

## Pandemic H1N1 2009 Influenza A Virus Induces Weak Cytokine Responses in Human Macrophages and Dendritic Cells and Is Highly Sensitive to the Antiviral Actions of Interferons<sup>∇</sup>

Pamela Österlund,<sup>1\*</sup> Jaana Pirhonen,<sup>1</sup> Niina Ikonen,<sup>1</sup> Esa Rönkkö,<sup>1</sup> Mari Strengell,<sup>1</sup>  
Sanna M. Mäkelä,<sup>1</sup> Mia Broman,<sup>1</sup> Ole J. Hamming,<sup>2</sup> Rune Hartmann,<sup>2</sup>  
Thedi Ziegler,<sup>1</sup> and Ilkka Julkunen<sup>1</sup>

*Viral Infections Unit, Department of Vaccination and Immune Protection, National Institute for Health and Welfare, Helsinki, Finland,<sup>1</sup> and Center for Structural Biology, Department of Molecular Biology, Aarhus University, Aarhus, Denmark<sup>2</sup>*

Received 4 August 2009/Accepted 16 November 2009

**In less than 3 months after the first cases of swine origin 2009 influenza A (H1N1) virus infections were reported from Mexico, WHO declared a pandemic. The pandemic virus is antigenically distinct from seasonal influenza viruses, and the majority of human population lacks immunity against this virus. We have studied the activation of innate immune responses in pandemic virus-infected human monocyte-derived dendritic cells (DC) and macrophages. Pandemic A/Finland/553/2009 virus, representing a typical North American/European lineage virus, replicated very well in these cells. The pandemic virus, as well as the seasonal A/Brisbane/59/07 (H1N1) and A/New Caledonia/20/99 (H1N1) viruses, induced type I (alpha/beta interferon [IFN- $\alpha$ / $\beta$ ]) and type III (IFN- $\lambda$ 1 to - $\lambda$ 3) IFN, CXCL10, and tumor necrosis factor alpha (TNF- $\alpha$ ) gene expression weakly in DCs. Mouse-adapted A/WSN/33 (H1N1) and human A/Udorn/72 (H3N2) viruses, instead, induced efficiently the expression of antiviral and proinflammatory genes. Both IFN- $\alpha$  and IFN- $\beta$  inhibited the replication of the pandemic (H1N1) virus. The potential of IFN- $\lambda$ 3 to inhibit viral replication was lower than that of type I IFNs. However, the pandemic virus was more sensitive to the antiviral IFN- $\lambda$ 3 than the seasonal A/Brisbane/59/07 (H1N1) virus. The present study demonstrates that the novel pandemic (H1N1) influenza A virus can readily replicate in human primary DCs and macrophages and efficiently avoid the activation of innate antiviral responses. It is, however, highly sensitive to the antiviral actions of IFNs, which may provide us an additional means to treat severe cases of infection especially if significant drug resistance emerges.**

The novel swine origin 2009 influenza A (H1N1) virus was identified in April 2009, and it is currently causing the first influenza pandemic of the 21st century. The virus is a completely new reassortant virus (8, 38), and the majority of the human population does not have preexisting immunity against it. The case fatality rate of the current pandemic virus infection is still unclear, but it is estimated to be somewhat higher than that of seasonal influenza virus infections (8). In most cases, the pandemic 2009 A (H1N1) virus causes an uncomplicated respiratory tract illness with symptoms similar to those caused by seasonal influenza viruses. However, gastrointestinal symptoms atypical to seasonal influenza have been detected in a significant proportion of cases (4, 7, 35).

The pandemic 2009 (H1N1) influenza A virus originates from a swine influenza A virus strain. It underwent multiple reassortment events in pigs and then transferred into the human population (8, 38). The new virus has gene segments from the North American triple-reassortant and Eurasian swine H1N1 viruses (8, 38). Sequence analysis of this new pandemic virus revealed that hemagglutinin (HA), NP, and NS gene segments are derived from the classical swine viruses, PB1

from human H3N2, and PB2 and PA from avian viruses within the triple-reassortant virus (8). In addition, the NA and M segments originate from the Eurasian swine virus lineage. The pandemic 2009 (H1N1) virus is genetically and antigenically distinct from previous seasonal human influenza A (H1N1) viruses. Thus, the current seasonal influenza vaccines are likely to give little, if any, protection against pandemic 2009 A (H1N1) virus infection (12, 14). However, some evidence indicates that people born early in the 20th century have cross-neutralizing antibodies against the pandemic 2009 A (H1N1) viruses (12, 14).

At present, relatively little is known about the pathogenesis and transmission of the pandemic 2009 A (H1N1) virus in humans. Studies with ferrets revealed that the pandemic virus replicated better than seasonal H1N1 viruses in the respiratory tracts of the animals. This suggests that the virus is more pathogenic in ferrets than seasonal influenza viruses (19, 24). The respiratory tract is the primary infection site of all mammalian influenza viruses, and, indeed, the specific glycan receptors on the apical surface of the upper respiratory tract have been reported to bind HA of the 2009 A (H1N1) virus (19). In human lung tissue binding assays, 2009 A (H1N1) HA showed a glycan binding pattern similar to that of the HA from the pandemic 1918 A (H1N1) virus though its affinity to  $\alpha$ 2,6 glycans was much lower than that of the 1918 virus HA. The lower glycan binding properties of the pandemic 2009 A (H1N1) virus seemed to correlate with less-efficient transmission in ferrets compared to seasonal H1N1 viruses (19). Ac-

\* Corresponding author. Mailing address: Viral Infections Unit, Department of Vaccination and Immune Protection, National Institute for Health and Welfare, P.O. Box 30, FI-00271 Helsinki, Finland. Phone: 358-408670324. Fax: 358-206108355. E-mail: pamela.osterlund@thl.fi.

<sup>∇</sup> Published ahead of print on 25 November 2009.

cording to another study with ferrets, the transmission of the pandemic 2009 A (H1N1) virus via respiratory droplets was as efficient as that of a seasonal A (H1N1) virus (24). It is clear that, besides experimental infections in animal models, analyses of the characters and pathogenesis of the pandemic 2009 A (H1N1) virus infection in humans are urgently needed.

In the present study, we have focused on analyzing innate immune responses in primary human dendritic cells (DCs) and macrophages in response to an infection with one of the Finnish isolates of the pandemic 2009 A (H1N1) virus. DCs and macrophages reside beneath the epithelium of the respiratory organs, and these cells are thus potential targets for influenza viruses. From the epithelial cells influenza viruses spread in DCs and macrophages, which coordinate the development of an effective innate immune response against the virus (22, 34, 41). During influenza virus infection, DCs and macrophages secrete antiviral cytokines such as interferons (IFNs) and tumor necrosis factor alpha (TNF- $\alpha$ ) (3, 13, 26). Moreover, DCs and macrophages activate virus-destroying NK cells and T cells with the cytokines they secrete and via direct cell-to-cell contacts (9, 29, 33, 37). Here we show that the pandemic (H1N1) virus infects and replicates very well in human monocyte-derived DCs and macrophages. The pandemic virus as well as two recent seasonal H1N1 viruses induced a relatively weak innate immune response in these cells, as evidenced by a poor expression of antiviral and proinflammatory cytokine genes. However, like seasonal influenza A viruses, the pandemic 2009 (H1N1) virus was extremely sensitive to the antiviral actions of type I IFNs (IFN- $\alpha/\beta$ ). Interestingly, the pandemic 2009 (H1N1) virus was even more sensitive to antiviral IFN- $\lambda$ 3 than a seasonal A (H1N1) virus. Thus, IFNs may provide us with an additional means to combat severe pandemic influenza virus infections, especially if viral resistance against neuraminidase (NA) inhibitors begins to emerge.

## MATERIALS AND METHODS

**Differentiation of macrophages and DCs from peripheral blood-derived monocytes.** Human macrophages and DCs were differentiated from peripheral blood monocytes (PBLs) as described previously (2, 43). In brief, PBLs from healthy blood donors were fractioned by Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) gradient centrifugation. To obtain monocytes for macrophage cultures, the peripheral blood mononuclear cell (PBMC) fraction of PBLs was depleted of lymphocytes by allowing the cells to adhere to six-well cell culture plates (Falcon Multiwell; Becton Dickinson, Franklin Lakes, NJ). Macrophages were differentiated from purified monocytes by culturing the cells for 7 days in serum-free medium (Life Technologies, Gaithersburg, MD) supplemented with 10 ng/ml granulocyte macrophage colony-stimulating factor (GM-CSF) (BioSource, Camarillo, CA).

