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Prolonged Elevation of IL-1 in *Pseudomonas aeruginosa* Ocular Infection Regulates Macrophage-Inflammatory Protein-2 Production, Polymorphonuclear Neutrophil Persistence, and Corneal Perforation [1]

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Prolonged Elevation of IL-1 in *Pseudomonas aeruginosa* Ocular Infection Regulates Macrophage-Inflammatory Protein-2 Production, Polymorphonuclear Neutrophil Persistence, and Corneal Perforation[1]

Xiaowen L. Rudner, Karen A. Kernacki, Ronald P. Barrett, and Linda D. Hazlett[2]

The kinetics of IL-1 (α and β) production after *Pseudomonas aeruginosa* corneal infection was examined in susceptible (cornea perforates) C57BL/6J (B6) and resistant (cornea heals) BALB/cByJ (BALB/c) mice. IL-1α and -1β (mRNA and protein) were elevated in both mouse strains, and levels peaked at 1 day postinfection (p.i.). Significantly greater amounts of IL-1 protein were detected in B6 vs BALB/c mice at 1 and 3 days p.i. At 5 days p.i., IL-1α and -1β (mRNA and protein) remained elevated in B6, but began to decline in BALB/c mice. To test the significance of elevated IL-1 in B6 mice, a polyclonal neutralizing Ab against IL-1β was used to treat infected B6 mice. A combination of subconjunctival and i.p. administration of IL-1β polyclonal Ab significantly reduced corneal disease. The reduction in disease severity in infected B6 mice was accompanied by a reduction in corneal polymorphonuclear neutrophil number, bacterial load, and macrophage inflammatory protein-2 mRNA and protein levels. These data provide evidence that IL-1 is an important contributor to *P. aeruginosa* corneal infection. At least one mechanism by which prolonged and/or elevated IL-1 expression contributes to irreversible corneal tissue destruction appears to be by increasing macrophage inflammatory protein-2 production, resulting in a prolonged stimulation of polymorphonuclear neutrophil influx into cornea. In contrast, a timely down-regulation of IL-1 appears consistent with an inflammatory response that is sufficient to clear the bacterial infection with less corneal damage. *The Journal of Immunology*, 2000, 164: 6576–6582.

Pseudomonas aeruginosa is a common cause of corneal infection, especially in extended wear contact lens users and in immunocompromised patients (1). Both bacterial and host factors released from infiltrating inflammatory cells have been implicated as contributing to the rapidly progressing liquefactive stromal necrosis that occurs during the infection (2–6). *P. aeruginosa* corneal infection is characterized by an infiltration of inflammatory cells, which are predominantly polymorphonuclear neutrophils (PMN).[3] Although PMN are essential for eliminating viable bacteria and eventual restoration of corneal clarity, persistence of these cells in cornea may increase the severity of corneal stromal damage (2, 7–9).

Many cytokines and chemokines influence PMN influx into tissues, including the potent proinflammatory cytokine IL-1 (10). IL-1 is primarily produced by macrophages and monocytes but also by resident corneal cells (11–13). It has a wide range of activities, including mediation of the acute-phase response, chemotaxis, activation of inflammatory cells and APC (such as Langerhans cells and macrophages), and stimulation of neovascularization (10, 13–15). Prior studies in our laboratory have examined the role of IL-1 early after *P. aeruginosa* infection. Message for IL-1α and -1β was elevated as early as 12 h postinfection (p.i.) in the *P. aeruginosa*-infected cornea in outbred, resistant (cornea heals) Swiss ICR mice (16). Protein levels were not tested in this study. In addition, lack of up-regulation of ICAM-1 was associated with the dysregulation of IL-1β protein expression in the corneas of infected aged Swiss ICR mice (susceptible, cornea perforates) (17). However, further immune mechanistic studies with Swiss ICR mice would be limited due to the outbred nature of the animals. Therefore, to vigorously test the role of IL-1 in *P. aeruginosa* ocular infection, the genetic resistance vs susceptibility model was selected. The current broad hypothesis tested using this model predicts that balance between the induction of a protective vs a destructive response to corneal infection is critical. Furthermore, it is predicted more specifically that prolonged elevation of IL-1 expression contributes to corneal destruction by continued stimulation of PMN influx into cornea. To test this, susceptible C57BL/6J (B6) and resistant BALB/cByJ (BALB/c) mice were tested for IL-1α and -1β mRNA and protein expression after *P. aeruginosa* infection. In addition, IL-1β Ab (polyclonal Ab (pAb)) was administered to susceptible B6 mice to determine whether this treatment prevented or lessened stromal destruction.

Materials and Methods

Infection of mice

Eight-week-old female BALB/c (resistant, cornea heals) and B6 (susceptible, cornea perforates) mice (The Jackson Laboratory, Bar Harbor, ME) were used in this study. Before corneal infection, mice were anesthetized with isoflurane (Aerrane; Anaquest, Madison, WI) and placed beneath a stereoscopic microscope at ×40 magnification. Using a sterile 25 5/8-gauge needle, the central cornea of the left eye was scarified with three 1-mm incisions. Random eyes were routinely examined histologically to

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[3] Abbreviations used in this paper: PMN, polymorphonuclear neutrophil; pAb, polyclonal Ab; B6, C57BL/6J; BALB/c, BALB/cByJ; p.i., postinfection; MIP-2, macrophage-inflammatory protein-2; MPO, myeloperoxidase.

