IL-23 Enhances Host Defense against Vaccinia Virus Infection Via a Mechanism Partly Involving IL-17[1]

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To investigate roles of IL-23 in viral infection, we have engineered recombinant vaccinia virus (VV) expressing IL-12 (VV-IL-12) and expressing IL-23 (VV-IL-23). We found VV-IL-23 was less virulent in BALB/c mice than wild-type VV (VV-WT), indicating that IL-23 enhances resistance to VV. VV-specific CTL activity in VV-IL-23-infected mice was slightly higher than activity in VV-WT-inoculated mice, although antiviral Ab production and NK activity were not increased. IL-12/23p40-deficient mice survived the infection with VV-IL-23, indicating that IL-23 promotes VV resistance independently of IL-12. The mechanism of the IL-23-mediated resistance was distinct from that of the IL-12-regulated resistance because IFN-γ-deficient mice did not eliminate VV-IL-12, but did eradicate VV-IL-23. These data indicate that IFN-γ is essential for the IL-12-mediated resistance, but dispensable for the IL-23-regulated resistance. Because IL-17 is a key in the IL-23-regulated resistance to bacteria, we hypothesized an involvement of IL-17 in the resistance to VV. Treatment with an anti-IL-17 mAb resulted in a significant increase of viral titers in VV-IL-23-infected IFN-γ-deficient mice. In addition, VV-IL-17 was less virulent than VV-WT in BALB/c mice, and IL-17-deficient mice were more sensitive to VV-WT than control mice. However, the effect of neutralization with an anti-IL-17 mAb was limited, and IL-17-deficient mice survived the infection with VV-IL-23. Taken together, these data suggest that the IL-23/IL-17 axis plays a certain but subdominant role in the IL-23-mediated resistance to VV. Unveiling of an alternative pathway in the IL-23-regulated resistance might provide a novel strategy against infectious pathogens without side effects of autoimmunity. ***The Journal of Immunology,*** **2007, 179: 3917–3925.**

Interleukin-12 is a heterodimeric proinflammatory cytokine composed of a 35-kDa L chain (p35) and a 40-kDa H chain (p40). IL-12 leads to the development of IFN-γ-producing Th1 cells, and therefore, this cytokine is considered to be a key regulator of cell-mediated immunity (1). A novel IL-12-associated cytokine, IL-23 is also a heterodimer formed by the p40 subunit of IL-12 and a new subunit, p19 (2). Furthermore, the receptor for IL-23 consists of one subunit of the IL-12 heterodimeric receptor, IL-12Rβ1, and a new receptor subunit, IL-23R (3). As a consequence of the structural similarity, it was originally considered that IL-23 would regulate Th1-mediated immune responses as well as IL-12. In fact, early studies demonstrated that IL-23 stimulated the production of IFN-γ and enhanced the activity of cellular immunity including CTLs (4–6). However, there is now increasing evidence that IL-23 is functionally distinct from IL-12 in the regulation of T cell-mediated immunity.

Although IL-12 promotes the differentiation of IFN-γ-producing Th1 cells and thereby generates resistance to intracellular infections, it was previously believed that IL-12-induced Th1 cells also triggered autoimmune diseases such as experimental allergic encephalomyelitis (EAE)[3] and collagen-induced arthritis. However, Cua and colleagues (7, 8) have demonstrated that mice deficient in either the IL-23p19 subunit or the IL-12/23p40 subunit are resistant to EAE and collagen-induced arthritis, whereas IL-12p35-deficient mice remain susceptible to these diseases. These data indicate that IL-23, not IL-12, is essential for the development of these autoimmune diseases. Furthermore, it was initially shown that IL-23 stimulated naive precursor cells to differentiate into a new $CD4^+$ T cell subset termed Th17 in the absence of IFN-γ and IL-4 (9). However, recent papers (10–12) have revealed that TGF-β and IL-6 drive the differentiation of naive T cells into Th17 cells. Although IL-23 does not induce the Th17 differentiation, this cytokine is essential for Th17 cells to expand and acquire full effector function (10–12). Because IL-23R is not expressed on naive T cells, TGF-β and IL-6 act to up-regulate IL-23R expression on them, and thereby conferring responsiveness to IL-23. Th17 cells produce IL-17 (IL-17A), IL-17F, IL-6, and TNF-α but not IFN-γ or IL-4 and, therefore, this subset is different from the classical Th1 or Th2 subset (9, 13). IL-17 has pleiotropic activities including the induction of a variety of proinflammatory cytokines, chemokines, and adhesion molecules on various cell types. IL-17 is detected in serum of patients with rheumatoid arthritis, multiple

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[3] Abbreviations used in this paper: EAE, experimental allergic encephalomyelitis; VV, vaccinia virus; VV-IL-12, VV expressing IL-12; VV-IL-23, VV expressing IL-23; VV-IL-17, VV expressing IL-17; VV-WT, wild-type VV; sc, single chain; KO, knockout; MOI, multiplicity of infection.

sclerosis, systemic lupus erythematosus, and asthma, suggesting that its involvement in the development of various human autoimmune diseases (14). In addition, IL-17-deficient mice are resistant to the development of collagen-induced arthritis (15) and EAE (16), suggesting a pathogenic role of IL-17 in these autoimmune diseases. Thus, the IL-23/Th17/IL-17 axis rather than the IL-12/Th1/IFN-γ axis is likely to be associated with T cell-mediated autoimmune diseases. However, the physiological function of IL-23 remains largely unknown.

Several groups have investigated a role of IL-23 in resistance to infection with several pathogens. It was shown that IL-12/23p40-, IL-23p19-, IL-12p35-, and IL-17R-deficient mice were sensitive to *Klebsiella pneumonia* (17), indicating that both of the IL-12/IFN-γ and IL-23/IL-17 axes are critical for the host defense against *K. pneumoniae*. However, IL-23 was induced sooner than IL-12 in this infection. Furthermore, IL-23p19-deficient mice dramatically reduced IL-17 production and showed substantial mortality from a normally sublethal dose of *K. pneumoniae*, despite normal IFN-γ production. In addition, administration of recombinant IL-17 restored bacterial control in IL-23p19-deficient mice, suggesting that IL-17 induced by IL-23 plays a significant role in the early host defense against *K. pneumoniae* (17). The IL-23/IL-17 axis is also necessary for host protection against *Citrobacter rodentium* (11). Thus, IL-23 seems to be important for host protection against several bacteria, and IL-17 plays a critical role in the IL-23-mediated resistance. However, it is unknown whether the IL-23/IL-17 axis is required for host defense against viral infection.

In the present study, we have engineered recombinant vaccinia virus (VV) expressing IL-12 (VV-IL-12) and IL-23 (VV-IL-23), and investigated a role of the IL-23/IL-17 axis for host defense against virus in comparison with the IL-12/IFN-γ axis.

Materials and Methods

Cell lines

The mouse mastocytoma cell line P815 (H-2^d), the Moloney murine leukemia virus-induced lymphoma YAC-1, the mouse fibroblast cell line NIH 3T3, and the human embryonic kidney cell line HEK 293T were obtained from the ATCC. The African green monkey-derived kidney cell lines CV-1 and BS-C-1, and the human osteosarcoma, thymidine kinase-defective cell line C143 were provided by Dr. T. Shioda (Osaka University, Osaka, Japan). These cell lines were cultured in DMEM with 10% FCS.

Construction of recombinant viruses

VV-IL-12, VV-IL-23, and VV expressing murine IL-17 (VV-IL-17) were generated as described (18). In brief, murine IL-17 cDNA without its leader sequence was generated by RT-PCR from total RNA of Con A-activated spleen cells of BALB/c mice (sense) 5′-AAGCTTGCGGCTA CAGTGAAGGCA-3′ (antisense) 5′-TCTAGATTAGGCTGCCTGGCG GACA-3′ and was subcloned into the pCR2.1 vector (Invitrogen Life Technologies). Following digestion with *Hin*dIII and *Xba*I, murine IL-17 cDNA was cloned into the p3xFLAG-CMV-9 (Sigma-Aldrich) vector (p3xFLAG-IL-17). This expression vector encodes the preprotrypsin signal peptide and the 3xFLAG-epitope-tag sequence upstream of the multiple cloning region and, hence, expresses a secreted N-terminal 3xFLAG fusion protein in mammalian cells. Conversely, the murine IL-12 (p3xFLAG-IL-12) and murine IL-23 (p3xFLAG-IL-23) expression plasmids, which include murine single-chain (sc)IL-12 and murine scIL-23 genes, respectively, had previously been constructed (6). The scIL-12 and scIL-23 genes are composed of p40 genetically fused with p35 and p19, respectively (6). The biological activity of each single-chain cytokine was shown to be almost similar to that of the native heterodimeric cytokine (4, 5).

