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Regulation of Virus-Induced IL-12 and IL-23 Expression in Human Macrophages[1]

Jaana Pirhonen,[2] Sampsa Matikainen, and Ilkka Julkunen

IL-23 is a novel cytokine that promotes the proliferation of naive and memory T cells and stimulates their IFN-γ production. Besides functional similarities, IL-23 bears structural resemblance to IL-12. Biologically active IL-23 is a heterodimer whose p40 subunit is identical to IL-12p40 while its p19 subunit is distantly related to IL-12p35. In the present study we demonstrate that human monocyte-derived macrophages are able to produce IL-23 in response to virus infection. Sendai virus stimulates the expression of p19 and p40 mRNAs in macrophages. Furthermore, it enhances p35 mRNA expression and the production of IL-12. Influenza A virus, in contrast, fails to stimulate IL-12 or IL-23 expression in macrophages. IL-12 and IL-23 contribute to the IFN-γ-inducing activity that cell culture supernatant from Sendai virus-infected macrophages show in NK-92 cells. The induction of IFN-γ production occurs in concert with IFN-αβ and IL-18, which are also secreted from the virus-infected cells. The IFN-γ-inducing activity is inhibited by IL-4, which down-regulates the transcription of *p19* and *p40* genes and the secretion of IFN-αβ, IL-12, and IL-18. IFN-γ, in contrast, up-regulates the p19 and p40 mRNA expression in Sendai virus infection. Thus, IL-4 and IFN-γ serve as opposing factors in the regulation of IFN-γ-inducing cytokines, including IL-23, in macrophages. ***The Journal of Immunology*****, 2002, 169: 5673–5678.**

Macrophages play a key role in the development of innate and adaptive immune responses against viral infections. Activated macrophages elicit direct antiviral effects through phagocytosis and secretion of cytokines that inhibit virus replication and promote inflammation. Indirect effects of virus-infected macrophages leading to humoral and cellular immune responses are mediated by their Ag presentation and, again, by soluble cytokines. Of multiple macrophage-derived cytokines, IFN-αβ, IL-12, and IL-18 are particularly important in the interactions between the innate and adaptive arms of immunity. IFN-αβ possesses antiviral activity against several viruses and, like, IL-12 and IL-18, has proinflammatory and chemotactic properties (1–3). First, IFN-αβ, IL-12, and IL-18 direct the immune response against viruses toward cell-mediated immunity. They enhance the generation and activity of cytotoxic NK and T cells and favor Th1 differentiation. In response to synergistic actions of IFN-αβ, IL-12 and IL-18 NK and T cells produce IFN-γ (4–8), which, consecutively, is critically required for macrophage activation (9).

Recently, a new IFN-γ-inducing cytokine, IL-23, was discovered (10). Functional IL-23 is a heterodimer that shares its p40 subunit with IL-12. Another subunit of IL-23 is p19, a novel protein that is distantly related to p35 of IL-12. Biological properties of IL-23 result from interaction of p19p40 complex with IL-12Rβ1, the p40-specific component of IL-12R, and an unique p19p40-specific receptor component termed IL-23R (10, 11). Like engagement of IL-12R by IL-12, binding of IL-23 to its receptor complex activates Janus kinase-Stat signaling cascade (10, 11). Not surprisingly, IL-23 has biological activities comparable to though distinct from IL-12. Similar to IL-12, IL-23 enhances proliferation of T cells and increases their IFN-γ production (10). Abnormal phenotype of p19 transgenic mice resembles that of IL-12 transgenic mice (12), and this suggests more functional similarities between IL-23 and IL-12.

