The Leader Protein of Theiler's Virus Interferes with Nucleocytoplasmic Trafficking of Cellular Proteins

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The leader protein of Theiler's virus was previously shown to block the production of alpha/beta interferon by infected cells. Here, we observed that expression of the leader protein in infected cells triggered subcellular redistribution of a nucleus-target green fluorescent protein. It enhanced redistribution of the nuclear polypyrimidine tract-binding protein but had no influence on the localization of the nuclear splicing factor SC-35. The leader protein also interfered with trafficking of the cytoplasmic interferon regulatory factor 3, a factor critical for transcriptional activation of alpha/beta interferon genes.

Theiler's murine encephalomyelitis virus (TMEV or Theiler's virus) is a picornavirus responsible for infections of the central nervous system of the mouse (21). Persistent strains of TMEV, such as DA and BeAn, induce a chronic, progressive, demyelinating disease in susceptible mice. These viruses have a striking capacity to persist in the central nervous system in spite of a strong and specific immune response (4, 14, 17).

The leader protein (L) produced by Theiler's virus plays an important role in viral persistence (23). This protein is a 76-amino-acid-long protein containing a zinc-binding motif (3). It was reported to inhibit alpha/beta interferon (IFN) production early after viral infection (11, 23). The zinc finger motif of the protein appears to be critical for IFN production inhibition *in vitro* and for persistence of the DA1 virus *in vivo* (23).

As inhibition of IFN production by the leader protein has been found to occur at the transcriptional level (22), the leader protein is thought to antagonize the activity of one of the transcription factors necessary for the activation of the IFN genes. A likely candidate for inhibition by the leader protein is IFN regulatory factor 3 (IRF-3), a cellular factor required for the transcriptional activation of immediate-early IFN subtypes.

We observed in this work that the leader protein of TMEV disturbs the subcellular localization of nuclear and cytoplasmic cellular proteins, notably that of IRF-3.

Perturbation of the subcellular localization of IRF-3 by the leader protein of TMEV. IRF-3 normally resides in the cytoplasm of the cell (24). Upon viral infection or in the presence of double-stranded RNA, IRF-3 becomes phosphorylated, dimerizes, and subsequently translocates to the nucleus of the cell, where it binds to the promoters of immediate-early IFN genes to activate their transcription (19).

Since IRF-3 was a likely target for the leader protein of TMEV, we examined the presence and the localization of IRF-3 in infected cells by immunofluorescence assays.

Detection of IRF-3 involved a primary polyclonal rabbit antibody (Zymed; 51-3200), a secondary antibody coupled to

horseradish peroxidase (Dako; P0448), and an amplification step (tyramide signal amplification-fluorescein isothiocyanate kit; NEN). Detection of viral antigen was performed with a murine monoclonal antibody directed against the VP1 protein of TMEV (F12B3 monoclonal antibody; kind gift of Michel Brahic, Pasteur Institute, Paris, France) and a secondary antibody coupled to either R-phycoerythrin (Dako; R0480) or Alexa Fluor 594 (Molecular Probes; A-11032).

L929 cells were infected with KJ6 and TM659, derivatives of the DA1 strain (16) carrying a capsid adapted to L929 cells (9, 23). KJ6 produces the wild-type L protein, while TM659 produces a mutant L protein, called L^{cs}, with a disrupted zinc finger (23). Nine hours after infection with 2 PFU of these viruses per cell, viral antigen and IRF-3 were labeled and cells were examined by double immunofluorescence assay (Fig. 1).

In 89% of cells infected with TM659, the L^{cs} mutant virus, IRF-3 exhibited a cytoplasmic localization as in uninfected cells. Eleven percent of cells positive for viral antigen exhibited a clear nuclear localization of IRF-3, compatible with IRF-3 activation (Fig. 1). This low percentage of infected cells showing nuclear accumulation of IRF-3 is in agreement with the fact that Theiler's virus appears to be a poor IFN inducer compared to other viruses (V. van Pesch et al., unpublished observations).