Murine IL-17, scIL-12, and scIL-23 genes linked to a nucleotide sequence comprised of the preprotrypsin signal sequence and the 3xFLAG-tag sequence were isolated by PCR amplification from p3xFLAG-IL-17, p3xFLAG-IL-12, and p3xFLAG-IL-23, respectively. Primers used include the following: murine IL-12, IL-17, and IL-23 (sense) 5′-GGATCCGC CACCATGTCTGCACTTCTGATCCTAGCT-3′ and (antisense for murine IL-12) 5′-CTCGAGTCAGGCGGAGCTCAGATAGC-3′, (antisense for murine IL-17) 5′-CCCGGGTTAGGCTGCCTGGCGGACA-3′, and (antisense for murine IL-23) 5′-GAGTCAAGCTGTTGGCACTAA GGGCTC-3′. The amplified genes were then inserted into the transfer vector pNZ68K2 (18). VV-IL-12, VV-IL-17, and VV-IL-23 were then generated by homologous recombination between wild-type VV (VV-WT) (WR strain) and the transfer vector, purified by three cycles of plaque cloning with C143 cells in the presence of BrdU, and propagated in CV-1 cells (18).

Expression of scIL-12 and scIL-23 fusion proteins

293T cells were infected with VV at a multiplicity of infection (MOI) of 3 for 1.5 h, and incubated for 2 days at 37°C. Fusion proteins in the culture supernatants were then immunoprecipitated using the anti-FLAG M2 mAb (Sigma-Aldrich) and protein G-Sepharose (Amersham Biosciences), as previously described (6). Following immunoprecipitation, Western blotting of immunoprecipitated proteins was performed (6). Briefly, the fusion proteins were separated by electrophoresis on a 12% SDS-PAGE under reducing conditions, and blotted onto a nitrocellulose membrane. The blot was stained with 5 μg/ml M2 mAb (Sigma-Aldrich) for 1 h at room temperature, followed by secondary staining with peroxidase-conjugated anti-mouse IgG Ab. The protein bands were developed by the BCIP/NBT phosphatase substrate system (Kirkegaard & Perry Laboratories).

Tyrosine phosphorylation of STAT4 triggered by either murine IL-12 or murine IL-23 was detected as described before (6). In brief, 293 T cells expressing either murine IL-12Rβ1 and murine IL-12Rβ2, or murine IL-12Rβ1 and murine IL-23R, were stimulated for 45 min with the culture supernatant of 293 T cells infected with VV at various concentrations of 0.2, 2.0, and 20%. Cells were then subjected to Western blotting using anti-STAT4 (Santa Cruz Biotechnology) and anti-phosphorylation-STAT4 (Zymed Laboratories) Abs.

Preparation of recombinant IL-12 and IL-23 proteins

Recombinant murine scIL-12 and recombinant scIL-23 were prepared as described (19). In brief, human embryonic kidney 293F cells were transiently transfected with p3xFLAG-IL-12 or p3xFLAG-IL-23 by using 293 fection (Invitrogen Life Technologies). After 3 days, culture supernatants were harvested and 3xFLAG-tagged recombinant scIL-12 and scIL-23 were purified by affinity chromatography using anti-FLAG affinity gel (Sigma-Aldrich). The concentration of 3xFLAG-tagged recombinant scIL-12 was determined using murine recombinant IL-12 as a standard in ELISA. Protein concentration of 3xFLAG-tagged recombinant scIL-23 was determined by titration in Western blotting with anti-FLAG (M2) mAb using 3xFLAG-tagged scIL-12 prepared as a standard.

Mice and infection

BALB/c and C57BL/6 mice were purchased from Japan Charles River Breeding Laboratory. IL-12/23p40 gene knockout (KO) mice (20) of the BALB/c background were purchased from The Jackson Laboratory. IFN-γ gene KO mice (21) and IL-17 gene KO mice (22) were previously described and were backcrossed eight generations to C57BL/6 and BALB/c, respectively. Six- to 8-wk-old mice were used for all experiments. Mice were housed in appropriate animal care facilities at Saitama Medical University (Saitama, Japan), and handled according to international guidelines for experiments with animals.

Each mouse was infected i.p. with 5×10^6 PFU of VV for the ovary VV titer assay or 2×10^7 PFU of VV for the cytotoxic assay, the intracellular cytokine staining assay, and detection of VV-specific Abs. To plot survival curves, 6–12 mice in each group were infected i.p. with either 2×10^8 or 5×10^8 PFU of VV, and were examined daily for weight loss and mortality.

Ovary VV titer assay

Mice were sacrificed on certain days after infection with 5×10^6 PFU of VV, and viral titers in ovaries were measured as described before (23). Six to twelve mice were used in each group. All titrations were performed in duplicates, and the average PFU per mouse was calculated. Some mice were treated in vivo by i.p. injection with 70 μg of neutralizing anti-mouse IL-17 mAb (clone 50104.11; R&D Systems) or a relevant isotype control at days 0, 2, and 7 postinfection with VV.

Cytotoxic assay

At the indicated time points following infection with 2×10^7 PFU of VV, mice were sacrificed, and spleen cells were prepared for effector cells. Cytotoxic activities of VV-specific CTLs and NK cells were measured in standard ^{51}Cr release assays. For preparation of virus-infected targets in CTL assays, P815 cells were infected with VV at an MOI of 3 for 1.5 h at 37°C, washed three times, and incubated in RPMI 1640 containing 10%

FCS overnight at 37°C. For detection of NK cell-mediated lysis, YAC-1 cells were used for targets. Target cells (1×10^6 cells) were labeled with 100 μCi of $Na_2{}^{51}CrO_4$ for 30 min at 37°C. After washing three times, the labeled target cells were plated in wells of a round-bottom 96-well plate at 1×10^4 cells/well with or without effector cells at various E:T ratios. After a 4-h incubation at 37°C, supernatant from each well was harvested and the radioactivity was counted. The results were calculated as the mean of a triplicate assay. The percentage of specific lysis was calculated according to the formula: Percentage of specific lysis = $((cpm_{sample} - cpm_{spontaneous})/(cpm_{maximum} - cpm_{spontaneous})) \times 100$, where spontaneous release represents the radioactivity released by target cells in the absence of effectors, and maximum release represents the radioactivity released by target cells lysed with 5% Triton X-100. At least three mice per group were used in each experiment. The experiment was repeated three times.

Intracellular cytokine staining

Intracellular cytokine staining was performed as previously described (23). Briefly, spleen cells of three to five mice per group infected with VV were pooled and resuspended in RPMI 1640 containing 10% FCS. In each well of a 96-well round-bottom plate, 2×10^6 spleen cells were incubated with 1×10^5 cells of either VV-infected P815 or naive P815 in the presence of 0.2 μl/well brefeldin A (GolgiPlug; BD Biosciences) for 5 h at 37°C. The cells were then washed once and incubated for 10 min at 4°C with the rat anti-mouse CD16/CD32 mAb (Fc Block; BD Biosciences) at a concentration of 1 μg/well. Following incubation, cells were stained with FITC-conjugated rat anti-mouse CD8α mAb (clone 53-6.7; BD Biosciences) at a concentration of 0.5 μg/well for 30 min at 4°C. After washing twice, the cells were fixed and permeabilized using the Cytofix/Cytoperm kit (BD Biosciences), and stained with PE-conjugated rat anti-mouse IFN-γ (clone XMG1.2; BD Biosciences) mAb. After washing, flow cytometric analyses were performed. The experiment was repeated three times.