While IFN-αβ and IL-18 are shown to be essential for the immunity to viruses (1, 13), IL-12 is proposed to play a modest part in the antiviral resistance. In mice IL-12 does contribute to early control of several virus infections, including that of influenza A (14–16), whereas certain viruses, like measles virus and HIV, can selectively inhibit IL-12 production and induce immunosuppression (17–19). The role of IL-23 in microbial infections is still an unexplored area. Human and murine dendritic cells secrete IL-23 after activation with LPS (10), but nothing is known about IL-23 expression during viral infections. We have previously shown that influenza A and Sendai virus infections in macrophages generate different expression patterns of IFN-αβ and IL-18 (20). Sendai virus is a better enhancer of IFN-αβ production, whereas influenza A infection results in a more pronounced IL-18 secretion that is partly regulated by IFN-αβ through caspase activation (21). In this report, we demonstrate that Sendai virus-infected human primary macrophages also produce IL-12 and IL-23. Sendai virus enhances mRNA expression of p19, p35, and p40 subunits of IL-12 and IL-23. Thus, Sendai virus is a potential stimulator of four distinct IFN-γ-inducing cytokines in macrophages. Influenza A virus infection, instead, does not induce p19, p35, or p40 mRNA expression, which supports the concept that IL-12- and IL-23-independent, but IFN-αβ- and IL-18-dependent, mechanisms of IFN-γ production are operative in influenza A virus infection.

Materials and Methods

Isolation of peripheral blood-derived macrophages

Macrophages from healthy blood donors were isolated and purified as previously described (20). In brief, to obtain macrophages PBLs were subjected to Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) gradient centrifugation and depleted from lymphocytes by allowing monocytes to adhere onto six-well cell culture plates (Falcon Multiwell; BD Biosciences,

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Franklin Lakes, NJ). Monocytes were grown into differentiated macrophages by culturing them for 2 wk in serum-free medium (Life Technologies, Gaithersburg, MD) supplemented with GM-CSF (10 ng/ml; Schering-Plough, Innishannon, Ireland).

Virus stocks and infections

Human pathogenic influenza A virus (strain Beijing/353/89, H3N2) and murine Sendai virus (strain Cantell) were grown as previously described (20). Macrophages were infected with 250 hemagglutination units per milliliter of influenza A and 150 hemagglutination units per milliliter of Sendai virus in serum-free medium. After adsorption for 1 h, virus inoculum was removed and the cells were washed with PBS and fed with RPMI 1640 medium containing 10% FCS. The cells and cell culture supernatants were harvested 3–24 h after the infection and samples for Northern blotting, ELISA, and biological IFN analyses were prepared. Each sample represents a pool of separately infected macrophages from four to six different donors.

Cytokines and Abs

Highly purified human leukocyte IFN-α was provided by the Finnish Red Cross Blood Transfusion Service (Helsinki, Finland). Recombinant human IL-4 and IL-12 were purchased from R&D Systems (Abingdon, U.K.), and recombinant human IL-18 was from Hayashibara Biochemical Laboratories (Okayama, Japan). Neutralizing Abs against human IFN-$\alpha\beta$ and anti-IL-18 Ab have been described previously (20, 22). Anti-IL-12 Ab was from R&D Systems. To obtain anti-IL-23 Ab for Western blotting, rabbits were immunized with purified glutathione *S*-transferase-p19 fusion protein (three injections of 20 μg at 0, 2, and 6 wk). The fusion protein was manufactured as follows. The coding sequence of *p19* was obtained from total macrophage cellular RNA by RT-PCR (TaqMan RT system; Promega, Madison, WI) using *Bgl*II sites containing oligonucleotides CAGGGCAGATCTGTGCCTGGGGGCAGC and AGGGTTAGATCTCC ATGGGCAAAGACC. The amplified fragment of *p19* was ligated into *Bam*HI site of the pGEM-3zf(+) vector (Promega). After sequence analysis, the *p19* insert was subcloned into the pGEX-2T vector (Pharmacia Biotech). *p19* was expressed in *Escherichia coli* B strain BL21(DE3) as a glutathione *S*-transferase fusion protein and purified in a preparative SDS-PAGE (Prep-Cell; Bio-Rad, Richmond, CA).

