptional activity in the nucleus.

Results

THOV ML suppresses IRF3-dependent promoter activation

We recently identified the ML protein of THOV as an IFN antagonist that blocks induction of IFN in infected cells (Hagmaier et al., 2003). ML is a C-terminally extended version of THOV M protein containing 38 additional amino acids. To analyze the activity of ML independent of the viral context, cDNA expression plasmids encoding THOV M, THOV ML, or an empty vector were transfected into 293 cells together with a firefly luciferase (FF-Luc) reporter construct under the control of the IFNβ promoter (Yoneyama et al., 1998). Infection of the transfected cells with *Newcastle disease virus* (NDV), a strong IFN inducer (Bazzigher et al., 1992), led to the expression of FF-Luc, indicating activation of the IFNβ promoter (Fig. 1A). Coexpression of ML resulted in a 5-fold reduction of promoter activity compared to the control, thereby confirming the IFN antagonistic activity of ML (Hagmaier et al., 2003). M expression, by contrast, had no inhibitory effect.

To elucidate the molecular basis of this inhibitory activity, we concentrated on IRF3 because this is the most critical cellular transcription factor that is essential for virus-induced IFNβ expression, whereas the other transcription factors NFκB and AP-1 have only enhancing effects (Wathelet et al., 1998). One of the earliest steps of IRF3

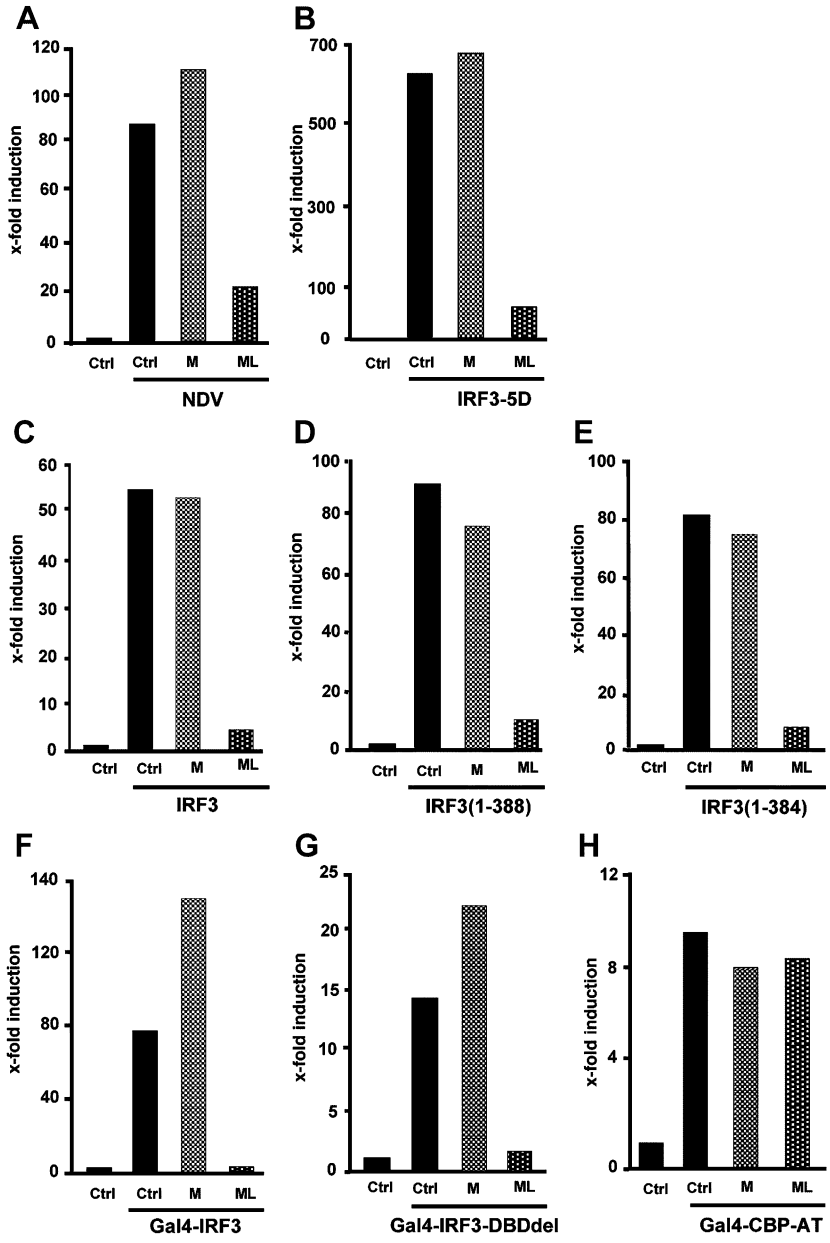

Fig. 1. ML suppresses the activity of IRF3. Human 293 cells were transfected with a reporter plasmid carrying the FF-Luc gene under the control of the IFNβ promoter (p125Luc; A and B) or under the control of an artificial promoter containing three IRF3 response elements (p55C1BLuc, C–E). In addition, a reporter plasmid carrying the REN-Luc gene under the control of the SV40 promoter together with expression plasmids encoding THOV M, ML, or empty plasmid (Ctrl) was cotransfected. (A) At 5 h post-transfection, the cells were infected with 1 PFU per cell of NDV for 18 h and analyzed for reporter gene expression. (B–E) Cells were cotransfected with different expression plasmids encoding IRF3 or IRF3 mutants. (F–H) To analyze the involvement of the IRF3 DNA-binding domain, cells were cotransfected with a reporter plasmid carrying the FF-Luc gene under the control of the Gal4 promoter together with pRL-SV40 and M/ML expression plasmids. Activation of the Gal4 promoter was achieved by cotransfection of a Gal4-IRF3 construct (F), a Gal4-IRF3 fusion protein with deletion of the DNA-binding domain, Gal4-IRF3-DBDdel (G), or a Gal4-CBP-AT construct (H). Twenty-four hours post-transfection, cell lysates were analyzed by dual luciferase assay. FF-Luc activity was normalized to REN-Luc activity and is indicated as fold induction compared to that of the untreated control.

activation is its phosphorylation at specific C-terminal Ser/Thr residues (Lin et al., 1998; Yoneyama et al., 1998). Expression of an IRF3 construct in which five phosphorylation sites are converted into phosphomimetic aspartic acid residues, IRF3(5D) (Lin et al., 1998), led to a strong activation of the IFNβ promoter, independent of additional stimuli (Fig. 1B). Similar to the situation with NDV-infected cells, expression of ML but not of M strongly inhibited IRF3(5D)-mediated reporter gene expression (Fig. 1B). This indicates that ML is able to suppress the transcriptional activity at a step downstream of the activation of IRF3.