Detection of VV-specific Abs

Titers of VV-specific serum Abs were determined by ELISA as described before (23). In brief, each well of a 96-well flat-bottom plate (model no. 3590; Costar) was coated with VV-infected cell lysate diluted in 0.05 M carbonate-bicarbonate buffer (pH 9.6) at 5×10^7 PFU/ml. The plate was then fixed with 2% paraformaldehyde and washed three times with PBS containing 0.05% Tween 20 (PBS-Tween 20). After blocking, 100 μl of diluted mouse serum was added to each well, and the plate was incubated for 1 h at 37°C. After washing three times with PBS-Tween 20, HRP-conjugated goat anti-mouse IgM, IgG1, or IgG2a Ab (Sigma-Aldrich) diluted 1/5000 in blocking buffer was added, and the plate was incubated for 1 h at 37°C. After washing five times with PBS-Tween 20, 100 μl of *o*-phenylenediamine dihydrochloride substrate (Sigma-Aldrich) was added to each well. The reaction was stopped with 50 μl/well of 6 N H_2SO_4 per well and the plate was read at 492 nm. Four to six mice per group were used in the experiments.

Cytokine ELISA

Spleen cells of naive mice were infected in vitro with VV at an MOI of 3, and cultured in wells of a 96-well round-bottom plate at 1×10^6/well for 2 days at 37°C. In some experiments, naive spleen cells were cultured with either murine recombinant IL-12 or murine recombinant IL-23 at various concentrations for 2 days at 37°C. Culture supernatants were then harvested and screened for the presence of murine IFN-γ and murine IL-17 by ELISA as described (23). Capture Abs, biotinylated detection Abs, and recombinant cytokines were purchased from BD Biosciences. Quantitative ELISA for murine IFN-γ and murine IL-17 was performed using paired mAbs specific for corresponding cytokines according to the manufacturer's instruction. Briefly, an ELISA plate was coated with a capture Ab for each cytokine and incubated overnight at 4°C. The plate was washed with PBS-Tween 20 and blocked with 10% calf serum in PBS for 2 h at room temperature. After washing, serially diluted samples and a recombinant standard were added to the plate and incubated at 4°C overnight. The plate was washed four times followed by the addition of cytokine-specific detection Abs. After 1 h of incubation at room temperature, HRP-conjugated avidin (BD Biosciences) was added. The color was developed by adding *o*-phenylenediamine dihydrochloride substrate, and the reaction was stopped with H_2SO_4. The concentration of each cytokine was calculated by reading the plates at 492 nm. Data represent three to five mice per group and are given as mean values ± SEM.

Detection of IL-17 secreted from VV-IL-17-infected cells

The 293 T cells were infected with either VV-IL-17 or VV-WT at an MOI of 3 for 1.5 h, and incubated for 2 days at 37°C. Murine IL-17 in the culture supernatants was then quantitated by ELISA as described. The biological activity of murine IL-17 was also measured by its ability to induce murine IL-6 production by NIH 3T3 as also described (24). Briefly, NIH 3T3 cells (2.5×10^6/well) were cultured with the culture supernatants of VV-IL-17- or VV-WT-infected 293T cells in wells of a 24-well plate. After incubation for 24 h at 37°C, 50 μl of supernatant was removed and assayed for murine IL-6 using ELISA as described. Capture and biotinylated detection Abs and recombinant murine IL-6 were purchased from BD Biosciences.

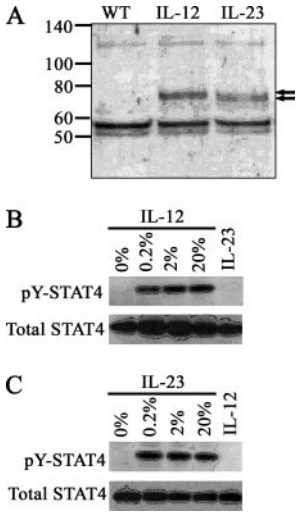

FIGURE 1. Detection of the murine scIL-12 and scIL-23 fusion proteins secreted from VV-infected cells. *A*, 293 T cells were infected with VV-WT (WT), VV-IL-12 (IL-12), or VV-IL-23 (IL-23). Proteins secreted in the supernatants were immunoprecipitated with the anti-FLAG mAb. The immunoprecipitated proteins were then subjected to Western blot analysis with the anti-FLAG mAb. The positions of protein molecular mass markers in kilodaltons are shown. Arrows indicate the bands of the murine scIL-12 (76 kDa) and scIL-23 (74 kDa) fusion proteins. *B* and *C*, STAT tyrosine phosphorylation assay. 293T cells expressing either murine IL-12Rβ1 and IL-12Rβ2 (*B*), or murine IL-12Rβ1 and IL-23R (*C*) were stimulated for 45 min with the culture supernatant containing either murine scIL-12 (IL-12) (*B*) or scIL-23 (IL-23) (*C*) at final concentrations of 0, 0.2, 2, and 20%. As negative controls, the cells were stimulated for 45 min with the culture supernatant containing either murine scIL-23 (IL-23) (*B*) or scIL-12 (IL-12) (*C*) at a final concentration of 20%. The cells were then subjected to Western blotting using anti-STAT4 (Total STAT4) and anti-phosphorylation-STAT4 (pY-STAT4) Abs.

Statistical analyses

Statistical analyses were performed with Student's *t* test. A value of $p < 0.05$ was considered statistically significant.

Results

Secretion of scIL-12 and scIL-23 from VV-infected cells

VV-IL-12 and VV-IL-23 were generated by homologous recombination between VV-WT and the transfer vector. The 293T cells were then infected with VV-WT, VV-IL-12, or VV-IL-23, and culture supernatants were harvested. Fusion proteins secreted into the culture supernatants were immunoprecipitated and subjected to Western blot analysis using the anti-FLAG mAb. As shown in Fig. 1*A*, the scIL-12 and scIL-23 fusion proteins were detected in lanes as single polypeptide bands of 76 and 74 kDa, respectively. Furthermore, STAT4 in 293T cells expressing the IL-12R composed of IL-12Rβ1 and IL-12Rβ2 was phosphorylated on tyrosine in response to scIL-12, but not by scIL-23 (Fig. 1*B*). In contrast, scIL-23 induced STAT4 tyrosine phosphorylation in 293 T cells expressing the IL-23R consisting of IL-12Rβ1 and IL-23R (Fig. 1*C*). These data indicate that scIL-12 and scIL-23 proteins secreted from VV-infected cells are functional.

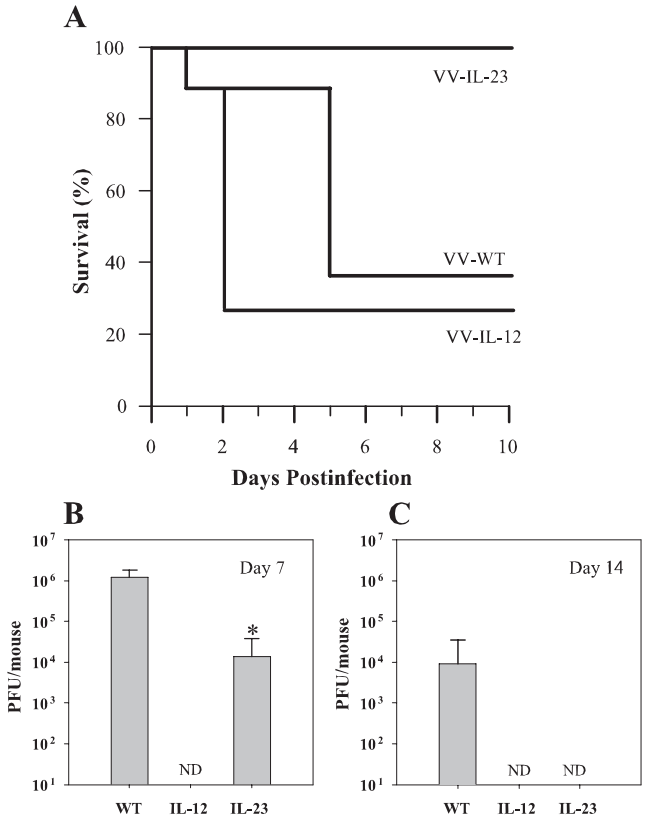

FIGURE 2. IL-23 delivered by VV-IL-23 enhances resistance to VV infection in BALB/c mice. BALB/c mice were infected i.p. with 2×10^8 PFU (*A*) or 5×10^6 PFU (*B* and *C*) of VV-WT, VV-IL-12, or VV-IL-23. *A*, Eight mice per group were monitored daily for mortality. *B* and *C*, BALB/c mice inoculated with VV-WT (WT), VV-IL-12 (IL-12), or VV-IL-23 (IL-23) were sacrificed at days 7 (*B*) and 14 (*C*) postinfection, and viral titers in ovaries were measured. From 8 to 12 mice were used in each group, and all titrations were performed in duplicates. Data are representative of one of three independent experiments, and results are shown as the mean PFU ± SEM. ND, Not detected. *, $p < 0.001$ compared with VV-WT.