Cytokine ELISAs

The amounts of IL-1β, IL-12, IL-18, TNF-α, and IFN-γ in cell culture supernatants were determined by specific ELISAs. IL-1β and TNF-α ELISAs were purchased from R&D Systems, and IL-12 and IFN-γ ELISAs were from Nordic Biosite (Täby, Sweden). IL-18 ELISA was obtained from Fujisaki Institute (Hayashibara Biochemical Laboratories) (23).

Biological assay for IFN-$\alpha\beta$

Macrophage culture supernatants were assayed for the presence of IFN-$\alpha\beta$ in Hep2 cells by vesicular stomatitis virus plaque reduction (24). The results are expressed as international units per milliliter using an international control IFN-α preparation as a standard.

IFN-γ-inducing activity of macrophage-derived cytokines

The IFN-γ-inducing activity of macrophage-derived cytokines was measured in a NK-92 cell line (ATCC CRL-2407) that has characteristics of human NK cells. Cell culture supernatants from virus-infected macrophages were incubated with NK-92 cells (3×10^6 cells/ml) in 24-well cell culture plates (Falcon Multiwell; BD Biosciences). After a 20-h incubation, NK-92 cell supernatants were collected and the amount of IFN-γ in the supernatants was determined by ELISA. To dissect the effect of individual cytokines, different combinations of neutralizing Abs against IFN-$\alpha\beta$, IL-12, and IL-18 were added into macrophage supernatants before subjecting them onto NK-92 cells.

Western blot analysis

Western blot samples from virus-infected macrophages were separated (30 μg of protein per lane) on 15% SDS-PAGE with the Laemmli buffer system. Proteins separated on gels were transferred onto Immobilon-P membranes (Millipore, Bedford, MA) with an Isophor electrotransfer apparatus (Hoeffer Scientific Instruments, San Francisco, CA) at 200 mA for 2 h. The membranes were blocked with PBS containing 5% nonfat milk. The blots were stained with anti-p19 Ab for 1 h at room temperature, followed by secondary staining (1 h at room temperature) with peroxidase-conjugated goat anti-rabbit IgG (Bio-Rad). The protein bands in filters were visualized by the ECL system (Amersham, Little Chalfont, U.K.).

RNA isolation and Northern blot analysis

Total cellular RNA from macrophages was isolated by guanidium isothiocyanate lysis followed by centrifugation through a CsCl cushion. Aliquots of RNA (20 μg) were size-fractionated on 1% formaldehyde-agarose gels and blotted to Hybond-N membranes (Amersham). The membranes were hybridized with human *p35* and *p40* (25), and *p19* cDNA probes. The *p19* probe was cloned from total cellular RNA obtained from macrophages by RT-PCR using oligonucleotides TGCAAAGGATCCACCAGGGTCTGA (sense) and TGAGTGGGATCCTTGAGCTGCTGC (antisense). The probes were labeled with [α-^{32}P]dCTP (3000 Ci/mmol; Amersham) by a random-primed DNA labeling kit purchased from Boehringer Mannheim (Mannheim, Germany). Hybridizations were performed at 42°C in a solution containing 50% formamide, 5× Denhardt's solution, 5× standard saline citrate phosphate/EDTA, and 0.5% SDS. After washing with 1× SSC supplemented with 0.1% SDS at room temperature and once at 60°C for 30 min, the filters were exposed to Kodak AR X-Omat films (Eastman Kodak, Rochester, NY) at −70°C in intensifying screens. For controlling equal RNA loading, rRNA bands were visualized by EtBr staining.

