INFECTION AND IMMUNITY, Jan. 1998, p. 376–379 Vol. 66, No. 1
0019-9567/98/$04.00+0

Early Cytokine and Chemokine Gene Expression during *Pseudomonas aeruginosa* Corneal Infection in Mice

KAREN A. KERNACKI, DENNIS J. GOEBEL, MICHAEL S. POOSCH, AND LINDA D. HAZLETT*
Department of Anatomy/Cell Biology, Wayne State University School of Medicine, Detroit, Michigan 48201

Received 22 July 1997/Returned for modification 15 September 1997/Accepted 13 October 1997

Using a multiprobe RNase protection assay, we examined cytokine and chemokine mRNAs that were expressed after corneal infection with *Pseudomonas aeruginosa* in mice. Cytokines that were upregulated included interleukin-1α (IL-1α) and -1β, IL-1 receptor antagonist, IL-6, IL-11, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, macrophage colony-stimulating factor, stem cell factor, lymphotoxin β, transforming growth factor β1, and tumor necrosis factor alpha. Chemokine transcripts that were upregulated included Eotaxin; gamma-interferon-inducible protein 10; monocyte chemoattractant protein 1; macrophage inflammatory proteins 1α, 1β, and 2; and RANTES. Peak expression of these cytokines and chemokines was observed between 1 and 3 days after infection. These responses returned to or approached baseline preinfection levels by 7 days after ocular challenge. Identification of the various cytokines and chemokines upregulated during corneal infection provides important information relevant to unraveling the pathogenesis induced by this bacterium and provides hope that specific molecules can be targeted for therapy.

Pseudomonas aeruginosa is a leading cause of corneal infections, especially in users of soft contact lenses and in immunocompromised or aged individuals (17). The infection is characterized by rapid liquefactive necrosis that often proceeds to corneal perforation despite optimum antibiotic chemotherapy (24). *P. aeruginosa* produces several exoproteins, such as elastase, alkaline protease, and exotoxin A, which have been implicated as mediators of corneal dissolution (23), but the mechanisms leading to stromal destruction are not well defined. Likewise, factors released from infiltrating inflammatory and resident corneal cells are thought to contribute to the tissue pathology (13, 23). Possible contributing host factors include cytokines and chemokines released by these cell types that can either augment or prolong the inflammatory response, a consequence required to contain the infection but also one which may be potentially devastating to maintenance of corneal clarity.

Based upon past studies (11), we hypothesize that a delicate balance exists between the induction of a protective and that of a destructive response to ocular infection. This may be reflected in the specific pattern or timing of cytokines and chemokines that are induced at early time points after infection. The current studies were employed to examine the mRNA expression of a wide variety of cytokines and chemokines in outbred mice to target potential factors involved in the tissue destruction seen during corneal infection. To this end, we have employed a multiprobe nuclease protection assay to detect and semiquantitate mRNAs of the various cytokines and chemokines (5). The multiprobe protection approach was chosen for these studies because of its relative sensitivity and specificity and its capacity to simultaneously detect several mRNA species in a single sample of total RNA. Identification of the particular mRNAs that are upregulated will then allow the design of single probes which will subsequently be used to detect and quantitate the individual mRNAs in infected corneal tissues. Moreover, the single probes will be useful for in situ hybridization applications to identify which specific cells produce the cytokines and chemokines.

Young adult (6- to 8-week-old) outbred Swiss ICR mice (Harlan Sprague-Dawley, Indianapolis, Ind.) were used for the initial studies. Other outbred mice, Swiss Webster (Harlan Sprague-Dawley), were used in a separate experiment to confirm the Swiss ICR mouse data. Before infection, the mice were lightly anesthetized with isoflurane (AErrane; Anaquest Inc., Liberty Corner, N.J.) and placed beneath a stereoscopic dissection microscope at an ×40 magnification. The central corneal surface of the left eye was scarified with three 1-mm incisions with a sterile 26-gauge needle, taking care not to penetrate the anterior chamber. Following scarification, 5 μl of a bacterial suspension containing 10^6 CFU of *P. aeruginosa* ATCC 19660 (American Type Culture Collection, Rockville, Md.), prepared as described previously (14), was topically applied to the wounded cornea. For collection of corneal tissues, mice were sacrificed by cervical dislocation. Following sacrifice, corneal tissues were collected, quick-frozen in liquid nitrogen, and stored at −70°C prior to extraction of total RNA. Animals in this study were humanely treated in accordance with the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research.

