IL-17 receptor signaling influences virus-induced corneal inflammation

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Abstract: IL-17 has been associated with selected inflammatory and autoimmune diseases. We characterized the expression of this proinflammatory cytokine following HSV-1 corneal infection and investigated whether IL-17R signaling modulated the host response to the viral pathogen at early time-points postinfection. IL-17 was elevated in the murine cornea 24 h after high-dose virus infection and subsequently persisted at low levels during the first week. Immunofluorescent studies showed that the IL-17R was expressed by cultured mouse corneal fibroblasts. Exposure of corneal cells to IL-17 led to production of IL-6 and MIP-2 in vitro and in vivo, indicating that the IL-17R was functional. Mice lacking IL-17R displayed significantly reduced neutrophil infiltration and corneal opacity. However, this effect was transient, as corneal pathology and neutrophil influx resembled that of wild-type (WT) hosts 4 days postinfection. HSV-1 growth and clearance in IL-17R$^{-/-}$ hosts were similar to that of the WT controls. Infection of IFN-γ gene knockout mice was associated with elevated IL-17 levels and accelerated corneal opacity, suggesting that IFN-γ negatively regulated IL-17 expression. Collectively, our results establish that IL-17 is rapidly produced in the cornea after HSV-1 infection and is regulated at least in part by IFN-γ. The absence of IL-17 signaling results in a transient decrease in the expression of proinflammatory mediators, neutrophil migration, and corneal pathology, but control of virus growth in the cornea and trigeminal ganglia is not compromised. Thus, IL-17 actively influences early virus-induced corneal inflammation. *J. Leukoc. Biol.* 83: 401–408; 2008.

Key Words: *HSV-1 · neutrophil · IFN-γ*

INTRODUCTION

HSV-1 infection of the cornea triggers an intense inflammatory response. In humans, repeated episodes of infection can occur as a result of virus reactivation from a latent state [1]. Repeated episodes of recurrent infection can lead to herpes stromal keratitis (HSK) and profound visual impairment. A cascade of cellular and molecular events orchestrates this TH1-mediated inflammation [2]. To sort out the various components operative after HSV-1 corneal infection, investigators have extensively used the mouse as a host [2]. In this model, one prominent feature is the rapid and extensive influx of neutrophils. These cells are thought to help limit virus replication as well as contribute to tissue pathology [3, 4]. The factors that control neutrophil recruitment and activation remain incompletely defined. Prior studies in our laboratory have indicated that the chemokines MIP-2 and MIP-1α play key roles in the recruitment of neutrophils into the cornea and that cytokines IL-1α and IL-6 are important in the generation of these chemoattractants [5, 6].

There is growing evidence that IL-17 is an important participant in neutrophil mobilization [7]. IL-17, also known as IL-17A, is the prototype member of the IL-17 family, which consists of six structurally related, homodimeric glycoproteins [8]. The receptor for human IL-17 is 69% identical to mouse IL-17R and is expressed by most cell types in both species [9].

The cytokine is secreted principally by T lymphocytes in man and mouse [10, 11]. Recent studies have provided evidence that the T cell subset producing IL-17 is clearly distinct from TH1 and TH2 cells [12, 13]. TGF-β, together with IL-6, is required for the differentiation of this T cell subset via induction of IL-23R expression [14–16]. IL-23 is thought to be essential to expand committed TH17 effectors.

IL-17 has been designated as proinflammatory, as it induces the expression of many mediators of inflammation [8, 17]. This cytokine has been associated with various chronic inflammatory conditions including rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, and cancer [17]. In particular, studies of chronic inflammatory lung disorders have documented the role that IL-17 plays in neutrophil recruitment and activation [7]. The cytokine appears to act by stimulating local cells in the lung to produce and release a number of mediators that act on neutrophils, including IL-8, IL-6, and GM-CSF.

Recently, Maertzdorf et al. [18] reported that message for IL-17 was expressed in corneas from HSK patients. In addition, cultured human corneal stromal fibroblasts (HCF) constitutively expressed IL-17R mRNA. Although exposure of HCF to exogenous IL-17 alone had little effect, IL-17 in the pres-

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ence of TNF-α and/or IFN-γ resulted in marked secretion of a number of proinflammatory mediators, including IL-6 and IL-8.

To date, IL-17 responses have been largely associated with extracellular bacterial and fungi infections [19, 20]. It is not known whether IL-17 influences the host response following HSV-1 corneal infection. Here, we report that the cytokine is rapidly induced and that mouse corneal fibroblasts express a functional IL-17R. The consequences of IL-17:IL-17R signaling in vivo were investigated by conducting experiments in mice deficient for the IL-17R gene. Recent studies have shown that IFN-γ can negatively affect IL-17 expression [12, 13]. Therefore, the influence of IFN-γ on IL-17 corneal expression was also examined.

MATERIALS AND METHODS

Animals

Male and female IL-17R-deficient mice on a C57Bl/6 background were obtained from Amgen Inc. (Thousand Oaks, CA, USA) for the establishment of a breeding colony. The genotype of the IL-17R$^{-/-}$ offspring was confirmed by PCR. RAG, IFN-γ$^{-/-}$, as well as sex- and aged-matched control C57Bl/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). All animals were cared for under specific pathogen-free conditions in the University of South Alabama (Mobile, AL, USA) animal facility under National Institutes of Health (NIH) guidelines.