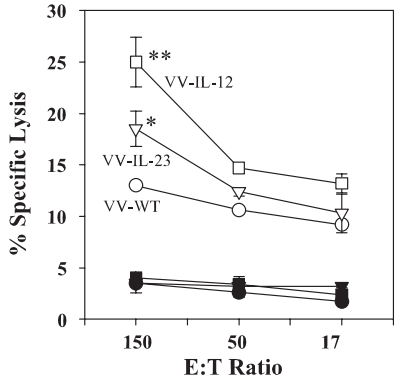

FIGURE 3. Activity of VV-specific CTLs in mice infected with VV-IL-12 and VV-IL-23. Spleen cells were prepared from mice at 2 wk after infection with 2×10^7 PFU of VV-WT (circles), VV-IL-12 (squares), or VV-IL-23 (inverted triangles). ^{51}Cr release assays were then performed to detect VV-specific CTL activity in spleen cells at various E:T ratios, using P815 cells infected with (open symbols) or without (filled symbols) VV-WT as targets. Data are representative of three independent and reproducible experiments. Results are shown as the mean ± SEM of triplicate wells. *, $p < 0.05$ compared with VV-WT; **, $p < 0.05$ compared with VV-IL-23.

IL-23 delivered by VV-IL-23 enhances resistance to VV infection in mice

To investigate the effect of IL-23 on resistance to VV infection in comparison with IL-12, BALB/c mice were infected i.p. with a high dose (2×10^8 PFU) of VV-WT, VV-IL-12, or VV-IL-23 and were monitored daily for mortality. As shown in Fig. 2*A*, five of eight VV-WT-injected mice died by day 5 postinfection, whereas all mice ($n = 8$) survived the infection with VV-IL-23. These findings demonstrate that IL-23 enhances host defense against VV in mice. In contrast, six of eight mice succumbed to the infection with 2×10^8 PFU of VV-IL-12 by day 2 postinfection (Fig. 2*A*), presumably due to the IL-12-associated toxicity at a high concentration of IL-12 (25). We next examined viral load in ovaries of mice at days 7 (Fig. 2*B*) and 14 (Fig. 2*C*) after inoculation with a sublethal dose (5×10^6 PFU) of VV. As shown in Fig. 2*B*, VV-IL-23-infected mice harbored nearly 100-fold less PFU of virus than VV-WT-infected mice at day 7 postinfection. Furthermore, VV-IL-23-injected mice completely cleared VV from ovaries at day 14 postinfection, although VV-WT-infected mice still held high virus titers on the same day (Fig. 2*C*). These data indicate IL-23 delivered by VV-IL-23 leads to rapid clearance of the virus from infected mice. Conversely, no virus was detected in ovaries of mice inoculated with a sublethal dose (5×10^6 PFU) of VV-IL-12 even at day 7 postinfection (Fig. 2*B*), indicating the potent activity of IL-12 in the clearance of VV.

Effect of IL-23 on cellular and humoral immunity to VV

Because $CD8^+$ CTLs play a critical role in the host defense against VV (26, 27), we tested the cytolytic activity of VV-specific CTLs in mice infected with 2×10^7 PFU of VV-WT, VV-IL-12, or VV-IL-23. As expected, the activity of VV-specific CTLs in VV-IL-12-infected mice was significantly higher than activity in VV-WT-injected mice (Fig. 3*A*), indicating that IL-12 enhanced the induction of VV-specific CTLs. In contrast, IL-23 delivery by VV resulted in a slight increase of VV-specific CTL induction (Fig. 3). We next examined the number of Ag-induced intracellular IFN-γ-positive $CD8^+$ T cells in spleen cells of mice infected with 2×10^7 PFU of VV (Fig. 4). Because spleen cells were stimulated in vitro with VV-WT-infected P815 cells for only 5 h, the possibility of substantial in vitro expansion of responder cells is precluded (28). At day 7 postinfection, the frequency of IFN-γ-positive $CD8^+$ T cells in mice infected with VV-IL-12 (11.94%) was ~2-fold higher than that in mice infected with-VV-WT (5.69%). In contrast, percentage of IFN-γ-producing $CD8^+$ T cells in VV-IL-23-infected mice (7.69%) was modestly increased when compared with that in VV-WT-infected mice (5.69%). These data suggest that IL-12 delivery by VV is likely to be more efficient for the induction of VV-specific CTLs than IL-23 delivery. However, these data also suggest that the VV-specific CTL activity slightly enhanced by IL-23 may be at least partly responsible for the attenuation of virulence of VV-IL-23.

It was indicated that humoral immunity was essential to protect against VV infection (26, 27). Therefore, antiviral IgM and IgG Abs were monitored in sera of mice after infection with a sublethal dose of VV-WT, VV-IL-12, or VV-IL-23 (Fig. 5, *A–C*). Levels of VV-specific IgG1 and IgG2a Abs in VV-IL-23-infected mice were almost equal to those in VV-WT-inoculated mice at days 7 (data not shown) and 14 (Fig. 5, *A* and *B*) postinfection. In contrast, VV-IL-12-inoculated mice produced much less VV-specific IgG1 Ab than VV-WT-injected mice (Fig. 5*A*), whereas there was no major difference in the production of VV-specific IgG2a Ab between the two groups (Fig. 5*B*). These data suggest that the effect of IL-23 on Ab production is likely to be different from that of

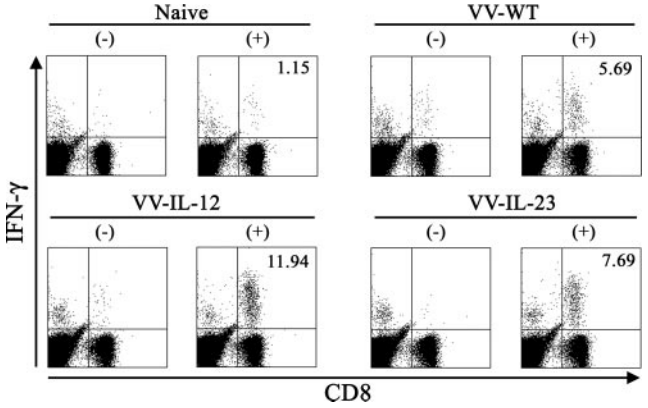

FIGURE 4. Intracellular IFN-γ staining of VV-specific $CD8^+$ T cells in mice infected with VV-WT, VV-IL-12, or VV-IL-23. BALB/c mice were infected with 2×10^7 PFU of either VV-WT, VV-IL-12, or VV-IL-23, and spleen cells were prepared at day 7 postinfection. Spleen cells of noninfected mice (Naive) were used as a negative control. Spleen cells were then stimulated with VV-infected P815 (+) or noninfected P815 (−) cells for 5 h. After stimulation, cells were stained for their surface expression of CD8 (*x*-axis) with FITC-conjugated mAb and for their intracellular expression of IFN-γ (*y*-axis) with PE-conjugated mAb. All lymphocytes were gated and analyzed on a FACScan flow cytometer using CellQuest software (BD Biosciences). Values shown in *upper right quadrant* indicate the percentage of $CD8^+$ cells that are positive for intracellular IFN-γ. Each experiment used 3–5 mice per group, and spleen cells of mice per group were pooled. Data shown are representative of three independent and reproducible experiments.