Paradoxically, IRF-3 appeared to be partially translocated to the nucleus of 95% of L929 cells infected with KJ6 even if these cells failed to produce detectable IFN- β or IFN- α 4 mRNA or IFN activity (23). However, closer examination of IRF-3 localization showed that the nuclear pattern of IRF-3 labeling was heterogeneous compared to that seen in cells infected with the L^{cs} mutant virus (Fig. 1). This observation suggests that the leader protein might trigger IRF-3 redistribution between the cytoplasm and the nucleus, possibly by affecting the integrity of the nuclear structure.

Similarly, IRF-3 redistribution was observed in murine BALB/3T3 cells infected with the wild-type DA1 virus but not in cells infected with the corresponding L^{cs} mutant virus called TM598 (Fig. 2A) (23). Similar data were obtained at different time points, suggesting that the differences observed between wild-type and the L^{cs} mutant viruses did not merely reflect a difference in the kinetics of cell infection by these viruses.

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FIG. 1. Perturbation of endogenous IRF-3 subcellular localization by the leader protein. Endogenous IRF-3 and viral antigen were detected by double immunofluorescence assay, in L929 cells infected for 9 h with KJ6 or TM659. The left panel shows endogenous IRF-3 staining in a cell infected with TM659, the virus expressing the mutant L^{cys} protein. Eleven percent of cells positive for viral antigen displayed the clear nuclear translocation of IRF-3 shown in the figure, whereas 89% of infected cells conserved the cytoplasmic staining seen in uninfected cells (data not shown). After infection with the KJ6 virus, expressing the wild-type L protein, 95% of cells positive for viral antigen showed IRF-3 redistribution through the cytoplasm and the nucleus (right panel). Note the conspicuous nuclear IRF-3 labeling with unstained nucleoles, visible in TM659-infected cells (left), and the heterogeneous nuclear IRF-3 labeling seen in KJ6-infected cells (right).

To further monitor the traffic of IRF-3, we used the pGFP-mIRF-3 plasmid expressing a fusion protein between murine IRF-3 and the green fluorescent protein (GFP). This plasmid, constructed by Tak Mak (University of Toronto, Toronto, Canada), was kindly forwarded by Peter Palese (Mount Sinai School of Medicine, New York, N.Y.) (20).

BALB/3T3 cells, stably transfected with pGFP-mIRF-3, were infected with DA1 or TM598 at a multiplicity of infection of 10 PFU per cell. Six hours after infection, cells were fixed and viral antigen was labeled as described above (Fig. 3).

In 98% of the cells infected with the wild-type DA1 virus, GFP-mIRF-3 fluorescence was redistributed between the cytoplasm and the nucleus, whereas in cells infected with the mutant TM598 virus, GFP-mIRF-3 fluorescence remained clearly cytoplasmic in 97% of the cells (Fig. 2B and 3).

Redistribution of a cytoplasmic protein lacking a nuclear localization signal (NLS). Taken together, our data indicate that the leader protein triggers an aberrant redistribution of the IRF-3 protein to the nucleus, without activation of IFN gene transcription.

Translocation of IRF-3 to the nucleus is due to the presence of an NLS. When IRF-3 becomes phosphorylated in response to viral infection, it is retained in the nucleus by interaction with the CBP/P300 coactivator (12, 13, 24).

We wondered whether the leader protein could trigger the redistribution of IRF-3 between the cytoplasm and the nucleus, independently of the NLS.

Therefore, we monitored the traffic of a GFP-mIRF-3 fusion in which we mutated the NLS sequence. Kumar et al. reported that the mutation of two amino acids (K77N and R78G) inactivated the NLS function of human IRF-3 (12). Site-directed mutagenesis was used to introduce these mutations in the

FIG. 2. Redistribution of cytoplasmic and nuclear proteins by the leader protein in BALB/3T3 cells. Histograms show the percentages of infected cells showing nuclear, cytoplasmic, or both cytoplasmic and nuclear IRF-3 staining. (A) Endogenous IRF-3 detection in cells infected for 6 h with the indicated viruses. (B and C) Localization of the GFP-mIRF-3 fusion protein (B) and of the mutant protein lacking the NLS (C). Cells were counted 6 h after infection with DA1 (L^{wt}) or TM598 (L^{cys}). (D) Localization of the NLS-eGFP fusion protein in cells infected for 10 h with the indicated viruses. For each experiment, IRF-3 localization was observed by fluorescence microscopy in 100 cells positive for viral antigen. Histograms show the means and standard errors of the means for three independent experiments done in parallel for DA1 and TM598.

murine IRF-3 protein encoded by the pGFP-mIRF-3 plasmid. The mutated construct was called pGFP-mIRF-3#NLS.