Intracorneal HSV-1 infection

Corneal infections were performed using an intrastromal route to inject the desired dose of HSV-1 strain RE. A pilot hole was produced through the corneal epithelium into the stroma using a 30-G disposable needle. A 32-G/30-cm needle attached to a repeating dispenser (Hamilton, Reno, NV, USA) was then inserted, and HSV-1 was injected intrastromally in a volume of 1 μl. The infectious dose was varied, ranging from 2×10^4 PFU to 3×10^6 PFU, depending on the experiment.

Corneal opacity scores

Eyes were monitored in a coded manner for corneal opacity by observation under the dissecting microscope and graded as follows: 0 = clear cornea, 1 = slight corneal haze, 2 = moderate corneal opacity, 3 = severe corneal opacity but iris visible, 4 = severe corneal opacity with iris obscured, and 5 = necrotizing stromal keratitis.

RT-PCR analysis for IL-17

In experiments where mRNA up-regulation was investigated, corneas and trigeminal ganglia (TG) from groups of three uninfected and three HSV-1-infected (1.5×10^5 PFU/cornea) wild-type (WT) mice were excised and pooled. The pooled tissue was homogenized for 30 s in 1 ml RNAwiz (Ambion Inc., Austin, TX, USA), and total RNA was extracted following the manufacturer's protocol. Contaminating genomic DNA was removed by DNase treatment using RNA-free DNase (Ambion Inc.), while total RNA purity and quantitation were performed using the SmartSpec 3000 spectrophotometer (Bio-Rad, Hercules, CA, USA). The MessageSensor RT kit from Ambion Inc. was used to convert 0.5 μg total RNA to cDNA for real-time PCR analysis using the Bio-Rad iCycler IQ system. Primers were generated for IL-17 and each chemokine or receptor using Beacon Designer Version 2 software (Premier Biosoft, Palo Alto, CA, USA), and PCR reactions were performed using iQ SYBR Green Supermix in a 96-well plate format (both by Bio-Rad). GAPD mRNA levels were used to normalize template-loading variations, and negative RT reactions were performed to ensure the absence of genomic DNA contamination in the samples. The latter was confirmed by analysis of the melt curves produced during real-time PCR reactions.

Virus titration

Individual corneal and TG lysates were titrated for infectious virus on Vero cell monolayers in a 48-h plaque assay.

Corneal fibroblast cell culture

Sixteen corneas from eight uninfected WT mice were excised, pooled, and incubated at 37°C and 5% CO_2 in 5 mM EDTA/PBS for 20 min. After this time, epithelial sheets were removed with forceps under the dissecting microscope. The stromal layer of each cornea was minced and incubated in 3000 U/ml collagenase type I (Sigma Chemical Co., St. Louis, MO, USA) at 37°C and 5% CO_2 for 1 h. The digested stromal layers were pooled and washed repeatedly in DMEM containing 20% FBS. The resultant cells were cultured in a T25 tissue-culture flask in a minimal volume of DMEM + 20% FBS medium. After 1 week of culture, cells were trypsinized and redistributed to the same flask to achieve uniform growth. At 90% confluency, cells were passaged to chamber slides at low density for immunofluorescent staining with 1 μg/ml goat anti-mouse IL-17R antibody (R&D Systems, Minneapolis, MN, USA) and a FITC-conjugated secondary. Alternatively, cells were transferred to 12-well tissue-culture plates at a density of 1×10^4 cells/well for stimulation studies. The cells were serum-starved for 3 days and then stimulated for 24 h with 1 or 10 ng/ml IL-17 (R&D Systems) or medium as a control. The cell supernatants were collected after 24 h of incubation and assayed for the desired mediators by ELISA.

Cytospin slide analysis of cells infiltrating the infected cornea

Corneas from C57Bl/6 mice were harvested at 2 and 4 days post-HSV-1 intracorneal infection at 2×10^4 PFU/cornea. Individual corneas were treated with 0.5 mM EDTA/PBS for 20 min at 37°C with 5% CO_2 to permit the removal of all limbal tissue and the epithelial sheet. Remaining stromal tissue was minced and incubated in the presence of collagenase type I (3000 U/ml) for 1 h at 37°C, 5% CO_2. The resultant cells were pipetted through a 10-μl pipette tip to disrupt any residual tissue clumps. Cells were washed in RPMI + 5% FBS and counted. Cells from individual corneas were cytospun onto a 2-cm grid slide. Each cornea was plated as two independent, 7 mm-diameter cytospin spots. Cells were differentially stained with HEMA 3 (Biochemical Sciences, Inc., Swedesboro, NJ, USA) to permit neutrophil versus mononuclear cell counts to be determined per cornea. The cells were counted under 450× magnification using the 2 mm^2 subdivisions of the grid as a guide. Slides were counted in a coded manner. Cells within three 2 mm^2 subdivisions of each cytospin spot were counted to obtain the mean number of infiltrating cells per cornea.

Cytokine and chemokine protein assay

Corneas were excised at the indicated times and individually frozen in a 0.5-ml vol RPMI 1640. Alternatively, to improve detection of IL-17 and IFN-γ, pools of four corneas in 0.4 ml medium were prepared. After thawing, the corneal samples were processed by homogenation (30 s in a tissue-tearer, Biospec Products, Bartlesville, OK, USA) and sonication (15 s). Samples were clarified by centrifugation at 150 *g* for 10 min, and supernatants were assayed for chemokines by ELISA (R&D Systems). The limits of detection for each ELISA were as follows: IL-17 < 5 pg/ml, IFN-γ < 2 pg/ml, MIP-2 < 1.5 pg/ml, MIP-1α < 1.5 pg/ml, and IL-6 < 2 pg/ml.