For DC cultures, the PBMC fraction was subjected to an additional centrifugation on a Percoll gradient (Amersham Biosciences, Uppsala, Sweden) and then the remaining lymphocytes were depleted with anti-CD3 and anti-CD19 magnetic beads (Dyna, Oslo, Norway). The monocyte-enriched cell fraction was further purified by adhering the cells onto cell culture plates. DCs were differentiated by culturing monocytes in complete RPMI medium in the presence of 10% fetal calf serum (FCS) (Integro BV, Dieren, the Netherlands), 10 ng/ml GM-CSF, and 20 ng/ml interleukin-4 (IL-4) (R&D Systems, Abingdon, United Kingdom). After 1 week of culturing, the cells showed the typical morphology and gene expression pattern for macrophages or DCs (18), and the cells were used in virus infection experiments.

**Influenza A viruses.** Pandemic 2009 influenza A virus strain A/Finland/553/2009 was isolated from a 22-year-old male patient who returned from Mexico in early May 2009. The sequencing of the virus indicated it to be almost identical to typical North American/European pandemic viruses. A/Finland/553/2009 and the other influenza viruses A/Brisbane/59/07 (H1N1), A/New Caledonia/20/99

(H1N1), A/WSN/33 (H1N1) (WHO World Influenza Centre, Mill Hill, London, United Kingdom), and A/Udorn/72 (H3N2) (kindly provided by Robert M. Krug, University of Texas at Austin, Austin, TX) (25) were all grown in embryonated chicken eggs as previously described (32). The hemagglutination titers of the virus stocks were 16, 128, 256, 128, and 256, respectively, and the passage histories of the stock viruses were E3, E5/E2, E/E3, E/E1, and E/E1, respectively. The original passage histories of the older virus strains (i.e., A/New Caledonia/20/99, A/WSN/33, and A/Udorn/72) are not known. The infectivity of the virus stocks in human DCs and macrophages was determined by infecting the cells with different virus doses and analyzing the percentages of virus-infected cells with flow cytometry. The viruses were used in infection experiments with doses giving comparable multiplicities of infection (MOI) based on the infectivity in DCs. The propagation of the A/Finland/553/2009 virus stock and the infection experiments with this virus were carried out under biosafety level 3 (BSL-3) conditions.

**Virus infection experiments.** To study the kinetics of virus infection in macrophages and DCs, the cells were infected with A/Finland/553/2009, A/Brisbane/59/07, A/New Caledonia/20/99, A/WSN/33, or A/Udorn/72 viruses at MOI of 5 and the infection was allowed to proceed for 1 to 48 h. Alternatively, the cells were infected with different virus doses for 20 h. In the IFN priming experiment the cells were pretreated with IFNs (see below), followed by infection at MOI of 2. The cells were harvested at 18 h after infection, and samples for flow cytometry and quantitative reverse transcription-PCR (qRT-PCR) analysis were prepared. Each sample represents a pool of separately infected macrophages or DCs from two to four different donors, and all experiments were carried out one to four times. The Madin-Darby canine kidney (MDCK) cell line was used as a control cell model for measuring the replication efficiency of A/Finland/553/2009 virus. The MDCK cells were infected with different virus doses for 20 h.

**IFN priming of virus-infected cells.** Recombinant human IFN- $\alpha$ 2 and IFN- $\beta$  were purchased from Schering-Plough (Kenilworth, NJ). Recombinant human IFN- $\lambda$ 1 and IFN- $\lambda$ 3 were produced in *Escherichia coli* and purified to homogeneity as described previously (6). In IFN priming experiments DCs and macrophages were treated with 1, 10, or 100 IU/ml of IFN- $\alpha$  or IFN- $\beta$  or with 1, 10, or 100 ng/ml of IFN- $\lambda$ 1 or IFN- $\lambda$ 3 for 16 h before the cells were infected with influenza viruses.

**Flow cytometric analysis (fluorescence-activated cell sorting [FACS]).** Flow cytometry was used to analyze the purity of monocyte-derived macrophages and DCs and the infectivities of influenza viruses in the cells. In the IFN priming experiments, the antiviral effect was monitored by measuring the expression of IFN- $\alpha/\beta$ -inducible MxA protein (26). The cell samples for the flow cytometric analysis were prepared as described previously (44). The cells obtained from different blood donors were infected separately and pooled after the virus infections. The cell samples were fixed with 4% paraformaldehyde for 15 min at room temperature. The expression of viral proteins and MxA was measured using cross-reactive rabbit anti-influenza A virus H1N1 glycoprotein-specific antibodies (15) or rabbit anti-human MxA protein-specific antibodies (31) followed by secondary anti-rabbit fluorescein isothiocyanate (FITC) antibodies (Caltag Laboratories, Burlingame, CA). The fluorescence-labeled cells were analyzed with a FACSCanto II flow cytometer using FACSDiva software (Becton Dickinson, San Diego, CA). The purity of DC and macrophage populations was confirmed by staining uninfected cells with phycoerythrin (PE)-anti-CD2, PE-Cy7-anti-CD14 (Becton Dickinson), FITC-anti-CD80, PE-anti-CD83, allophycocyanin (APC)-anti-CD86 (Caltag Laboratories), and APC-H7-anti-HLA-DR antibodies (Becton Dickinson).

**qRT-PCR.** The virus-infected cells from different blood donors were harvested and pooled, and total cellular RNA was isolated using the RNeasy Mini kit (Qiagen, Valencia, CA), including DNase digestion (RNase-free DNase kit; Qiagen). One microgram of total RNA was transcribed to cDNA by using the TaqMan reverse transcriptase kit (Applied Biosystems) with random hexamers as primers. The cDNA was amplified by PCR using TaqMan Universal PCR Mastermix and a commercial gene expression assay (Applied Biosystems) with primers and probes for IFN- $\alpha$ 1 (Hs 00256882\_s1), IFN- $\beta$  (Hs00277188\_s1), IFN- $\lambda$ 1 (Hs00601677\_g1), IFN- $\lambda$ 2 (Hs00820125\_g1), CXCL10 (Hs00171042\_m1), TNF- $\alpha$  (Hs00174128\_m1), and MxA (Hs00182073\_m1) from Applied Biosystems. The probe for IFN- $\lambda$ 2 detects also IFN- $\lambda$ 3, since the proteins show 96% amino acid homology with each other (16). The influenza A virus M1 gene-specific primer-probe pair that detects a highly conserved sequence in the M genes of all influenza A viruses was designed by Ward and colleagues and was used with minor modifications (42). The data were normalized to 18S rRNA with the TaqMan endogenous control kit (Applied Biosystems). Cytokine gene expression data are presented as relative gene expression in relation to that for the unstimulated sample in order to calculate the fold change achieved by the stimulation. Viral gene expression data are presented as relative copy numbers of the M1 RNA molecules.

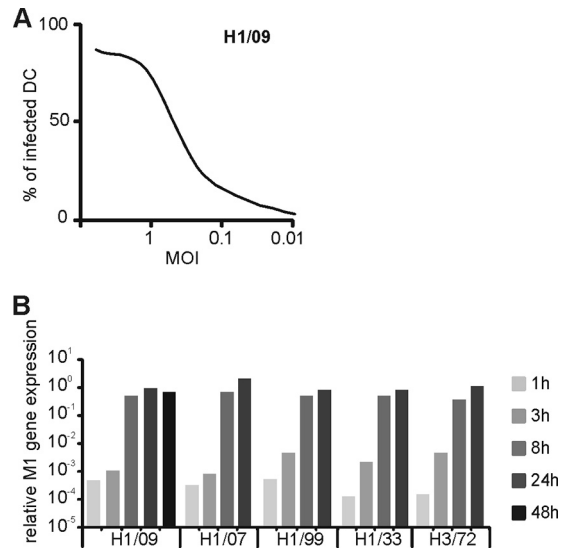
**ELISA.** The secreted levels of CXCL10 and TNF- $\alpha$  were analyzed from cell culture supernatants using antibody pairs from BD Pharmingen (San Diego, CA). IFN- $\lambda$ 1 and IFN- $\lambda$ 2 were measured with a Duoset enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN). ELISAs to detect human IFN- $\beta$  and IFN- $\alpha$  were supplied by PBL Biomedical Laboratories (Piscataway, NJ).