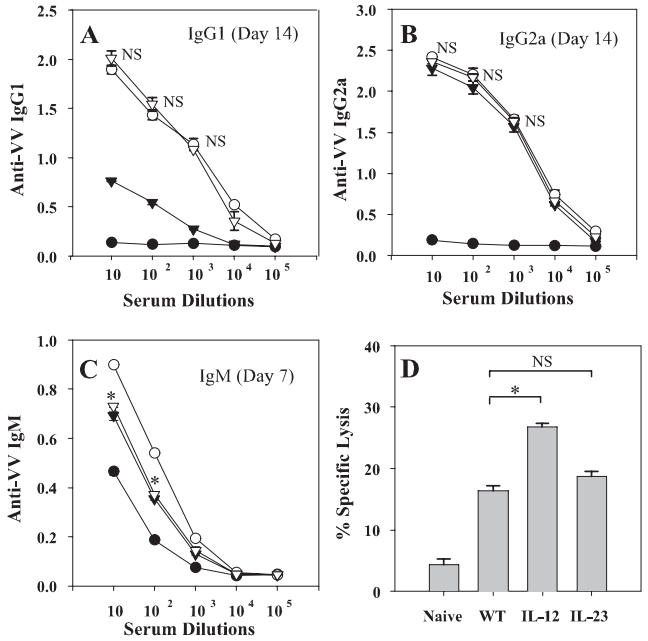

FIGURE 5. VV-specific IgG and IgM Ab production and NK activity in mice infected with either VV-WT, VV-IL-12 or VV-IL-23. *A–C*, BALB/c mice were infected i.p. with 2×10^7 PFU of VV-WT (○), VV-IL-12 (▼), or VV-IL-23 (▽), and serum of each mouse was collected at days 7 (*C*) and 14 (*A* and *B*) postinfection. Serum of noninfected mice (●) was used as negative controls. Titers of anti-VV IgG1 (*A*), IgG2a (*B*), and IgM (*C*) in serially diluted sera were determined by a solid-phase ELISA. Data are shown as the average ± SEM of four to six mice per group. The experiment was repeated twice with similar results. *, $p < 0.05$ compared with VV-WT. *D*, NK cell-mediated cytolytic activity. BALB/c mice were infected i.p. with 2×10^7 PFU of either VV-WT (WT), VV-IL-12 (IL-12), or VV-IL-23 (IL-23). Spleen cells were prepared at day 2 postinfection and used as effector cells in standard ^{51}Cr YAC-1 cell-killing assays. Spleen cells of noninfected mice (Naive) were used as a negative control. Data are shown as the mean ± SEM. The experiment was repeated three times with similar results, and at least three mice per group were used in each experiment. *, $p < 0.05$ compared with VV-WT.

IL-12 (29). Conversely, levels of anti-VV IgM Ab in both VV-IL-12-infected mice and VV-IL-23-infected mice were slightly lower than those in VV-WT-inoculated mice (Fig. 5*C*). Taken together, these data suggest that the increased resistance to VV caused by either IL-12 or IL-23 could not be explained by humoral immunity.

Because NK cells contribute to the recovery from VV infection (30), NK cell-mediated lysis was examined. The cytolytic capacity of splenic NK cells in VV-IL-12-inoculated mice was higher than that in VV-WT-infected mice. However, a significant increase of splenic NK activity was not observed in VV-IL-23-inoculated mice when compared with VV-WT-injected mice (Fig. 5*D*), suggesting that the attenuation of VV-IL-23 is likely to be independent of the activity of NK cells.

VV-IL-23-infected cells produce both IFN-γ and IL-17

It is well known that IFN-γ is a key factor in the IL-12-mediated antiviral response (1). In contrast, it has been reported that IL-17 plays a critical role in the IL-23-dependent resistance to some bacteria such as *K. pneumoniae* (17) and *C. rodentium* (11). Therefore, we wished to evaluate roles of IFN-γ and IL-17 in resistance to VV-IL-12 and VV-IL-23. Firstly, we tested whether VV-IL-12 and VV-IL-23 could stimulate lymphocytes to produce IFN-γ and/or IL-17. Naive spleen cells of BALB/c mice were infected in vitro with VV-WT, VV-IL-12, or VV-IL-23 and cultured for 2 days at 37°C. Culture supernatants were then screened for the presence of IFN-γ and IL-17 by ELISA (Fig. 6, *A* and *B*). VV-IL-12-infected cells secreted large amounts of IFN-γ (21.8 ng/ml), whereas VV-IL-23-infected cells produced much less but significant amounts of IFN-γ (1.4 ng/ml) (Fig. 6*A*). In contrast, VV-IL-12-infected cells did not produce IL-17 at all, but IL-23 delivered by VV-IL-23 led to a high production of IL-17 (760 pg/ml) (Fig. 6*B*). Similar patterns were observed when spleen cells were incubated with various concentrations of recombinant scIL-12 and scIL-23 (Fig. 6, *C* and *D*). As shown in Fig. 6*C*, large amounts of IFN-γ were secreted from cells incubated with recombinant scIL-12 even at a low concentration of 10 pg/ml, whereas recombinant scIL-23 stimulated IFN-γ production much less effectively than recombinant scIL-12. In contrast, recombinant scIL-23 induced IL-17 production in a dose-dependent manner, whereas recombinant scIL-12 did not stimulate secretion of IL-17 at all (Fig. 6*D*).

Effect of IL-23 on resistance to VV in IL-12/23p40-deficient mice

We next examined the effect of IL-23 on resistance to VV in IL-12/23p40-deficient mice. Firstly, BALB/c mice and IL-12/23p40 KO mice of the BALB/c background were infected i.p. with a high dose (2×10^8 PFU/mouse) of either VV-WT or VV-IL-23 and were monitored for mortality. As shown in Fig. 7*A*, all IL-12/23p40 KO mice ($n = 6$) succumbed to the infection with VV-WT by day 7 postinfection, whereas three of seven BALB/c mice survived the infection with VV-WT, demonstrating that IL-12/23p40 KO mice are more susceptible to the infection with VV-WT than are BALB/c mice. However, five of six IL-12/23p40 KO mice survived the infection with VV-IL-23, indicating that IL-23 promotes host defense against VV infection even in the absence of IL-12. Secondly, viral titers in ovaries of IL-12/23p40-deficient

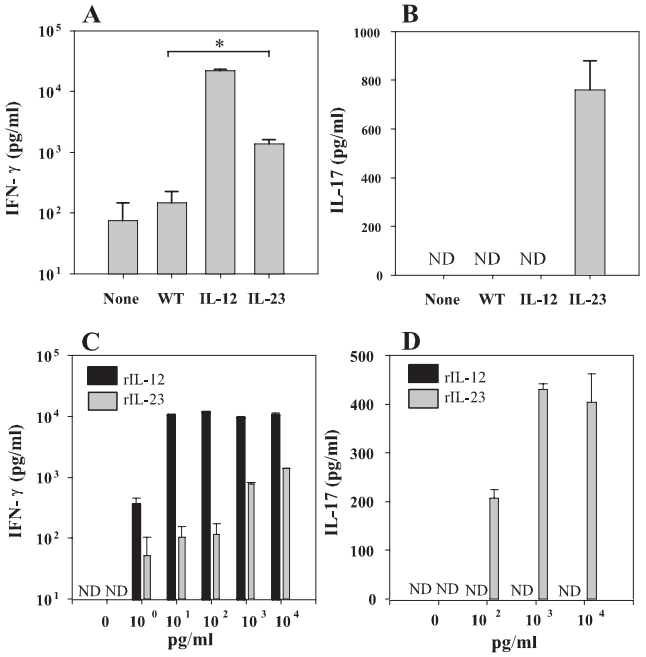

FIGURE 6. VV-IL-23-infected lymphocytes produce both IFN-γ and IL-17. *A* and *B*, Naive spleen cells of BALB/c mice were infected in vitro with VV-WT (WT), VV-IL-12 (IL-12), or VV-IL-23 (IL-23). Noninfected spleen cells (None) were used as a negative control. *C* and *D*, Naive spleen cells of BALB/c mice were cultured with either recombinant IL-12 or recombinant IL-23 at various concentrations. After 2 days of incubation, amounts of IFN-γ (*A* and *C*) and IL-17 (*B* and *D*) in the culture supernatants were quantitated by ELISA. Data are shown as the mean ± SEM of three to five mice per group. The experiment was repeated three times with similar results. ND, Not detected. *, $p < 0.01$.