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ensure that the wounds were shallow and penetrated no deeper than the superficial corneal stroma. A 5-μl bacterial suspension containing 1.0×10^6 CFU of *P. aeruginosa* (American Type Culture Collection strain 19660) prepared as described previously (18) was topically applied onto the scarified cornea. Eyes were examined macroscopically at 24 h p.i. and at times described below to ensure that all mice were similarly infected and to monitor the course of disease, respectively. All animals were treated humanely and in full compliance with the Association for Research in Vision and Ophthalmology resolution on usage and treatment of animals in research.

Quantitation of corneal IL-1α and -1β mRNA

RNase protection assays were used to quantitate corneal levels of IL-1α and -1β mRNA. IL-1α and -1β cDNA clones were generated by RT-PCR using total RNA from *P. aeruginosa*-infected corneas as the template for the reverse transcription reaction. PCR primers were designed (MacVector Software; Oxford Molecular, Madison, WI) to amplify nt 304–548 of murine IL-1α (accession number X01450) and nt 294–474 of murine IL-1β (accession number M15131). *Eco*RI and *Xba*I restriction sites were added to 5′ ends of primers to facilitate ligation of PCR products to the pGEM-3Z vector. ^{32}P-labeled IL-1α and -1β antisense RNA probes and unlabeled IL-1α and -1β sense-strand RNAs were generated from the cDNA clones by in vitro transcription. Sense-strand RNAs were used to produce standard curves to quantitate amounts of respective mRNA in cornea.

Corneas were collected from BALB/c and B6 mice before and at 6 and 12 h and 1, 3, and 5 days p.i. Immediately after collection, corneas were flash-frozen in liquid nitrogen and stored at −70°C until RNA extraction. Eight corneas were pooled, and total RNA was extracted using RNA STAT-60 (Tel-Test, Friendswood, TX) according to the manufacturer's instructions. Five micrograms of total RNA from each sample was hybridized overnight at 56°C to 300 pg of the IL-1α and -1β riboprobes. Similarly, various concentrations of the unlabeled sense-strand standards (5–250 pg) were hybridized to the same amount of riboprobe. After hybridization, samples were digested with 1000 U of T1 nuclease (Life Technologies, Gaithersburg, MD). Nuclease-protected fragments were resolved on a 4.5% urea-containing sequencing gel. Protected bands were observed by exposing the dried gel to x-ray film and were quantitated using an AlphaImager 2000 documentation and analysis system (Inotech, San Leandro, CA). This experiment was performed at least three times to ensure reproducibility of the data. Results from two separate experiments are shown and reported as pg cytokine mRNA/5 μg total RNA.

Quantitation of corneal IL-1α and -1β protein

Protein for IL-1α and -1β was determined using ELISA kits (R&D Systems, Minneapolis, MN). For these studies, individual corneas ($n = 3$ at each time point) were collected from mice before and at 12 h and 1, 3, and 5 days p.i. as described above. Before storage at −70°C, the total weight of each cornea was determined. Immediately before analysis, samples were thawed and homogenized in 0.5 ml of 0.1% Tween 20-PBS with a glass Kontes pestle (Fischer, Itasca, IL). Samples were centrifuged at $5000 \times g$ for 10 min, and an aliquot of each supernatant was assayed for IL-1α and -1β protein. The sensitivity of the ELISA was 2.5 pg/ml for IL-1α and 3.0 pg/ml for IL-1β. ELISA experiments were performed in duplicate to ensure reproducibility of the data. Results are reported as pg cytokine/mg cornea.

IL-1β neutralization

Rabbit anti-murine IL-1β pAb was purchased from PeproTech (Rocky Hill, NJ). The lyophilized powder (500 μg) was reconstituted in 0.5 ml of water as recommended by the manufacturer. B6 mice ($n = 5$) were anesthetized with Aerrane and were injected with IL-1β pAb subconjunctivally (10 μg; 1 day before infection) and i.p. (150 μg; 1 day before and 1 and 3 days after infection). Control mice ($n = 5$) received an equal volume of PBS subconjunctivally and i.p. at the same times. The neutralization experiments were repeated similarly three times to ensure reproducibility of the data.

Ocular response to infection

After *P. aeruginosa* corneal infection in IL-1β pAb- vs PBS-treated mice, ocular disease was graded using the following established scale (19): 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. To observe eyes whose lids were sealed, mice were anesthetized with isoflurane, and sterile PBS was applied to the lids to permit their careful partial opening, without inducing corneal perforation. A mean clinical score was calculated for each group of mice ($n = 5$ for each group) to express disease severity. This was done by summation of the ocular disease scores for each group divided by the total number of mice scored at each time point (18).

Histopathology

For histopathological analysis, eyes were enucleated at 5 days p.i. from three mice from each group (IL-1β pAb- vs PBS-treated). Eyes were immediately immersed in PBS, rinsed, and placed in a fixative containing 1% osmium tetroxide, 2.5% glutaraldehyde, and 0.2 M Sorenson's phosphate buffer (pH 7.4) (1:1:1) at 4°C for a total of 3 h. Eyes were transferred into fresh fixative after 1.5 h and then dehydrated in graded ethanols and embedded in Epon-araldite as described previously (18, 20). Thick sections (1.5 μm) were cut, stained with a modified Richardson's stain, and observed. Representative sections were photographed with a Zeiss Axiophot photomicroscope (Carl Zeiss, Morgan Instruments, Cincinnati, OH) equipped with bright field optics using Ilford pan F film (Mobberley, Cheshire, U.K.).

Quantitation of PMN in cornea

A myeloperoxidase (MPO) assay (20–21) was used to quantitate the total number of PMN infiltrating the cornea after infection. At 3, 5, and 7 days p.i., three corneas from each group were collected for MPO analysis. Corneas were excised at the limbus with a sterile razor blade, and noncorneal tissue was removed by dissection. After collection, individual corneas were homogenized with a glass Kontes pestle in 1.0 ml of 50 mM phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethyl-ammonium bromide, freeze-thawed three times, and centrifuged at $14{,}000 \times g$ for 10 min to remove cellular debris. An aliquot of the supernatant (0.1 ml) was added to 2.9 ml of the 50 mM phosphate buffer containing *o*-dianisidine dihydrochloride (16.7 mg/100 ml) and hydrogen peroxide (0.0005%). The change in absorbance at 460 nm was continuously monitored for 5 min using a Genesis 2 spectrophotometer (Spectronics, Rochester, NY). The slope of the line was determined for each individual sample and used to calculate the number of units of MPO in the tissue. One unit of MPO activity is defined as that degrading 1 μmol of peroxide per minute (21). To ensure reproducibility of the data, the MPO assay was repeated once similarly. Results are reported as units of MPO/cornea ± SEM.

Quantitation of viable bacteria in cornea

At 5 and 7 days after infection, three corneas from each experimental group were collected for the determination of viable bacteria in infected cornea. Individual corneas were homogenized in sterile 0.9% NaCl containing 0.25% BSA (22). A total of 100 μl of each sample was diluted serially 1:10 in the same solution and plated in triplicate on peptone-tryptic soy agar plates (Difco, Detroit, MI). Plates were 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. This experiment was repeated once to ensure reproducibility of the data. Results are reported as $\log_{10}$ number of CFU/cornea ± SEM.

Quantitation of corneal macrophage-inflammatory protein-2 (MIP-2) level in IL-1β pAb-treated B6 mice

A RNase protection assay and ELISA were used to quantitate MIP-2 in IL-1β pAb- vs PBS-treated B6 mice at 5 and 7 days p.i. MIP-2 probe was prepared from cDNA obtained by RT-PCR using total RNA from *P. aeruginosa*-infected mouse corneas as the template. Five micrograms of total RNA from each group was hybridized to ^{32}P-labeled MIP-2 probe as described above and in previous work (20). The level of MIP-2 protein in each cornea was determined using an ELISA kit (R&D Systems). This was done according to the manufacturer's instructions as described above and as reported previously (20).

Statistical analysis

An unpaired, two-tailed Student *t* test was used to determine statistical significance for ELISA, mean clinical scores, MPO assays, and bacterial plate counts. Mean differences were considered significant at the confidence level of $p \leq 0.05$.

Table I. *IL-1α and IL-1β mRNA levels in P. aeruginosa-infected corneas*[a]

	A				B			
	IL-1α		IL-1β		IL-1α		IL-1β	
Time Postinfection	B6	BALB/c	B6	BALB/c	B6	BALB/c	B6	BALB/c
0 h	36.3	41.2	0.0	0.0	6.7	6.0	0.0	0.0
6 h	36.3	43.2	0.0	0.0	8.3	15.7	0.0	0.0
12 h	49.1	48.8	2.5	8.5	45.9	50.3	10.3	10.1
1 day	114.0	110.1	101.0	101.5	104.74	79.2	55.5	59.17
3 days	96.2	112.2	100.8	104.5	87.4	60.5	62.12	61.9
5 days	101.0	59.1	121.4	61.8	88.6	30.7	70.58	27.5

[a] Results are reported as pg IL-1α or IL-1β mRNA per 5 μg total corneal RNA sample.

Results

IL-1 mRNA level after P. aeruginosa *corneal infection*

Corneal IL-1α and -1β mRNA levels were quantitated in BALB/c and B6 mice before and after *P. aeruginosa* challenge. This was done to determine whether there were differences in regulation of the proinflammatory cytokines that could contribute to the disparate response to infection observed between the two mouse strains (20). IL-1α and -1β mRNA levels were measured before and at 6 and 12 h and 1, 3, and 5 days p.i. using RNase protection assays. Data from two separate but similar experiments are shown in Table I. A low level of IL-1α mRNA was detected in uninfected corneas (time 0) as well as in corneas at 6 h p.i. in both mouse strains. In contrast, IL-1β mRNA was not detected at any of these times in either group of mice under the assay conditions tested. Both IL-1α and -1β mRNA levels began to rise by 12 h p.i., and peak mRNA expression of both cytokines was detected in each mouse strain at 1 day p.i. By 5 days p.i., both IL-1α and -1β mRNA began to decline in resistant BALB/c mice, but the level of each cytokine remained elevated in B6 mice.