**Nucleotide sequence accession numbers.** Viral sequences are available in GenBank under accession numbers GQ183633 and -34, GQ223437 to -39, GQ283487 to -95, and GQ328864 to -69).

## RESULTS

**Replication of the pandemic 2009 influenza A (H1N1) virus in human DCs.** In order to study the ability of pandemic 2009 influenza A (H1N1) viruses to replicate and induce innate immune responses in human primary leukocytes, we isolated and characterized a collection of pandemic 2009 (H1N1) viruses. Altogether, 25 virus isolates were molecularly and phenotypically characterized. Three of the viruses were almost completely sequenced (A/Finland/553/2009, A/Finland/554/2009, and A/Finland/555/2009; GenBank accession numbers GQ183633 and GQ183634, GQ223437 to GQ223439, GQ283487 to GQ283495, and GQ328864 to GQ328869), while for the rest of the viruses only HA and NA segments were identified. All virus strains isolated in Finland exhibited high conservation of their amino acid sequences to the initial isolates (A/California/04/2009 and A/California/07/2009). The percentages of identity of the Finnish isolates with the Californian isolates were above 99.3% and 99.6% in their HA and NA genes, respectively (data not shown). Thus, all our viruses were genetically and phenotypically very much alike and represented typical North American/European pandemic influenza A (H1N1) virus strains. For our experiments we selected A/Finland/553/2009 virus, which was isolated in mid-May 2009 from a 22-year-old male traveler returning from Mexico. The patient suffered from a mild upper respiratory tract infection, received oseltamivir treatment, and recovered completely.

Dendritic cells are readily infected by influenza viruses and play an important role in regulating host innate and adaptive immune responses against viruses (10). To reveal the ability of the pandemic 2009 (H1N1) influenza A virus to infect and replicate in human DCs, we determined the infectivity titer of the virus stock. We analyzed with flow cytometry the expression of viral glycoproteins on the cell surface of human monocyte-derived DCs infected with different doses of the virus (Fig. 1A) and found that the pandemic virus infected and replicated well in DCs. To compare the replication efficiency of the pandemic virus in human DCs with those of the seasonal influenza viruses, we determined also the infectivity titers of A/Brisbane/59/07 (H1N1), A/New Caledonia/20/99 (H1N1), A/WSN/33 (mouse-adapted H1N1), and A/Udorn/72 (H3N2) virus stocks. We infected DCs with different influenza A virus strains at MOI of 5, followed by analysis of viral M1 gene expression at different time points after infection. The pandemic 2009 (H1N1) influenza A virus replicated in human DCs as efficiently as the other H1N1 (Brisbane/07, New Caledonia/99, and WSN/33) and H3N2 (Udorn/72) type viruses (Fig. 1B). The DCs infected with all these virus strains expressed viral RNA with equal kinetics. The maximal levels of viral RNA were reached at 8 h postinfection. However, the pandemic 2009 (H1N1) virus did not induce a cytopathic effect (CPE) in



**FIG. 1.** Infectivity and replication of the pandemic 2009 influenza A (H1N1) virus in primary human DCs. (A) To study the infectivity of the pandemic 2009 (H1N1) influenza A virus, human monocyte-derived DCs from four blood donors were infected separately with different doses (starting from 1 HA unit/ml) of A/Finland/553/2009 strain of the virus (H1/09) for 18 h. The expression of viral glycoproteins was detected by flow cytometry using cross-reacting H1N1 glycoprotein-specific rabbit anti-H1N1 antibodies. The proportion of infected cells was analyzed, and the multiplicity of infection (MOI) was determined as 0.5 in the dilution where 50% of cells were infected. (B) To study the kinetics of virus replication, DCs were infected with a pandemic A/Finland/553/2009 virus (H1/09) and seasonal influenza viruses A/Brisbane/59/07 (H1/07), A/New Caledonia/20/99 (H1/99), A/WSN/33 (H1/33), and A/Udorn/72 (H3/72) at MOI of 5. After 1, 3, 8, 24, or 48 h of infection, the cells from different donors were harvested and pooled, and total cellular RNA samples for quantitative RT-PCR were prepared. Due to significant cell death, 48-h samples were not available for the cells infected with seasonal viruses, and thus the 48-h time point is missing. The expression of viral M1 RNA was measured by qRT-PCR, and the values were normalized to 18S rRNA and presented as relative copy numbers of M1 RNA.

DCs as rapidly as the other viruses did. While the seasonal influenza viruses caused a strong CPE at 24 h postinfection and the total RNA levels were reduced to 25% of those of control cells, the pandemic H1N1 virus required 48 h to induce a clear CPE with corresponding reduction in total cellular RNA levels. The 48-h time point was omitted for seasonal viruses (Fig. 1B) due to massive cell death and dramatic loss of total cellular RNA. The reduced capacity of the pandemic 2009 (H1N1) virus to induce CPE may explain the moderate pathological changes observed in animal experiments in the sites of virus replication (19, 24).

**The pandemic 2009 A (H1N1) virus induces a weak expression of antiviral and proinflammatory cytokines in human DCs.** To analyze the capacity of the replicating pandemic 2009 (H1N1) virus to induce IFN and other cytokine responses, DCs were infected with different H1N1 and H3N2 influenza viruses. The gene expression and protein levels of antiviral type I and type III IFNs, NK and Th1 type cell-recruiting CXCL10, and proinflammatory TNF- $\alpha$  were quantified by qRT-PCR and ELISA, respectively.