Results

Sendai virus stimulates p19, p35, and p40 gene expression in human macrophages

Influenza A and Sendai viruses differ in their ability to stimulate IFN-$\alpha\beta$ and IL-18 production in human macrophages, and this is reflected by the dissimilar IFN-γ-inducing activity in T and NK cells (7, 8, 20). We have further studied the regulation of IFN-γ-inducing cytokines in macrophages during virus infection and analyzed the expression of IL-12 and IL-23. mRNA for IL-12 or IL-23 was not basally expressed in macrophages, but infection with Sendai virus activated gene expression of p35 and p40 subunits of IL-12 as well as expression of IL-23-specific subunit p19 (Fig. 1). p19 mRNA expression was induced at 6 h after Sendai virus infection and it persisted up-regulated up to 24 h, whereas the expression of p35 and p40 mRNAs peaked already at 3 h postinfection. Influenza A virus, in contrast, was not able to enhance p19, p35, or p40 mRNA expression in macrophages (Fig. 1). It also failed to stimulate secretion of p35p40, the biologically active IL-12 heterodimer that was produced in response to Sendai virus infection. In fact, during Sendai virus infection macrophages produced as much as 1800 pg/ml IL-12 (Fig. 2). Similarly, only Sendai virus was able to stimulate expression of p19 protein (Fig. 3).

Virus-induced cytokines from macrophages stimulate IFN-γ production in NK-92 cells

IFN-$\alpha\beta$, IL-12, IL-18, and IL-23 all posses IFN-γ-inducing activity (4, 7, 10, 15, 26). To test which of these cytokines are involved

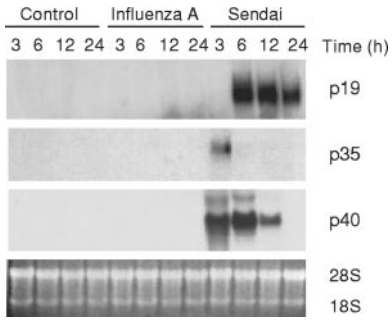

FIGURE 1. Sendai, but not influenza A, virus infection induces IL-23 p19, IL-12 p35, and IL-12 p40 mRNA expression in human macrophages. Macrophages were infected with influenza virus strain A/Beijing/353/89 (H3N2) or Sendai virus strain Cantell. The cells were harvested at indicated times, and total cellular RNA was collected. Pooled RNA samples (20 μg/lane) representing macrophages from six individual blood donors were subjected to Northern blot analysis with *p19*, *p35*, and *p40* cDNA probes. Ethidium bromide staining of rRNA bands was used to control equal RNA loading. The experiment was repeated four times with similar results.

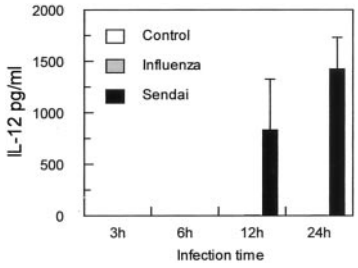

FIGURE 2. IL-12 is secreted from macrophages in response to Sendai virus infection. Cell culture supernatants from influenza A or Sendai virus-infected cells were collected at indicated time points, and the amount of IL-12 p70 in the supernatants was measured by ELISA. The mean ± SD of four separate experiments is shown.

in IFN-γ stimulation during virus infection we stimulated NK-92 cells with supernatants from influenza A or Sendai virus-infected macrophages. Both influenza A and Sendai virus infections induced a release of factors that resulted in IFN-γ production from NK-92 cells (Fig. 4). When supernatants from influenza A virus-infected macrophages were treated with neutralizing anti-IFN-αβ Abs the IFN-γ-inducing activity was completely abrogated. Anti-IL-18 Abs also diminished IFN-γ production, whereas anti-IL-12 Abs had no effect on IFN-γ induction by influenza A supernatants. IFN-α also mediated Sendai virus-induced IFN-γ activity. However, anti-IFN-αβ Abs in supernatants of Sendai virus-infected macrophages only partially inhibited IFN-γ production from NK-92 cells. Treatment of supernatants with anti-IL-12 and anti-IL-18 Abs showed that in the case of Sendai virus IFN-γ-inducing cytokines include also IL-12 and IL-18.