To confirm that the function of NLS had indeed been inactivated in the latter construct, BALB/3T3 cells, stably transfected with pGFP-mIRF-3 or pGFP-mIRF-3#NLS, were treated with 2.5 μ g of polyriboinosinic-polyribocytidylic acid [poly(IC)]. Thirty hours after transfection of poly(IC), fluorescence clearly accumulated in the nucleus of many cells expressing the GFP-mIRF-3 construct and remained cytoplasmic in cells expressing GFP-mIRF-3#NLS (Fig. 3). Accordingly, 9 h 30 min after infection of these cells with the Italian strain of Newcastle disease virus (NDV; 10 50% egg infective doses per cell), 54% of cells expressing wild-type GFP-mIRF-3 exhibited partial (most cells) or complete GFP-mIRF-3 nuclear translocation. In contrast, NDV triggered nuclear translocation of GFP-mIRF-3#NLS in less than 3% of the cells (Fig. 3). Mutation of the NLS in this construct thus inhibited both poly(IC)- and NDV-mediated IRF-3 translocation to the nucleus, as expected.

BALB/3T3 cells stably transfected with the pGFP-mIRF-3#NLS construct were then infected with the DA1 or TM598 virus, as described above. Six hours after infection with the

FIG. 3. Redistribution of a cytoplasmic protein, IRF-3, lacking an NLS. BALB/3T3 cells stably expressing GFP-mIRF-3 (top row) or GFP-mIRF-3#NLS (third row) were left untreated and uninfected (leftmost panels, NI), treated for 30 h with 2.5 μ g of poly(IC), infected for 8 h with NDV, or infected for 6 h with DA1 or TM598 as indicated. Immunofluorescence assays for viral capsid antigen were performed in the case of DA1 and TM598 infections. White arrows point to cells positive for viral antigen. Green fluorescence of GFP-mIRF-3 and GFP-mIRF-3#NLS fusions is shown. Note that, upon poly(IC) transfection or NDV infection, only the IRF-3 fusion containing the NLS was translocated to the nucleus, whereas upon infection with DA1 both fusion proteins were redistributed.

wild-type DA1 virus, we observed a clear fluorescence redistribution between the cytoplasm and the nucleus in 87% of infected cells, in spite of the NLS mutation. Again, infection with the TM598 L^{cys} mutant virus failed to relocate IRF-3. The L protein could thus trigger the nucleocytoplasmic redistribution of a cytoplasmic protein, even if this protein was lacking an NLS (Fig. 2C and 3).

Redistribution of nuclear proteins. Other picornaviruses were reported to induce an early alteration of nucleocytoplasmic trafficking. For instance, poliovirus has been shown to trigger the relocation of nuclear host proteins such as the polypyrimidine tract-binding protein (PTB) (1) or the La autoantigen (15) to the cytoplasm where these proteins interact with viral RNA. Poliovirus, coxsackievirus B3, and human rhinovirus 14 were also shown to trigger the relocation of an NLS-enhanced GFP (eGFP) fusion to the cytoplasm (2, 7, 8). To determine whether the L protein could also interfere with the nucleocytoplasmic traffic of a nuclear protein, we monitored the localization of an NLS-eGFP protein in cells infected with Theiler's virus.

To construct a plasmid expressing an NLS-eGFP fusion, the sequence coding for the simian virus 40 T-antigen NLS (PKKKRKVE) was cloned, in frame, at the 5' end of the eGFP gene (Clontech, Becton Dickinson). The constructed plasmid,

called pSD5, expresses the NLS-eGFP fusion under the control of a tetracycline-inducible promoter (5). BALB/3T3 cells expressing rtTA (6), the activator of tetracycline-inducible promoters, were transfected with the NLS-eGFP-expressing plasmid. Twenty-four hours after treatment with 5 μ g of doxycycline/ml to activate NLS-eGFP expression, these cells were infected with the DA1 or TM598 virus at a multiplicity of infection of 10 PFU per cell. Ten hours after infection, localization of NLS-eGFP was examined in infected cells.