Interestingly, the pandemic 2009 (H1N1) virus as well as the

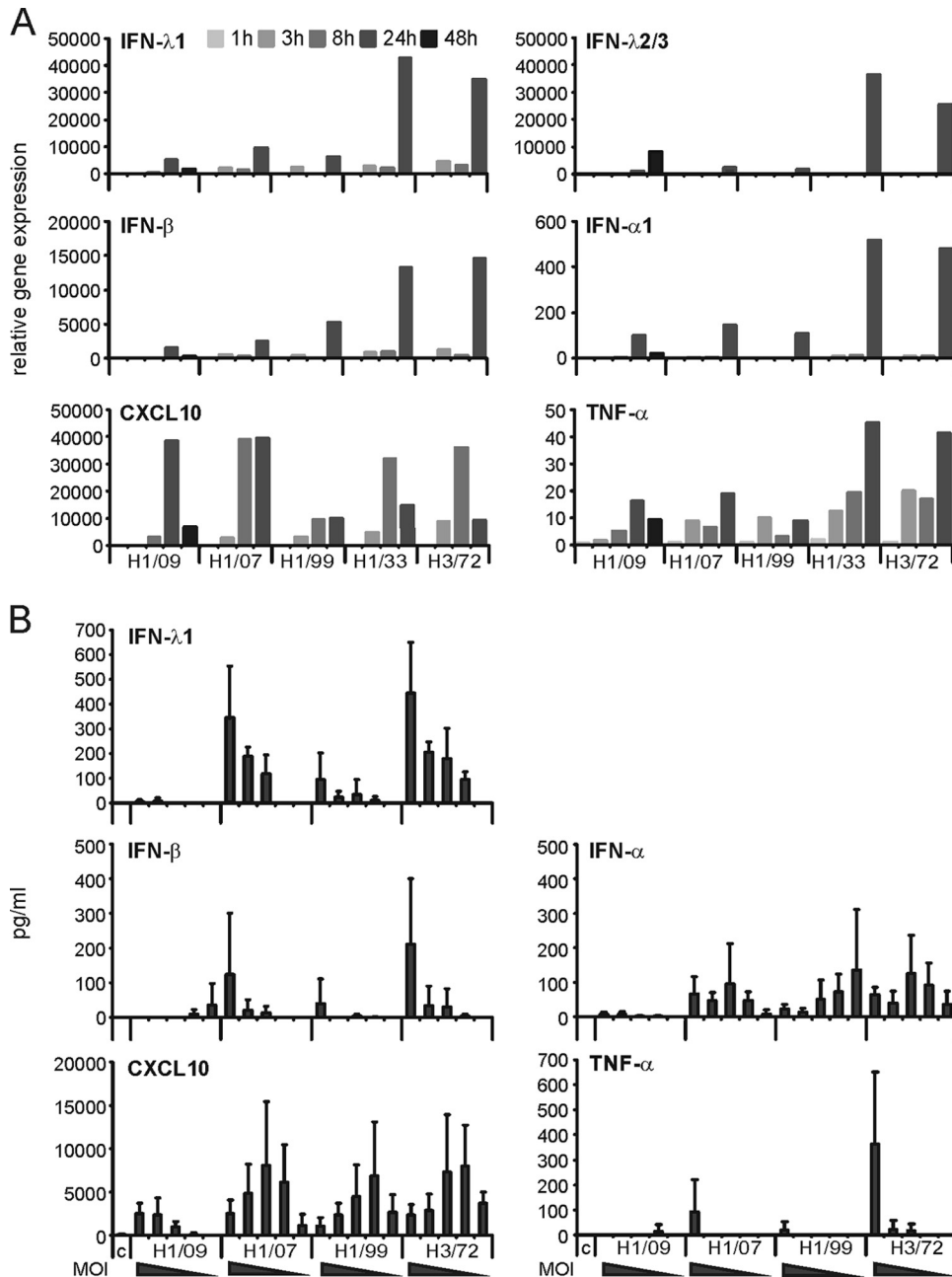


FIG. 2. The pandemic 2009 and seasonal influenza A (H1N1) viruses induce weak cytokine gene expression in DCs. (A) DCs were infected with influenza viruses A/Finland/553/2009 (H1/09), A/Brisbane/59/07 (H1/07), A/New Caledonia/20/99 (H1/99), A/WSN/33 (H1/33), and A/Udorn/72 (H3/72) at MOI of 5. Cells from different blood donors were collected at 1 h to 48 h after infection and pooled, and cellular RNA was isolated. Virus-induced cytokine expression was analyzed by quantitative RT-PCR with primers and probes for IFN-λ1, IFN-λ2/3, IFN-β, IFN-α1, CXCL10, and TNF-α. The values were normalized to 18S rRNA and presented as relative gene expression in relation to the RNA sample obtained from uninfected control cells. (B) Protein levels of IFN-λ1, IFN-β, IFN-α, CXCL10, and TNF-α measured by ELISA from the supernatants of DCs after 24 h of infection with different influenza A viruses at decreasing doses (MOI of 5, 1, 0.2, 0.04, and 0.008, respectively). The data are presented as mean values with standard deviations calculated from the results from 3 different experiments, each having cells from 2 to 4 different donors. c, control supernatant of cells without stimulation.

recent seasonal H1N1 Brisbane/07 and New Caledonia/99 viruses induced relatively weak mRNA expression of IFNs (Fig. 2A). The expression of IFN-α1, IFN-β, IFN-λ1, and IFN-λ2/3 genes was significantly higher in cells infected with the Udorn/72 H3N2 virus or the mouse-adapted WSN/33 H1N1 virus. The production of IFN protein induced by the pandemic

2009 (H1N1) virus was almost undetectable, whereas the seasonal H1N1 viruses and H3N2 virus induced clearly measurable amounts of IFN-α, IFN-β, and IFN-λ1 (Fig. 2B). The IFN-λ2/3 levels in DCs infected with any of the influenza A viruses remained undetectable.

All the studied influenza A viruses induced the expression of

the CXCL10 gene although the New Caledonia/99 virus showed the weakest ability to induce CXCL10 mRNA (Fig. 2A). The seasonal H1N1 and H3N2 viruses induced maximal CXCL10 mRNA expression already at 8 h postinfection, while the pandemic 2009 (H1N1) virus did not activate the expression of the CXCL10 gene until 24 h after infection. Thus, the 2009 A (H1N1) virus exhibited a marked delay in the expression of the CXCL10 gene. CXCL10 protein expression was stimulated by all the influenza A viruses studied although the amounts of CXCL10 remained lower in the cell supernatants collected from the cells infected with the pandemic H1N1 virus (Fig. 2B). This seems to correlate with the late expression of CXCL10 mRNA in pandemic H1N1 virus-infected DCs (Fig. 2A).

The production of proinflammatory TNF- $\alpha$  is low in DCs infected with H3N2 influenza A virus compared to that in cells infected with Sendai virus (26). Here we show that the pandemic 2009 (H1N1) virus and the other H1N1 viruses studied induced even lower expression of the TNF- $\alpha$  gene than the H3N2 type virus Udorn/72 (Fig. 2A). Also the amount of TNF- $\alpha$  protein remained lower in the H1N1 virus-infected DC culture supernatants than in the Udorn/72 H3N2 influenza virus-infected cells (Fig. 2B). Collectively, these findings demonstrate that the pandemic 2009 (H1N1) influenza virus, like the other recent H1N1 viruses, fails to efficiently activate IFN and TNF- $\alpha$  gene expression in human monocyte-derived DCs. Moreover, the pandemic 2009 (H1N1) influenza virus managed to induce only marginal production of antiviral and proinflammatory cytokines in DCs.

**The pandemic 2009 (H1N1) virus induces stronger type I IFN and CXCL10 responses in macrophages than in DCs.** Macrophages, which are rapidly recruited into the lungs during infection, play an important role in the pulmonary immune defense against influenza viruses (28). We have observed that primary human macrophages secrete a number of antiviral and proinflammatory cytokines in response to infection with seasonal influenza A viruses (20, 29, 33, 36). Moreover, highly pathogenic influenza viruses such as the avian H5N1 influenza A virus can stimulate a hyperinduction of IFNs and proinflammatory cytokines in macrophages (5). Thus, we compared the abilities of the pandemic 2009 (H1N1) virus to replicate and induce cytokine response in human DCs and macrophages.

Comparable numbers of DCs or macrophages were infected with decreasing doses of the pandemic (H1N1) influenza virus. The highest virus dose represented MOI of 10 in DCs. After 20 h of infection cellular RNA was isolated and the expression of the influenza A virus-specific M1 gene was quantitated by qRT-PCR. In order to evaluate the efficiency of viral replication, we infected MDCK cells with the pandemic (H1N1) virus at the same doses as a reference. We observed that the virus replicated with similar efficiencies in macrophages and DCs, and in both cell types viral M1 RNA expression was comparable to that seen in MDCK cells (Fig. 3A). The expression of IFN- $\alpha$  and IFN- $\beta$  genes and especially that of CXCL10 (up to 100-fold) were higher in macrophages than in DCs (Fig. 3B). Between the two cell types there were only marginal differences in the expression of IFN- $\lambda$ 1, IFN- $\lambda$ 2/3, and TNF- $\alpha$  genes. These findings indicate that macrophages are highly susceptible to the pandemic 2009 (H1N1) virus and that they