Prior to analysis, total RNA was extracted from uninfected and infected corneal tissues according to the method of Chomczynski and Sacchi (4). Detection and semiquantitation of a variety of murine cytokine and chemokine mRNAs were accomplished with the multiprobe RNase protection assay system from Pharmingen (San Diego, Calif.), with some minor modifications. Briefly, a mixture of [^{32}P]CTP-labelled antisense riboprobes was generated from a panel of different cytokine or chemokine templates. These panels also included templates for the murine housekeeping genes encoding glyceraldehyde-3-phosphate dehydrogenase and L32 (a murine ribosomal protein) (28), to ensure equal loading of total RNA onto the gels. A predetermined amount of total corneal RNA was hybridized overnight at 56°C with 300 pg of the ^{32}P-anti-

* Corresponding author. Mailing address: Department of Anatomy/Cell Biology, Wayne State University School of Medicine, 540 E. Canfield Ave., Detroit, MI 48201. Phone: (313) 577-1061. Fax: (313) 577-3125. E-mail: lhazlett@med.wayne.edu.

sense riboprobe mixture. After hybridization, the samples were digested with 2,500 U of T_1 nuclease (Gibco-BRL, Gaithersburg, Md.). Nuclease-protected RNA fragments were purified by ethanol precipitation. After purification, the samples were resolved on a 4.5% polyacrylamide sequencing gel. Protected bands were observed after exposure of the gels to Fuji X-ray film (Fisher, Itasca, Ill.). The specific cytokine and chemokine bands were identified on the basis of their individual migration patterns in comparison with the undigested probes. The bands were quantitated by densitometric analysis with an MDX Persen S1 II densitometer and Image Quant densitometric software (Molecular Dynamics, Sunnyvale, Calif.) to determine the relative amounts of mRNA in the corneal tissues. The scanned intensities of the bands from the RNA in the infected corneal tissues were compared to those of uninfected corneal tissues. A ratio of signal (area scanned × optical density) from infected corneal tissue to that from uninfected corneal tissue was calculated to determine the extent of upregulation of a particular mRNA species.

For these studies, all five of the currently available mouse cytokine and chemokine template sets were used to examine corneal cytokine and chemokine gene expression. Preliminary experiments were employed to determine the amount of total corneal RNA required to detect cytokine- and chemokine-specific bands. For the CK2 panel, 5 μg of total RNA was sufficient for the detection and subsequent densitometric analysis of the mRNAs. Twenty micrograms of total RNA was required for the other four panels.

For these studies, corneal tissues were obtained from Swiss ICR mice before infection and at 6 and 12 h and 1, 3, 5, and 7 days after infection. Before collection of corneal tissues for RNA isolation, ocular grades for the individual mice were determined, as described previously (14). This ensured that animals with similar disease responses were used for an individual sample which consisted of pooled corneas from 15 to 20 mice. In all of the panels examined, the intensities of the bands of the two housekeeping genes encoding L32 and glyceraldehyde-3-phosphate dehydrogenase were similar, indicating that similar amounts of total RNA were loaded onto the gels. Corneal tissues from mice that had received only a corneal abrasion without bacterial inoculation were collected at 24 h after wounding and demonstrated responses similar to those of the unwounded, uninfected controls (data not shown).