As expected, in uninfected cells, NLS-eGFP fluorescence was essentially confined to the nucleus (Fig. 4). Partial redistribution of fluorescence could be observed in 79% of the cells infected with the wild-type virus but in less than 8% of cells infected with the virus mutated in the L protein (Fig. 2D and 4). Identical results were obtained in BHK-21 cells (data not shown).

We also analyzed redistribution of endogenous PTB, since this nuclear protein was reported to be relocated to the cytoplasm of cells infected with poliovirus, which does not express a protein related to the leader protein. PTB is known to interact with the internal ribosome entry site of Theiler's virus to activate translation (10, 18). Therefore, we monitored PTB redistribution in L929 cells infected with 2.5 PFU of viruses KJ6 and TM659 per cell. PTB was immunolabeled with a

FIG. 4. Redistribution of NLS-eGFP but not of SC-35. (A) BALB/3T3 cells expressing NLS-eGFP were infected with the DA1 and TM598 viruses. Ten hours after infection, cells were fixed and stained for viral antigen (VP1). White arrows point to cells positive for viral antigen. NLS-eGFP green fluorescence (left panels) was redistributed to the cytoplasm in 79% of cells infected with the DA1 virus but remained cytoplasmic in 92% of cells infected with the TM598 virus. (B) HeLa cells infected with viruses DA1 and TM598 for 10 h were fixed and stained for the nuclear SC-35 splicing factor (left panels) and for viral antigen by using an anti-2A rabbit polyclonal antibody (right panels). White arrows point to cells positive for viral antigen. SC-35 staining was unaffected by viral infection.

mouse monoclonal anti-PTB antibody (Zymed; 32-4800) and a secondary antibody coupled to Alexa Fluor 488 (Molecular Probes; A-11017) (Fig. 5).

Interestingly, both wild-type and L-mutant viruses could trigger PTB redistribution to the cytoplasm. However, redistribution was

much more rapid and extensive when the virus expressed the leader protein. For unknown reasons, many cells infected by the L^{cys} mutant showed a punctate cytoplasmic distribution of PTB with the majority of PTB left in the nucleus.

We next studied redistribution of the SC-35 splicing factor in

FIG. 5. Redistribution of PTB. L929 cells were left uninfected (NI) or were infected with KJ6 or TM659. At 4 h, 5 h 30 min, and 7 h after infection, cells were labeled for endogenous PTB. Viral antigen labeling done 7 h postinfection (hpi) showed that more than 98% of cells were infected in this experiment. Histograms present the means and standard errors of the means of 150 cell counts from triplicate infection experiments (B). Representative fields of PTB labeling are shown above (A). Note the conspicuous nuclear staining for uninfected cells and for cells infected for 4 h with TM659 and the extensive redistribution of PTB for cells infected with KJ6 at this time point. At 5 h 30 min and 7 h postinfection, several cells infected with TM659 displayed a punctate PTB cytoplasmic staining, often in addition to nuclear staining.

infected HeLa cells by using a mouse monoclonal antibody (Sigma; S4045). In these cells, SC-35 localizes to characteristic nuclear speckles. This localization was unaffected in cells infected with either wild-type or mutant viruses (Fig. 4), in contrast to localization of PTB, which showed clear redistribution in these cells after infection with the wild-type virus (data not shown).

In this study, we observed that infection by Theiler's virus considerably disturbed nucleocytoplasmic trafficking of cellular proteins. It can promote the redistribution of a nuclear protein to the cytoplasm and, conversely, of a cytoplasmic protein to the nucleus. Wild-type leader protein expression was necessary to observe redistribution of NLS-eGFP or GFP-mIRF-3#NLS and facilitated relocation of PTB to the cytoplasm. As in the case of poliovirus infection (7), Theiler's virus infection failed to relocate SC-35, a nonshuttling splicing factor.

Thus, perturbation of nucleocytoplasmic trafficking appears to be at least partly mediated by the leader protein. One might argue that mutation of the leader could affect replication of the virus in IFN-competent cells, thereby decreasing expression of another viral protein potentially responsible for trafficking perturbation. However, real-time reverse transcription-PCR data indicate that replication levels of wild-type and L-mutant viruses do not differ significantly (less than twofold) during the first cycle of cell infection (data not shown). Moreover, no correlation was observed between the intensity of viral antigen labeling and the extent of NLS-eGFP or GFP-mIRF-3 protein redistribution.