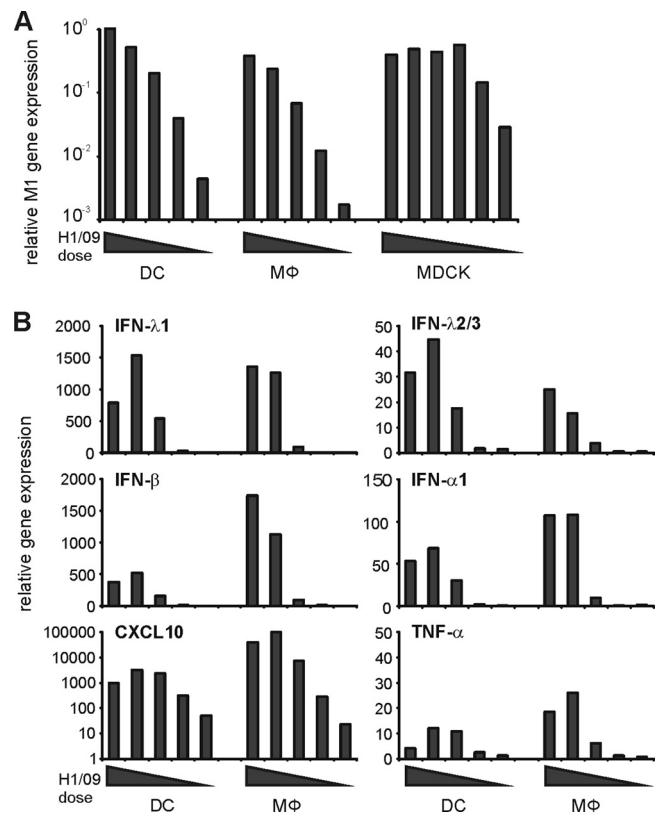


FIG. 3. Comparison of pandemic influenza A (H1N1) virus-induced cytokine responses in monocyte-derived DCs and macrophages. Monocyte-derived human DCs and macrophages and MDCK cells were infected with decreasing doses (representing MOI of 10, 2, 0.4, 0.08, or 0.016 in DCs) of A/Finland/553/2009 virus. Cells were collected at 20 h after infection and pooled, cellular RNA was isolated, and qRT-PCR was carried out. (A) Virus replication in DCs, macrophages, and MDCK cells as measured by quantifying influenza virus M1 gene expression by qRT-PCR. The values were normalized to 18S rRNA and presented as relative copy numbers. (B) Virus dose-dependent expression of IFN- $\lambda$ 1, IFN- $\lambda$ 2/3, IFN- $\beta$ , IFN- $\alpha$ 1, CXCL10, and TNF- $\alpha$  mRNAs in DCs and macrophages as analyzed by qRT-PCR. The values were normalized to 18S rRNA, and the fold inductions were calculated from the uninfected control cells.

express more antiviral and proinflammatory cytokines than DCs.

**Antiviral activity of type I and type III IFNs against the 2009 A (H1N1) virus.** IFNs, which consist of type I (IFN- $\alpha$ / $\beta$ ), type II (IFN- $\gamma$ ), and type III (IFN- $\lambda$ 1 to - $\lambda$ 3) IFN family members, have an important role in regulating direct and indirect antiviral responses in the host (16, 46). Type I and type III IFNs, especially, contribute to innate antiviral responses against influenza viruses (23, 27, 30, 39). In order to determine the sensitivity of pandemic 2009 (H1N1) virus to the antiviral actions of IFNs, DCs and macrophages were pretreated with IFN- $\alpha$ , IFN- $\beta$ , IFN- $\lambda$ 1, or IFN- $\lambda$ 3 prior to infection with the pandemic 2009 or seasonal H1N1 Brisbane/07 viruses. The antiviral activity of different IFN types was analyzed as a reduction in viral glycoprotein expression by flow cytometry or as viral M1 gene-specific RNA expression by quantitative RT-PCR.

We observed that IFN- $\alpha$  and IFN- $\beta$  displayed substantial

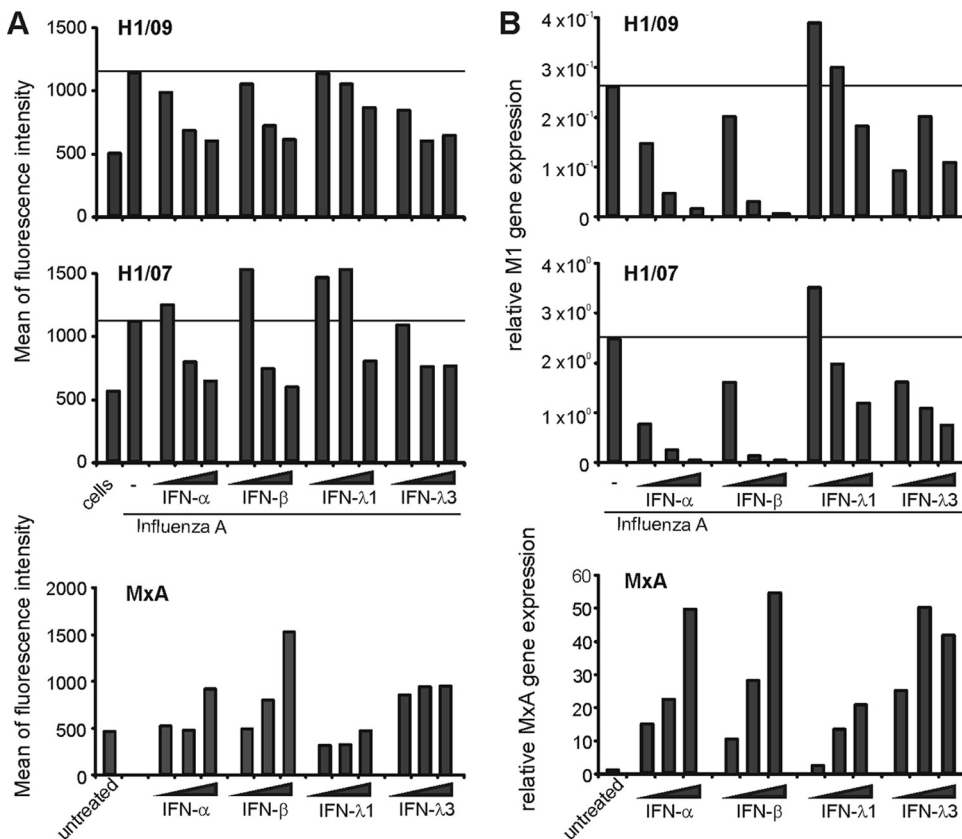


FIG. 4. Pandemic 2009 (H1N1) influenza A virus is highly sensitive to the antiviral activity of IFNs. Monocyte-derived human DCs and macrophages from four different donors were primed with different doses of IFN- $\alpha$  (1, 10, or 100 IU/ml), IFN- $\beta$  (1, 10, or 100 IU/ml), IFN- $\lambda$ 1 (1, 10, or 100 ng/ml), or IFN- $\lambda$ 3 (1, 10, or 100 ng/ml) for 16 h, followed by infection with pandemic A/Finland/553/2009 (H1/09) and seasonal A/Brisbane/59/07 (H1/07) influenza virus at MOI of 2 for 18 h. The effect of IFNs on the replication of the viruses and MxA gene expression was analyzed at the protein level by FACS in DCs (A) and at the mRNA level by qRT-PCR in macrophages (B). (A) Expression of cell surface influenza A virus glycoproteins in DCs after priming the cells with different doses of IFN- $\alpha$ , IFN- $\beta$ , IFN- $\lambda$ 1, or IFN- $\lambda$ 3. To control the effectiveness of IFN priming, induction of MxA protein was measured in a sample of cells prior to the infection. The expression of viral glycoproteins and MxA protein was detected by FACS, and the results are expressed as the means of fluorescence intensities. (B) Expression of the influenza A virus M1 gene in IFN- $\alpha$ -, IFN- $\beta$ -, IFN- $\lambda$ 1-, or IFN- $\lambda$ 3-primed, virus-infected macrophages. Cellular MxA gene expression was analyzed in macrophages obtained before virus infection. The values were normalized to 18S rRNA and presented as relative MxA gene expression in relation to the RNA sample obtained from untreated (no IFN stimulation) and uninfected control cells or as relative copy number of M1 RNA.

antiviral activity against both studied influenza A viruses. The expression of viral glycoproteins on DCs was almost completely eliminated already with 10 IU/ml of type I IFNs (Fig. 4A). IFN- $\lambda$ s had a weaker antiviral activity than type I IFNs. There was, however, a clear reduction in viral glycoprotein expression, especially in the IFN- $\lambda$ 3-pretreated and pandemic virus-infected cells, while the corresponding decrease of seasonal virus glycoprotein expression was not detected (Fig. 4A). We also studied the antiviral effects of IFNs in macrophages and observed a very similar inhibition of virus replication in response to the IFN pretreatment (Fig. 4B). The expression of the viral M1 gene was almost completely inhibited by IFN- $\alpha$  and IFN- $\beta$ , while IFN- $\lambda$ 1 and IFN- $\lambda$ 3 pretreatment had a weaker inhibitory effect on virus replication in macrophages (Fig. 4B).

To ensure the biological activity of IFNs in DCs and macrophages, we measured the expression of the MxA gene in uninfected cells. The MxA gene is specifically and sensitively under the regulation of type I IFNs (30). Both IFN- $\alpha$  and IFN- $\beta$  induced a dose-dependent expression of MxA (Fig. 4).

IFN- $\lambda$ s, too, induced the expression of the MxA gene dose dependently, but the induction was weaker than the type I IFN-induced expression. These data support the concept that type I IFNs have a superior antiviral activity over type III IFNs in human primary macrophages and DCs.