IFN-γ up-regulates virus-induced p19, p35, and p40 mRNA expression

IFN-γ is suggested to enhance IFN-αβ, IL-12, and IL-18 expression in macrophages (27–29). We studied the effect of IFN-γ on IL-23 production by treating macrophages with IFN-γ (10 IU/ml) 20 h before influenza A or Sendai virus infection. IFN-γ alone could not stimulate p19 mRNA expression, but priming the cells with IFN-γ strongly enhanced the Sendai virus-induced expression of p19 mRNA (Fig. 5). Similarly, p35 and p40 gene expression was increased in IFN-γ-pretreated and Sendai virus-infected macrophages. However, IFN-γ priming could not activate *p19*, *p35*, or *p40* gene expression in influenza A virus-infected cells.

IL-4 down-regulates virus-induced cytokine expression

Macrophage functions are negatively controlled through the activity of IL-4 (30). During influenza A and Sendai virus infections IL-4 had substantial effects on cytokine expression in macrophages. Treatment of macrophages with IL-4 for 0.5–20 h before virus inoculum reduced p19 and p40 mRNA expression in Sendai virus-infected cells (Fig. 6). The down-regulation of IL-12 gene expression through diminished p40 mRNA expression was followed by reduced IL-12 protein release in response to Sendai virus infection (Fig. 7). In addition, secretion of IFN-αβ, IL-1β, and IL-18 was considerably diminished in IL-4-primed macrophages. This effect was seen during both Sendai and influenza A virus infections (Fig. 7). Virus-induced TNF-α production was not significantly affected by IL-4 (Fig. 7).

The down-regulatory effect of IL-4 on IFN-αβ, IL-12, IL-18, and IL-23 expression was seen as a lower IFN-γ-inducing activity. Supernatants from IL-4-treated and virus-infected macrophages induced less IFN-γ from NK-92 cells than IL-4-nontreated and virus-infected control cells (Fig. 8). The reduction in IFN-γ amounts was comparable to the reduction provoked by anti-IFN-αβ, anti-IL-12, and anti-IL-18 Abs (Fig. 4).

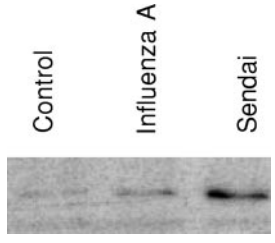

FIGURE 3. Sendai virus infection enhances IL-23 p19 protein expression in macrophages. Macrophages were infected with influenza A or Sendai viruses. Cells were collected at 18 h postinfection and protein samples were prepared. Proteins (30 μg/lane) were separated on 15% SDS-PAGE and Western blotted. Blots were stained with anti-p19 Ab.

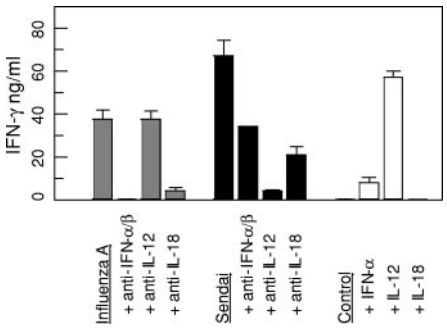

FIGURE 4. Supernatants from virus-infected macrophages stimulate IFN-γ production in NK-92 cells. Macrophages were infected with influenza A or Sendai viruses, and 20 h after infections cell culture supernatants were collected. The supernatants were left untreated or were treated with neutralizing anti-IFN-αβ, anti-IL-12, and anti-IL-18 Abs before subjecting them onto NK-92 cells. IFN-γ-inducing activity of macrophage supernatants was measured as IFN-γ production from NK-92 cells by ELISA. The mean ± SD of three separate experiments is shown.