Statistical analysis

The Mann-Whitney's U test and Student's *t*-test were used for the statistical evaluation of the results. The level of confidence at which the results were judged significant was $P < 0.05$.

RESULTS

Expression of IL-17 in the HSV-1-infected cornea

Preliminary RT-PCR studies revealed that IL-17 mRNA was up-regulated (five- to 14-fold) in corneas of C57BL/6 mice 24 h

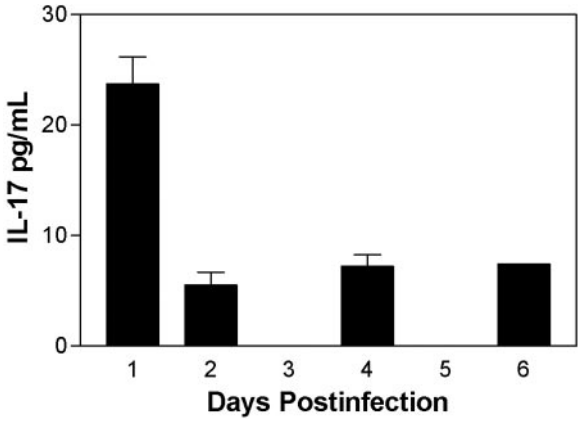

Fig. 1. IL-17 expression in the HSV-1-infected cornea. WT mice were infected intracorneally with 3×10^6 PFU HSV-1. On the indicated days postinfection, pools of four corneas were prepared, and lysates thereof were assayed for IL-17 by ELISA. The means of two to three samples per time-point are shown.

after HSV-1 intracorneal infection (data not shown). **Figure 1** shows that IL-17 protein was present in the cornea at 24 h postinfection and persisted at low levels during the first week of infection. No IL-17 protein was detected in normal corneas or in corneas 12 h after infection.

IL-17R is expressed and functional on mouse corneal fibroblasts

Immunofluorescent experiments were conducted initially to determine whether the receptor for IL-17 was present on mouse corneal cells. To this end, fibroblasts prepared from excised C57Bl/6 corneas were grown on chamber slides and stained with antibody to mouse IL-17R 24 h later. **Figure 2** shows that the stromal fibroblasts stained positively for IL-17R but did not react with control Ig. To test whether the receptor was functional, corneal fibroblasts were stimulated with IL-17. It was found that recombinant mouse (rm)IL-17 induced the cells to produce and secrete the proinflammatory cytokine IL-6 and the neutrophil chemoattractant MIP-2 in a dose-response manner (**Fig. 3**). These results document that cultured murine corneal fibroblasts express a functional IL-17R.

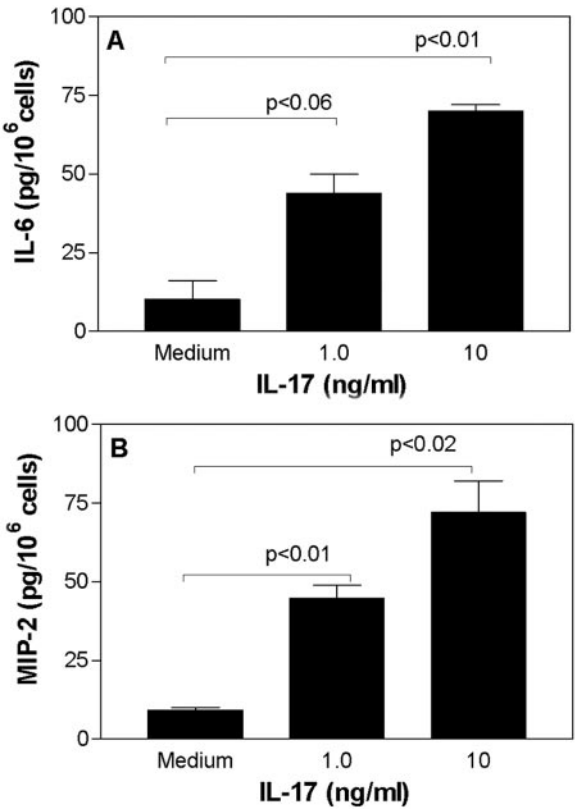

Fig. 3. The IL-17R is functional on cultured mouse corneal fibroblasts, which were isolated and cultured from WT C57Bl/6 mice and treated with 1 or 10 ng/ml rmIL-17 in serum-free conditions. Nonstimulated cells were exposed to medium only. After 24 h of stimulation, supernatants were harvested and assayed by ELISA to determine the levels of secreted IL-6 (A) and MIP-2 (B).