**DISCUSSION**

The novel pandemic swine origin 2009 influenza A (H1N1) virus has spread all over the world faster than any previous pandemic influenza virus. Efficient transmission of the virus among the human population raises the concern of the possibility of hundreds of millions of people becoming infected, with many of the individuals suffering from severe forms of infection. The pandemic 2009 A (H1N1) virus is a completely new reassortant virus, with gene segments originating from different swine and avian influenza viruses. Therefore, the human population appears to have limited immunity against the virus (8). Since the world is encountering a completely new type of virus, we found it relevant and urgent to study the virus-host

relationships in human cells. Our analyses revealed that the pandemic (H1N1) virus replicated efficiently in primary human monocyte-derived DCs and macrophages, where it induced a weak innate immune response. However, the virus was very sensitive to the antiviral activity of various types of IFNs. These characteristics of the 2009 pandemic (H1N1) influenza A virus resemble very much those of the recent human seasonal H1N1 type viruses.

Analysis of the replication of the pandemic (H1N1) virus revealed that it can readily infect primary human DCs and macrophages and that it replicates with a speed comparable to that of the seasonal human H1N1 and H3N2 viruses. Within a couple of hours after infection, viral gene expression was taking place at high levels, and there were no major differences between the studied viruses. This indicates that the pandemic virus can efficiently bind to, enter, and initiate its replication in human DCs and macrophages. The data also showed that these cell types had no inherent resistance to the pandemic virus, since the cells underwent apoptotic/necrotic cell death starting at 24 h after the infection.

In influenza virus infection the host resistance to the virus is dependent on innate IFN-mediated and other cytokine-mediated immune responses, which play a part in restricting the replication of the virus and initiating the adaptive immune responses. In the present study, we demonstrated that the pandemic 2009 (H1N1) virus induced only a weak antiviral response, as evidenced by a weak induction of IFN- $\alpha$ , IFN- $\beta$ , IFN- $\lambda$ 1, and IFN- $\lambda$ 2/3 genes (Fig. 2). The expression of TNF- $\alpha$ , too, remained at a low level in primary human DCs. A robust cytokine response is generally associated with highly pathogenic influenza viruses such as the pandemic 1918 A (H1N1) and avian H5N1 viruses. The highly pathogenic H5N1 avian influenza causes lethal infections with severe viral pneumonia in humans (40). Histological findings in the mouse model indicate a massive infiltration of immune cells, such as neutrophils, macrophages, and DCs, into the injured lung tissues, leading to enhanced tissue damage (28). It is also suggested that, in fatal human cases of the H5N1 infections, a virus-induced "cytokine storm" contributes to the severity of the disease (5). Moreover, in human macrophages the highly pathogenic H5N1 virus induces very strong cytokine and IFN responses (13). However, in spite of the large genetic and antigenic distances of the pandemic 2009 A (H1N1) virus from the seasonal human H1 viruses, the pandemic virus-induced cytokine responses were still similar to those seen with seasonal human H1N1 viruses. Consistent with observations presented here we have previously reported that H3N2 influenza A virus could induce relatively weak IFN and TNF- $\alpha$  responses in human DCs (26). It was of interest that 2009 A (H1N1) virus induced an even weaker innate immune response than H3N2 type Udorn/72 virus, although it is notable that the Udorn/72 and WSN/33 influenza strains are well-adapted laboratory strains, which could have affected to their capabilities to induce immune responses (Fig. 2). These results, taken together, suggest that the pandemic 2009 A (H1N1) virus could escape the innate immune defense by impeding cytokine responses. This phenomenon is characteristic of influenza viruses in general since they have an ability to inhibit the host's antiviral responses by multiple mechanisms (11, 17).

The CXCL10 gene was induced in DCs, and there were

apparently no major differences between the analyzed virus strains. The kinetics of CXCL10 gene expression in the 2009 A (H1N1) virus-infected cells was, however, slower than that induced by seasonal H1N1 or H3N2 viruses. This also led to lower secretion of CXCL10 protein from pandemic H1N1 virus-infected DCs. It is unclear whether the delayed production of NK and Th1 cell-recruiting CXCL10 in DCs has an effect on the activation of innate and adaptive cellular responses during 2009 A (H1N1) virus infection. Although DCs seem to produce CXCL10 late during the infection, it is likely that the efficient CXCL10 production in macrophages (Fig. 3) would compensate for the late production in DCs and ensure proper cellular responses to the pandemic virus infection. Previously, it was shown that an H3N2 type virus induces efficient IFN and TNF- $\alpha$  responses in human macrophages (29). In the present study we observed a slightly more pronounced expression of IFN- $\alpha$  and IFN- $\beta$  genes in macrophages than in DCs in response to the pandemic 2009 virus infection (Fig. 3). The data suggest that macrophage-derived chemokines and cytokines have an important role in inducing antiviral and inflammatory responses also in the case of pandemic 2009 (H1N1) virus infection. Further evidence for an important role of immune cell-derived cytokines comes from recent observations, which show that lung epithelial cells (A549 cell line) require IFN- $\alpha$  and/or TNF- $\alpha$  priming before efficient influenza A virus-induced cytokine production takes place (21, 45).

IFNs can inhibit the replication of a wide variety of viruses. The pandemic 2009 A (H1N1) influenza virus also turned out to be sensitive to the antiviral actions of IFN- $\alpha$  and IFN- $\beta$  (Fig. 4). Interestingly, the 2009 A (H1N1) virus was also sensitive to the antiviral actions of IFN- $\lambda$ s (especially that of IFN- $\lambda$ 3), even to a greater extent than the seasonal H1N1 virus. Type III IFNs have similar antiviral properties as type I IFNs, although they seem to be more multifunctional immune regulators than type I IFNs (1, 6, 16, 27). However, the expression of the IFN- $\lambda$  receptor is rather restricted, and the relatively weak response to IFN- $\lambda$ s in DCs and macrophages could well be due to low receptor expression in these cell types. Recent evidence from mice suggests that IFN- $\lambda$ s have an important role in anti-influenza defense, especially in the lungs (23, 39). Obviously more work is needed to test the response of primary human lung epithelial cells to IFN- $\lambda$ s and the therapeutic benefits of IFN- $\lambda$  for patients infected with the 2009 A (H1N1) virus. Since type I and type III IFNs showed very good or moderate *in vitro* antiviral activity against the pandemic 2009 A (H1N1) virus, respectively, they could provide additional means to treat patients with severe forms of pandemic influenza virus infections, especially if the virus develops resistance against neuraminidase inhibitors.

So far, all experimental studies on the pandemic 2009 A (H1N1) virus have been carried out with early isolates of the virus. The currently circulating 2009 A (H1N1) viruses are genetically very uniform, and only limited genetic evolution has been observed (38). The pandemic 2009 A (H1N1) viruses are very distinct from the seasonal human A (H1N1) viruses, but according our present study, the cytokine responses to both these viruses are rather mild. However, there is a great concern that the 2009 (H1N1) virus could evolve by a selective pressure into a more pathogenic variant in humans. Thus, systematic worldwide surveillance of circulating pandemic and seasonal

influenza viruses is still important in monitoring the evolution of the viruses and characterizing their geno- and phenotypic features in detail. Phenotypic characterization would include pathogenesis studies on experimental animals and human cell culture systems, as presented here.

In summary, the present study demonstrates that the pandemic 2009 A (H1N1) virus replicates efficiently in primary human immune cells and that the virus induces a very similar cytokine-mediated immune response as the seasonal H1N1 influenza viruses do. Although the pandemic virus at least partially evades the host innate immune response by interfering with cytokine gene expression in human DCs and macrophages, the virus is highly sensitive to the antiviral actions of IFNs. Thus, IFNs may provide an additional means to treat patients suffering from severe pandemic influenza virus infections, especially if significant resistance against oseltamivir and other antiviral drug emerges.

#### ACKNOWLEDGMENTS

This study was partially funded by the Medical Research Council of the Academy of Finland, the Sigrid Juselius Foundation, and the Ministry of Health and Social Affairs.

We thank Riitta Santanen, Hanna Valtonen, Sari Maljanen, Mari Aaltonen, Valtteri Järvinen, and Tuulia Niska for expert technical assistance.