Other picornaviruses were shown to perturb nucleocytoplasmic trafficking (2, 7, 8), but none of them encodes a protein related to the leader protein of Theiler's virus. It is likely, in the case of TMEV, that other proteins cooperate with the leader protein in the perturbation of the nucleocytoplasmic trafficking. Indeed, PTB relocation to the cytoplasm occurred to some extent in cells infected with the L^{lys} mutant. Unfortunately, direct influence of the leader could not be tested, as this protein turned out to be extremely toxic to cells when expressed separately.

The leader protein of Theiler's virus shares 35% identity with the leader protein encoded by encephalomyocarditis virus (EMCV) (16). In particular, the zinc finger is conserved in the L proteins of all the cardioviruses. The leader protein of EMCV has been credited with several functions including shut-off of protein synthesis and IFN production inhibition (25, 26). However, it is not clear whether the leader proteins of TMEV and EMCV act in a similar fashion. Unlike EMCV, TMEV is not considered to shut off host protein synthesis extensively. However, we observed that the leader protein of TMEV was toxic to cells when expressed alone and that cells infected with a virus expressing the wild-type L rounded up earlier than did cells infected with the L mutant in spite of a similar replication level, suggesting that L could participate in cytopathogenicity. Thus, it is possible that the TMEV leader induces a light shutoff which might be the cause of protein redistribution. Alternatively, perturbation of nucleocytoplasmic trafficking could be the cause of L toxicity, or the two phenomena could be independent. Further work will be required to identify the direct target of the leader protein.

The L protein was previously shown to antagonize the production of alpha/beta IFN (23). This inhibition was shown to

occur at least in part at the transcriptional level (22). IRF-3 is the major factor involved in transcriptional activation of immediate-early IFN genes that are first activated after viral infection (i.e., IFN- α 4 and IFN- β in the mouse). IRF-3 is constitutively present in the cell cytoplasm of uninfected cells, ensuring very rapid activation of IFN genes upon viral infection of the cell. By disturbing nucleocytoplasmic trafficking early after infection, viruses might have found a way to prevent this rapid induction of alpha/beta IFN production and to be protected from the powerful antiviral action of these cytokines.

In addition, nucleocytoplasmic trafficking perturbation might be viewed as an efficient mechanism of immune response evasion, as the expression of many early effectors of the immune response depends on the nuclear translocation of cellular proteins.

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REFERENCES

- Back, S. H., Y. K. Kim, W. J. Kim, S. Cho, H. R. Oh, J. E. Kim, and S. K. Jang. 2002. Translation of polioviral mRNA is inhibited by cleavage of polypyrimidine tract-binding proteins executed by polioviral 3C^{pro}. *J. Virol.* **76**:2529–2542.
- Belov, G. A., A. G. Evstafieva, Y. P. Rubtsov, O. V. Mikitas, A. B. Vartapean, and V. I. Agol. 2000. Early alteration of nucleocytoplasmic traffic induced by some RNA viruses. *Virology* **275**:244–248.
- Chen, H. H., W. P. Kong, and R. P. Roos. 1995. The leader peptide of Theiler's murine encephalomyelitis virus is a zinc-binding protein. *J. Virol.* **69**:8076–8078.
- Drescher, K. M., L. R. Pease, and M. Rodriguez. 1997. Antiviral immune responses modulate the nature of central nervous system (CNS) disease in a murine model of multiple sclerosis. *Immunol. Rev.* **159**:177–193.
- Gossen, M., and H. Bujard. 1992. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc. Natl. Acad. Sci. USA* **89**:5547–5551.
- Gossen, M., S. Freundlieb, G. Bender, G. Muller, W. Hillen, and H. Bujard. 1995. Transcriptional activation by tetracyclines in mammalian cells. *Science* **268**:1766–1769.
- Gustin, K. E., and P. Sarnow. 2001. Effects of poliovirus infection on nucleocytoplasmic trafficking and nuclear pore complex composition. *EMBO J.* **20**:240–249.
- Gustin, K. E., and P. Sarnow. 2002. Inhibition of nuclear import and alteration of nuclear pore complex composition by rhinovirus. *J. Virol.* **76**:8787–8796.
- Jnaoui, K., and T. Michiels. 1998. Adaptation of Theiler's virus to L929 cells: mutations in the putative receptor binding site on the capsid map to neutralization sites and modulate viral persistence. *Virology* **244**:397–404.
- Kaminski, A., S. L. Hunt, J. G. Patton, and R. J. Jackson. 1995. Direct evidence that polypyrimidine tract binding protein (PTB) is essential for internal initiation of translation of encephalomyocarditis virus RNA. *RNA* **1**:924–938.
- Kong, W. P., G. D. Ghadge, and R. P. Roos. 1994. Involvement of cardiovirus leader in host cell-restricted virus expression. *Proc. Natl. Acad. Sci. USA* **91**:1796–1800.
- Kumar, K. P., K. M. McBride, B. K. Weaver, C. Dingwall, and N. C. Reich. 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.
- Lin, R., C. Heybroeck, P. M. Pitha, and J. Hiscott. 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.
- Lipton, H. L. 1975. Theiler's virus infection in mice: an unusual biphasic disease process leading to demyelination. *Infect. Immun.* **11**:1147–1155.

15. **Meerovitch, K., Y. V. Svitkin, H. S. Lee, F. Lejbkowitz, D. J. Kenan, E. K. Chan, V. I. Agol, J. D. Keene, and N. Sonenberg.** 1993. La autoantigen enhances and corrects aberrant translation of poliovirus RNA in reticulocyte lysate. *J. Virol.* **67**:3798–3807.
16. **Michiels, T., V. Dejong, R. Rodrigus, and C. Shaw-Jackson.** 1997. Protein 2A is not required for Theiler's virus replication. *J. Virol.* **71**:9549–9556.
17. **Monteyne, P., J. F. Bureau, and M. Brahic.** 1997. The infection of mouse by Theiler's virus: from genetics to immunology. *Immunol. Rev.* **159**:163–176.
18. **Pilipenko, E. V., T. V. Pestova, V. G. Kolupaeva, E. V. Khitrina, A. N. Poperechnaya, V. I. Agol, and C. U. Hellen.** 2000. A cell cycle-dependent protein serves as a template-specific translation initiation factor. *Genes Dev.* **14**:2028–2045.
19. **Servant, M. J., B. Tenoever, and R. Lin.** 2002. Overlapping and distinct mechanisms regulating IRF-3 and IRF-7 function. *J. Interferon Cytokine Res.* **22**:49–58.
20. **Talon, J., C. M. Horvath, R. Polley, C. F. Basler, T. Muster, P. Palese, and A. Garcia-Sastre.** 2000. Activation of interferon regulatory factor 3 is inhibited by the influenza A virus NS1 protein. *J. Virol.* **74**:7989–7996.
21. **Theiler, M., and S. Gard.** 1940. Encephalomyelitis of mice. I. Characteristics and pathogenesis of the virus. *J. Exp. Med.* **72**:49–67.
22. **van Pesch, V., and T. Michiels.** 2003. Characterization of interferon-alpha 13, a novel constitutive murine interferon-alpha subtype. *J. Biol. Chem.* **278**:46321–46328.
23. **van Pesch, V., O. van Eyll, and T. Michiels.** 2001. The leader protein of Theiler's virus inhibits immediate-early alpha/beta interferon production. *J. Virol.* **75**:7811–7817.
24. **Yoneyama, M., W. Suhara, Y. Fukuhara, M. Fukuda, E. Nishida, and T. Fujita.** 1998. Direct triggering of the type I interferon system by virus infection: activation of a transcription factor complex containing IRF-3 and CBP/p300. *EMBO J.* **17**:1087–1095.
25. **Zoll, J., J. M. Galama, F. J. van Kuppeveld, and W. J. Melchers.** 1996. Mengovirus leader is involved in the inhibition of host cell protein synthesis. *J. Virol.* **70**:4948–4952.
26. **Zoll, J., W. J. Melchers, J. M. Galama, and F. J. van Kuppeveld.** 2002. The mengovirus leader protein suppresses alpha/beta interferon production by inhibition of the iron/ferritin-mediated activation of NF- κ B. *J. Virol.* **76**:9664–9672.