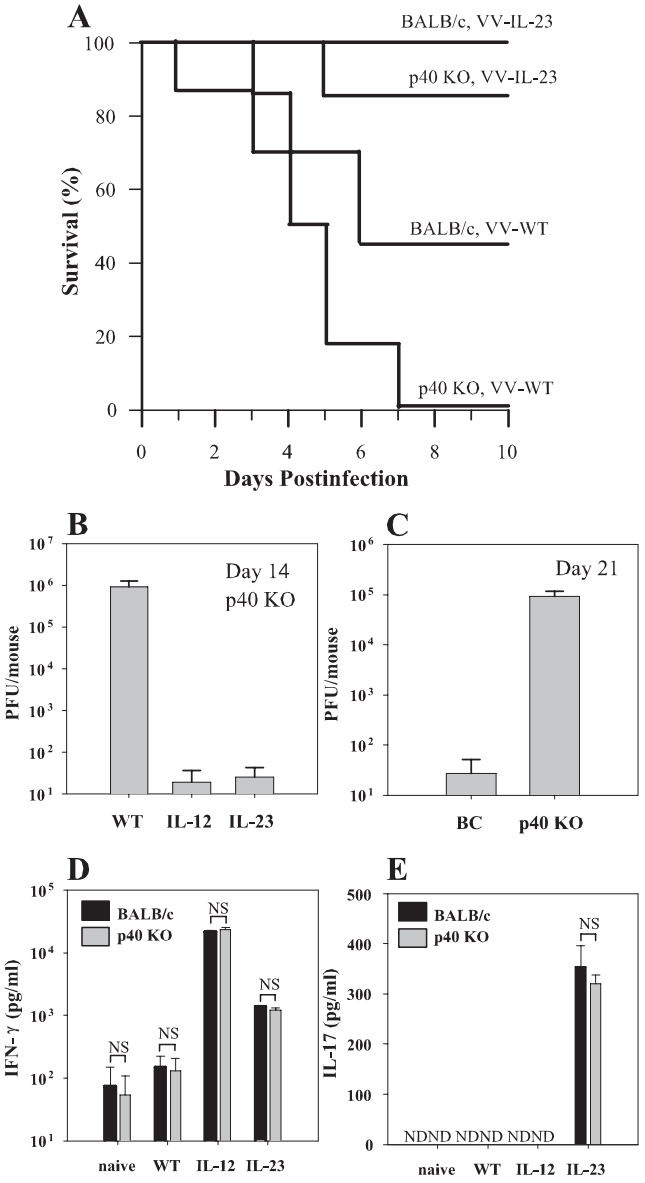

FIGURE 7. IL-23 delivered by VV-IL-23 enhances resistance to VV infection in IL-12/23p40-deficient mice. *A*, IL-12/23p40-deficient mice and BALB/c mice were infected i.p. with 2×10^8 PFU of VV-WT or VV-IL-23, and were monitored daily for mortality. *B*, IL-12/23p40-deficient mice infected with 5×10^6 PFU of VV-WT (WT), VV-IL-12 (IL-12), or VV-IL-23 (IL-23) were sacrificed at day 14 postinfection, and viral titers in ovaries were measured. From 8 to 12 mice were used in each group, and all titrations were performed in duplicates. Data are shown as the mean PFU ± SEM. *C*, BALB/c mice (BC) and IL-12/23p40-deficient mice (p40 KO) were infected with 5×10^6 PFU of VV-WT and sacrificed at day 21 postinfection. Viral titers in ovaries were then measured. Data shown are the mean PFU ± SEM. *D* and *E*, Naive spleen cells of either BALB/c or IL-12/23p40-deficient (p40 KO) mice were infected in vitro with VV-WT (WT), VV-IL-12 (IL-12), or VV-IL-23 (IL-23). Noninfected spleen cells (Naive) were used as a negative control. After 2 days of incubation, culture supernatants were screened for the presence of IFN-γ (*D*) and IL-17 (*E*) by ELISA. Data are shown as the mean ± SEM of three to five mice per group. Each experiment was repeated three times with similar results. ND, Not detected.

mice were measured after inoculation of a sublethal dose (5×10^6 PFU) of VV (Fig. 7*B*). Both VV-IL-12-infected and VV-IL-23-infected IL-12/23p40 KO mice almost cleared the virus at day 14 postinfection, although high virus titers were retained in VV-WT-inoculated IL-12/23p40 KO mice on the same day (Fig. 7*B*). Taken together, IL-23 delivered by VV-IL-23 is capable of enhancing the resistance to VV independently of IL-12 and vice versa. Based on the data of viral titers at day 21 postinfection with VV-WT (Fig. 7*C*), it was confirmed that IL-12/23p40 KO mice were far more sensitive to VV infection than were BALB/c mice, indicating that IL-12 and/or IL-23 play a critical role in resistance to VV infection. VV-IL-12-infected spleen cells of IL-12/23p40 KO mice produced IFN-γ as much as those of BALB/c mice (Fig. 7*D*), indicating that IL-12 delivered by VV-IL-12 efficiently stimulated to produce IFN-γ even in the absence of IL-12/23p40. In contrast, VV-IL-23-infected spleen cells derived from IL-12/23p40 KO mice secreted significant amounts of both IFN-γ (Fig. 7*D*) and IL-17 (Fig. 7*E*) as well as VV-IL-23-infected BALB/c spleen cells.

IL-23 enhanced the resistance to VV in IFN-γ-deficient mice

We then investigated the host defense against VV-IL-23 in the absence of IFN-γ. IFN-γ KO mice of the C57BL/6 background and control mice (C57BL/6) were infected with 2×10^8 PFU/mouse of either VV-WT or VV-IL-23 for monitoring survival rates (Fig. 8*A*). Because C57BL/6 mice are more resistant to VV than BALB/c mice (M. Matsui, unpublished observations), it is understandable that only one of eight C57BL/6 mice infected with VV-WT died, and all C57BL/6 mice ($n = 8$) infected with VV-IL-23 survived the infection. In contrast, all IFN-γ KO mice ($n = 12$) succumbed to the infection with VV-WT by day 6 postinfection, indicating that IFN-γ plays a crucial role in resistance to VV infection. However, all IFN-γ KO mice ($n = 8$) survived the infection with VV-IL-23, showing that IL-23 delivered by VV-IL-23 enhances the host defense against VV infection even in the