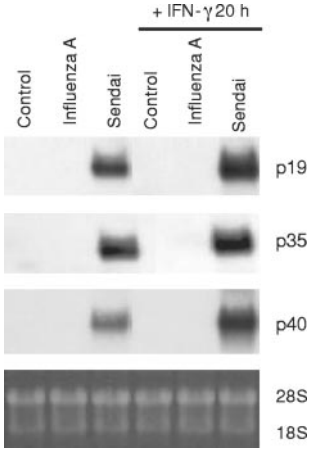

FIGURE 5. IFN-γ up-regulates IL-23 p19, IL-12 p35, and IL-12 p40 mRNA expression in Sendai virus-stimulated macrophages. Macrophages were treated for 20 h with IFN-γ (10 IU/ml) and sequentially infected with influenza A or Sendai virus. The cells were harvested at 6 h after the infection and total cellular RNA was isolated. Northern blotting analysis was performed with *p19*, *p35*, and *p40* cDNA probes. The results are representative of three different experiments with similar results.

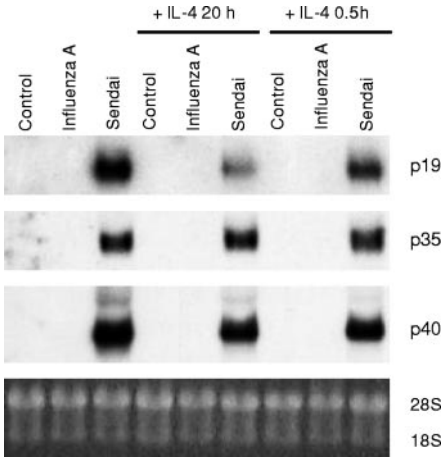

FIGURE 6. IL-4 down-regulates IL-23 p19 and IL-12 p40 mRNA expression in Sendai virus-stimulated macrophages. Macrophages were treated with 10 ng/ml IL-4 for 20 or 0.5 h before stimulation with influenza A or Sendai viruses. Total RNA was extracted from the cells at 6 h after the stimulations, and Northern blot analysis was performed with *p19*, *p35*, and *p40* cDNA probes. The results are representative of three different experiments with comparable results.

Discussion

Macrophages contribute to innate and adaptive immune responses against viruses by secreting IFN-$\alpha\beta$, IL-12, and IL-18, which synergistically induce IFN-γ production in NK and T cells (4–8). Recently, a novel IFN-γ-inducing cytokine IL-23 was discovered (10), and this is the first report demonstrating that IL-23 expression is stimulated by virus infection. We have studied regulation of cytokine expression in human primary macrophages during influenza A and Sendai virus infections and reported that these viruses differ in their ability to stimulate IFN-$\alpha\beta$ and IL-18 production (20, 21). Now we show that Sendai, but not influenza A, virus induces IL-12 and IL-23 expression in macrophages, and we show how macrophage-derived cytokines enhance IFN-γ production in NK cells. In addition, the roles of IFN-γ and IL-4 in the regulation of IL-12 and IL-23 activity are defined.

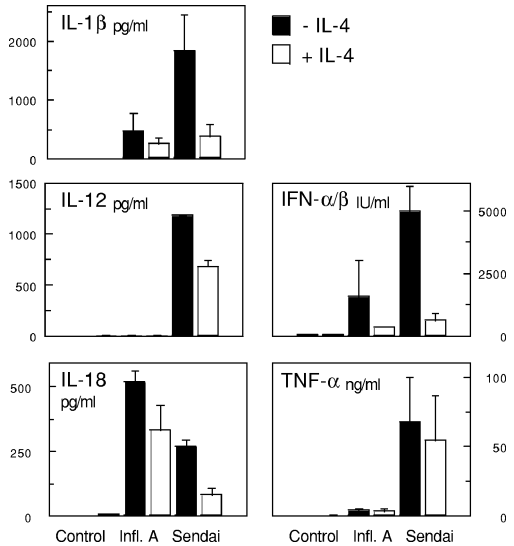

FIGURE 7. IL-4 limits virus-induced IFN-$\alpha\beta$, TNF-α, IL-1β, IL-12, and IL-18 production from macrophages. Macrophages were primed with IL-4 (10 ng/ml) for 20 h and infected with influenza A or Sendai viruses for another 20 h before the cell culture supernatants were harvested. The amounts of secreted cytokines were determined with biological assay (IFN-$\alpha\beta$) or ELISAs (TNF-α, IL-1β, IL12, and IL-18). The mean ± SD of three separate experiments is shown.