IL-1 protein levels during P. aeruginosa *corneal infection*

The amount of IL-1α and -1β protein also was determined in uninfected and infected corneas of resistant vs susceptible mice by ELISA. Data from a representative experiment are shown in Figs. 1 and 2. A low level of IL-1α but no -1β protein was detected in uninfected corneal tissue in both mouse strains (data not shown). In BALB/c mice, IL-1α and -1β was detected in the cornea as early as 12 h p.i. The level of each cytokine remained relatively constant in BALB/c mice from 12 h to 5 days p.i. In B6 mice, the level of both IL-1α and -1β protein began to rise by 12 h p.i., peaked at 1 day, and then gradually declined thereafter. The amount of IL-1 was significantly greater in B6 vs BALB/c mice at 1 (IL-1α, $p = 0.0124$; IL-1β, $p = 0.0002$) and 3 days p.i. (IL-1α, $p = 0.0059$; IL-1β, $p = 0.0404$). Although differences between levels of the two cytokines in B6 and BALB/c were not statistically significant at 5 days p.i., the concentrations of IL-1α and -1β in B6 mice were 1.5- and 1.2-fold greater, respectively, than in BALB/c mice.

Neutralization of IL-1β in susceptible B6 mice

Because both IL-1α and -1β were produced in significantly greater amounts from 1 to 3 days p.i. in B6 when compared with BALB/c mice, we hypothesized that IL-1 promoted the destructive inflammation observed in the cornea of susceptible B6 mice. Therefore, we next tested whether the susceptibility phenotype of B6 mice could be altered by administration of a neutralizing IL-1β pAb. B6 mice were injected subconjunctivally with 10 μg of IL-1β pAb 1 day before and i.p. with 150 μg 1 day before and 1 and 3 days after ocular challenge. The ocular response to infection was examined from 1 to 7 days p.i. Mean clinical scores for the IL-1β pAb- and PBS-treated mice were calculated and are shown in Fig. 3. Both groups initially (1 day p.i.) displayed similar disease grades. Significant differences between IL-1β pAb- and PBS-treated mice were observed at 3, 5, and 7 days p.i. ($p = 0.0001$ at all three time points, respectively). Representative eyes from both groups were photographed using a slit lamp at 5 days p.i., and these data are shown in Fig. 4. IL-1β pAb-treated mice showed only slight opacity covering the anterior segment of the eye (Fig. 4*A*), whereas PBS-treated mice exhibited centrally thinned and/or a perforated corneas (Fig. 4*B*).

Corneas from IL-1β pAb- and PBS-treated mice also were examined histopathologically at 5 days p.i. Marked differences that were consistent with mean clinical scores and slit lamp data were noted between the two groups (Fig. 5). Specifically, in IL-1β pAb-treated mice, the corneal epithelium was thinned with wide intercellular spaces between the cells. Nevertheless, the epithelium remained intact from limbus to limbus and contained few inflammatory cells. In the stroma, inflammatory cells were seen spread throughout as well as clustered into densely packed foci of cells. Also, few infiltrating cells were commonly observed in the peripheral portion of the anterior chamber between the iris and the corneal endothelium. The corneal epithelial basal lamina, Descement's membrane, and the endothelium also appeared intact. In PBS-treated control mice, total dissolution of the corneal stroma

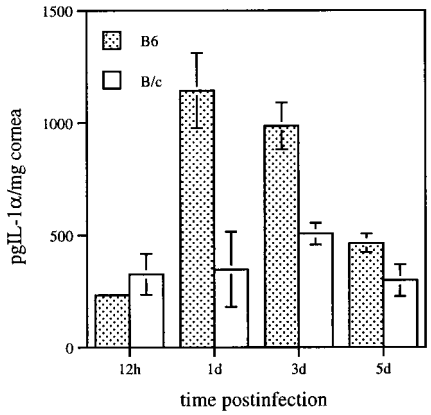

FIGURE 1. Corneal IL-1α protein levels in B6 and BALB/c mice after *P. aeruginosa* ocular challenge. Individual corneas were analyzed for IL-1α by ELISA at 12 h to 5 days p.i. Results are reported as pg IL-1α protein/mg cornea ± SEM ($p = 0.3809$, 0.0124, 0.0059, and 0.0891 at 12 h and 1, 3, and 5 days p.i., respectively).

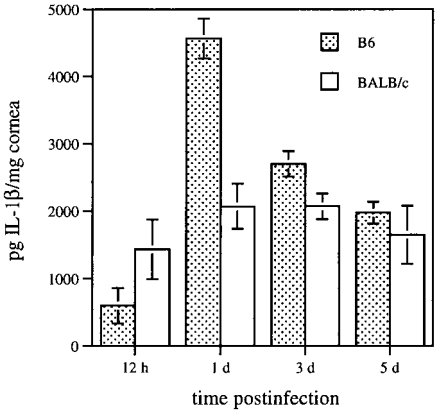

FIGURE 2. Corneal IL-1β protein levels in B6 and BALB/c mice after *P. aeruginosa* ocular challenge. Individual corneas were analyzed for IL-1β by ELISA at 12 h to 5 days p.i. Results are reported as pg IL-1β protein/mg cornea ± SEM ($p = 0.1374$, 0.0002, 0.0404, and 0.4938 at 12 h and 1, 3, and 5 days p.i., respectively).

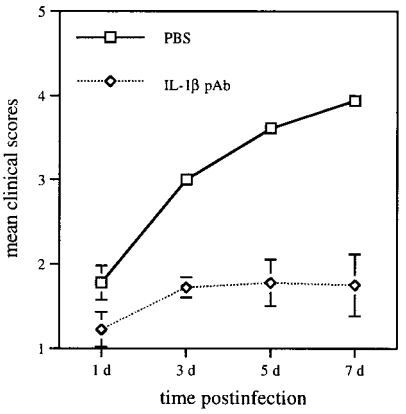

FIGURE 3. Ocular disease response in IL-1β pAb- and PBS-treated B6 mice. Ocular disease grades were averaged at individual times after infection. Results are reported as mean clinical score ± SEM. Significant differences were observed at 3, 5, and 7 days p.i. ($p = 0.0001$ at all three time points, respectively).

was observed, consistent with corneal perforation that was routinely observed in B6 mice within 7 days p.i. The corneal epithelium was denuded centrally, the stroma was thinned with extensive destruction of collagen fibers/extracellular matrix, and numerous free bacteria were commonly observed. A heavy infiltration of inflammatory cells was noted in the peripheral stroma and anterior chamber. Necrotic and/or apoptotic inflammatory cells also were observed superficially in the stroma near large foci of free bacteria.

Quantitation of PMN and viable bacteria in P. aeruginosa*-infected cornea of IL-1β pAb-treated B6 mice*

Treatment with IL-1β pAb significantly reduced ocular disease grades at 3–7 days p.i. as determined by mean clinical score data. To ascertain whether these data correlated with reduced PMN number in the cornea of IL-1β pAb- vs PBS-treated B6 mice, MPO assays were used. Data from a representative experiment are shown in Fig. 6. At 3 and 7 days p.i., MPO activity was significantly decreased in IL-1β pAb- vs PBS-treated mice ($p = 0.0182$ and 0.0227, respectively). At 5 days p.i., MPO activity also was reduced in pAb-treated mice, but these data were not significant ($p = 0.4392$).

Because treatment with IL-1β pAb reduced PMN number, we next tested whether this decrease in inflammatory cells resulted in a significantly increased number of viable bacteria in infected cornea. To accomplish this, direct plate count was used to quantitate bacterial load in the cornea of these two groups of mice. The mean $\log_{10}$ number of viable bacteria per cornea (±SEM) from one of two similar experiments is shown in Fig. 7. Although a slight decrease in bacterial load was observed in IL-1β pAb- vs PBS-treated mice at 5 days p.i., these data were not statistically significant. However, at 7 days p.i., there were significantly fewer CFU bacteria ($p = 0.0006$) in IL-1β pAb- vs PBS-treated mice. These data indicate that treatment with IL-1β pAb resulted in reduction in the number of corneal PMN number at 7 days p.i. without a concomitant increase in bacterial load.

MIP-2 mRNA and protein level in IL-1β pAb-treated B6 mice

Because PMN number was reduced after IL-1β pAb treatment, we next tested whether this effect was mediated by IL-1 regulation of other cytokines/chemokines. MIP-2, a potent PMN chemoattractant and the mouse homologue of IL-8 (23), was shown recently to be associated with the recruitment and persistence of PMN in the infected cornea (20). Therefore, we used RNase protection assay and ELISA to test whether the reduction in PMN number observed in IL-1β pAb-treated mice was associated with a concomitant decrease in MIP-2 chemokine production. Although the amount of MIP-2 mRNA transcript (data not shown) and protein (Fig. 8) was decreased by 31.7% and 30.0%, respectively, in IL-1β pAb- vs PBS-treated mice at 5 days p.i., these data were not significant. At 7 days p.i., a significant decrease in the amount of MIP-2 protein (60% reduction, $p = 0.0245$) was observed in IL-1β pAb-treated cornea (Fig. 8). These data confirmed that reduction in PMN number in the cornea of IL-1β pAb-treated mice was directly associated with down-regulation of MIP-2 expression.

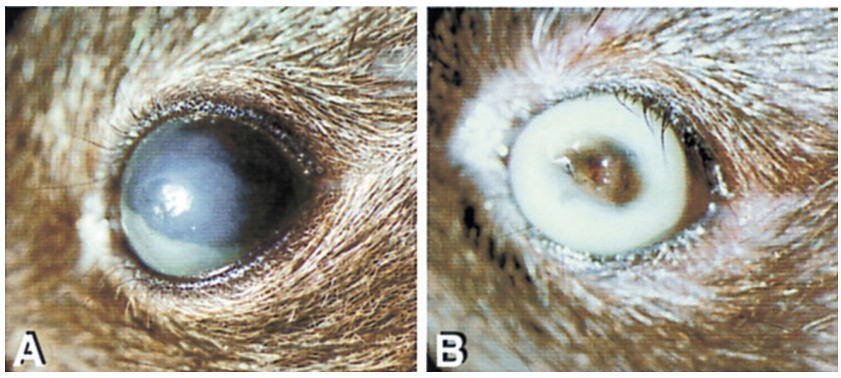

FIGURE 4. Slit lamp photomicrographs of *P. aeruginosa*-infected eyes in IL-1β pAb- and PBS-treated B6 mice. Representative eyes from both groups of mice were photographed at 5 days p.i. using a slit lamp (total magnification, ×25). Only slight opacity was observed in the IL-1β pAb-treated eye (*A*), whereas corneal perforation was evident in the eye of a PBS-treated animal (*B*).

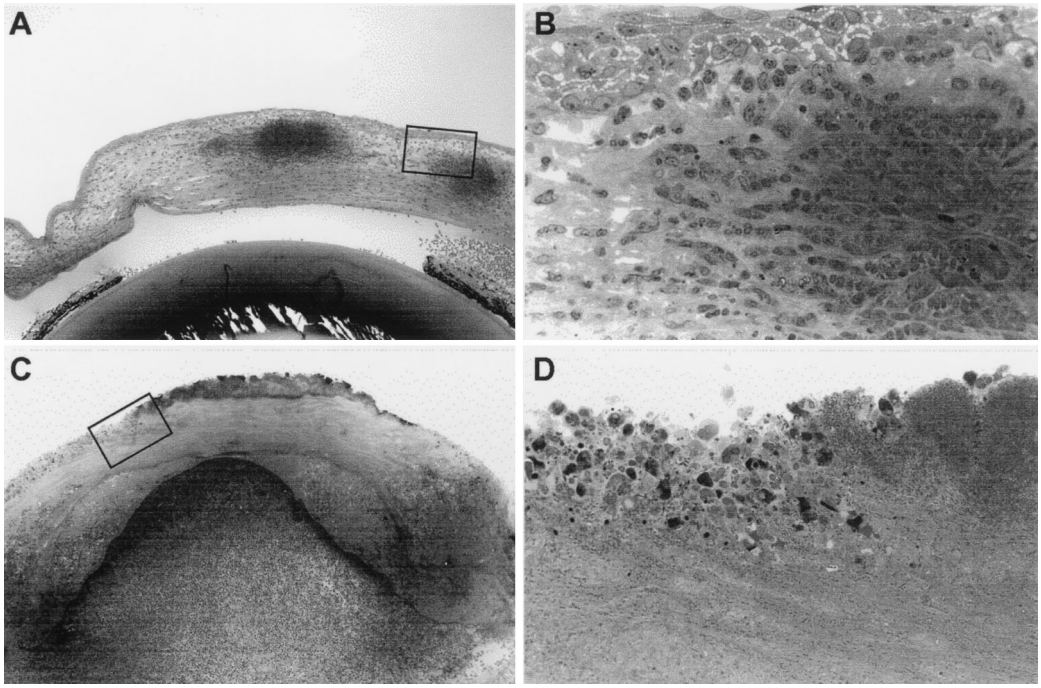

FIGURE 5. Light microscopic histopathology of *P. aeruginosa*-infected corneas from B6 mice treated with IL-1β pAb (*A* and *B*) or PBS (*C* and *D*) at 5 days p.i. Boxes in *A* and *C* ($\times$40) denote regions of cornea shown at a higher magnification in *B* and *D* ($\times$400), respectively.

Discussion

P. aeruginosa corneal infection is characterized by rapid liquefactive necrosis, and frequently the cornea perforates within 24–48 h p.i. (1). This extensive corneal damage often occurs despite antibiotic sterilization of the cornea (7). Such events suggest that, in large part, the corneal destruction observed is mediated by the host inflammatory response, which may remain up-regulated by the persistence of bacterial products (e.g., LPS) in the cornea. In the study reported herein, we examined the role of IL-1 in regulation of the host inflammatory response after *P. aeruginosa* corneal infection. IL-1 is a potent multifunctional cytokine that plays a central role in inflammation and immunity. In concert with other cytokines such as TNF-α, IL-1 initiates the host immune response (12). IL-1 also directs the secretion of other cytokines, including TNF-α, IL-6, and IFN-γ (12). In the eye, increased IL-1 activity has been shown previously to correlate with the severity of inflammation in several experimental models, including the injured vitamin A-deficient rat cornea (24), the herpes-infected cornea (25), and corneal allograft rejection (26). In addition, previous studies from this laboratory showed that differences in IL-1β protein expression early after infection (6–48 h p.i.) were observed in the outbred aged (susceptible, cornea perforates) vs young (resistant, cornea heals) Swiss ICR mice (17). Based upon these studies, we postulated that increased expression of IL-1 in cornea may play a significant regulatory role in the destructive host response to *P. aeruginosa* corneal infection. As a corollary to this, we hypothesized that an appropriate balance of IL-1 expression in the cornea is consistent with an inflammatory response, which would be sufficient to reduce the bacterial load yet did not produce irreversible corneal destruction.

Using RNase protection assays and ELISA, we document that message for both IL-1α and -1β was elevated in susceptible B6 and resistant BALB/c mice within 12 h after infection and reached peak levels of expression (mRNA and protein) at 1 day p.i. Although the mRNA level of each cytokine remained elevated in B6 mice, the message for these cytokines began to decline in BALB/c mice by 5 days p.i. In addition, although IL-1α and -1β protein concentrations were significantly higher in B6 vs BALB/c mice at both 1 and 3 days p.i., protein levels were relatively constant in resistant BALB/c mice through 5 days p.i. In B6 mice, the prolonged and elevated expression of IL-1 was consistent with an increased severity of inflammation, extensive stromal damage, and corneal perforation. Thus, it appears that the susceptibility of B6 mice to *P. aeruginosa* ocular infection is associated with an aberrant regulation of the proinflammatory cytokine IL-1. In contrast, the data from resistant BALB/c mice suggest that 1) an appropriate up- and down-regulation of IL-1 contributes to wound healing and the reestablishment of corneal integrity, or 2) BALB/c mice express more effective negative regulators of IL-1 activity (e.g., IL-1 receptor antagonist) than B6 mice do.

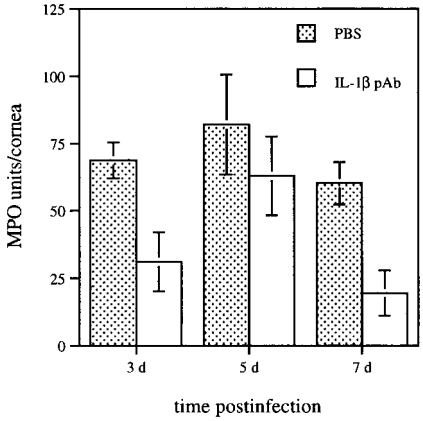

FIGURE 6. Corneal MPO activity in IL-1β pAb- and PBS-treated B6 mice after *P. aeruginosa* challenge. Three individual corneas were collected from each group at 3, 5, and 7 days p.i. for analysis. Results are reported as units MPO/cornea $\pm$ SEM (p = 0.0182, 0.4392, and 0.0227 at 3, 5, and 7 days p.i., respectively).

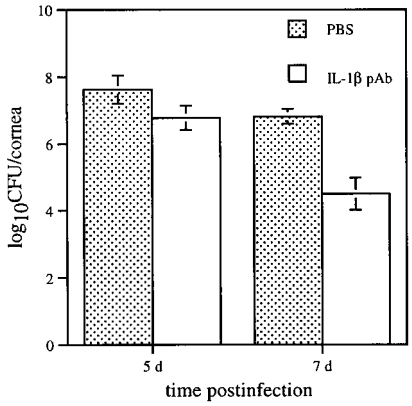

FIGURE 7. Bacterial counts in IL-1β pAb- and PBS-treated B6 mouse cornea after *P. aeruginosa* challenge. Three individual corneas were used from each group at 5 and 7 days p.i. for analysis. Results are reported as $\log_{10}$ number of CFU/cornea ± SEM ($p = 0.5318$ and 0.0006 at 5 and 7 days p.i., respectively).

IL-1α and -1β bind to the same receptor and have very similar if not identical biological properties. To examine the biological relevance of elevated IL-1 in corneal infection, we reduced total IL-1 activity in B6 mice using an IL-1-specific neutralizing pAb against IL-1β. We hypothesized that reduction in the level of either IL-1α or -1β in B6 mice would reduce total IL-1 activity sufficiently to promote an inflammatory response that would allow clearance of bacteria from cornea without severe corneal disruption. Alternately, because IL-1 receptor antagonist is structurally related to IL-1β and also binds to the same receptor sites as the two molecular forms of IL-1 (27), it is possible that the level of bound antagonist, coupled with reduction in IL-1β, together may have resulted in ameliorated disease. The latter hypothesis remains to be tested. To directly test the first hypothesis, IL-1β pAb was injected subconjunctivally and systemically. The progress of ocular disease was monitored from 1 to 7 days p.i., and mean clinical scores were calculated. By 5–7 days p.i., it was apparent that the corneas of IL-1β pAb-treated B6 mice exhibited significantly less severe corneal disease than did those of PBS-injected control animals. These macroscopic data (both mean clinical scores and slit lamp) were confirmed by histopathology studies, which provided morphological evidence that less stromal damage was induced in the cornea of IL-1β pAb- vs PBS-treated mice. As a final measure to confirm that pAb neutralization of IL-1β resulted in a decrease in the concentration of IL-1β in the cornea of pAb-treated mice, ELISA analysis was performed. These studies (data not shown) confirmed that there was a significant decrease ($p = 0.0168$) in IL-1β protein in the cornea of pAb- vs PBS-treated control mice at 7 days p.i.

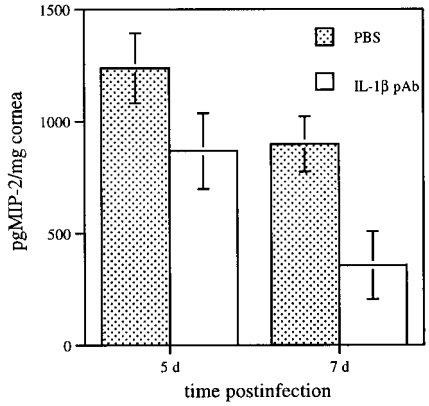

FIGURE 8. Corneal MIP-2 protein levels in IL-1β pAb- and PBS-treated B6 mice after *P. aeruginosa* ocular challenge. Three individual corneas were analyzed for MIP-2 by ELISA at 5 and 7 days p.i. Results are reported as pg MIP-2 protein/mg cornea ± SEM ($p = 0.1602$ and 0.0245 at 5 and 7 days p.i., respectively).

Because administration of IL-1β pAb significantly reduced ocular disease, the next series of studies were performed to elucidate the possible mechanism(s) by which this occurred. Among the multifaceted roles of IL-1 is its ability to mediate PMN influx to inflamed sites (13). In this regard, infiltration of PMN is a central feature in the ocular pathogenesis of *P. aeruginosa* (8, 9). Furthermore, recent work from this laboratory has shown that the persistence of an increased number of PMN in cornea at later times p.i. (5 and 7 days) correlated with the development of corneal perforation in susceptible B6 mice (20). Therefore, we next examined whether reduction in ocular disease after IL-1β pAb treatment coincided with down-regulation in the number of PMN in the cornea. Mice treated with IL-1β pAb vs PBS demonstrated fewer PMN in the cornea from 3 to 7 days p.i. with significant decreases at both 3 and 7 days p.i. These data demonstrate that IL-1β can directly or indirectly regulate a PMN response during *P. aeruginosa* infection and that a decrease in the number of PMN after IL-1β pAb treatment correlates with less stromal damage.

We next explored the mechanism by which IL-1β pAb mediated PMN down-regulation in IL-1β pAb-treated B6 mice. Recent work from this laboratory has shown that MIP-2 is a mediator of corneal PMN infiltration and that persistence of this chemokine correlated with the susceptible phenotype of B6 mice (20). Furthermore, other investigators have shown that IL-1 induced MIP-2 production in different inflammatory models, such as the HSV-1-infected cornea (25), and in the injured lung (28). Therefore, we hypothesized that in our susceptible model IL-1β pAb treatment down-regulated expression of MIP-2, which in turn led to fewer PMN in the cornea. To test this hypothesis, MIP-2 mRNA and protein levels were determined in the cornea of IL-1β pAb- vs PBS-treated B6 mice. By 7 days p.i., a significant reduction of MIP-2 protein expression was found in IL-1β pAb-treated cornea. These data suggest that at least one function of IL-1β is to up-regulate MIP-2 production in the cornea of susceptible B6 mice. The decrease in PMN number in the cornea of IL-1β pAb-treated mice appears due, at least in part, to a down-regulation of this chemokine. Thus, from data reported herein, it appears that IL-1, released early after *P. aeruginosa* infection, provides an initial warning signal to induce the expression of MIP-2, which then augments infiltration of PMN to the infection site. Confirming reports from another model also support this conclusion. In the HSV-1-infected cornea, neutralization of IL-1α or -1β resulted in substantial inhibition of MIP-2 production (25).

Because PMN play an important role in bacterial clearance, one would predict that the decrease in PMN number in IL-1β pAb-treated mice would be associated with enhanced bacterial growth in cornea. To determine whether IL-1β pAb-treated mice were capable of effectively clearing bacteria from infected tissue, viable bacteria were quantitated in the cornea of IL-1β pAb- vs PBS-treated mice. Slightly fewer viable bacteria were detected in the cornea of pAb- vs PBS-treated mice at 5 days p.i. but, unexpectedly, by 7 days p.i. the difference was significant between the two groups. These data imply either that IL-1β pAb-treated B6 mice are capable of more efficient clearance of viable bacteria from the cornea or that bacterial growth and spreading is reduced in the absence of extensive breakdown of stromal proteins by PMN.

In summary, we have investigated the role of IL-1 in *P. aeruginosa* corneal infection in two inbred mouse strains, one susceptible, the other resistant. Our data have shown that the levels of both IL-1α and -1β are elevated after infection in both mouse strains. However, significantly higher concentrations of IL-1α and -1β were seen at 1 and 3 days p.i., and the level of expression remained elevated at 5 days p.i. in susceptible B6 vs resistant BALB/c mice. We also have shown that administration of IL-1β pAb subconjunctivally and i.p. offered an effective means of reducing host-mediated stromal destruction in susceptible mice. Such treatment decreased MIP-2 expression and resulted in a reduction in PMN number at later times p.i. Based on the evidence provided herein, we suggest that prolonged and elevated IL-1 expression leads to an increase in MIP-2 production, which induces persistence of PMN influx and ultimately corneal perforation in susceptible mice. In contrast, balanced regulation of IL-1 facilitates sufficient MIP-2 production to attract a cellular infiltrate that is sufficient to eliminate the bacteria with less corneal damage.

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