IL-17 stimulates the production of proinflammatory mediators in vivo

To test whether the IL-17R was also functional in vivo, IL-17 was injected directly into the mouse cornea, and corneas were excised 6 h later, and lysates thereof were assayed for IL-6 and MIP-2. **Figure 4** shows that in vivo stimulation of corneal tissue with IL-17 resulted in production of modest but significant amounts of IL-6 and MIP-2. IL-1α is a well-established, early warning cytokine active in the cornea [21]. It was found that IL-1α acted synergistically with IL-17 to up-regulate IL-6

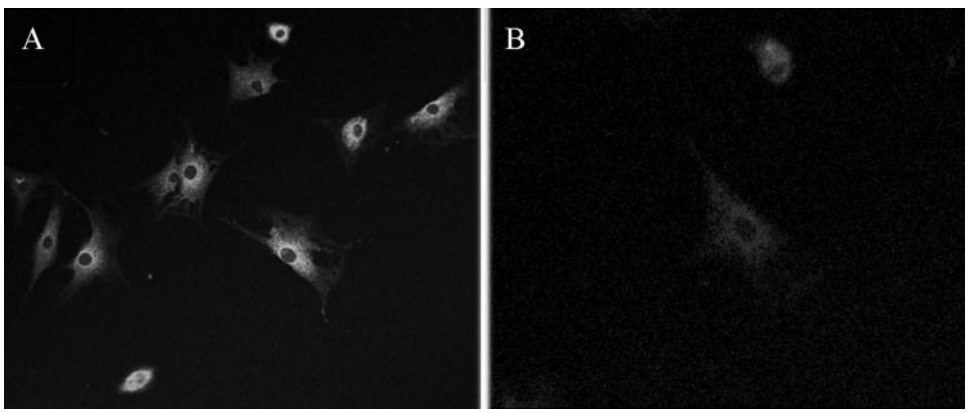

Fig. 2. The IL-17R is present on cultured mouse corneal fibroblasts. WT mouse corneal fibroblast cells were cultured in chamber slides for 24 h prior to staining with goat anti-mouse IL-17R antibody (A) or control goat anti-serum (B). Images were captured digitally from the ×40 oil immersion objective of a confocal microscope.

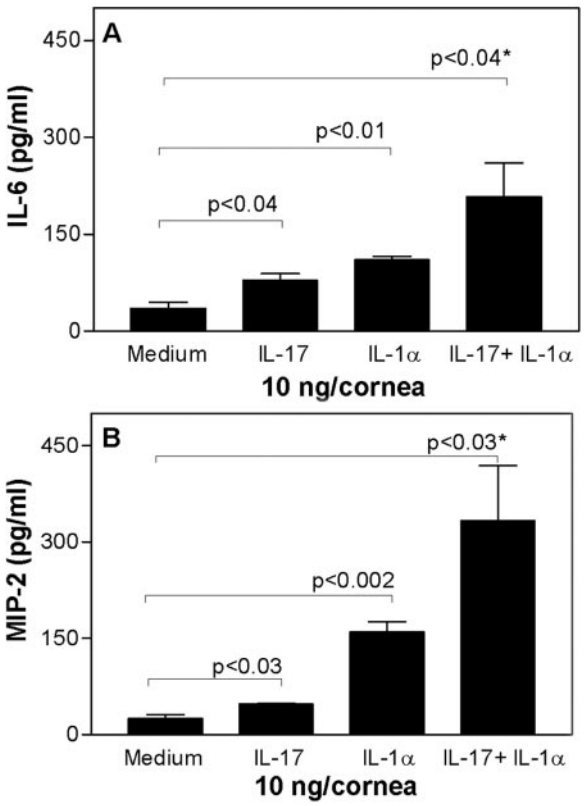

Fig. 4. IL-17 functions in vivo to produce proinflammatory mediators. WT mice (n=3–4) were injected intracorneally with 10 ng/cornea rmIL-17 or IL-1α alone or in combination to test for synergy. Medium alone was used as a control. Six hours postinjection, mice were killed, corneas harvested, and lysates produced for ELISA assay of proinflammatory mediators, IL-6 (A) and MIP-2 (B). *, The result is greater than additive, where a combination of IL-17 and IL-1α was given.

production by >2.5-fold and MIP-2 by sevenfold relative to IL-17 stimulation alone (Fig. 4).

Initial corneal opacity and neutrophil influx are diminished in HSV-1-infected IL-17R$^{-/-}$ hosts

The foregoing results suggested that IL-17 may influence proinflammatory mediator expression in the HSV-1-infected cornea. To investigate this possibility, studies were conducted in IL-17R-deficient mice. These mice and their corresponding WT controls were infected intracorneally with HSV-1 and monitored for corneal opacity. On day 2 postinfection, it was observed that corneal pathology in the WT hosts was significantly greater than that in the IL-17R$^{-/-}$ mice (**Fig. 5A**). Microscopic examination of the cells recovered by collagenase digestion of the cornea revealed that neutrophil recruitment in the receptor KO hosts was only 18% of that observed in the controls (Fig. 5B). In contrast, the mononuclear cell counts in the WT animals were similar to that of the IL-17R$^{-/-}$ mice (Fig. 5B). When this analysis was repeated at Day 4 postinfection, a clear distinction in corneal pathology was no longer evident. At this later time-point, neither corneal opacity (Fig. 5C) nor number of infiltrating neutrophils (Fig. 5D) for the two groups was significantly different. This continued to be the case for corneal opacity over the remaining 11 days of observation.