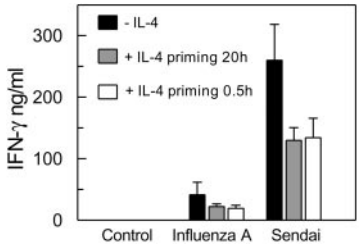

FIGURE 8. Priming of virus-infected macrophages with IL-4 reduces the IFN-γ-inducing activity of macrophage supernatants. Macrophages were stimulated with IL-4 (10 ng/ml) for 0.5 or 20 h and infected with influenza A or Sendai viruses. Twenty hours postinfection the cell supernatants were collected and subjected onto NK-92 cells. IFN-γ induction by macrophage supernatants in NK-92 cells was measured by IFN-γ-specific ELISA. The mean ± SD of three separate experiments is shown.

Biologically active IL-12 is a 70-kDa heterodimer composed of two covalently linked subunits, p40 and p35. p35 and p40 are encoded by two separate genes whose expression is independently regulated at the transcriptional level (31, 32). While the *p35* gene is expressed in most cells, *p40* expression is restricted to a few cell types, such as dendritic cells, monocytes, and macrophages (33). In contrast, activated phagocytic cells express ~10- to 100-fold more p40 than p35 mRNA, which leads to overproduction of free p40 chain compared with the p35-containing IL-12. Abundant production of p40 chain may, however, have a physiological function; p40 chains form a homodimer that serves as an IL-12 antagonist by competing for IL-12R (34–36). Recently, Oppmann et al. (10) discovered a new function for excessive p40. p40 combines with a novel protein called p19 to form biologically active cytokine IL-23.

Regulation of IL-23 activity is still an open question. mRNA for the p19 component of IL-23 is expressed at least in endothelial and hematopoietic cells, including B and T cells, macrophages, and dendritic cells (10). However, expression of p19 via mRNA on its own does not elicit a biological response (12), and purified p19 protein is not sufficient to increase IFN-γ expression in hematopoietic cells (10). It seems that the formation of functional IL-23 requires synthesis of both p19 and p40 subunits within the same cell, and the p40 component is a limiting factor in IL-23 production. Therefore, it is logical that the physiological source of active IL-23 could be p40-expressing dendritic cells and macrophages, which are the major producers of IL-12 as well. At the moment it is known that LPS stimulation of murine and human dendritic cells can result in IL-23 production (10). Our present study with human macrophages demonstrates that virus-infected macrophages are potential producers of IL-23.

Influenza A and Sendai virus infections in macrophages result in the secretion of IFN-$\alpha\beta$ and IL-18 (7, 20). In addition, Sendai, but not influenza A, virus stimulates macrophages to produce IL-12 (Fig. 2). IL-12 production is preceded by increased expression of p35 and p40 mRNA (Fig. 1). Interestingly, Sendai virus induces also the transcription of *p19* gene (Fig. 1) and the expression of the p19 protein (Fig. 3), suggesting that macrophages are able to produce IL-23 as well. We tested the biological activity of the virus-induced cytokines by measuring the capacity of macrophage supernatants to induce IFN-γ expression in NK-92 cells. Supernatants from both influenza A and Sendai virus-infected macrophages stimulate high IFN-γ production from NK-92 cells (Fig. 4). Treatment of influenza A supernatants with neutralizing anti-IFN-$\alpha\beta$ Abs abrogates completely the IFN-γ production, and