#### REFERENCES

- Ank, N., H. West, C. Bartholdy, K. Eriksson, A. R. Thomsen, and S. R. Paludan. 2006. Lambda interferon (IFN-lambda), a type III IFN, is induced by viruses and IFNs and displays potent antiviral activity against select virus infections in vivo. *J. Virol.* **80**:4501–4509.
- Cantell, K., and J. Pirhonen. 1996. IFN-gamma enhances production of IFN-alpha in human macrophages but not in monocytes. *J. Interferon Cytokine Res.* **16**:461–463.
- Coccia, E. M., M. Severa, E. Giacomini, D. Monneron, M. E. Remoli, I. Julkunen, M. Cella, R. Lande, and G. Uze. 2004. Viral infection and Toll-like receptor agonists induce a differential expression of type I and lambda interferons in human plasmacytoid and monocyte-derived dendritic cells. *Eur. J. Immunol.* **34**:796–805.
- Dawood, F. S., S. Jain, L. Finelli, M. W. Shaw, S. Lindstrom, R. J. Garten, L. V. Gubareva, X. Xu, C. B. Bridges, and T. M. Uyeki. 2009. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. *N. Engl. J. Med.* **360**:2605–2615.
- de Jong, M. D., C. P. Simmons, T. T. Thanh, V. M. Hien, G. J. Smith, T. N. Chau, D. M. Hoang, N. V. Chau, T. H. Khanh, V. C. Dong, P. T. Qui, B. V. Cam, Q. Ha do, Y. Guan, J. S. Peiris, N. T. Chinh, T. T. Hien, and F. Farrar. 2006. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. *Nat. Med.* **12**:1203–1207.
- Dellgren, C., H. H. Gad, O. J. Hamming, J. Melchjorsen, and R. Hartmann. 2009. Human interferon-lambda3 is a potent member of the type III interferon family. *Genes Immun.* **10**:125–131.
- Fraser, C., C. A. Donnelly, S. Cauchemez, W. P. Hanage, M. D. Van Kerkhove, T. D. Hollingsworth, J. Griffin, R. F. Baggaley, H. E. Jenkins, E. J. Lyons, T. Jombart, W. R. Hinsley, N. C. Grassly, F. Balloux, A. C. Ghani, N. M. Ferguson, A. Rambaut, O. G. Pybus, H. Lopez-Gatell, C. M. Alpujch-Aranda, I. B. Chapela, E. P. Zavala, D. M. Guevara, F. Checchi, E. Garcia, S. Hugonnet, and C. Roth. 2009. Pandemic potential of a strain of influenza A (H1N1): early findings. *Science* **324**:1557–1561.
- Garten, R. J., C. T. Davis, C. A. Russell, B. Shu, S. Lindstrom, A. Balish, W. M. Sessions, X. Xu, E. Skepner, V. Deyde, M. Okomo-Adhiambo, L. Gubareva, J. Barnes, C. B. Smith, S. L. Emery, M. J. Hillman, P. Rivailier, J. Smagala, M. de Graaf, D. F. Burke, R. A. Fouchier, C. Pappas, C. M. Alpujch-Aranda, H. Lopez-Gatell, H. Olivera, I. Lopez, C. A. Myers, D. Faix, P. J. Blair, C. Yu, K. M. Keene, P. D. Dotson, Jr., D. Boxrud, A. R. Sambol, S. H. Abid, K. St George, T. Bannerman, A. L. Moore, D. J. Stringer, P. Blevins, G. J. Demmler-Harrison, M. Ginsberg, P. Kriner, S. Waterman, S. Smole, H. F. Guevara, E. A. Belongia, P. A. Clark, S. T. Beatrice, R. Donis, J. Katz, L. Finelli, C. B. Bridges, M. Shaw, D. B. Jernigan, T. M. Uyeki, D. J. Smith, A. I. Klimov, and N. J. Cox. 2009. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. *Science* **325**:197–201.
- Gerosa, F., A. Gobbi, P. Zorzi, S. Burg, F. Briere, G. Carra, and G. Trinchieri. 2005. The reciprocal interaction of NK cells with plasmacytoid or myeloid dendritic cells profoundly affects innate resistance functions. *J. Immunol.* **174**:727–734.
- Grayson, M. H., and M. J. Holtzman. 2007. Emerging role of dendritic cells in respiratory viral infection. *J. Mol. Med.* **85**:1057–1068.
- Hale, B. G., R. E. Randall, J. Ortin, and D. Jackson. 2008. The multifunctional NS1 protein of influenza A viruses. *J. Gen. Virol.* **89**:2359–2376.
- Hancock, K., V. Veguilla, X. Lu, W. Zhong, E. N. Butler, H. Sun, F. Liu, L. Dong, J. R. Devos, P. M. Gargiullo, T. L. Brammer, N. J. Cox, T. M. Tumpey, and J. M. Katz. 2009. Cross-reactive antibody responses to the 2009 pandemic H1N1 influenza virus. *N. Engl. J. Med.* **361**:1945–1952.
- Hui, K. P., S. M. Lee, C. Y. Cheung, I. H. Ng, L. L. Poon, Y. Guan, N. Y. Ip, A. S. Lau, and J. S. Peiris. 2009. Induction of proinflammatory cytokines in primary human macrophages by influenza A virus (H5N1) is selectively regulated by IFN regulatory factor 3 and p38 MAPK. *J. Immunol.* **182**:1088–1098.
- Itoh, Y., K. Shinya, M. Kiso, T. Watanabe, Y. Sakoda, M. Hatta, Y. Muramoto, D. Tamura, Y. Sakai-Yagawa, T. Noda, S. Sakabe, M. Imai, Y. Hatta, S. Watanabe, C. Li, S. Yamada, K. Fujii, S. Murakami, H. Imai, S. Kakugawa, M. Ito, R. Takano, K. Iwatsuki-Horimoto, M. Shimajima, T. Horimoto, H. Goto, K. Takahashi, A. Makino, H. Ishigaki, M. Nakayama, M. Okamatsu, D. Warshauer, P. A. Shult, R. Saito, H. Suzuki, Y. Furuta, M. Yamashita, K. Mitamura, K. Nakano, M. Nakamura, R. Brockman-Schneider, H. Mitamura, M. Yamazaki, N. Sugaya, M. Suresh, M. Ozawa, G. Neumann, J. Gern, H. Kida, K. Ogasawara, and Y. Kawaoka. 2009. In vitro and in vivo characterization of new swine-origin H1N1 influenza viruses. *Nature* **460**:1021–1025.
- Julkunen, I., A. Hautanen, and J. Keski-Oja. 1983. Interaction of viral envelope glycoproteins with fibronectin. *Infect. Immun.* **40**:876–881.
- Kotenko, S. V., G. Gallagher, V. V. Baurin, A. Lewis-Antes, M. Shen, N. K. Shah, J. A. Langer, F. Sheikh, H. Dickensheets, and R. P. Donnelly. 2003. IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. *Nat. Immunol.* **4**:69–77.
- Krug, R. M., W. Yuan, D. L. Noah, and A. G. Latham. 2003. Intracellular warfare between human influenza viruses and human cells: the roles of the viral NS1 protein. *Virology* **309**:181–189.
- Lehtonen, A., H. Ahlfors, V. Veckman, M. Miettinen, R. Lahesmaa, and I. Julkunen. 2007. Gene expression profiling during differentiation of human monocytes to macrophages or dendritic cells. *J. Leukoc. Biol.* **82**:710–720.
- Maines, T. R., A. Jayaraman, J. A. Belser, D. A. Wadford, C. Pappas, H. Zeng, K. M. Gustin, M. B. Pearce, K. Viswanathan, Z. H. Shriver, R. Raman, N. J. Cox, R. Sasisekharan, J. M. Katz, and T. M. Tumpey. 2009. Transmission and pathogenesis of swine-origin 2009 A(H1N1) influenza viruses in ferrets and mice. *Science* **325**:484–487.
- Matikainen, S., J. Pirhonen, M. Miettinen, A. Lehtonen, C. Govenius-Vintola, T. Sareneva, and I. Julkunen. 2000. Influenza A and Sendai viruses induce differential chemokine gene expression and transcription factor activation in human macrophages. *Virology* **276**:138–147.
- Matikainen, S., J. Siren, J. Tissari, V. Veckman, J. Pirhonen, M. Severa, Q. Sun, R. Lin, S. Meri, G. Uze, J. Hiscott, and I. Julkunen. 2006. Tumor necrosis factor alpha enhances influenza A virus-induced expression of antiviral cytokines by activating RIG-I gene expression. *J. Virol.* **80**:3515–3522.
- Moltedo, B., C. B. Lopez, M. Pazos, M. I. Becker, T. Hermesh, and T. M. Moran. 2009. Cutting edge: stealth influenza virus replication precedes the initiation of adaptive immunity. *J. Immunol.* **183**:3569–3573.
- Mordstein, M., G. Kochs, L. Dumoutier, J. C. Renaud, S. R. Paludan, K. Klucher, and P. Staeheli. 2008. Interferon-lambda contributes to innate immunity of mice against influenza A virus but not against hepatotropic viruses. *PLoS Pathog.* **4**:e1000151.
- Munster, V. J., E. de Wit, J. M. van den Brand, S. Herfst, E. J. Schrauwen, T. M. Bestebroer, D. van de Vijver, C. A. Boucher, M. Koopmans, G. F. Rimmelzwaan, T. Kuiken, A. D. Osterhaus, and R. A. Fouchier. 2009. Pathogenesis and transmission of swine-origin 2009 A(H1N1) influenza virus in ferrets. *Science* **325**:481–483.
- Noah, D. L., K. Y. Twu, and R. M. Krug. 2003. Cellular antiviral responses against influenza A virus are countered at the posttranscriptional level by the viral NS1A protein via its binding to a cellular protein required for the 3' end processing of cellular pre-mRNAs. *Virology* **307**:386–395.
- Osterlund, P., V. Veckman, J. Siren, K. M. Klucher, J. Hiscott, S. Matikainen, and I. Julkunen. 2005. Gene expression and antiviral activity of alpha/beta interferons and interleukin-29 in virus-infected human myeloid dendritic cells. *J. Virol.* **79**:9608–9617.
- Osterlund, P. I., T. E. Pietila, V. Veckman, S. V. Kotenko, and I. Julkunen. 2007. IFN regulatory factor family members differentially regulate the expression of type III IFN (IFN-lambda) genes. *J. Immunol.* **179**:3434–3442.
- Perrone, L. A., J. K. Plowden, A. Garcia-Sastre, J. M. Katz, and T. M. Tumpey. 2008. H5N1 and 1918 pandemic influenza virus infection results in early and excessive infiltration of macrophages and neutrophils in the lungs of mice. *PLoS Pathog.* **4**:e1000115.
- Pirhonen, J., T. Sareneva, M. Kurimoto, I. Julkunen, and S. Matikainen. 1999. Virus infection activates IL-1 beta and IL-18 production in human macrophages by a caspase-1-dependent pathway. *J. Immunol.* **162**:7322–7329.