We repeated the foregoing experiment using a 2×10^5 PFU challenge dose and obtained similar results; i.e., the WT corneal opacity score at Day 2 but not at Day 4 postinfection was significantly greater (P<0.001) than that seen in the IL-17R KO hosts. Collectively, these results suggest that IL-17

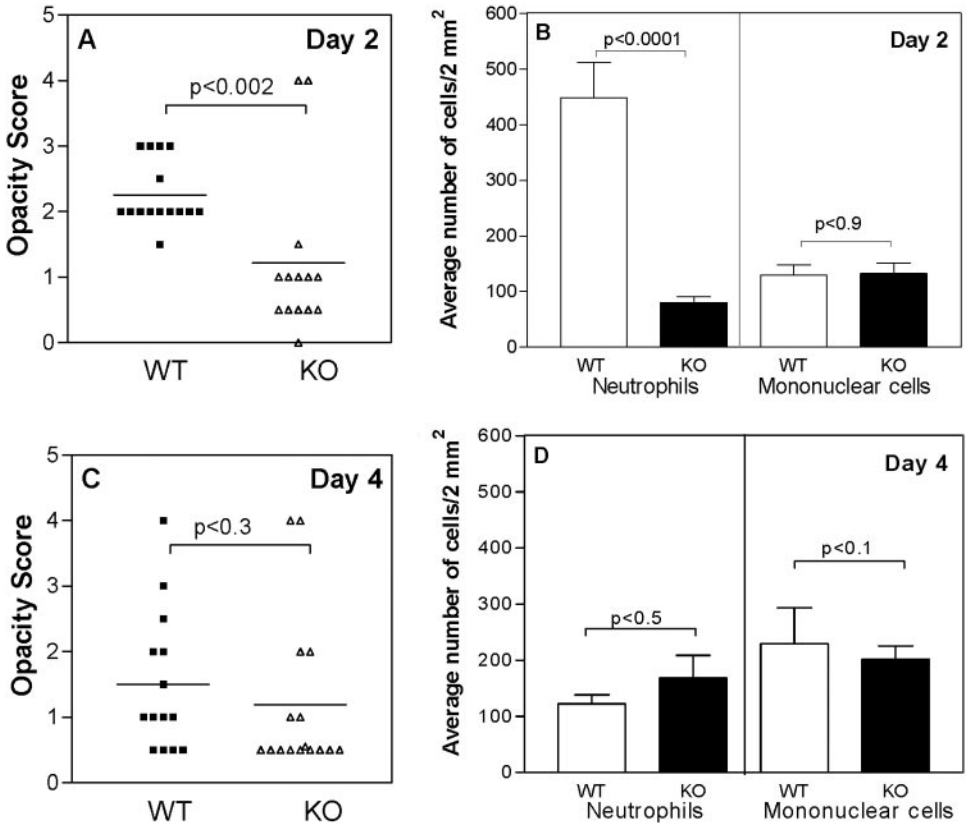

Fig. 5. Corneal opacity and cellular infiltrates are reduced initially in the IL-17R$^{-/-}$ mouse. WT and IL-17R$^{-/-}$ [knockout (KO)] mice (n=14–15) were infected intracorneally with 2×10^4 PFU HSV-1 and examined microscopically for the development of corneal opacity (A and C). At each time-point, mice (n=6–8) were killed, and corneas were harvested for the quantitation of infiltrating cells (B and D). A single cell suspension was produced by collagenase I digestion of the cornea, and cell populations were counted after differential staining of cytospin slides of the individual corneal samples.

signaling markedly affected only the initial phase of neutrophil influx and corneal pathology.

Altered chemokine expression in the HSV-1-infected IL-17R$^{-/-}$ host

Previous studies in our laboratory had established that the chemokines MIP-2 and MIP-1α were important promoters of neutrophil influx into the HSV-1-infected cornea [5]. Therefore, we investigated whether the deficiency in initial neutrophil recruitment in the IL-17R$^{-/-}$ hosts was associated with reduced chemokine production. It was found that corneas collected on Day 2 postinfection from IL-17R$^{-/-}$ hosts had significantly lower levels of MIP-2 than did WT mice (**Fig. 6A**). Similarly, MIP-1α levels were reduced in the receptor-deficient mice. This difference was not evident at Day 4 postinfection for MIP-2, and MIP-1α levels were higher in the IL-17R$^{-/-}$ corneas compared with the WT controls (Fig. 6B).

HSV-1 growth in the IL-17R$^{-/-}$ mouse cornea

In an earlier report, it was observed that neutrophil-depleted mice were considerably more susceptible to HSV-1 corneal infection [4]. We tested whether the IL-17R$^{-/-}$ animals displayed reduced resistance to virus replication. **Figure 7** shows that at Days 2 and 4 postinfection, virus titers in the receptor KO hosts were indistinguishable from that of WT controls. At

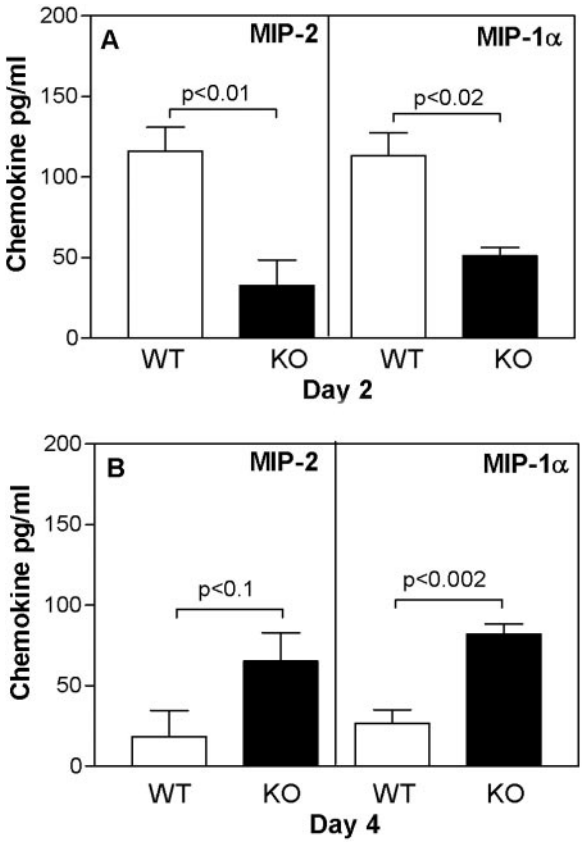

Fig. 6. Reduced neutrophil chemoattractant levels in the IL-17R$^{-/-}$ mouse cornea. WT and KO mice (n=5–6) were infected with 2 × 10^4 PFU HSV-1 by intracorneal injection. On Days 2 (A) and 4 (B) postinfection, corneas were harvested and individually assayed for MIP-2 and MIP-1α by ELISA.

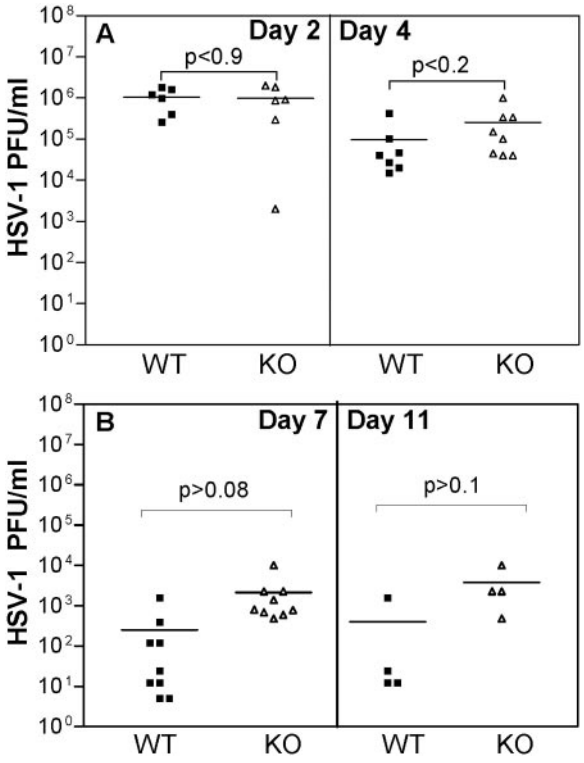

Fig. 7. Virus growth is not elevated in the IL-17R$^{-/-}$ mouse cornea. WT and KO mice (n=4–9) were infected with 2 × 10^4 PFU HSV-1 by intracorneal injection. On Days 2 and 4 (A) and 7 and 11 (B) postinfection, corneas were harvested and individually assayed for virus titer by plaque assay on Vero cells.

subsequent time-points (Days 7 and 11 postinfection), there was a suggestion that the IL-17R$^{-/-}$ mice were less efficient at suppressing virus growth in the cornea, but statistically significant differences were not seen. At Day 13 postinfection, all 11 WT and nine of the 10 IL-17R$^{-/-}$ corneas were infectious virus-free. Similarly, little or no infectious virus was detected in the TG of WT and receptor KO hosts on Day 13 (data not shown).

The foregoing results were confirmed in a second experiment in which a tenfold higher virus challenge dose was given, and tissues were assayed for infectious virus content at Day 11 postinfection. Again, the IL-17R$^{-/-}$ mice cleared HSV-1 from the cornea and TG as efficiently as WT hosts. In addition, none of the mice in either group displayed signs of encephalitis over the 21-day observation period. Thus, the kinetics of HSV-1 clearance in IL-17R$^{-/-}$ mice was comparable with that of the controls.

Evidence IFN-γ regulates early IL-17 expression in the cornea

Recent studies have indicated that IFN-γ can function as an inhibitor of IL-17 synthesis in vivo [12, 13]. **Figure 8A** shows that this immunoregulatory cytokine was expressed in abundance in WT corneas 24 h after virus infection and as expected, was not detected in IFN-γ gene-depleted hosts. When the same samples were assayed for IL-17, we found significantly (P<0.04) increased levels in IFN-γ$^{-/-}$ mice in com-

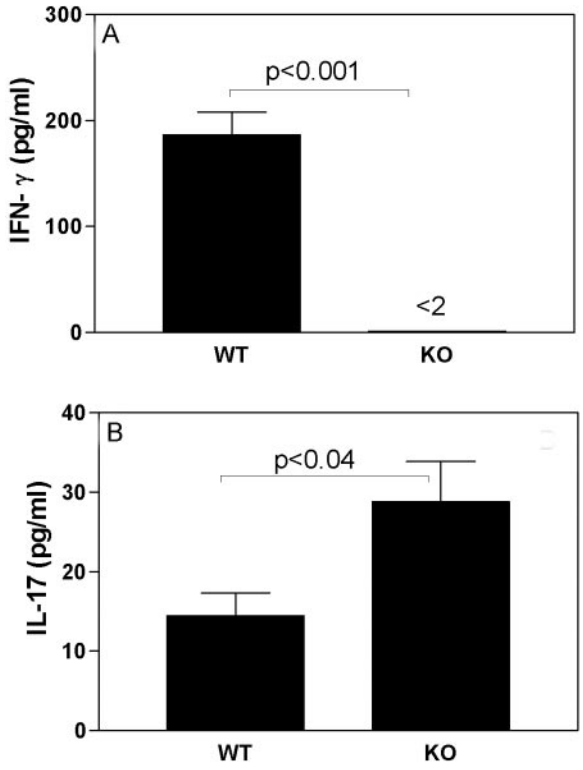

Fig. 8. Elevated IL-17 expression in the HSV-1-infected cornea of IFN-$\gamma^{-/-}$ mice. WT and IFN-γ KO hosts were infected intracorneally with 3×10^6 PFU HSV-1. Twenty-four hours postinfection, pools of three to four corneas were prepared, and lysates thereof were assayed for IFN-γ and IL-17. The means of six samples are shown.

parison with that expressed by the WT controls (Fig. 8B). This suggests that IFN-γ directly or indirectly functions as a negative regulator of early IL-17 expression in the HSV-1-infected cornea.

We next tested whether enhanced IL-17 expression in the IFN-$\gamma^{-/-}$ mice was associated with accelerated corneal disease. **Figure 9A** shows that corneal opacity was clearly accelerated in the IFN-$\gamma^{-/-}$ hosts beginning 2 days postinfection. Additional experiments revealed that the IFN-$\gamma^{-/-}$ mice had significantly increased ($P<0.03$) expression of MIP-2 (Fig. 9B). Greater levels of IL-6 were also seen, although not statistically significant (Fig. 9C). The accelerated inflammation in the IFN-$\gamma^{-/-}$ hosts is compatible with the view that IL-17 influences early corneal opacity.

DISCUSSION

This study assesses the role of IL-17 and its receptor in the immunopathology of HSV-1 ocular infection. Our results clearly document that this proinflammatory cytokine is rapidly expressed in the infected cornea and that IL-17R is present and functional on mouse corneal fibroblasts. Several lines of evidence established that ligand-receptor interaction significantly influenced the host response to virus infection. Thus, exposure of corneal fibroblasts to IL-17 resulted in production of the proinflammatory mediators IL-6 and MIP-2 in vitro and in vivo. These mediators have previously been linked to virus-induced corneal inflammation [5, 6]. Further, the absence of the IL-17R was associated with reduced MIP-2 and IL-6 levels, decreased neutrophil infiltration, and diminished corneal opacity.

In this HSV-1 ocular infection model, IL-17 clearly accelerated corneal inflammation. However, the cytokine's effect was transient, as 4 days postinfection, the eyes of IL-17R$^{-/-}$ mice displayed corneal pathology comparable with the WT controls. Why might this be? Recent studies have shown that the T cell subset producing IL-17 is distinct from that producing IFN-γ [12, 13], a cytokine promoting the TH1 subset [22]. Importantly, IFN-γ has been shown to negatively regulate IL-17 expression, apparently by suppressing IL-23 synthesis [12], which is critical for the amplification and survival of IL-17-producing cells. In the HSV-1-infected cornea, IFN-γ levels were an order of magnitude higher than that of IL-17 and thus, might be expected to down-regulate IL-17 production. Consistent with this, we observed that IFN-γ KO mice had elevated levels of IL-17 relative to the WT controls and displayed accelerated development of corneal opacity. Interestingly, previous reports on experiment autoimmune encephalomyelitis [23–27] and collagen-induced arthritis [28] also noted that higher IL-17 levels and greater disease are seen in IFN-$\gamma^{-/-}$ mice compared with WT mice. A caveat for this hypoth-

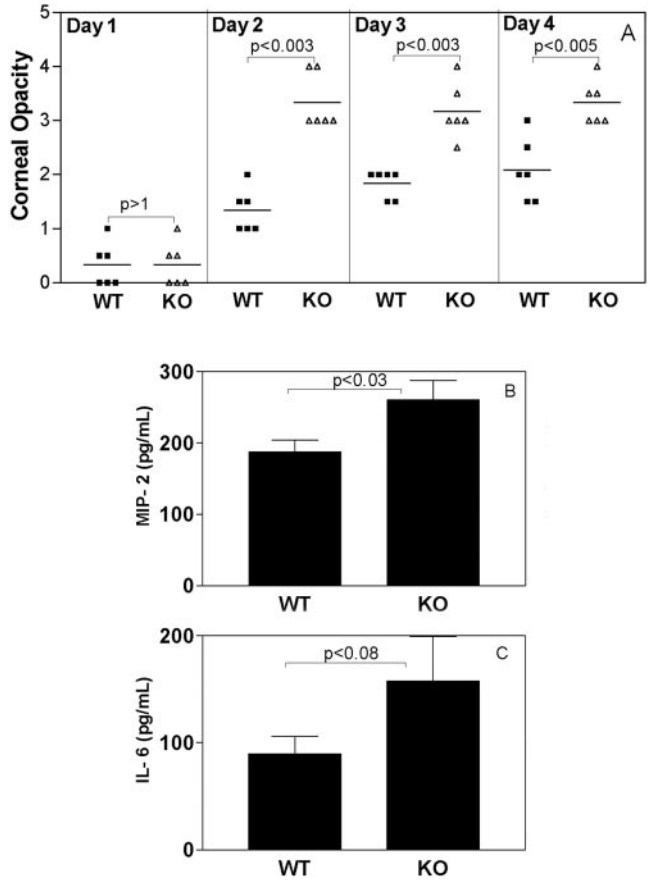

Fig. 9. Accelerated corneal opacity in IFN-$\gamma^{-/-}$ mice. WT and IFN-γ KO mice ($n=6$) were infected intracorneally with 2×10^5 PFU HSV-1 and monitored for ocular disease (A). At 48 h postinfection, individual corneal lysates ($n=6$) were prepared and assayed for MIP-2 (B) and IL-6 (C) by ELISA.

esis is that IFN-γ KO mice have a number of defects in host defense, and so, the heightened corneal opacity may be a consequence of additional factors besides elevated IL-17 production.