anti-IL-18 Abs diminish it. As expected, neutralizing anti-IL-12 Abs have no effect on influenza A virus-induced IFN-γ activity. This implies that, in the case of influenza A virus, the stimulators of IFN-γ are IFN-$\alpha\beta$ and IL-18. The fact that anti-IFN-$\alpha\beta$ Abs alone are sufficient to inhibit IFN-γ stimulation is explained by a synergistic effect of IFN-α and IL-18. IL-18 on its own is a weak inducer of IFN-γ (Fig. 4), but together with IFN-α it has a strong effect on T and NK cells (7, 8). In addition, in influenza A virus-infected macrophages IFN-α is involved in the regulation of IL-18 secretion (21). IFN-γ induction by Sendai virus supernatants is also diminished with anti-IFN-$\alpha\beta$ Abs, but more pronounced inhibition requires neutralization of IL-12 and IL-18 as well (Fig. 4). However, even a combined administration of anti-IFN-$\alpha\beta$, anti-IL-12, and anti-IL-18 Abs cannot entirely block Sendai virus-induced IFN-γ activity (data not shown). Therefore, it is conceivable that, in addition to IFN-$\alpha\beta$, IL-12, and IL-18, IL-23 takes part in Sendai virus-induced IFN-γ production. Possible synergistic actions of IL-23 with IFN-$\alpha\beta$, IL-12, and/or IL-18 warrant further investigation.

The induction of IFN-γ production by IFN-$\alpha\beta$ and IL-12 involves a potential autostimulatory loop. In macrophages IFN-γ has been shown to have a stimulatory effect on IFN-α and IL-12 release in response to bacterial products (27, 37–40). In Sendai virus-infected macrophages IFN-γ enhances IFN-α production (41); moreover, the virus-induced p19 and p40 mRNA expression is up-regulated by IFN-γ priming (Fig. 5). This suggests that a positive feedback mechanism acts on IL-23 and IFN-γ expression during virus infection.

Another key regulator of cytokine expression in myeloid cells is IL-4 (30). It inhibits virus-induced IFN-α production (42–44) and modulates expression of IL-12 (45, 46). IL-4 is also involved in the regulation of IL-12- and IL-18-induced IFN-γ production (47), but its role in suppressing IL-12 or IL-18 expression during virus infections is still inadequately characterized. Even less is known about the possible contribution of IL-4 to IL-23 expression and subsequent IFN-γ production. We studied the regulatory role of IL-4 in the expression of IFN-γ-inducing cytokines by treating macrophages with IL-4 before infecting them with influenza A or Sendai viruses. IL-4 diminishes the production of IFN-$\alpha\beta$ and IL-18 in response to either virus infection (Fig. 7). In a like manner, Sendai virus-induced IL-12 production is significantly reduced in IL-4-treated cells (Fig. 7). The reduced IL-12 production is apparently due to down-regulation of the *p40* gene because IL-4 during Sendai virus infection decreases mRNA expression of p40 but not that of p35 (Fig. 6). In addition, IL-4 decreases Sendai virus-induced p19 mRNA expression (Fig. 6). Consequently, IL-4-dependent down-regulation of IL-23 expression can occur through declined transcription of both *p19* and *p40* genes. The fact that even a short-term IL-4 stimulation is sufficient to inhibit IL-23 expression suggests that IL-4 acts on macrophages via a direct mechanism. The decreased production of IFN-$\alpha\beta$, IL-12, IL-18, IL-4, and probably also that of IL-23, is reflected in diminished IFN-γ production in NK cells stimulated by supernatants of virus-infected macrophages (Fig. 8).

In conclusion, our present study demonstrates that a novel IFN-γ-inducing cytokine, IL-23, is involved in the cross-talk of immune cells during viral infection. Sendai virus infection in macrophages stimulates high expression of *p19* and *p40* genes, which encode for the two subunits of the IL-23 heterodimer. The expression of *p19* and *p40* is up-regulated by IFN-γ and down-regulated by IL-4, the latter of which inhibits the production of IFN-$\alpha\beta$, IL-12, and IL-18 as well. Through limiting the expression of these macrophage-derived cytokines, IL-4 counteracts an IFN-γ-driven positive feedback mechanism that operates in macrophages and NK and T cells. Further studies on cytokine-mediated cellular interactions will certainly bring new perspectives on the development of immune responses against viruses.

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