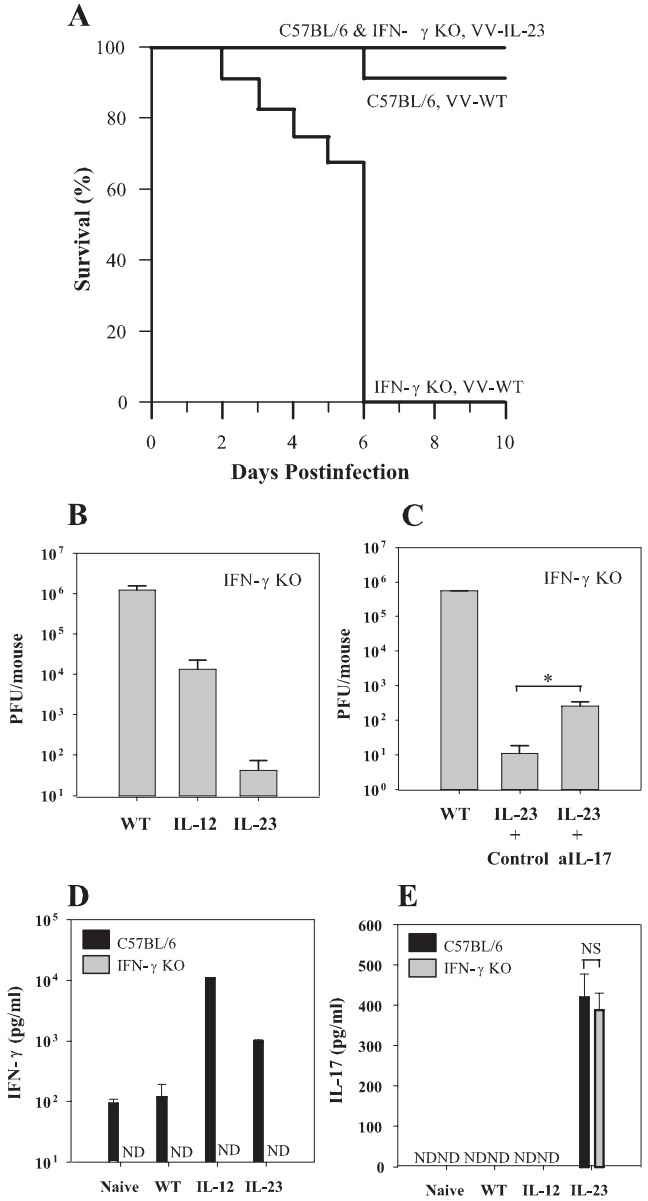

FIGURE 8. IL-23 delivered by VV-IL-23 enhances resistance to VV infection in IFN-γ-deficient mice. *A*, IFN-γ-deficient (IFN-γ KO) mice and C57BL/6 mice were infected i.p. with 2×10^8 PFU of VV-WT or VV-IL-23, and were monitored daily for mortality. *B*, IFN-γ-deficient mice infected with 5×10^6 PFU of VV-WT (WT), VV-IL-12 (IL-12), or VV-IL-23 (IL-23) were sacrificed at day 14 postinfection, and viral titers in ovaries were measured. Six to twelve mice were used in each group, and all titrations were performed in duplicates. Data are shown as the mean PFU ± SEM. *C*, IFN-γ -deficient mice were infected with 5×10^6 PFU of VV-WT (WT) or VV-IL-23 (IL-23). VV-IL-23-infected mice were then administered with 70 μg of a neutralizing anti-IL-17 mAb (aIL-17) or a relevant isotype control Ab (Control) at days 0, 2, 7 postinfection with VV. Viral titers in ovaries were then measured at day 14 postinfection. Four mice were used in each group, and all titrations were performed in duplicates. Data are shown as the mean PFU ± SEM. *, $p < 0.01$. *D* and *E*, Naive spleen cells of either C57BL/6 or IFN-γ-deficient (IFN-γ KO) mice were infected in vitro with VV-WT (WT), VV-IL-12 (IL-12), or VV-IL-23 (IL-23). Noninfected spleen cells (Naive) were used as a negative control. After 2 days of incubation, culture supernatants were screened for the presence of IFN-γ (*D*) and IL-17 (*E*) by ELISA. Data are shown as the mean± SEM of three to five mice per group. The experiment was repeated three times with similar results. ND, Not detected.

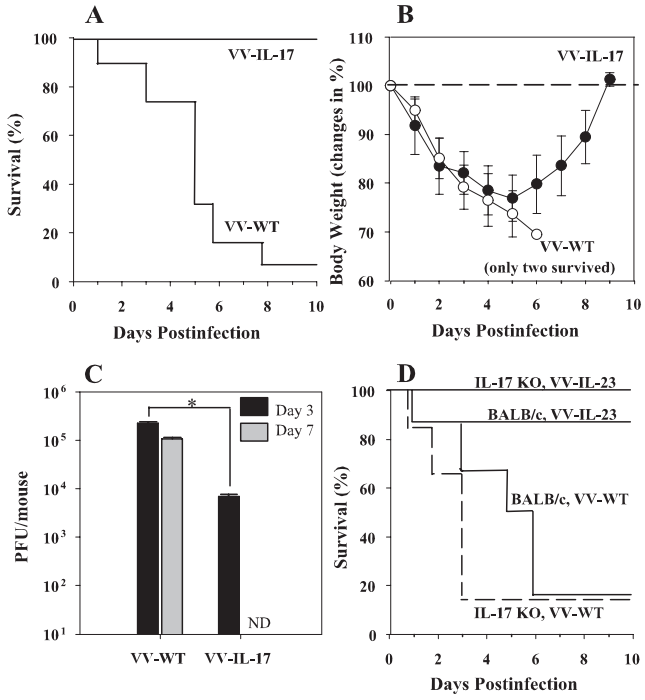

FIGURE 9. Role of IL-17 in resistance to VV infection. *A* and *B*, Twelve BALB/c mice per group were infected i.p. with 5×10^8 PFU of VV-WT or VV-IL-17. *A*, Mice were monitored daily for mortality. *B*, Changes in body weight of mice infected with VV-WT (open symbols) or VV-IL-17 (solid symbols) were calculated as the percentage of the mean weight per group in comparison with starting body weight. Data are shown as the mean ± SEM. *C*, BALB/c mice infected with 5×10^6 PFU of VV-WT or VV-IL-17 were sacrificed at day 3 (■) or 7 (▨) postinfection, and virus titers in ovaries of mice were measured. Six mice were used in each group, and all titrations were performed in duplicates. Data are shown as the mean PFU ± SEM. Nd, Not detected. *, $p < 0.01$. *D*, IL-17-deficient (IL-17 KO) mice and BALB/c mice were infected i.p. with 5×10^8 PFU of VV-WT or VV-IL-23, and were monitored daily for mortality. Six mice were used in each group. Data of VV-WT-infected IL-17 KO mice were shown as a dashed line for highlighting.

absence of IFN-γ. Viral titers in ovaries of IFN-γ KO mice were then examined after inoculation of a sublethal dose of VV (Fig. 8*B*). It was confirmed that C57BL/6 mice did not resolve VV-WT in ovaries, but did eliminate VV-IL-23 as well as VV-IL-12 at day 14 postinfection in the same manner as BALB/c mice (data not shown). In accordance with the survival data (Fig. 8*A*), IFN-γ KO mice nearly cleared VV-IL-23 in ovaries at day 14 postinfection, whereas high virus titers were still retained in ovaries of IFN-γ KO mice inoculated with either VV-WT or VV-IL-12 (Fig. 8*B*). These data indicate that IL-23 promotes resistance to VV even in the absence of IFN-γ and strongly suggest that the mechanism of the IL-23-mediated resistance to VV infection is distinct from that of the IL-12-regualted resistance in which IFN-γ plays a critical role. It was reported that IL-17 plays a critical role in the IL-23-dependent resistance to bacteria such as *K. pneumoniae* (17) and *C. rodentium* (11). To examine contribution of IL-17 in the IL-23-mediated resistance, VV-IL-23-infected IFN-γ KO mice were administered with a neutralizing anti-IL-17 mAb. As shown in Fig. 8*C*, treatment with the anti-IL-17 mAb, but not with a control mAb, resulted in a slight but significant increase of viral titers in ovaries at day 14 postinfection, suggesting an involvement of IL-17 in the IL-23-associated host defense against VV in the absence of IFN-γ. It was confirmed that spleen cells of IFN-γ KO

mice infected with VV-WT, VV-IL-12, or VV-IL-23 did not produce IFN-γ at all (Fig. 8*D*). In contrast, VV-IL-23-infected spleen cells of IFN-γ KO mice produced IL-17 as much as VV-IL-23-infected spleen cells of C57BL/6 mice (Fig. 8*E*).