Phosphorylation of IRF3 occurs on two C-terminal amino acid clusters. Five Ser/Thr residues between amino acids 396–405 (Lin et al., 1998) and two serine residues at

amino acids 385/386 (Yoneyama et al., 1998) were described as the responsible targets of the activating protein kinases. Therefore, we constructed deletion mutants of IRF3 lacking 39 or 43 amino acids of the C terminus called IRF3(1–388) and IRF3(1–384). These truncated variants of IRF3 are active upon overexpression together with an IRF3-responsive FF-Luc reporter construct containing three IRF3-binding elements (Yoneyama et al., 1998). Overexpression of wild-type IRF3 or the C-terminal deletion mutants strongly stimulated reporter gene expression (Figs. 1C, D, and E), albeit to lower levels than those in IRF3(5D) transfection experiments. ML, but not M, was able to suppress this activation independent of the presence of IRF3 C-terminal phosphorylation sites. These results suggest that the effect of ML on IRF3 transcriptional activity is independent of the phosphorylation state of the IRF3 C-terminus and further implies that the C-terminal regulatory moiety of IRF3 is not a target of ML action.

Suppression of IRF3 by THOV ML is independent of the DNA-binding capacity of IRF3

After dimerization and association with transcriptional coactivators, IRF3 is thought to bind to cognate DNA elements and stimulate promoter activity (Wathelet et al., 1998). We therefore tested whether the block of IRF3-dependent promoter activation by ML is based on an inhibition of IRF3 DNA binding. To uncouple transcriptional activation by IRF3 from its DNA-binding property, we used a Gal4-IRF3 fusion protein that transactivates reporter gene expression by binding to Gal4 promoter elements (Fitzgerald et al., 2003). As expected, the full-length Gal4-IRF3 fusion construct induced reporter gene expression under the control of the Gal4-promoter (Fig. 1F). Coexpression of THOV M led to an increase in reporter gene expression, while coexpression of THOV ML completely abrogated transactivation by Gal4-IRF3 (Fig. 1F). Accordingly, we tested the effect of ML on a Gal4-IRF3 construct that lacks the complete IRF3 DNA-binding domain (amino acids 7–107). Coexpression of ML again suppressed transactivation by the Gal4-IRF3 fusion protein (Fig. 1G). Since IRF3 activity is dependent on the recruitment of cotransactivators like CBP to the promoter region, we tested whether transactivation by CBP was also inhibited by coexpression of ML. As shown in Fig. 1H, neither THOV M nor ML had any effect on the activity of a Gal4-CBP fusion protein, indicating that THOV ML does not affect CBP function. Therefore, THOV ML appears to specifically affect IRF3 function independent of its DNA-binding properties.

THOV ML prevents virus-induced IRF3 dimerization and interaction with CBP

Upon activation, IRF3 forms homodimers that are required for DNA binding and interaction with transcriptional coactivators (Iwamura et al., 2001). To test the effect of ML on these steps of IRF3 activation, 293 cells were infected with recombinant THOVs that either encode the ML protein THOVML+ or do not encode ML THOVML− (Hagmaier et al., 2003). To analyze the oligomerization state of IRF3, lysates of infected cells were separated by nondenaturating gel electrophoresis, and IRF3 monomers and dimers were detected by Western blot analysis using an IRF3-specific antibody. As shown in Fig. 2A, THOVML infection induced IRF3 dimerization, whereas in uninfected cells, no IRF3 dimers could be detected. Surprisingly, in cells infected with THOVML+, IRF3 predominantly accumulated in the monomeric fraction and only weak dimer formation was detectable. Detection of the viral NP suggests comparable infection with the two different recombinant viruses (Fig. 2B). In addition, we directly tested the effect of recombinant ML on the homo-oligomerization of IRF3, independent of virus infection. 293 cells were cotransfected with cDNA constructs encoding the constitutively activated IRF3 variant IRF3(5D) together with M and ML expression plasmids. Analysis of the cell lysates 24 h later showed a

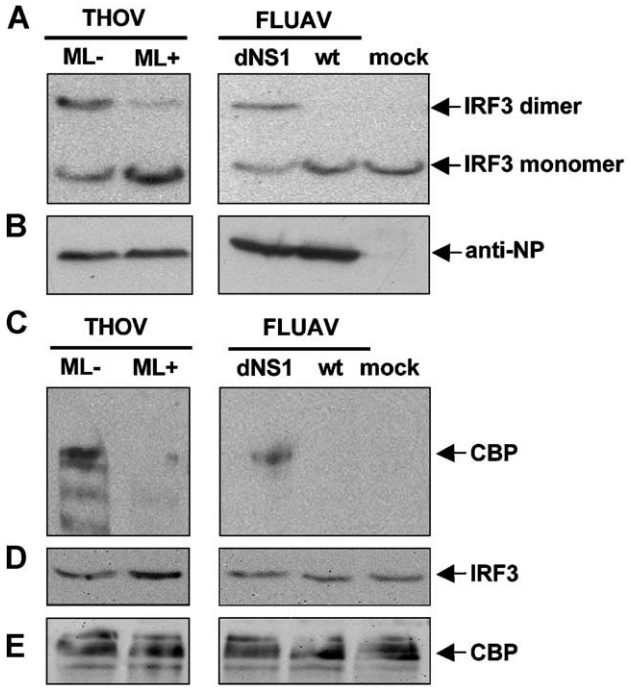

Fig. 2. ML prevents IRF3 dimerization and association with CBP. 293 cells were infected with 5 PFU per cell of THOVML−, THOVML+, FLUAV(wt), FLUAVdNS1, or were mock infected for 20 h. (A) Cell lysates were analyzed for monomers and dimers of IRF3 by non-denaturing gel electrophoresis followed by Western blotting using an IRF3-specific antibody. (B) Viral infection was monitored using an antibody directed against THOV NP or FLUAV NP. (C) The same cell lysates were used for coimmunoprecipitation with a monoclonal IRF3-specific antibody and the immunocomplexes were analyzed by Western blot with a CBP-specific antibody. (D) IRF3 present in the immunocomplex was detected with a polyclonal rabbit antiserum directed against IRF3. (E) The total amount of CBP in individual cell lysates was monitored using the CBP-specific antibody.

spontaneous formation of IRF3(5D) dimers, as expected. Consistent with our experiments with infected cells, expression of recombinant ML suppressed IRF3(5D) dimerization whereas expression of M had no effect (data not shown).