The analyses for each of the five panels were performed at least three times with similar results. Representative panels are shown in Fig. 1. Tables 1 to 3 show the results of densitometric analysis of the various bands in the different panels. Uninfected corneal tissues demonstrated detectable levels for migration inhibition factor (MIF), transforming growth factor β1 (TGF-β1) and TGF-β2, and tumor necrosis factor alpha (TNF-α). The responses for MIF and TGF-β2 were essentially unchanged over the 7-day period after infection. Transcripts for the proinflammatory-immunoregulatory cytokines (Fig. 1A and Table 1) interleukin-1α (IL-1α) and IL-1β, IL-1 receptor antagonist (IL-1Ra), IL-6, lymphotoxin β, TGF-β1, and TNF-α increased as early as 6 to 12 h after infection, peaked at 1 to 3 days, and began to decrease or approach uninfected baseline levels by 5 to 7 days. Increases of the hematopoietic cytokines (Fig. 1B and Table 2) IL-11, granulocyte-macrophage colony-stimulating factor, macrophage colony-stimulating factor, granulocyte colony-stimulating factor, and stem cell factor were detected as early as 6 h after infection. Peak responses for these cytokines were observed at 1 day, and these levels began to decrease or approach preinfection levels by 5 to 7 days. Upregulation of the transcripts for the chemokines (Fig. 1C and Table 3) RANTES; Eotaxin (Eotxn); macrophage inflammatory protein 1β (MIP-1β), MIP-1α, and MIP-2; gamma-interferon (IFN-γ)-inducible protein 10 (IP-10); and monocyte chemoattractant protein 1 (MCP-1) was found as early as 6 h after infection and peaked at 1 to 3 days. By 7 days, the bands for MIP-1β, -1α, and -2; MCP-1; and IP-10 were still readily observed, whereas the bands for RANTES and Eotxn were undetectable. Some of the bands in Fig. 1B and C were very faint, and routine photographic methods did not provide good visual representation. However, the bands were measurable by densitometry. Other cytokines and chemokines that were probed for but not detected in these studies included IL-2, IL-3, IL-4, IL-5, IL-7, IL-10, IL-13, IL-15, IFN-γ and IFN-β, TNF-β, lymphotactin, and TCA-3.

Since the majority of tissue damage in the murine model of

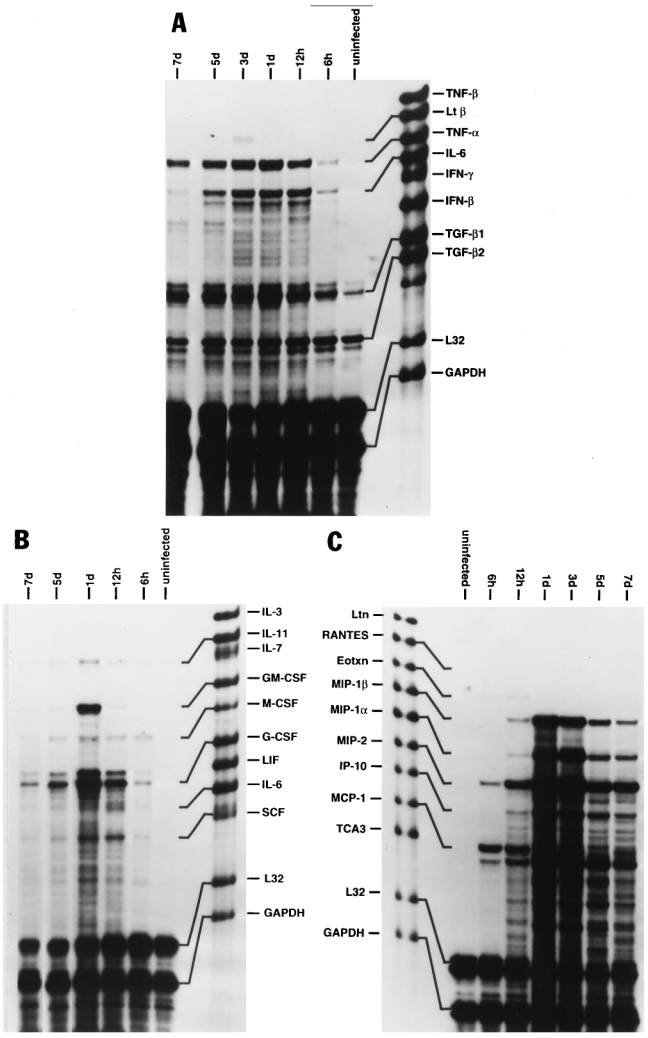

FIG. 1. CK3, CK4, and CK5 panel RNase protection assays. Twenty micrograms of total RNA collected from mice prior to or at 6 and 12 h and 1, 3, 5, and 7 days after infection was hybridized to the CK3 (A), CK4 (B), or CK5 (C) probe template set. RNase-protected fragments were analyzed on polyacrylamide gels which were subsequently exposed to X-ray film to visualize protected bands. The lanes on the far right (A and B) or left (C) show the undigested probe template set which serves as a size reference to which the protected fragments were compared. Ltβ, lymphotoxin β; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GM-CSF, granulocyte-macrophage colony-stimulating factor; M-CSF, macrophage colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; LIF, leukemia inhibitory factor; SCF, stem cell factor; Ltn, lymphotactin; TCA3, homolog of human I-309.