IL-17 is made primarily by sensitized T cells expressing α/β TCRs [10, 11], and these cells would not be expected to be in the cornea 24 h postinfection. Recent studies in our laboratory have revealed that relative to WT hosts, IL-17 is reduced significantly 24 h postinfection in mice with a deletion in the δ gene expressed by γ/δ T cells. Conversely, in the same time-frame, IL-17 is not suppressed in Rag-1 mice, which lack T and B cells [29]. Thus, our preliminary data suggest that the early appearing IL-17 may be made by γ/δ T cells. These cells are known to participate in innate immune responses.

Although IL-17 can activate a variety of proinflammatory mediators, it has been particularly associated with promotion of neutrophil migration. In the HSV-1-infected cornea, MIP-2 and MIP-1α are critical chemokines promoting the rapid recruitment of neutrophils into the infected murine cornea [5]. Consistent with this, we found that mice lacking IL-17R and therefore, not able to support IL-17 signaling, had significantly reduced chemokine levels and reduced neutrophil influx. Furthermore, reduced neutrophil migration was associated with diminished corneal opacity. Our results parallel those seen in models of bacterial and parasite infection. Ye et al. [30] found that absence of IL-17R was associated with reduced MIP-2 in bronchoalveolar lavage fluid and a significant delay in neutrophil recruitment into the *Klebsiella pneumoniae*-infected lung. Similarly, defective neutrophil migration was observed in IL-17R-deficient hosts infected orally with *Toxoplasma gondii* [31]. Cua and Kastelen [32] have proposed that a key role of IL-17 is to generate a massive neutrophil burst when the need arises for an immediate and robust inflammatory response, such as after a high infection pathogen-challenge dose.

In our hands, IL-17 added directly to murine neutrophils in culture did not stimulate MIP-2 or IL-6 production (S. J. Molesworth-Kenyon and R. N. Lausch, unpublished observation). Thus, the likely scenario is that IL-17 acts indirectly to promote neutrophil recruitment. That is, IL-17 is envisioned to bind to receptors on mouse corneal fibroblasts inducing or more likely, enhancing the induction and secretion of chemokines such as MIP-2 by early warning cytokines [14]. These chemokines in turn chemoattract neutrophils and other cells to accumulate in the HSV-1-infected cornea. Infiltration of these proinflammatory cells promotes the development of corneal opacity. Whether IL-17 can act directly on resident corneal cells to alter their function will require further investigation.

Previous neutrophil depletion studies documented that these cells helped suppress HSV-1 replication in the cornea and nervous tissues and prevented induction of fatal encephalitis [4]. Thus, we predicted that IL-17R-deficient mice would be more susceptible to HSV-1 challenge. However, these gene KO mice did not display elevated HSV-1 virus titers in the cornea, and virus clearance was similar to that seen in the WT controls. This outcome may be attributed to the fact that neutrophil migration into the IL-17R$^{-/-}$ cornea was only transiently impaired, with decreased numbers being no longer evident 4 days postinfection. Additional proinflammatory cytokines such as IL-1α and IL-6 are rapidly released following HSV-1 corneal infection [33], and these mediators are known to promote MIP-2 and MIP-1α expression [5, 6]. Indeed, the latter was elevated significantly 4 days after infection. Thus, there may be rapid compensation for the absence of IL-17 signaling, resulting in sufficient time for host defense mechanisms to limit virus spread and growth in ocular and nervous tissue.

To date, IL-17 has been associated primarily with host resistance to bacteria and fungi [19, 20]. What role IL-17 plays in the outcome of virus infection is just beginning to be investigated. Patera et al. [34] reported that mice infected i.p. with vaccinia virus containing the gene for IL-17 displayed elevated virus titers in the ovaries 1–3 days postinfection, enhanced early mortality, and reduced NK cell cytotoxicity. Respiratory syncytial virus infection in STAT1 KO mice resulted in elevated IL-17, increased virus growth, and enhanced lung pathology [35]. Thus, as in HSV-1 infection, IL-17 has been associated with increased tissue damage.

Nakae et al. [36] reported that IL-17-deficient mice were impaired in their ability to generate antigen-specific cells active in selective hypersensitivity responses as well as T cell-dependent antibody production. Impaired acquired immunity was not evident in our ocular infection model, because as noted above, HSV-1 was cleared in IL-17R$^{-/-}$ mice with the same kinetics seen in WT controls. Furthermore, HSV-1-induced corneal opacity, a T cell-dependent immunopathological response, was comparable with that in the WT controls at Day 4 and beyond.

In summary, IL-17R signaling following HSV-1 corneal infection promotes chemokine production and neutrophil influx, which in turn, is reflected in enhanced corneal opacity. These responses occur rapidly after infection, are transient in nature, and appear to be regulated in part by IFN-γ. Thus, IL-17 signaling clearly impacts the early host response in this ocular infection model.

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