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IL-18 Contributes to Host Resistance Against Infection with Pseudomonas aeruginosa Through Induction of IFN-γ Production

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Pseudomonas aeruginosa keratitis destroys the cornea in susceptible (B6), but not resistant (BALB/c) mice. To determine mechanisms mediating resistance, the role of IFN-γ, IL-12, and IL-18 was tested in BALB/c mice. RT-PCR analysis detected IFN-γ mRNA expression levels in cornea that were significantly increased at 1–7 days postinfection. IL-18 mRNA was detected constitutively in cornea and, at 1–7 days postinfection, levels were elevated significantly, while no IL-12 mRNA was similarly detected. To test whether IL-18 contributed to IFN-γ production, mice were treated with anti-IL-18 mAb. Treatment decreased corneal IFN-γ mRNA levels, and bacterial load and disease increased/worsened, compared with IgG-treated mice. To stringently examine the role of IFN-γ in bacterial killing, knockout (+/−) vs wild-type (wt) mice also were tested. All corneas perforated, and bacterial load was increased significantly in +/− vs wt mice. Because disease severity was increased in IFN-γ−/− vs IL-18-neutralized mice, and since IL-18 also induces production of TNF, we tested for TNF-α in both groups. ELISA analysis demonstrated significantly elevated corneal TNF-α protein levels in IFN-γ−/− vs wt mice after infection. In contrast, RT-PCR analysis of IL-18-neutralized vs IgG-treated infected mice revealed decreased corneal TNF-α mRNA expression. Next, to resolve whether TNF was required for bacterial killing, TNF-α was neutralized in BALB/c mice. No difference in corneal bacterial load was detected in neutralized vs IgG-treated mice. These data provide evidence that IL-18 contributes to the resistance response by induction of IFN-γ and that IFN-γ is required for bacterial killing. The Journal of Immunology, 2002, 168: 5756–5763.

In this regard, another cytokine, IL-18, shares some of the properties of IL-12, including inducing production of IFN-γ by T cells, NK cells, and NKT cells (10–13). IL-18 is produced by macrophages and dendritic cells and, like IL-1, is released as an inactive precursor, requiring cleavage by IL-1β-converting enzyme/caspase-1 for its maturation (14–16). IL-18 is a costimulus for IFN-γ production in the setting of microbial stimulation of macrophage cytokines such as IL-12 and may synergize with IL-12 to drive Th1 T cell development (7, 17, 18). In BALB/c mice, the mechanism(s) of resistance, including control of bacterial load in the cornea, remains incompletely defined. Because IFN-γ is an important regulatory cytokine of host defense, often required for development of innate resistance and control of other microbial pathogens such as Toxoplasma gondii (19) and Chlamydia pneumoniae (20), we began the pathogenesis studies described in this study by testing for IFN-γ. We also tested BALB/c mice for IL-12 and IL-18 mRNA expression in cornea before and following P. aeruginosa ocular challenge. In addition, a neutralizing, anti-IL-18 mAb was administered to BALB/c mice to determine whether this treatment modified the resistance phenotype. The role of IFN-γ and TNF-α in bacterial killing also was tested in IL-18 and/or TNF-α-neutralized and IFN-γ−/− mice that endogenously lacked the cytokine.

Materials and Methods

Infection of mice

Eight-week-old female BALB/c and IFN-γ−/− mice on the BALB/c background (The Jackson Laboratory, Bar Harbor, ME) were used in this study. For corneal infection, mice were anesthetized (Aerrane; Anaquest, Madison, WI) and placed beneath a stereoscopic microscope, and the cornea was scarified, as described before (3, 21). A 5-μl bacterial suspension containing 1 × 10^6 CFU/μl P. aeruginosa strain 19660 (American Type Culture Collection, Manassas, VA), prepared as described before (3), was topically applied onto the scarified cornea. Eyes were examined macroscopically at...
1 day postinfection (p.i.)\(^3\) and at times described below to ensure that all mice were similarly infected and to monitor the course of disease. Animals were treated humanely and in full compliance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

**Ocular response to infection**

After bacterial infection, corneal disease was graded using an established scale (22): 0, clear or slight opacity partially covering the pupil; +1, slight opacity fully covering the entire anterior segment; +2, dense opacity partially or fully covering the pupil; +3, dense opacity covering the entire anterior segment; and +4, corneal perforation. A mean clinical score was calculated for each group of mice (\(n = 5\)/group/treatment) to express disease severity. Five mice from each group, along with a similar number of controls, were examined at 1–5 days p.i. in the IL-18 mAb neutralization and in the IFN-\(\gamma\)– studies.