TABLE 1. Densitometric analysis of the CK2 and CK3 panels[a]

Cytokine	Panel	Uninfected	Value at h:		Value at day:			
			6	12	1	3	5	7
IL-1α	CK2	1.0 (123)	2.1	7.3	34.3	ND	14.1	5.0
IL-1β	CK2	1.0 (93)	2.1	13.4	181.4	ND	153.9	118.8
IL-1Ra	CK2	1.0 (85)	2.5	5.5	150.5	ND	146.7	89.5
MIF	CK2	1.0 (5,770)	1.2	1.0	1.4	ND	1.1	0.9
Ltβ	CK3	1.0 (139)	1.4	2.8	3.0	6.6	4.3	1.8
TNF-α	CK3	1.0 (568)	3.3	17.1	33.7	26.7	16.1	11.6
IL-6	CK3	1.0 (143)	17.0	104.4	105.4	102.1	45.1	8.9
TGF-β1	CK3	1.0 (4,137)	3.0	5.0	8.4	4.9	5.3	4.9
TGF-β2	CK3	1.0 (13,396)	1.1	1.6	1.6	1.1	0.9	1.4

[a] The sizes of the individual bands on the autoradiographs in Fig. 1 were quantitated by densitometric analysis to yield a signal that reflects the size of the area scanned × the band intensity. The ratio of signal from infected corneal tissue to the signal from uninfected tissue is shown. In the uninfected tissue column, a value of 1.0 was assigned to this sample and the number in parentheses indicates the signal observed for uninfected corneal tissue. When a signal of <150 is reported, a band for this sample was not visually observed. ND, densitometric analyses were not determined for this time point. Ltβ, lymphotoxin β.

infection is found at 5 to 7 days after infection, we also examined the cytokine and chemokine profiles in a second group of outbred mice, Swiss Webster, at 5 and 7 days after infection to confirm the Swiss ICR mouse data. The cytokine and chemokine profiles for these mice were similar to those observed for the Swiss ICR mice (data not shown).

The current study detected the upregulation of several proinflammatory, immunoregulatory, and hemopoietic cytokines as well as various chemokines after corneal infection with *P. aeruginosa* by a multiprobe RNase protection assay. This method permits qualitative analysis of various cytokine and chemokine mRNAs; however, direct quantitation of the individual transcripts is not possible with the multiprobe assay. These cytokine and chemokine profiles were confirmed in two different groups of outbred mice, Swiss ICR and Swiss Webster.

Potential sources for these cytokines and chemokines include infiltrating polymorphonuclear leukocytes, macrophages, B cells, and T cells as well as resident corneal epithelial, stromal, and endothelial cells (3, 6, 12, 18, 20, 21, 25, 26). Our data suggest that resident corneal cells initiate the inflammatory response in the infected cornea since some of the mRNAs are significantly upregulated as early as 6 h after infection, prior to the infiltration of significant numbers of polymorphonuclear leukocytes or macrophages (7, 8, 11). By 1 day after infection, it is likely that the observed responses are from both resident corneal and infiltrating inflammatory cells.

TABLE 2. Densitometric analysis of the CK4 panel[a]

Cytokine	Uninfected	Value at h:		Value at day:		
		6	12	1	5	7
IL-11	1.0 (128)	2.1	7.2	22.9	8.9	3.0
GM-CSF	1.0 (144)	19.2	101.1	208.2	55.5	43.9
M-CSF	1.0 (149)	5.1	4.9	11.1	7.9	4.2
G-CSF	1.0 (70)	2.2	12.9	195.2	5.3	2.5
SCF	1.0 (123)	2.3	8.1	17.3	4.5	2.2

[a] The sizes of the individual bands on the autoradiographs in Fig. 1 were quantitated by densitometric analysis to yield a signal that reflects the size of the area scanned × the band intensity. The ratio of signal from infected corneal tissue to the signal from uninfected tissue is shown. In the uninfected tissue column, a value of 1.0 was assigned to this sample and the number in the parentheses indicates the signal observed for uninfected corneal tissue. When a signal of <150 is reported, a band for this sample was not visually observed. GM-CSF, granulocyte-macrophage colony-stimulating factor; M-CSF, macrophage colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; SCF, stem cell factor.