Role of IL-17 in resistance to VV infection

To further examine the role of IL-17 in resistance to VV infection, we have constructed VV-IL-17 and investigated the susceptibility of BALB/c mice to VV-IL-17. It was confirmed that IL-17 was detected in the culture supernatant of VV-IL-17-infected 293T cells by ELISA, and IL-17 in the culture supernatant could induce IL-6 production from NIH 3T3 cells (data not shown). BALB/c mice were injected i.p. with 5×10^8 PFU/mouse of either VV-WT or VV-IL-17 for monitoring survival rates (Fig. 9*A*) and changes in body weight (Fig. 9*B*). As shown in Fig. 9*B*, VV-IL-17-infected mice looked very sick on the first few days after infection, but all of the mice ($n = 12$) eventually recovered from illness (Fig. 9A and B). In contrast, eleven of twelve mice injected with VV-WT succumbed to the infection (Fig. 9, *A* and *B*). Furthermore, VV-IL-17-infected BALB/c mice cleared VV from ovaries at day 7 postinfection with 5×10^6 PFU, whereas VV-WT-infected mice did not clear the virus (Fig. 9*C*). These results demonstrate that IL-17 promotes host resistance to VV infection, and therefore, support the data in Fig. 8*C* showing an involvement of IL-17 in the IL-23-mediated defense against VV. We next tested resistance to VV-WT and VV-IL-23 in IL-17-deficient mice. BALB/c mice and IL-17 KO mice of the BALB/c background were infected i.p. with 5×10^8 PFU/mouse of either VV-WT or VV-IL-23 and were monitored for mortality. As shown in Fig. 9*D*, IL-17 KO mice were more sensitive to VV-WT than control BALB/c mice, showing that IL-17 is necessary for host defense against VV infection. In contrast, all of VV-IL-23-infected IL-17 KO mice survived the infection, indicating that IL-17 is dispensable for the IL-23-mediated clearance of VV.

Discussion

In the current study, we demonstrated that IL-23 delivered by VV-IL-23 enhanced resistance to VV infection in BALB/c mice as well as IL-12 (Fig. 2). A similar activity of IL-23 was observed in IL-12/23p40-deficient mice infected with VV-IL-23 as well (Fig. 7, *A* and *B*), indicating that IL-23 enhances resistance to VV infection independently of IL-12. In other words, IL-23 could compensate for the lack of IL-12 in the host defense against VV. However, the mechanism of the IL-23-mediated resistance to VV infection is different from that of the IL-12-regulated resistance because IFN-γ-deficient mice did not eliminate VV-IL-12, but almost did eradicate VV-IL-23 in ovaries at day 14 postinfection (Fig. 8*B*). VV-IL-23-infected cells produced a certain amount of IFN-γ (Fig. 6*A*), and furthermore, several early studies pointed out that IL-23 stimulated the production of IFN-γ to induce various immune responses including CTL responses (2, 5, 6, 31). However, the current data indicate that IFN-γ is dispensable for the IL-23-regulated resistance to VV, although it does not mean that IFN-γ induced by IL-23 is totally useless in resistance to VV infection. According to the survival rate of IFN-γ KO mice infected with VV-WT (Fig. 8*A*), IFN-γ plays a critical role in host defense against VV infection, and hence, IL-23-induced IFN-γ should be beneficial for the viral clearance. Rather, it is possible to assume that some other factors could play a dominant role and compensate for the absence of IFN-γ in the IL-23-regulated resistance to VV. In contrast, IFN-γ plays a critical role in the IL-12-mediated resistance to VV (Fig. 8*B*). In fact, VV-IL-12-infected cells secreted large amounts of IFN-γ (Fig. 6*A*). However, viral titers of VV-IL-12-infected IFN-γ KO mice were ~100 times lower than those of VV-WT-infected IFN-γ KO mice (Fig. 8*B*). This finding could be explained by the IFN-γ-independent activity of IL-12 including direct effects on T cells and NK cells acting as a growth factor and an enhancer of cytotoxicity as well as inducing other cytokines (32).

Because the IL-23/IL-17 axis is known to be required for the host protection against *K. pheumoniae* (17) and *C. rodentium* (11), and further VV-IL-23-infected cells produced considerable amounts of IL-17 (Fig. 6*B*), it was quite possible to expect that IL-17 might play a major role in the IL-23-regulated resistance to VV infection. In fact, treatment with a neutralizing anti-IL-17 mAb resulted in a significant increase of viral titers in ovaries of VV-IL-23-infected IFN-γ KO mice (Fig. 8*C*), suggesting an involvement of IL-17 in the IL-23-regulated defense against VV at least in the absence of IFN-γ. It is presumed that the anti-viral IL-17 was secreted from Th17 cells, which were differentiated by TGF-β and IL-6 and were expanded to acquire fully effective function by IL-23 (10–12). However, the effect of neutralization with anti-IL-17 mAb was limited (Fig. 8*C*) although we cannot rule out the possibility that IL-17 in the mice was not fully neutralized with the mAb. These data suggest that something else directly or indirectly induced by IL-23 should play a major role in the IL-23-regulated resistance to VV infection. For example, it has recently been reported that Th17 cells secrete IL-22 together with IL-17 (33, 34). IL-22 in conjunction with IL-17 synergistically functions in host defense as well as in the pathogenesis of autoimmune diseases (33, 34). Actually, activated T cells from HIV-1-exposed, but uninfected individuals overproduce IL-22, suggesting that IL-22 participates in an innate anti-HIV-1 host resistance (35). It might be possible to presume that IL-22 is associated with the IL-23-driven enhancement of the resistance to VV. In addition, a potent inflammatory cytokine, IL-17F might play a major role in the IL-23-mediated resistance because Th17 cells produce IL-17F (36) as well as IL-17 (IL-17A) and IL-22.

To test whether IL-17 participates in host defense against VV infection, we have engineered VV-IL-17 and compared it with VV-WT. As shown in Fig. 9, *A–C*, VV-IL-17 was less virulent than VV-WT in BALB/c mice, indicating that IL-17 promotes resistance to VV infection. It has been reported that the IL-23/Th17/IL-17 axis plays an essential role in neutrophil recruitment (37). In the case of influenza virus infection, neutrophils as well as macrophages are accumulated at the site of infection soon after the viral infection, and either type of cell is capable of phagocytosing virus-infected, apoptotic cells (38). Virus-infected, apoptotic cells phagocytosed are then digested together with virus by degrading enzymes that exist in lysosomes of phagocytes. Hence, the influx of neutrophils induced by IL-17 may contribute to the rapid clearance of VV in VV-IL-17-infected mice. In the current study, VV-IL-17 induced viral clearance by day 7 of infection (Fig. 9*C*), whereas VV-IL-23 took 14 days to clear the virus (Fig. 2*C*). It may be possible to assume that VV-IL-17-infected cells could secrete large amounts of IL-17 at the site of infection faster than VV-IL-23-infected cells, and thereby, could induce the rapid influx of neutrophils, which may lead to the faster clearance of the virus. In addition, IL-17 KO mice were likely to be more sensitive to VV-WT than BALB/c mice (Fig. 9*D*), indicating that IL-17 is important in host defense against VV. However, IL-17 KO mice survived the infection with VV-IL-23 (Fig. 9*D*), demonstrating that IL-17 does not play a dominant role in the IL-23-regulated resistance to VV infection. Taken together, IL-23 may be working in the resistance to VV predominately through other effector molecules expressed by Th17 cells such as IL-22 or IL-17F although IL-17 (IL-17A) is likely to play at least a certain role in the IL-23-mediated resistance to VV.

It was previously shown that IL-23 and the subsequent IL-17 pathway play an essential role in resistance to infection with certain extracellular bacteria (11, 17). In contrast, Cua et al. (39) proposed that the IL-23/Th17 axis has physiologically been designed for the induction of immediate inflammatory response against catastrophic breaches of pathogens in the initial phase of mucosal infection. Our current data show a significant role of IL-23 in host defense against VV for the first time. Furthermore, IL-17 participates in the IL-23-regulated resistance to VV infection, but IL-17 is unlikely to play a dominant role in it. Our data provide a possibility that there might be an alternative pathway in the mechanism of IL-23-regulated resistance to VV infection. Therefore, our data suggest that it might be possible to use the adjuvant activity of IL-23 for the prophylactic and therapeutic strategies against infectious pathogens without side effects of autoimmunity caused by the IL-23/Th17/IL-17 axis.

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Disclosures

The authors have no financial conflict of interest.

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