30. Ronni, T., S. Matikainen, T. Sareneva, K. Melen, J. Pirhonen, P. Keskinen, and I. Julkunen. 1997. Regulation of IFN-alpha/beta, MxA, 2',5'-oligoadenylate synthetase, and HLA gene expression in influenza A-infected human lung epithelial cells. *J. Immunol.* **158**:2363–2374.
31. Ronni, T., K. Melen, A. Malygin, and I. Julkunen. 1993. Control of IFN-inducible MxA gene expression in human cells. *J. Immunol.* **150**:1715–1726.
32. Ronni, T., T. Sareneva, J. Pirhonen, and I. Julkunen. 1995. Activation of IFN-alpha, IFN-gamma, MxA, and IFN regulatory factor 1 genes in influenza A virus-infected human peripheral blood mononuclear cells. *J. Immunol.* **154**:2764–2774.
33. Sareneva, T., S. Matikainen, M. Kurimoto, and I. Julkunen. 1998. Influenza A virus-induced IFN-alpha/beta and IL-18 synergistically enhance IFN-gamma gene expression in human T cells. *J. Immunol.* **160**:6032–6038.
34. Seo, S. H., R. Webby, and R. G. Webster. 2004. No apoptotic deaths and different levels of inductions of inflammatory cytokines in alveolar macrophages infected with influenza viruses. *Virology* **329**:270–279.
35. Shinde, V., C. B. Bridges, T. M. Uyeki, B. Shu, A. Balish, X. Xu, S. Lindstrom, L. V. Gubareva, V. Deyde, R. J. Garten, M. Harris, S. Gerber, S. Vagasky, F. Smith, N. Pascoe, K. Martin, D. Dufficy, K. Ritger, C. Conover, P. Quinlisk, A. Klimov, J. S. Bresee, and L. Finelli. 2009. Triple-reassortant swine influenza A (H1) in humans in the United States, 2005–2009. *N. Engl. J. Med.* **360**:2616–2625.
36. Siren, J., J. Pirhonen, I. Julkunen, and S. Matikainen. 2005. IFN-alpha regulates TLR-dependent gene expression of IFN-alpha, IFN-beta, IL-28, and IL-29. *J. Immunol.* **174**:1932–1937.
37. Siren, J., T. Sareneva, J. Pirhonen, M. Strengell, V. Veckman, I. Julkunen, and S. Matikainen. 2004. Cytokine and contact-dependent activation of natural killer cells by influenza A or Sendai virus-infected macrophages. *J. Gen. Virol.* **85**:2357–2364.
38. Smith, G. J., D. Vijaykrishna, J. Bahl, S. J. Lycett, M. Worobey, O. G. Pybus, S. K. Ma, C. L. Cheung, J. Raghvani, S. Bhatt, J. S. Peiris, Y. Guan, and A. Rambaut. 2009. Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. *Nature* **459**:1122–1125.
39. Sommereyns, C., S. Paul, P. Staeheli, and T. Michiels. 2008. IFN-lambda (IFN-lambda) is expressed in a tissue-dependent fashion and primarily acts on epithelial cells in vivo. *PLoS Pathog.* **4**:e1000017.
40. Subbarao, K., A. Klimov, J. Katz, H. Regnery, W. Lim, H. Hall, M. Perdue, D. Swayne, C. Bender, J. Huang, M. Hemphill, T. Rowe, M. Shaw, X. Xu, K. Fukuda, and N. Cox. 1998. Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. *Science* **279**:393–396.
41. Tyner, J. W., O. Uchida, N. Kajiwara, E. Y. Kim, A. C. Patel, M. P. O'Sullivan, M. J. Walter, R. A. Schwendener, D. N. Cook, T. M. Danoff, and M. J. Holtzman. 2005. CCL5-CCR5 interaction provides antiapoptotic signals for macrophage survival during viral infection. *Nat. Med.* **11**:1180–1187.
42. Ward, C. L., M. H. Dempsey, C. J. Ring, R. E. Kempson, L. Zhang, D. Gor, B. W. Snowden, and M. Tisdale. 2004. Design and performance testing of quantitative real time PCR assays for influenza A and B viral load measurement. *J. Clin. Virol.* **29**:179–188.
43. Veckman, V., M. Miettinen, S. Matikainen, R. Lande, E. Giacomini, E. M. Coccia, and I. Julkunen. 2003. Lactobacilli and streptococci induce inflammatory chemokine production in human macrophages that stimulates Th1 cell chemotaxis. *J. Leukoc. Biol.* **74**:395–402.
44. Veckman, V., M. Miettinen, J. Pirhonen, J. Siren, S. Matikainen, and I. Julkunen. 2004. Streptococcus pyogenes and Lactobacillus rhamnosus differentially induce maturation and production of Th1-type cytokines and chemokines in human monocyte-derived dendritic cells. *J. Leukoc. Biol.* **75**:764–771.
45. Veckman, V., P. Osterlund, R. Fagerlund, K. Melen, S. Matikainen, and I. Julkunen. 2006. TNF-alpha and IFN-alpha enhance influenza-A-virus-induced chemokine gene expression in human A549 lung epithelial cells. *Virology* **345**:96–104.
46. Zhang, S. Y., S. Boisson-Dupuis, A. Chappier, K. Yang, J. Bustamante, A. Puel, C. Picard, L. Abel, E. Jouanguy, and J. L. Casanova. 2008. Inborn errors of interferon (IFN)-mediated immunity in humans: insights into the respective roles of IFN-alpha/beta, IFN-gamma, and IFN-lambda in host defense. *Immunol. Rev.* **226**:29–40.