In parallel, we did the same analysis with two strains of FLUAV (A/PR/8/34) that either expressed or lacked the IFN-antagonistic protein NS1 (FLUAVdNS1) (Garcia-Sastre et al., 1998). While FLUAVdNS1 was a strong inducer of IRF3 activation and resulted in the formation of IRF3 dimers, wild-type FLUAV infection prevented IRF3 dimerization, similar to the situation in THOVML+-infected cells (Fig. 2A).

IRF3 dimers recruit the transcriptional coactivator CBP to gain full activity (Suhara et al., 2000; Weaver et al., 1998; Yang et al., 2002). Therefore, we considered that ML may not only prevent IRF3 dimerization but also the association with CBP. To test this hypothesis, cells were infected with the recombinant THOVs and FLUAVs for 20 h and then subjected to immunoprecipitation analysis using an IRF3-specific antibody. The precipitated immunocomplexes were analyzed by Western blot using CBP- and IRF3-specific antibodies. As shown in Fig. 2C, no interaction was observed between CBP and IRF3 in cells infected with the wild-type viruses THOVML+ and FLUAV. In contrast, the mutants THOVML− and FLUAVdNS1 both triggered the association of CBP with IRF3 (Fig. 2C). Fig. 2D indicates that comparable levels of IRF3 are present in the immunocomplexes. Using the anti-CBP antibody, we confirmed that equal amounts of CBP are present in individual cell lysates (Fig. 2E). These results demonstrate that the ML protein of THOV antagonizes IRF3 dimerization and association to CBP, similar to the NS1 protein of FLUAV.

THOV ML does not affect nuclear accumulation of IRF3

Nuclear translocation of IRF3 is a prerequisite for its stimulatory activity (Lin et al., 1998). To analyze the effect of ML on the nuclear accumulation of IRF3, we used two independent approaches. First, we transfected cells with THOV M or ML expression constructs together with a GFP-IRF3-encoding plasmid. Then the cells were infected with NDV to induce nuclear accumulation of the IRF3 fusion protein. The cells were then fixed, and the expression of M and ML was detected by immunofluorescence. As expected, in uninfected cells, GFP-IRF3 was detected in the cytoplasm whereas upon NDV infection GFP-IRF3 accumulated in the nucleus (Fig. 3A). Surprisingly, neither expression of M nor of ML disturbed the virus-induced nuclear accumulation of GFP-IRF3 (Fig. 3A). In a second set of experiments, we used the recombinant THOVs described above. Vero cells were infected with these viruses and the localization of endogenous IRF3 as well as the production of viral NP was analyzed by double immunofluorescence 24 h after infection. In uninfected cells, IRF3 was detected in the cytoplasm, whereas THOV infection led to the accumulation of IRF3 in the cell nucleus (Fig. 3B), indicating that THOV has the potential to trigger IRF3 activation. Interestingly, IRF3 nuclear accumulation was observed following infection with both the ML-expressing and the ML-lacking THOVs (Fig. 3B). These data suggest that despite the lack of IRF3 dimerization and the absence of IRF3/CBP interaction, infection with wild-type THOV induces nuclear translocation and accumulation of IRF3, indicating that expression of ML is not able to block this process.

Discussion

In this report, we describe how the THOV protein ML prevents IFN induction by targeting IRF3, the essential factor that stimulates early IFN production after virus infection. ML thereby interferes with the dimerization of IRF3 and its association with the transcriptional coactivator CBP, but not with the nuclear accumulation of IRF3.

Virus infection represents a stress signal that activates the innate immune system. This results in the rapid expression and secretion of type I IFNs that are responsible for the induction of antiviral host response (Goodbourn et al., 2000; Stark et al., 1998). One of the earliest stress responses after virus infection is the activation of IRF3, the essential factor for IFNβ expression (Collins et al., 2004; Sato et al., 2000). IRF3 is constitutively expressed and resides in the cytoplasm until activation. According to the current model (Servant et al., 2002; Yoneyama et al., 2002), the cell responds to virus infection with the activation of diverse Ser/Thr protein kinases that activate IRF3. Phosphorylated IRF3 then dimerizes, translocates into the nucleus, and associates with the CBP coactivator. This holocomplex then binds to IRF3-responsive promoters and stimulates transcription.

Our data clearly show that the ML protein of THOV prevents activation of IRF3 at a late stage by interfering with its dimerization and association with the coactivator molecule CBP. In contrast, the nuclear translocation of IRF3 was not affected by ML. IRF3 contains an N-terminal DNA-binding domain (7–107) and a C-terminal transactivation domain (110–427) and is normally in an inactive conformation (Servant et al., 2002; Yang et al., 2002). The C-terminal part is divided into a linker region (110–198), an IRF-association domain (199–308), and a C-terminal autoinhibitory/serine-rich domain (385–427). The linker region is the target of MAP- and DNA-dependent protein kinases (Karpova et al., 2002; Servant et al., 2001) and contains nuclear import and export signals. The IRF-association domain is responsible for dimerization and association with CBP, whereas the C-terminal autoinhibitory/serine-rich domain is the target of virus-activated protein kinases (VAK) (Lin et al., 1998; Yoneyama et al., 1998). C-terminal phosphorylation results in structural changes that allow dimerization of IRF3 and interaction with CBP via the IRF-association domain (Qin et al., 2003; Takahasi et al., 2003).

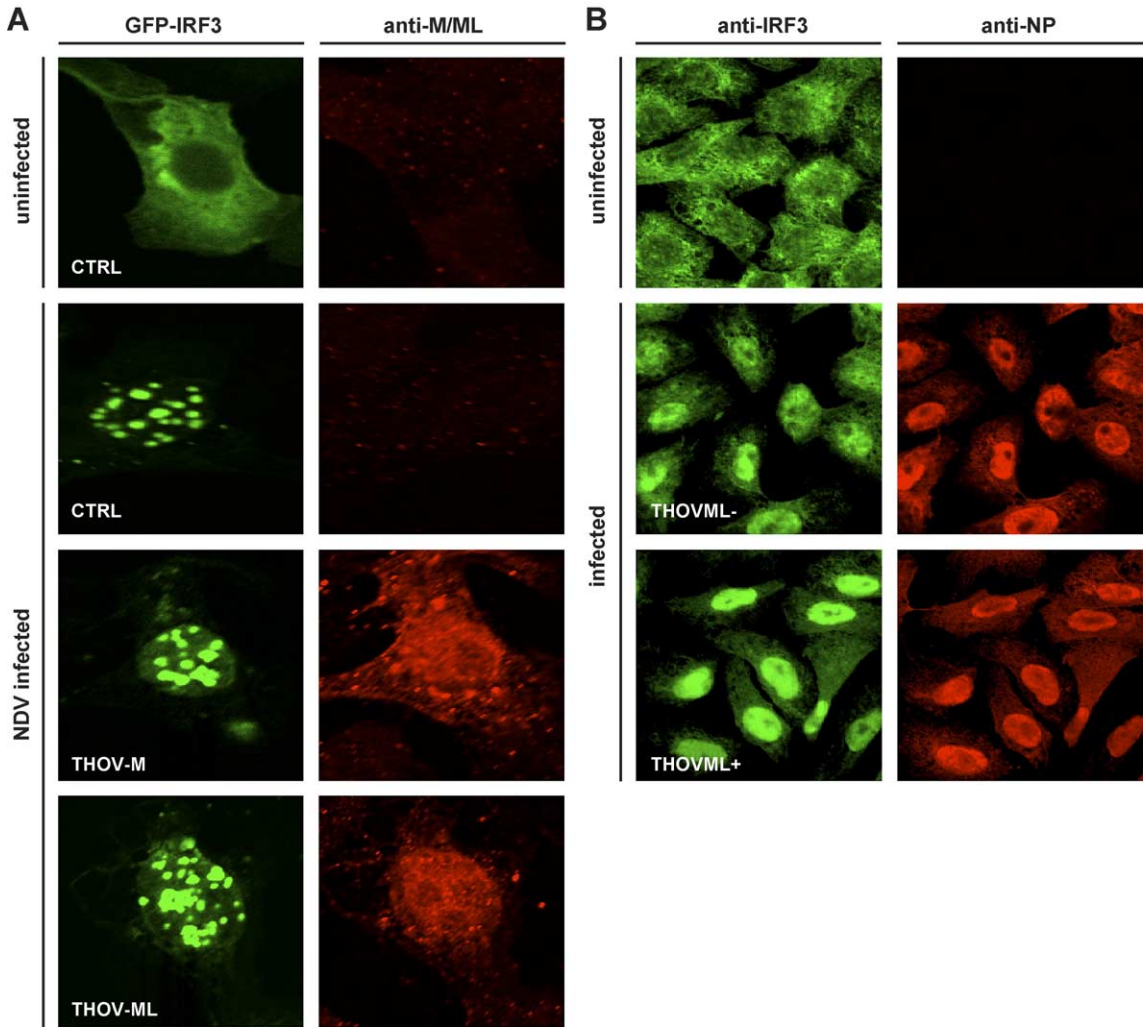

Fig. 3. ML does not prevent nuclear accumulation of IRF3. (A) Vero cells were transfected with a GFP-IRF3 expression plasmid together with expression plasmids encoding THOV M, THOV ML, or empty vector (Ctrl). At 6 h post-transfection, cells were infected with 1 PFU per cell of NDV or mock infected. At 20 h postinfection, expression of M and ML protein was detected by immunofluorescence using an antibody directed against THOV M/ML. GFP-IRF3 (green) and M or ML (red) localizations were detected by confocal microscopy. (B) Vero cells were infected with 5 PFU per cell of THOV expressing ML (THOVML+), lacking ML (THOVML−), or mock infected. At 24 h postinfection, cells were fixed and analyzed for localization of endogenous IRF3 (green) using an IRF3-specific antibody. THOV infection was detected by using an antibody directed against THOV NP (red).

Neither substitution of the IRF3 DNA-binding domain by an unrelated DNA-binding domain nor deletion of the autoinhibitory C-terminal region prevented the inhibitory effect of ML on IRF3 function, indicating that phosphorylation by VAK or binding to the IRF3 response element is not the target of ML action. In agreement with this, ML was also able to inhibit the constitutively active mutant of IRF3 (IRF3-5D), in which a cluster of Ser/Thr residues whose phosphorylation is required for IRF3 activation was replaced by Asp, a phosphomimetic amino acid. Since ML does not interfere with nuclear translocation of IRF3, the nuclear translocation signal and the putative phosphorylation sites within the linker domain seem not to be affected by ML. In contrast, ML had a strong effect on IRF3 homo- and hetero-oligomerization with CBP, suggesting that ML affects the function of the C-terminal IRF-association domain. Therefore, a straightforward explanation for the suppression of IFNβ promoter activation by THOV would be the existence of an interaction of ML with the IRF3-association domain, resulting in a block of IRF3 dimerization and of IRF3 binding to CBP. However, coimmunoprecipitation experiments did not reveal any direct interaction between ML and IRF3 (data not shown), suggesting that these two proteins do not interact or that these interactions are weak or only transient. According to Kumar et al. (2000), IRF3 nuclear accumulation is a result of the interaction of IRF3 homodimers with CBP. It is interesting to note that in our experiments with THOV ML, IRF3 nuclear accumulation can occur independently of these interactions.

While the precise mechanism of blocking IRF3-mediated transactivation by ML remains to be elucidated, it is clear that the C-terminal part of ML plays a critical role. The M and ML proteins of THOV are expressed from the

same genomic segment. ML is expressed from a colinear transcript whereas M is encoded by a spliced mRNA of segment 6 (Kochs et al., 2000). Therefore, the ML protein represents a 38 amino acid C-terminal elongated version of the M protein (Hagmaier et al., 2003). Both M and ML are structural components of the THOV particle. In contrast to M, however, ML does not perform M protein functions like inhibition of viral polymerase activity and assistance in formation of viral particles (Hagmaier et al., 2004; Wagner et al., 2000). Mutational analysis of ML showed that the entire C-terminal half of ML is necessary for its IFN antagonistic activity, indicating that a complex structure of the ML protein distinct from that of the M protein is crucial for its IFN antagonistic activity (Hagmaier et al., 2004).

IRF3 is the target of many different viral IFN antagonists (Goodbourn et al., 2000; Levy and Garcia-Sastre, 2001; Weber et al., 2003). In contrast to THOV ML, the proteins NS1 of FLUAV and E3L of vaccinia virus target an early step of IRF3 activation. Both proteins are thought to prevent activation of the respective IRF3 kinases through sequestration of dsRNA, a potent activator of these kinases (Talon et al., 2000; Wang et al., 2000; Xiang et al., 2002). For the NS1 protein, additional antagonistic effects on later events in the IFN-induced antiviral host response have been described and appear to be mediated by a general inhibition of the host mRNA processing (Donelan et al., 2003; Krug et al., 2003; Seo et al., 2002). Furthermore, VP35 of *Ebola virus* (Basler et al., 2003), NS1/NS2 of bovine respiratory syncytial virus (Bossert et al., 2003), and NS3/4A serine protease of hepatitis C virus (Foy et al., 2003) inhibit IRF3 activation most likely by targeting cellular components that stimulate IRF3 phosphorylation and therefore prevent IRF3 activation and nuclear translocation.

Similar to THOV, several other viruses target late steps of IRF3 activation. Upon BVDV infection, IRF3 translocates into the nucleus but fails to induce IFN expression (Baigent et al., 2002). The E6 protein of human papillomavirus-16 directly binds to IRF3 and thereby prevents IRF3 function (Ronco et al., 1998). The ICP0 protein of *Herpes simplex virus* targets a late step of IRF3 activation and, like the THOV ML, is able to suppress transactivation by the constitutively active IRF3(5D) (Lin et al., 2004). The vIRF1 encoded by human *Herpes virus-8* (HHV8) and the adenovirus (AdV)-E1A protein prevent the formation of the holocomplex consisting of IRF3 and CBP. In contrast to ML that targets IRF3 dimerization but not CBP function (see Fig. 1H), the IFN antagonists of HHV-8 and AdV directly interact with CBP to inhibit the formation of the transcriptionally active IRF3 holocomplex (Juang et al., 1998; Lin et al., 2001).

Whether ML of THOV directly interacts with IRF3 or binds to an IRF3-associated cofactor will be the focus of our future efforts to elucidate the precise mechanism of ML action. However, our data clearly demonstrate that THOV uses a strategy to suppress IFN induction that is different from that of other orthomyxoviruses, revealing the amazingly broad variation of strategies that viruses have evolved to circumvent the induction of antiviral host response.

Materials and methods

Cells and viruses

293 cells and Vero cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS). For infection studies, virus stocks were diluted in DMEM supplemented with 2% FCS and 20 mM HEPES (pH 7.5). Recombinant *Thogoto viruses* (THOV) expressing ML (THOVML+) or lacking ML (THOVML−) were described previously (Hagmaier et al., 2003; Wagner et al., 2001). The *Newcastle disease virus* (NDV; strain H53) (Bazzigher et al., 1992) stock was grown on 10-day-old embryonated chicken eggs. *Influenza A virus* (FLUAV) A/PR/8/34 (FLUAVwt) and the recombinant influenza A/PR/8/34 with a deletion in the NS1 gene (FLUAVdNS1) were described previously (Garcia-Sastre et al., 1998). FLUAV stocks were diluted in phosphate-buffered saline (PBS) supplemented with 0.3% bovine serum albumin prior to infection.

Plasmids

THOV M and ML expression constructs under the control of the chicken β-actin promoter (pCAGGS) have been described previously (Hagmaier et al., 2003). Plasmids expressing a constitutively active IRF3, IRF3(5D) (Lin et al., 1998), and a fusion protein containing green fluorescent protein (GFP) N-terminally fused to IRF3, GFP-IRF3, have been described (Basler et al., 2003). N-terminally HA-tagged IRF3 constructs in pCAGGS were designed for expression of full-length human IRF3 (1–427) and C-terminally truncated forms, IRF3(1–388) and IRF3(1–384), lacking 39 and 43 C-terminal amino acids, respectively. The Gal4 promoter studies were performed using a p5xGal4-AdML-luc reporter plasmid (Wathelet et al., 1998) cotransfected with a Gal4-IRF3 fusion construct, pGal4-IRF3 (Wathelet et al., 1998) (kindly provided by Tom Maniatis), or a Gal4-CBP-acetyltransferase (AT) fusion construct, pcDNA3-Gal4-CBP-AT (Bordoli et al., 2001) (kindly provided by Richard Eckner). The Gal4-IRF3 fusion construct was deleted in its DNA-binding domain (between amino acid 7–107), yielding Gal4-IRF3-DBDdel.

Reporter plasmids carrying the firefly luciferase (FF-Luc) gene under the control of either the IFNβ promoter (p125Luc) or an artificial promoter containing three IRF3-binding sites (p55C1B-Luc) were kindly provided by Takashi Fujita (Yoneyama et al., 1998). The reporter plasmid pRL-SV40 carrying the Renilla luciferase gene (REN-Luc) under the control of the constitutive SV40 promoter was purchased from Promega.

Reporter gene assays

Transient transfection of 293 cells was performed by using 2 μl Dac-30 (Eurogentec)/μg DNA in 200 μl OPTIMEM (Gibco-BRL) as described (Hagmaier et al., 2003). Cells were transfected with 0.5 μg of either p125Luc or p55C1B-Luc, together with 0.05 μg of pRL-SV40, and 1 μg of the indicated THOV M or ML expression plasmids, in addition to 1 μg of the IRF3 expression constructs, when indicated. At 5 h post-transfection, cells were infected with 1 plaque forming unit (PFU) per cell of NDV or were left untreated. At 24 h post-transfection, cells were harvested and lysed in 200 μl of Passive Lysis Buffer (Promega). An aliquot of 20 μl was used to measure FF-Luc and REN-Luc activities as described by the manufacturer (Dual-Luciferase Reporter Assay System, Promega). FF-Luc activities were normalized to REN-Luc activities and are indicated as fold induction relative to the untreated control.

For reporter assays based on Gal4-driven reporter gene expression, 293 cells were transfected with 0.5 μg of p5xGal4-AdML-luc, 0.05 μg of control plasmid pRL-SV40, 1 μg of expression plasmids encoding Gal4-IRF3, Gal4-IRF3-DBDdel, or Gal4-CBP-AT fusion protein, and expression plasmids for THOV M, THOV ML, or empty vector. Twenty-four hours post-transfection, cells were lysed and a dual luciferase assay (Promega) was performed.

Immunofluorescence analysis

To analyze the subcellular localization of IRF3, Vero cells were grown on coverslips and transfected with 1 μg of GFP-IRF3 expression plasmid and 1 μg of expression plasmids for THOV M, THOV ML, or empty vector. Five hours post-transfection, cells were infected with 1 PFU per cell of NDV or were mock infected. At 20 h post infection, cells were fixed in 3% paraformaldehyd and permeabilized with 0.5% Triton X-100. Cells were stained using a polyclonal antibody specific for THOV M/ML and a Cy3-conjugated secondary antibody.

To examine the localization of endogenous IRF3 in Vero cells after infection with the recombinant THOVs, IRF3 was detected 24 h postinfection using an IRF3-specific polyclonal rabbit antibody FL-425 (Santa Cruz), and the signal was enzymatically amplified by the Tyramide Signal Amplification (TSA) system (Perkin-Elmer) according to the manufacturer's instructions. Infection with THOV was monitored using a mouse monoclonal antibody directed against THOV NP and a Cy3-conjugated secondary antibody. Samples were examined with a Leica confocal laser scanning microscope.

IRF3 dimerization assay

293 cells were infected with 5 PFU per cell of the viruses or were mock infected. At 20 h postinfection, cells were resuspended in 200 μl lysis buffer containing 50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, protease inhibitors (Roche) and phosphatase inhibitors (Calbiochem), vortexed, incubated on ice for 10 min, and centrifuged at 4 °C for 5 min at 10 000 × *g*. Aliquots of 10 μg protein were separated on a 7.5% nondenaturing polyacrylamide gel with 1% deoxycholate in the cathode buffer as described (Iwamura et al., 2001). IRF3 monomers and dimers were detected by Western Blot analysis using a polyclonal rabbit anti-IRF3 antibody FL-425 (Santa Cruz). To monitor virus infection, viral NP was detected by Western blot analysis using polyclonal rabbit antisera directed against THOV NP or FLUAV NP.

IRF3/CBP coimmunoprecipitation assay

The cell lysates used for IRF3 dimerization assays were also subjected to coimmunoprecipitation analysis using the monoclonal mouse anti-IRF3 antibody SL12 (Ronco et al., 1998) (kindly provided by P.M. Howley). The cell lysates were preadsorbed with protein G-Sepharose (Amersham Biosciences) for 1 h at 4 °C, then centrifuged at 10 000 × *g* for 5 min at 4 °C followed by incubation of the supernatants with protein G-Sepharose and 5 μl of the monoclonal anti-IRF3 antibody for 2 h at 4 °C. Sepharose beads were washed three times with lysis buffer and the bound proteins were subjected to Western Blot analysis using a polyclonal rabbit antibody directed against CBP, A-22 (Santa Cruz).

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References

Alexopoulou, L., Holt, A.C., Medzhitov, R., Flavell, R.A., 2001. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature 413, 732–738.

Algarte, M., Nguyen, H., Heylbroeck, C., Lin, R., Hiscott, J., 1999. IkB-mediated inhibition of virus-induced beta interferon transcription. J. Virol. 73, 2694–2702.

Baigent, S.J., Zhang, G., Fray, M.D., Flick-Smith, H., Goodbourn, S., McCauley, J.W., 2002. Inhibition of beta interferon transcription by noncytopathogenic bovine viral diarrhea virus is through an interferon regulatory factor 3-dependent mechanism. J. Virol. 76, 8979–8988.

Basler, C., Mikulasova, A., Martinez-Sobrido, L., Paragas, J., Mühlberger, E., Bray, M., Klenk, H.-D., Palese, P., Garcia-Sastre, A., 2003. The *Ebola virus* VP35 protein inhibits activation of interferon regulatory factor 3. J. Virol. 77, 7945–7956.

Bazzigher, L., Pavlovic, J., Haller, O., Staeheli, P., 1992. Mx genes show weaker primary response to virus than other interferon-regulated genes. Virology 186, 154–160.

Bordoli, L., Hüsser, S., Lüthi, U., Netsch, M., Osmani, H., Eckner, R., 2001. Functional analysis of the p300 acetyltransferase domain: the PHD finger of p300 but not of CBP is dispensable for enzymatic activity. Nucleic Acids Res. 29, 4462–4471.

Bossert, B., Marozia, S., Conzelmann, K., 2003. Nonstructural proteins NS1 and NS2 of bovine respiratory syncytial virus block activation of interferon regulatory factor 3. J. Virol. 77, 8661–8668.

Collins, S.E., Noyce, R.S., Mossman, K.L., 2004. Innate cellular response to virus particle entry requires IRF3 but not virus replication. J. Virol. 78, 1706–1717.

Dauber, B., Heins, G., Wolff, T., 2004. The influenza B virus nonstructural NS1 protein is essential for efficient viral growth and antagonizes beta interferon induction. J. Virol. 78, 1865–1872.

Davies, C.R., Jones, L.D., Nuttall, P.A., 1986. Experimental studies on the transmission cycle of *Thogoto virus*, a candidate orthomyxovirus, in *Rhipicephalus appendiculatus*. Am. J. Trop. Hyg. 35, 1256–1262.

Der, S.D., Zhou, A., Williams, B.R.G., Silverman, R.H., 1998. Identification of genes differentially regulated by interferon alpha, beta, or gamma using oligonucleotide arrays. Proc. Natl. Acad. Sci. U.S.A. 95, 15623–15628.

Diebold, S.S., Kaisho, T., Hemmi, H., Akira, S., Sousa, C.R., 2004. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science 303, 1529–1531.

Donelan, N.R., Basler, C.F., Garcia-Sastre, A., 2003. A recombinant influenza A virus expressing an RNA-binding-defective NS1 protein induces high levels of beta interferon and is attenuated in mice. J. Virol. 77, 13257–13266.

Dupuis, S., Jouanguy, E., Al-Hajjar, S., Fieschi, C., Al-Mohsen, I.Z., Al-Jumaah, S., Yang, K., Chapgier, A., Eidenschenk, C., Eid, P., Al-Ghonaium, A., Tufenkeji, H., Frayha, H., Al-Gazlan, S., Al-Rayes, H., Schreiber, R.D., Gresser, I., Casanova, J.L., 2003. Impaired response to interferon-alpha/beta and lethal viral disease in human STAT1 deficiency. Nat. Genet. 33, 388–391.

Erlandsson, L., Blumenthal, R., Eloranta, M.L., Engel, H., Alm, G., Weiss, S., Leanderson, T., 1998. IFN-beta is required for IFN-alpha production in mouse fibroblasts. Curr. Biol. 8, 223–226.

Fiette, L., Aubert, C., Müller, U., Huang, S., Aguet, M., Brahic, M., Bureau, J.F., 1995. *Theiler's virus* infection of 129Sv mice that lack the interferon alpha/beta or interferon gamma receptors. J. Exp. Med. 181, 2069–2076.

Fitzgerald, K.A., McWhirter, S.M., Faia, K.L., Rowe, D.C., Latz, E., Golenbock, D.T., Coyle, A.J., Liao, S.-M., Maniatis, T., 2003. IKKε and TBK1 are essential components of the IRF3 signaling pathway. Nat. Immunol. 4, 433–491.

Foy, E., Li, K., Wang, C., Sumpter, R., Ikeda, M., Lemon, S.M., Gale, M., 2003. Regulation of interferon regulatory factor-3 by the hepatitis C virus serine protease. Science 300, 1145–1148.

Garcia-Sastre, A., Egorov, A., Matassov, D., Brandt, S., Levy, D.E., Durbin, J.E., Palese, P., Muster, T., 1998. Influenza A virus lacking the NS1 gene replicates in interferon-deficient systems. Virology 252, 324–330.

Goodbourn, S., Didcock, L., Randall, R.E., 2000. Interferons: cell signalling, immune modulation, antiviral responses and virus countermeasures. J. Gen. Virol. 81, 2341–2364.

Graff, J.W., Mitzel, D.N., Weisend, C.M., Flenniken, M.L., Hardy, M.E., 2002. Interferon regulatory factor 3 is a cellular partner of rotavirus NSP1. J. Virol. 76, 9545–9550.

Grandvaux, N., Servant, M.J., tenOever, B., Sen, G.C., Balachandran, S., Barber, G.N., Lin, R., Hiscott, J., 2002. Transcriptional profiling of interferon regulatory factor 3 target genes: direct involvement in the regulation of interferon-stimulated genes. J. Virol. 76, 5532–5539.

Hagmaier, K., Jennings, S., Buse, J., Weber, F., Kochs, G., 2003. Novel gene product of *Thogoto virus* segment 6 codes for an interferon antagonist. J. Virol. 77, 2747–2752.

Hagmaier, K., Gelderblom, H.R., Kochs, G., 2004. Functional comparison of the two gene products of *Thogoto virus* segment 6. J. Gen. Virol. 85 (in press).

Haller, O., Kochs, G., 2002. *Thogotovirus*. In: Tidona, C.A., Darai, G. (Eds.), The Springer Index of Viruses. Springer-Verlag, Berlin, pp. 615–619.

Haller, O., Frese, M., Rost, D., Nuttall, P., Kochs, G., 1995. Tick-borne *Thogoto virus* infection in mice is inhibited by the orthomyxovirus resistance gene product Mx1. J. Virol. 69, 2596–2601.

Iwamura, T., Yoneyama, M., Yamaguchi, K., Suhara, W., Mori, W., Shiota, K., Okabe, Y., Namiki, H., Fujita, T., 2001. Induction of IRF-3/-7 kinase and NF-kappaB in response to double-stranded RNA and virus infection: common and unique pathways. Genes Cells 6, 375–388.

Jones, J.D., Nuttall, P.A., 1989. The effect of virus-immune hosts on *Thogoto virus* infection of the tick, *Rhipicephalus appendiculatus*. Virus Res. 14, 129–140.

Juang, Y.T., Lowther, W., Kellum, M., Au, W.C., Lin, R., Hiscott, J., Pitha, P.M., 1998. Primary activation of interferon A and interferon B gene transcription by interferon regulatory factor 3. Proc. Natl. Acad. Sci. U.S.A. 95, 9837–9842.

Karpova, A.Y., Trost, M., Murray, J.M., Cantley, L.C., Howley, P.M., 2002. Interferon regulatory factor-3 is an in vivo target of DNA-PK. Proc. Natl. Acad. Sci. U.S.A. 99, 2818–2823.

Kochs, G., Weber, F., Gruber, S., Delvendahl, A., Leitz, C., Haller, O., 2000. *Thogoto virus* matrix protein is encoded by a spliced mRNA. J. Virol. 74, 10785–10789.

Krug, R.M., Yuan, W., Noah, D.L., Latham, A.G., 2003. Intracellular warfare between human influenza viruses and human cells: the roles of the viral NS1 protein. Virology 309, 181–189.

Kumar, K.P., McBride, K.M., Weaver, B.K., Dingwall, C., Reich, N.C., 2000. Regulated nuclear-cytoplasmic localization of interferon regulatory factor 3, a subunit of double-stranded RNA-activated factor 1. Mol. Cell. Biol. 20, 4159–4168.

Levy, D.E., Garcia-Sastre, A., 2001. The virus battle: IFN induction of the antiviral state and mechanisms of viral evasion. Cytokine Growth Factor Rev. 12, 143–156.

Lin, R., Heylbroeck, C., Pitha, P.M., Hiscott, J., 1998. Virus-dependent phosphorylation of the IRF-3 transcription factor regulates nuclear translocation, transactivation potential, and proteasome-mediated degradation. Mol. Cell. Biol. 18, 2986–2996.

Lin, R., Genin, P., Mamane, Y., Sgarbanti, M., Battistini, A., Harrington, W.J., Barber, G.N., Hiscott, J., 2001. HHV-8 encoded vIRF-1 represses the interferon antiviral response by blocking IRF-3 recruitment of the CBP/p300 coactivators. Oncogene 20, 800–811.

Lin, R., Noyce, R.S., Collins, S.E., Everett, R.D., Mossman, K.L., 2004. The *Herpes simplex virus* ICP0 RING finger domain inhibits IRF3- and IRF7-mediated activation of interferon-stimulated genes. J. Virol. 78, 1675–1684.

Marie, I., Durbin, J.E., Levy, D.E., 1998. Differential viral induction of distinct IFN-alpha genes by positive feedback through IFN regulatory factor-7. EMBO J. 17, 6660–6669.

Mori, M., Yoneyama, M., Takashi, I., Takahashi, K., Fuyuhiko, I., Fujita, T., 2004. Identification of Ser 386 of interferon regulatory factor 3 as critical target for inducible phosphorylation that determines activation. J. Biol. Chem. 279, 9698–9702.

Müller, U., Steinhoff, U., Reis, L.F.L., Hemmi, S., Pavlovic, J., Zinkernagel, R.M., Aguet, M., 1994. Functional role of type I and type II interferons in antiviral defense. Science 264, 1918–1921.

Nuttall, P.A., Morse, M.A., Jones, L.D., Portela, A., 1995. Adaptation of members of the orthomyxoviridae family to transmission by ticks.

In: Gibbs, A.J., Calisher, C.H., Garcia-Arenal, F. (Eds.), Molecular Basis of Virus Evolution. Cambridge Univ. Press, Cambridge, England, pp. 416–425.

Pavlovic, J., Arzet, H.A., Hefti, H.P., Frese, M., Rost, D., Ernst, B., Kolb, E., Staeheli, P., Haller, O., 1995. Enhanced virus resistance of transgenic mice expressing the human MxA protein. J. Virol. 69, 4506–4510.

Peters, K.L., Smith, H.L., Stark, G.R., Sen, G.C., 2002. IRF-3-dependent, NFkappa B- and JNK-independent activation of the 561 and IFN-beta genes in response to double-stranded RNA. Proc. Natl. Acad. Sci. U.S.A. 99, 6322–6327.

Pichlmair, A., Buse, J., Jennings, S., Haller, O., Kochs, G., Staeheli, P., 2004. *Thogoto virus* lacking interferon-antagonistic protein ML is strongly attenuated in newborn Mx1-positive but not Mx1-negative mice. J. Virol. 78, 11422–11424.

Poole, E., He, B., Lamb, R.A., Randall, R.E., Goodbourn, S., 2002. The V proteins of simian virus 5 and other paramyxoviruses inhibit induction of interferon-beta. Virology 303, 33–46.

Qin, B.Y., Liu, C., Lam, S.S., Srinath, H., Delston, R., Correia, J.J., Derynck, R., Lin, K., 2003. Crystal structure of IRF-3 reveals mechanism of autoinhibition and virus-induced phosphoactivation. Nat. Struct. Biol. 10, 913–921.

Ronco, L.V., Karpova, A.Y., Vidal, M., Howley, P.M., 1998. Human papillomavirus 16 E6 oncoprotein binds to interferon regulatory factor-3 and inhibits its transcriptional activity. Genes Dev. 12, 2061–2072.

Samuel, C.E., 2001. Antiviral action of interferons. Clin. Microbiol. Rev. 14, 778–809.

Sato, M., Suemori, H., Hata, N., Asagiri, M., Ogasawera, K., Nakao, K., Nakaya, T., Katsuki, M., Noguchi, S., Tanaka, N., Taniguchi, T., 2000. Distinct and essential roles of transcription factors IRF-3 and IRF-7 in response to viruses for IFN-alpha/beta gene induction. Immunity 13, 539–548.

Schafer, S.L., Lin, R., Moore, P.A., Hiscott, J., Pitha, P.M., 1998. Regulation of type I interferon gene expression by interferon regulatory factor-3. J. Biol. Chem. 273, 2714–2720.

Seo, S.H., Hoffmann, E., Webster, R.G., 2002. Lethal H5N1 influenza viruses escape host anti-viral cytokine response. Nat. Med. 8, 950–954.

Servant, M.J., tenOever, B., LePage, C., Conti, L., Gessani, S., Julkunen, I., Lin, R., Hiscott, J., 2001. Identification of distinct signaling pathways leading to the phosphorylation of interferon regulatory factor 3. J. Biol. Chem. 276, 355–363.

Servant, M.J., Grandvaux, N., Hiscott, J., 2002. Multiple signaling pathways leading to the activation of interferon regulatory factor 3. Biochem. Pharmacol. 64, 985–992.

Sharma, S., tenOever, B.R., Grandvaux, N., Zhou, G.P., Lin, R., Hiscott, J., 2003. Triggering the interferon antiviral response through an IKK-related pathway. Science 300, 1148–1151.

Staeheli, P., 1990. Interferon-induced proteins and the antiviral state. Adv. Virus Res. 38, 147–200.

Stark, G.R., Kerr, I.M., Williams, B.R., Silverman, R.H., Schreiber, R.D., 1998. How cells respond to interferons. Annu. Rev. Biochem. 67, 227–264.

Suhara, W., Yoneyama, M., Iwamura, T., Yoshimura, S., Tamura, K., Namiki, H., Aimoto, S., Fujita, T., 2000. Analysis of virus-induced homomeric and heteromeric protein associations between IRF-3 and coactivator CBP/p300. J. Biochem. 128, 301–307.

Takahasi, K., Suzuki, N.N., Horiuchi, M., Mori, M., Suhara, W., Okabe, Y., Fukuhara, Y., Terasawa, H., Akira, S., Fujita, T., Inagaki, F., 2003. X-ray crystal structure of IRF-3 and its functional implications. Nat. Struct. Biol. 10, 922–927.

Talon, J., Horvath, C.M., Polley, R., Basler, C.F., Muster, T., Palese, P., Garcia-Sastre, A., 2000. Activation of interferon regulatory factor 3 is inhibited by the influenza A virus NS1 protein. J. Virol. 74, 7989–7996.

Van Regenmortel, M.H.V., Fauquet, C.M., Bishop, D.H.L., Carstens, E.B., Estes, M.K., Lemon, S.M., Maniloff, J., Mayo, M.A., McGeoch, D.J., Pringle, C.R., Wickner, R.B., 2000. Virus taxonomy, nomenclature of viruses. Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press, San Diego.

Wagner, E., Engelhardt, O.G., Weber, F., Haller, O., Kochs, G., 2000. Formation of virus-like particles from cloned cDNA of *Thogoto virus*. J. Gen. Virol. 81, 2849–2853.

Wagner, E., Engelhardt, O.G., Gruber, S., Haller, O., Kochs, G., 2001. Rescue of recombinant *Thogoto virus* from cloned cDNA. J. Virol. 75, 9282–9286.

Wang, X., Li, M., Zheng, H., Muster, T., Palese, P., Beg, A.A., Garcia-Sastre, A., 2000. Influenza A virus NS1 protein prevents the activation of NFkB and induction of type I IFN. J. Virol. 74, 11566–11573.

Wathelet, M.G., Lin, C.H., Parekh, B.S., Ronco, L.V., Howley, P.M., Maniatis, T., 1998. Virus infection induces the assembly of coordinately activated transcription factors on the IFN-β enhancer in vivo. Mol. Cell. Biol. 1, 507–518.

Weaver, B.K., Kumar, K.P., Reich, N.C., 1998. Interferon regulatory factor 3 and CREB-binding protein/p300 are subunits of double-stranded RNA-activated transcription factor DRAF1. Mol. Cell. Biol. 18, 1359–1368.

Weber, F., Kochs, G., Haller, O., Staeheli, P., 2003. Viral evasion of the interferon system: old viruses, new tricks. J. Interferon Cytokine Res. 23, 209–213.

Xiang, Y., Condit, R.C., Vijaysri, S., Jacobs, B., Williams, B.R.G., Silverman, R.H., 2002. Blockade of interferon induction and action by the E3L double-stranded RNA binding proteins of vaccinia virus. J. Virol. 76, 5251–5259.

Yang, H., Lin, C.H., Ma, G., Orr, M., Baffi, M.O., Wathelet, M.G., 2002. Transcriptional activity of interferon regulatory factor (IRF)-3 depends on multiple protein–protein interactions. Eur. J. Biochem. 269, 6142–6151.

Yoneyama, M., Suhara, W., Fukuhara, Y., Fukuda, M., Nishida, E., Fujita, T., 1998. Direct triggering of the IFN-alpha/beta system by virus infection: activation of a transcription factor complex containing IRF-3 and CBP/p300. EMBO J. 17, 1087–1095.

Yoneyama, M., Suhara, W., Fujita, T., 2002. Control of IRF-3 activation by phosphorylation. J. Interferon Cytokine Res. 22, 73–76.