**RT-PCR**

Infected corneas were removed from mice before infection and at 6 and 12 h and 1, 3, 5, and 7 days p.i., immediately frozen in liquid nitrogen, and stored at \(-70^\circ\text{C}\). Frozen tissue samples were homogenized in RNA STAT-60 (Tel-Test, Friendsville, TX), and total RNA was isolated following the manufacturer’s instruction. Total RNA (100 ng) was reverse transcribed using oligo(dt) primers (Life Technologies, Grand Island, NY) and reverse transcriptase (Life Technologies) in the presence of 10 U of RNase inhibitor (Promega, Madison, WI). Amplification of cDNA was conducted with Taq polymerase (Life Technologies) and specific primers for IFN-\(\gamma\); IL-12, IL-18, and TNF-\(\alpha\) (sense) and 5'-AGA GTG AAC ATT ACA GAT TTA TCC CCA-3' and 5'-CAC TCT TTC ATG GCC TCT GG-3' (antisense) for IFN-\(\gamma\); 5'-GTC AAC CTC ACC GTG GAC CAG C-3' (sense) and 5'-TGA ATA CTT CTA GTC TCT ACC GTG ACC ACC ACC ACA TTC TGT GGG-3' (antisense) for IL-12; 5'-ACC GAA TTC ACT GTA CAA CCG CAG TAA TAC GGA-3' (sense) and 5'-GCT CCT AGA GTG ACG AAC TAT ACC GAT TTA TTA CCA-3' (antisense) for IL-18; 5'-GCA AGC TTC GTG CCT TGT GCT ACT GAA CTT CGG-3' (sense) and 5'-GCT CTA GAA TGA GAT AGC AAA TCG CTT GCT GAC GG-3' (antisense) for TNF-\(\alpha\); and 5'-GGT GGC CGC TCT AGG CAC CAA-3' (sense) and 5'-CTC TTT GTG GTC ACG CAC GAT TCC TCT-3' (antisense) for \(\beta\)-actin, respectively. Control RT-PCR without reverse transcriptase during reverse transcription was done to confirm the lack of DNA contamination in the total RNA samples. A total of 20 \(\mu\)l of final PCR products was analyzed by electrophoresis on 1.2% agarose gels with SYBR Green I Nucleic Acid Gel Stain (Molecular Probes, Eugene, OR, and Invitrogen, Carlsbad, CA). The bands were visualized under UV transillumination and quantitated using an Alphalager 2000 Documentation and Analysis system (Alpha Innotech, San Leandro, CA). Integrated density values (IDV) for the IFN-\(\gamma\); IL-12, IL-18, and TNF-\(\alpha\) PCR products were corrected for the amount of \(\beta\)-actin on each sample. Data are expressed as the mean IDV of samples from five separate mice for each experimental time point.

**mAb treatment**

Neutralizing rat anti-mouse IL-18 (endotoxin <10 ng/ml Ab) and TNF-\(\alpha\) (endotoxin not measured) mAbs, both of IgG1 isotype, were purchased from Medical & Biological Laboratories (Naka-Ku Nagoya, Japan) and BioSource International (Camarillo, CA), respectively. BALB/c mice \((n = 5)/group\) were injected subconjunctivally 1 day before infection with 10 \(\mu\)l (10 \(\mu\)g/cornea) of anti-IL-18 or TNF-\(\alpha\) mAb. At 4 h and again at 2 days p.i., each mouse was injected i.p. with 150 \(\mu\)l (150 \(\mu\)g) of anti-IL-18 or TNF-\(\alpha\) mAb diluted in 0.05% PBS. Control mice similarly received an equal volume of rat IgG (Sigma-Aldrich, St. Louis, MO) diluted in 0.05% PBS.

**Quantitation of viable bacteria in cornea**

At 3 and 5 days p.i., three corneas from each experimental group (anti-IL-18 and TNF-\(\alpha\) mAb–vs rat IgG-treated mice and at 1, 3, and 5 days p.i. from IFN-\(\gamma\)– vs wild-type (wt) BALB/c mice) were collected, and the number of viable bacteria was quantified. For this, individual corneas were homogenized in sterile 0.9% NaCl containing 0.25% BSA (d). A total of 100 \(\mu\)l of each sample was diluted serially 1/10 in the same solution, plated in triplicate on *Pseudomonas* isolation agar (Difco, Detroit, MI) plates, and incubated overnight at 37°C. The number of viable bacteria in an individual cornea was determined by counting individual colonies on plates from the various dilutions and multiplying the number of colonies by the appropriate dilution. Results are reported as log\(_{10}\) number of CFU/cornea ± SEM.

**Histopathology of IFN-\(\gamma\)– vs wt BALB/c mice**

For histopathology, eyes from three mice of each group were enucleated at 5 days p.i., immersed in PBS, rinsed, and fixed in 1% osmium tetroxide, 2.5% glutaraldehyde, and 0.2 M Sorenson’s phosphate buffer (pH 7.4; 1:1:1) at 4°C for 3 h. Eyes were dehydrated in ethanol and embedded in Epon-araldite, and thick sections (1.5 \(\mu\)m) were cut, stained, observed, and photographed, as described before (3, 4, 21).

**Quantitation of TNF-\(\alpha\) protein**

TNF-\(\alpha\) protein levels were determined using an ELISA kit (R&D Systems, Minneapolis, MN), as described previously (21). Individual corneas \((n = 5)/group\) were collected from -/− and wt as well as TNF-\(\alpha\)-neutralized and rat IgG-treated control mice at 3 and 5 days p.i., the total weight of each cornea was determined, and samples were immediately analyzed. Samples were homogenized with a glass pestle ( Kontes; Fischer, I usca, IL) and centrifuged, and an aliquot of each supernatant was assayed for TNF-\(\alpha\) protein levels. The sensitivity of the assay was 5.1 pg/ml.

**Statistical analysis**

An unpaired, two-tailed Student’s t test was used to determine statistical significance for data from RT-PCR, mean clinical scores, bacterial counts, and ELISA analyses. Mean differences were considered significant at the confidence level of \(p = 0.05\). All experiments were repeated at least twice to ensure reproducibility, and representative data from a single experiment are shown.

**Results**

**IFN-\(\gamma\) mRNA expression in the cornea**

To determine whether IFN-\(\gamma\) was produced in the cornea of BALB/c mice before and after infection with *P. aeruginosa*, we tested for mRNA expression levels in uninfected and infected corneas using RT-PCR. Data from a representative experiment are shown in FIGURE 1.
shown in Fig. 1. IFN-γ mRNA transcripts were not detected in the uninfected (0 h) cornea nor at 6 or 12 h p.i. (data not shown) in BALB/c mice. Readily detectable low levels of IFN-γ mRNA were seen at 1 day p.i. in the cornea, and these levels continued to rise at 3 and peaked at 5 days p.i. By 7 days p.i., cytokine expression levels had decreased, but still remained significantly greater when compared with 3-day values. Statistically, IFN-γ mRNA levels in cornea were significantly increased (\( p = 0.02, 0.0006, 0.0001, \) and 0.001 at 1, 3, 5, and 7 days p.i., respectively) when compared with levels in the uninfected cornea.

**IL-12 and IL-18 mRNA expression**

Since mRNA for IFN-γ was detected in the infected cornea of BALB/c mice, we next tested the infected cornea for the presence of IL-12, a major IFN-γ-inducing cytokine. In preliminary (9) and the current experiments (Fig. 2), IL-12 p40 was not detectable at any time p.i. in the cornea of BALB/c mice. We then tested for the presence of IL-18, a cytokine, with functional similarities to IL-12 (10–13). Low levels of mRNA transcripts for IL-18 were constitutively expressed in the uninfected BALB/c mouse cornea (Fig. 3). At 6 and 12 h p.i., mRNA levels began to rise, but neither time point was significantly different from expression levels detected in the uninfected cornea. However, by 1 day p.i., IL-18 mRNA levels were significantly elevated, peaked at 3 days p.i., and remained significantly elevated at 5 and 7 days p.i. when compared with constitutive levels of expression (\( p = 0.664, 0.36, 0.001, 0.0001, 0.0001, 0.0001 \) at 6 and 12 h and 1, 3, 5, and 7 days p.i., respectively).

**Anti-IL-18 mAb treatment**

Because our data showed that both IL-18 and IFN-γ mRNA levels were significantly increased in the BALB/c mouse cornea after bacterial infection, the next series of in vivo studies tested the significance of these data. mAb neutralization of IL-18 was used to determine whether IL-18 induced IFN-γ production in the cornea after bacterial infection. Corneal IFN-γ mRNA expression was analyzed by RT-PCR at 3 and 5 days p.i., and the data are shown in Fig. 4. Treatment with anti-IL-18 mAb led to a significant decrease in IFN-γ (\( p = 0.007 \) and 0.02 at 3 and 5 days p.i., respectively) when compared with mRNA levels of cytokine in the cornea of rat IgG-treated control mice. Mean clinical scores in IL-18 mAb- vs rat IgG-treated mice showed a trend for disease worsening, but corneal perforation did not occur in the IL-18-neutralized mice (data not shown).

**Quantitation of viable bacteria in infected corneal tissue**

We next tested whether mAb neutralization of IL-18, resulting in decreased mRNA expression for IFN-γ, contributed to increased bacterial growth in the cornea. Direct plate count was used to quantitate bacterial load in the cornea of IL-18 mAb- vs rat IgG-treated mice at 3 and 5 days p.i. The mean log_{10} number of viable...
bacteria per cornea (±SEM) is shown in Fig. 5. A significant increase in bacterial load (1- to 2-log increase, \( p = 0.0014 \) and 0.002 at 3 and 5 days p.i., respectively) was observed in the cornea of anti-IL-18 mAb- vs rat IgG-treated mice.

Response of IFN-\( \gamma^{--} \) mice
To assure that IFN-\( \gamma \) expression was required for bacterial killing in resistant BALB/c mice, corneas of IFN-\( \gamma^{--} \) vs wt BALB/c mice were infected with \( P. \) aeruginosa, and ocular disease was graded. Mean clinical scores were significantly different at 3 and 5 days p.i. (\( p = 0.0003 \) and 0.0001 at 3 and 5 days p.i., respectively) in IFN-\( \gamma^{--} \) vs wt mice (Fig. 6). In addition, all of the corneas perforated in the \( \sim/\sim \) animals at 5 days p.i., in contrast with a +2 ocular disease grade observed in wt mice. Viable bacteria in the cornea of IFN-\( \gamma^{--} \) vs wt mice also were quantitated at 1, 3, and 5 days p.i. (Fig. 7). A significantly increased number of bacteria was detected in the \( \sim/\sim \) vs wt mouse cornea at all times tested (\( p = 0.011 \), 0.006, and 0.001 at 1, 3, and 5 days p.i., respectively).

Histopathology
At 5 days p.i., eyes from IFN-\( \gamma^{--} \) and wt BALB/c mice were enucleated and prepared for histopathology, and these data are shown in Fig. 8. The cornea of the \( \sim/\sim \) mice lacked epithelium; stromal cytoarchitecture was destroyed; and perforation, observed visually by mean clinical score grading, was confirmed. In contrast, the cornea of the wt mice had begun to reepithelialize, less stromal damage was apparent, and no perforation was observed. Inflammatory infiltrate, however, remained and was concentrated in the deep stroma and anterior chamber.

Role of other cytokines
Because the cornea of anti-IL-18 mAb-treated infected mice did not perforate and corneal perforation was routinely observed at 5 days p.i. in IFN-\( \gamma^{--} \) mice, we postulated that other proinflammatory cytokines must be affected in the \( \sim/\sim \) mice that contributed to corneal perforation. ELISA analysis (Fig. 9) revealed that the cornea of the \( \sim/\sim \) vs wt BALB/c mouse had significantly elevated TNF-\( \alpha \) protein levels at 3 days p.i. (\( p = 0.016 \)) that remained elevated, but not significantly, at 5 days p.i. (\( p = 0.197 \)). TNF-\( \alpha \) mRNA levels in cornea were then tested in IL-18-neutralized vs IgG-treated mice at similar times p.i. TNF-\( \alpha \) levels were significantly (\( p = 0.001 \) and 0.02, at 3 and 5 days p.i., respectively) decreased in IL-18- vs IgG-treated mouse cornea (Fig. 10).
Tumor necrosis factor-α

Data from the IFN-γ−/− experiment suggested that despite elevated levels of TNF-α, without IFN-γ, bacterial killing was impaired. In addition, data from the IL-18 neutralization study showed that in the absence of endogenous IFN-γ, −/− mice failed to control acute infection with T. gondii and no evidence for an IFN-γ-independent protective function was apparent. Similar results were reported recently showing that IFN-γ was necessary for development of innate resistance against infection with C. pneumoniae. In contrast, a previous study using mAb neutralization of IFN-γ in susceptible B6 mice suggested that prolonged and elevated expression of IFN-γ was associated with the susceptibility response and corneal perforation (3), and further work showed that significantly reduced levels of IFN-γ were equally deleterious (8). Since no information existed on the role of IFN-γ in the BALB/c mouse and development of the resistance phenotype, we tested for this cytokine. Our data showed that IFN-γ was detectable at 1, peaked at 5, and declined at 7 days p.i. in the infected cornea of these mice, suggesting that tighter regulation of IFN-γ might be important in

Discussion

IFN-γ is an important regulatory cytokine of host defense in both innate and acquired immunity (24–26). It plays a critical role in inflammation and regulates the antimicrobial/tumoricidal potential of macrophages by up-regulation of MHC class I and class II protein expression; enhanced production of macrophage-derived mediators such as TNF-α, IL-1, IL-6, IL-12, IL-18, and NO; and down-regulation of the synthesis of anti-inflammatory mediators such as IL-10 (27–30). Scharton-Kersten et al. (19) demonstrated that IFN-γ is necessary for development of innate resistance against infection with C. pneumoniae. In contrast, a previous study using mAb neutralization of IFN-γ in susceptible B6 mice suggested that prolonged and elevated expression of IFN-γ was associated with the susceptibility response and corneal perforation (3), and further work showed that significantly reduced levels of IFN-γ were equally deleterious (8). Since no information existed on the role of IFN-γ in the BALB/c mouse and development of the resistance phenotype, we tested for this cytokine. Our data showed that IFN-γ was detectable at 1, peaked at 5, and declined at 7 days p.i. in the infected cornea of these mice, suggesting that tighter regulation of IFN-γ might be important in

FIGURE 8. Histopathology. Corneal sections from −/− (A) and wt (B) BALB/c mice at 5 days p.i. are shown. The cornea of the −/− mouse was perforated, whereas the cornea of the wt mouse was reepithelialized with a more intact stroma. Magnification, ×72.

FIGURE 9. TNF-α protein levels. Corneas of −/− and wt BALB/c mice were tested for TNF-α protein levels. Elevated levels were seen in −/− vs wt mice at 3 and 5 days p.i. (p = 0.016 and 0.197), but were only significant at 3 days.

FIGURE 10. TNF-α mRNA expression in cornea after anti-IL-18 mAb treatment. Data shown are the mean IDV of three PCR of samples from five individual mice at each time point. Values represent the mean ± SEM, and significant differences were observed (p = 0.001 and 0.02 at 3 and 5 days p.i., respectively).
Results are reported as log 10 number of viable bacteria per cornea days p.i., respectively.

Perforation, in B6 mice, leads to resolution of disease in BALB/c vs tissue destruction and toxicitiy, and corneal perforation, as seen in B6, but not BALB/c mice. Next, because IL-18 is known to induce the synthesis of IFN-γ, often in collaboration with IL-12 (10, 24–26, 31), we tested for IL-18 expression in the uninfected and infected BALB/c mouse cornea. Rationale for these studies was provided by the experiments of Muller et al. (12), who demonstrated that IL-12-independent IFN-γ production in experimental Chagas’ disease is mediated by IL-18. Kawakami et al. (11) also recently reported that IL-18 contributes to host resistance against infection with Cryptococcus neoformans in mice with defective IL-12 synthesis through induction of IFN-γ synthesis. Furthermore, in IL-12 p40−/−-infected mice, low serum levels of IFN-γ (20–30% of that in wt mice) were detected after infection with C. neoformans, which further indicated the existence of IL-12-independent mechanisms for IFN-γ production and eradicating this pathogen.

After establishing elevated levels of IL-18 mRNA expression in the infected BALB/c mouse cornea, we used Ab neutralization to determine whether IL-18 induced production of IFN-γ. Neutralization of IL-18 significantly reduced IFN-γ mRNA compared with control mice (Fig. 4), implicating the importance of IL-18 in induction of IFN-γ in the infected cornea. Whether IL-18 neutralization induced systemic effects in draining cervical lymph nodes, for example, was not tested, due to the lack of T cell participation in corneal pathogenesis in these mice, but such effects cannot be ruled out by this study.

We next determined whether IFN-γ was required for bacterial killing, by quantitating bacteria in the cornea of anti-IL-12 mAb-treated mice. Viable bacterial number was increased ∼2 logs (Fig. 5) in mAb-neutralized vs control-treated mice, implying that IL-18 protects mice against P. aeruginosa infection by inducing IFN-γ production and bacterial killing in the cornea. To confirm the importance of IFN-γ in this model, IFN-γ−/− BALB/c mice also were tested. Mean clinical score data and histopathology showed that IFN-γ−/− vs wt mice were susceptible to P. aeruginosa infection, and by 5 days p.i., all of the infected corneas of the −/− mice had perforated, whereas the corneas of wt BALB/c had begun to recover. Furthermore, viable bacterial load increased significantly (1–2 logs) in the cornea of −/− vs wt BALB/c mice (Fig. 7). Together, these data further suggested that IFN-γ is critical in bacterial killing in P. aeruginosa-induced keratitis and development of the resistance response. However, the precise mechanism(s) whereby IFN-γ contributes to bacterial killing in this model remains untested. In this vein, other studies have provided direct evidence of IFN-γ killing of Legionella pneumophila after administering an adenovirus vector containing murine IFN-γ cDNA concomitant with the bacterial inoculum and showed a 10-fold decrease in lung bacterial CFU compared with controls (32). Also in studies with C. pneumoniae, IFN-γ was found necessary for control of bacterial load by increasing NO release and superoxide peroduction, both of which the authors concluded related to bacterial killing (20).

Nonetheless, a difference was noted between IL-18 mAb-neutralized vs −/− mice, namely, that although disease worsened in the Ab-neutralized mouse cornea, none of the corneas perforated, whereas all infected corneas in the IFN-γ−/− animals perforated after infection. Thus, we next predicted that although IFN-γ is important in clearance of P. aeruginosa in the cornea, other cytokines may also be required for killing. Others using IFN-γ−/− mice in a viral infection model (33) similarly concluded that although IFN-γ played an important role in the clearance of HSV resistance to P. aeruginosa infection. Direct comparative measurement of IFN-γ levels in infected cornea of B6 vs BALB/c mice would directly test this hypothesis, but has not yet been done. Nevertheless, together, these data support the tenet that controlled cytokine induction of IFN-γ is important in clearance of bacterial infection. Direct comparative measures of cytokines in infected cornea of B6 vs BALB/c mice (32) similarly concluded that although IFN-γ played an important role in the clearance of HSV. After establishing elevated levels of IL-18 mRNA expression in the infected BALB/c mouse cornea, we used Ab neutralization to determine whether IL-18 induced production of IFN-γ. Neutralization of IL-18 significantly reduced IFN-γ mRNA compared with control mice (Fig. 4), implicating the importance of IL-18 in induction of IFN-γ in the infected cornea. Whether IL-18 neutralization induced systemic effects in draining cervical lymph nodes, for example, was not tested, due to the lack of T cell participation in corneal pathogenesis in these mice, but such effects cannot be ruled out by this study.

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from the eye, the pathogenesis of herpetic stromal keratitis lesions involved additional cytokines. However, to the best of our knowledge, no other cytokines were tested in that study.

In this regard, although IL-18 exerts some of its proinflammatory effects through induction of IFN-γ (11, 34), recent data suggest that IL-18 also induces TNF-α production through stimulating activation of NF-κB (17), inducing production of not only TNF-α, but IL-1β and chemokines such as IL-8 and macrophage-inflammatory protein-1α (35). All of the latter cytokines, except TNF-α, have been shown previously to play critical roles in T cell chemotaxis and polymorphonuclear neutrophil (PMN) persistence following P. aeruginosa-induced corneal infection (21, 36, 37).

TNF-α has been shown to play a crucial role in response to tissue injury, infection, and inflammation (38). Sjegmuend et al. (39) demonstrated that neutralization of IL-18 reduced disease severity in murine colitis and reduced intestinal IFN-γ and TNF-α production. Moreover, similar results were reported by Neta et al. (17), who demonstrated that neutralization of IL-18 during lethal endotoxemia protected mice against the lethal effects of LPS, by reduction of TNF-α and PMN infiltration. IL-18 also up-regulates expression of adhesion molecules such as ICAM-1 (40), also shown to be of importance in PMN infiltration into cornea in ocular models of P. aeruginosa infection (41). Therefore, we next tested for TNF-α levels in IFN-γ−/− and IL-18-neutralized mice. ELISA analysis confirmed significantly elevated levels of TNF-α protein in cornea in IFN-γ−/− vs wt BALB/c mice at 3 days p.i. with levels remaining elevated, but not significant, at 5 days p.i. (Fig. 9). These data support the importance of IFN-γ in bacterial killing and imply that in the absence of endogenous IFN-γ, TNF-α alone, even at elevated levels, does not contribute to bacterial killing, but rather, may contribute to increased pathology and corneal perforation. In contrast, in IL-18-neutralized mice, RT-PCR revealed decreased levels of TNF-α at similar time points. Thus, neutralization of IL-18 significantly decreased TNF-α as well as IFN-γ levels when compared with levels in controls. To further test the role of TNF-α in bacterial killing, we neutralized TNF in BALB/c mice. No difference was detected in corneal bacterial load in mAb-neutralized vs IgG-treated mice, confirming that TNF-α is not critical for bacterial killing. In P. aeruginosa-induced models of pneumonia, TNF-α is regarded as somewhat a double-edged sword, and the role of the cytokine remains controversial. It has been reported as necessary for PMN recruitment and bacterial clearance in mice (42), but in a rat model, levels of IL-1α and TNF-α increased consistently following infection until death, implicating these cytokines in the pathogenesis of acute P. aeruginosa-induced pneumonia (43). We hypothesize, but have not tested, that tissue-specific mechanisms of bacterial killing (cornea vs lung) are a contributing factor to these disparate data in the mouse.

In summary, the data presented demonstrate that IFN-γ is produced in BALB/c mice following ocular bacterial challenge and that IL-18 plays an important role in inducing production of the cytokine. We also provide evidence that neutralization of IL-18 decreased both IFN-γ and TNF-α production in cornea and that neutralization of TNF-α does not significantly change bacterial load in the cornea when compared with IgG-treated mice. Data from the IFN-γ−/− studies also imply that elevated levels of TNF-α, in the absence of IFN-γ, fail to control bacterial load, and suggest that TNF-α contributes to corneal pathogenesis and perforation.

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