TABLE 3. Densitometric analysis of the CK5 panel[a]

Cytokine	Uninfected	Value at h:		Value at day:			
		6	12	1	3	5	7
RANTES	1.0 (65)	2.7	2.1	3.1	4.7	1.0	1.0
Eotxn	1.0 (70)	3.1	5.5	3.3	2.2	1.1	1.5
MIP-1β	1.0 (64)	3.0	35.3	355.4	297.9	115.7	55.5
MIP-1α	1.0 (40)	2.5	29.2	494.8	564.8	115.7	132.1
MIP-2	1.0 (131)	14.2	102.2	237.8	249.7	166.9	150.7
IP-10	1.0 (43)	3.9	43.7	374.8	335.4	238.7	107.3
MCP-1	1.0 (80)	29.3	90.1	308.9	308.9	191.2	168.1

[a] The sizes of the individual bands on the autoradiographs in Fig. 1 were quantitated by densitometric analysis to yield a signal that reflects the size of the area scanned × the band intensity. The ratio of signal from infected corneal tissue to the signal from uninfected tissue is shown. In the uninfected tissue column, a value of 1.0 was assigned to this sample and the number in the parentheses indicates the signal observed for uninfected corneal tissue. When a signal of <150 is reported, a band for this sample was not visually observed.

Our working hypothesis for this model is that an appropriate balance must exist in the timing and pattern of cytokine and chemokine gene expression to facilitate elimination of *P. aeruginosa*, yet protect the cornea from excessive tissue destruction. Studies by Walley et al. in a murine sepsis model demonstrated that there is a link between the severity of sepsis and the balance of inflammatory cytokines (27). Furthermore, genetic susceptibility to infectious or ocular autoimmune disease has been associated with the expression of different cytokine profiles (9, 19). We also have previously described impaired corneal cytokine responses (IL-1β and IFN-γ protein) and an inability to upregulate intercellular adhesion molecule in aged (susceptible) mice compared with young adult (resistant) mice (10). Likewise, several inbred strains of mice have been characterized as being susceptible or resistant to corneal infection with *P. aeruginosa* based on their ability to restore corneal clarity and ocular integrity (2). As this report has identified potential targets that modulate the corneal inflammatory response, current efforts are directed at designing individual probes for use in single-probe RNase protection assays. The design of these probes will permit quantitation of the individual mRNAs in corneal tissues and allow direct comparison of transcripts from different samples. Likewise, these probes will be invaluable in in situ hybridization studies to identify the cell types synthesizing the individual cytokines and chemokines. Use of these methods with the susceptible and resistant mouse models should provide insight into the roles that the individual members of this select panel of cytokines and chemokines play in the tissue destruction which occurs during infection.

We did not detect any of the T-cell- or NK-cell-derived cytokines in these studies. This was surprising since we previously identified by immunohistochemical staining IL-2R-positive T cells in these and in different inbred strains of mice by 5 days after corneal infection (15, 16). Likewise, previously we were able to detect IFN-γ protein in the Swiss ICR mice as early as 12 h postinfection, which may reflect a role for activated NK cells early during infection (11). We did, however, in the current studies detect very low levels of the chemokine RANTES in infected corneal tissues between 6 h and 3 days after infection. RANTES became undetectable by 5 days after infection. The RANTES data described herein provides additional evidence that activated T cells are present in infected corneal tissues. This conclusion is based on the studies of

Schall, who described the downregulation of RANTES mRNA levels upon activation of T cells (22). One possible reason for our inability to detect T-cell or NK-cell cytokines is that only small numbers of infiltrating lymphocytes migrate into the cornea after infection and the amount of mRNA in corneal tissue for these cytokines may be below the sensitivity limit for the RNase protection assay (7, 8). Others have reported the detection of T-cell cytokines in herpes simplex virus-infected corneal tissues (1), but lymphocytes were found in corneal tissues in larger numbers than in this model (20). Alternative, more sensitive competitive reverse transcription-PCR assays may be required for the detection of these cytokines.

In summary, the studies described in this report have identified several cytokines and chemokines that are upregulated as a result of corneal *P. aeruginosa* infection. This pattern of cytokine and chemokine expression was confirmed in two different types of outbred mice. Future studies will focus on the design of individual probes for single-probe RNase protection assays and in situ hybridization applications based on the data from the current studies. Together, these will provide tools to further examine the role of cytokines and chemokines in the progression and potential modulation of *P. aeruginosa* corneal infection.

This work was supported by grants R01EY02986 and P30 EY04068 from the National Eye Institute of the National Institutes of Health, Bethesda, