Supporting Information

Iwasaki and Yanagi 10.1073/pnas.1107382108

SI Materials and Methods

Cells and Viruses. NIH 3T3, L, HeLa, HEK293, and Vero cells constitutively expressing human signaling lymphocyte activation molecule (SLAM) (NIH 3T3/hSLAM, L/hSLAM, HeLa/hSLAM, HEK293/hSLAM, and Vero/hSLAM, respectively) (1, 2) were maintained in DMEM (Invitrogen) supplemented with 7.5% FBS and 500 μg/ml G418. 293T and L929 cells were maintained in DMEM supplemented with 7.5% FBS. VV5-4 cells (a derivative of CHO cells) were maintained in RPMI medium 1640 (MP Biomedicals) supplemented with 7.5% FBS. Recombinant measles virus (MV) based on the virulent IC-B strain was used, and the viruses encoding the IC-B strain were propagated in MDCK cells. At various time intervals, the infected cells were scraped into monolayers. At various time intervals, the infected cells were scraped into monolayers and support plasmids expressing the MV N, phospho- (P), and large (L) proteins were kindly provided by K. Komase, National Institute of Infectious Diseases, Tokyo, Japan. The IFN-α/β-inducible plasmid pJSRE-Luc (a gift from K. Ozato, National Institute of Child Health and Human Development, Bethesda, MD) has three tandem repeat sequences of the IFN-stimulated response element followed by the firefly luciferase gene. The plasmid pRL-TK has the herpes simplex virus thymidine kinase promoter followed by the Renilla luciferase gene.

Antibodies. Rabbit polyclonal antibodies against the Cre recombinase, STAT1 and phosphorylated STAT1, and mouse monoclonal antibodies against EGFP (JL-8) and β-actin (C-2) were purchased from EMD chemicals Inc., Santa Cruz Biotechnology, Cell Signaling Technology, TAKARA BIO Inc., and Santa Cruz Biotechnology, respectively. The serum of a patient with subacute sclerosing panencephalitis containing high-level antibodies against MV proteins including the nucleocapsid (N) protein (5) and rabbit serum against the recombinant SeV C protein (6) were kindly provided by M. B. A. Oldstone, The Scripps Research Institute, La Jolla, CA and A. Kato, National Institute of Infectious Diseases, Tokyo, Japan, respectively.

Plasmids. p(+)MV323-EGFP and p(+)MV323-Luci were described previously (7, 8). p(+)MV323-Cre was generated by replacing the transcriptional unit of EGFP of p(+)MV323-EGFP with that of the Cre recombinase fused with the nuclear localization signal in its amino terminus. p(+)MV323-EGFP-addCre was generated by replacing the PΔC and C genes of p(+) MV323-PΔC-addC (9) with the intact P gene and the sequence encoding the Cre recombinase fused with nuclear localization signal in its amino terminus, respectively. The recombinant MVs were generated from p(+)MV323-EGFP, p(+)MV323-Luci, p(+) MV323-Cre, and p(+)MV323-EGFP-addCre were designated MV-EGFP, MV-Luci, MV-Cre, and MV-addCre, respectively. Expression plasmids pCA7-SeV-C, pCA7-Cre, and pCA7-NS1 were generated by inserting the DNA fragments encoding the SeV C protein, Cre recombinase (gifts from A. Kato, National Institute of Infectious Diseases, Tokyo, Japan and T. Yagi, Osaka University, Osaka, Japan, respectively), and the NS1 protein from the influenza A virus PR8 strain into the expression vector pCA7, pCA7-SeV-Cm5, pCA7-SeV-Cm4*, pCA7-SeV-Y1, and pCA7-SeV-C-d194 were generated by cloning into the pCA7 vector DNA fragments encoding mutant SeV C proteins with amino acid (aa) substitutions K151A, E153A, and R154A, with aa substitutions K151A, E153K, and R157L, with amino-terminal truncation of 23 aa and with carboxyl-terminal truncation of 10 aa, respectively. The expression plasmids pCA7-IC-C and pCA7-IC-VAC encoding the MV C and V proteins, respectively, were reported previously (10). pCA7-IC-VAC does not direct the production of the C protein. To generate pCA7-loxP-SC, the plasmid encoding the puromycin resistance gene with the polyA signal sequence flanked by loxP sequences (a gift from C. Meno, Kyushu University, Fukuoka, Japan) was used. Two SV40 polyA signal sequences were tandemly inserted downstream of the original polyA signal to prevent readthrough transcription. The plasmids used for the MV minigenome assay [p18MGFLuc01 and support plasmids expressing the MV N, phospho- (P), and large (L) proteins] were kindly provided by K. Komase, National Institute of Infectious Diseases, Tokyo, Japan. The IFN-α/β-inducible plasmid pJSRE-Luc (a gift from K. Ozato, National Institute of Child Health and Human Development, Bethesda, MD) has three tandem repeat sequences of the IFN-stimulated response element followed by the firefly luciferase gene. The plasmid pRL-TK has the herpes simplex virus thymidine kinase promoter followed by the Renilla luciferase gene.

Virus Titration. Monolayers of Vero/hSLAM cells in 12-well cluster plates were infected with serially diluted virus samples. After 1 h of incubation at 37 °C, the virus samples were removed, and the cells were overlaid with DMEM containing 2% FBS and 0.75% agarose. At 6 d postinfection, the numbers of pfu were counted.

Comparison of Luciferase Activities with Viral Multiplication. Subconfluent NIH 3T3/hSLAM cells in six-well cluster plates were mock-treated or treated with IFN-α/α/D (Sigma) at the concentration of 10, 100, or 1,000 units/ml for 24 h. The cells were infected with MV-Luci at a multiplicity of infection (MOI) of 0.05. At 48 h postinfection, Renilla luciferase activities in part of the samples were measured using a Renilla luciferase assay system (Promega) and a luminometer Mithras LB 940 (Berthold Technologies). The rest of the samples were scraped into medium, and their viral titers were determined by plaque assay.

Measurement of MV Growth. Subconfluent monolayers of NIH 3T3/hSLAM, L/hSLAM, HeLa/hSLAM, or HEK293/hSLAM cells in 12-well cluster plates were transfected with 1 μg of pCA7 or pCA7-SeV-C using the lipofectamine 2000 reagent. In other experiments, NIH 3T3/hSLAM cells in 24-well cluster plates were transfected with 0.5 μg of pCA7, pCA7-SeV-C, pCA7-MV-C, or pCA7-MV-VAC; NIH 3T3/hSLAM and HeLa/hSLAM cells with the total 250 ng of pCA7-Cre, pCA7-loxP-SC, and pCA7 in various combinations (total amounts of transfected plasmids were adjusted by pCA7); NIH 3T3/hSLAM cells with various amounts (25, 100, and 400 ng) of expression plasmids encoding wild-type or mutant SeV C proteins (total amounts of transfected plasmids were adjusted to 400 ng by pCA7); NIH 3T3/hSLAM cells with 0.5 μg of pCA7, pCA7-SeV-C, or pCA7-NS1. At 24 or 48 h posttransfection, these cells were infected with MV-Luci at an MOI of 0.05, and at 48 h postinfection, enzymatic activities in infected cells were measured using a Renilla luciferase assay system and a luminometer Mithras LB 940.

Growth Kinetics. Monolayers of Vero/hSLAM, NIH 3T3/hSLAM, and 3SC cells on six-well cluster plates were infected with MV-EGFP, MV-Cre or MV-addCre at an MOI of 0.01 (Vero/hSLAM) or 0.05 (NIH 3T3/hSLAM and 3SC) and cultured in 2 mL of medium. At various time intervals, the infected cells were scraped into the medium and the viral titer was determined by plaque assay.

Western Blot Analysis. Western blot analysis was performed as described previously (11). The membranes were incubated with appropriate antibody, followed by incubation with horseradish peroxidase-conjugated anti-rabbit IgG (Invitrogen Life Technologies), anti-mouse IgG (Jackson ImmunoResearch Laboratories Inc.), or anti-human IgG antibodies (EY Laboratories). The Chemi Lumi One Super reagent (Nacalai Tesque) was used to elicit chemiluminescent signals, and the signals on the membranes were detected and visualized using a VersaDoc 3000 imager (Bio-Rad).


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**Fig. S1.** Comparison of luciferase activities with viral titers. NIH 3T3/hSLAM cells were mock-treated or treated with IFN-α at the concentration of 10, 100, or 1,000 units/mL for 24 h. The cells were infected with MV-Luci, and at 48 h postinfection, Renilla luciferase activities (left y axis, filled bars) and viral titers (right y axis, filled circles) of the respective samples were determined. Data represent the means ± SD of triplicate samples.

**Fig. S2.** IFN-antagonist activities of the SeV C and d194 proteins. Mouse L929 cells were transfected with pISRE-Luc and pRL-TK, together with pCA7 (Empty), various amounts (25, 100, and 400 ng) of the expression plasmid encoding the SeV C protein, or 400 ng of that encoding d194. Total amounts of transfected plasmids were adjusted by pCA7. At 36 h posttransfection, part of the respective samples were lysed and subjected to SDS/PAGE and Western blotting for the detection of the SeV C and d194 proteins. The cells were treated with IFN-α for 8 h, and the relative luciferase activities were determined as described in *Materials and Methods*. Data represent the means ± SD of triplicate samples.

**Fig. S3.** Effects of the SeV C and influenza A virus NS1 proteins on MV growth in mouse cells. NIH 3T3/hSLAM cells were transfected with pCA7 or expression plasmids encoding the SeV C or influenza A virus NS1 protein. At 24 h posttransfection, the cells were infected with MV-Luci, and at 48 h postinfection, *Renilla* luciferase activities in the infected cells were measured. The luciferase activity in the cells transfected with the empty vector was set to 1.0. Data represent the means ± SD of triplicate samples.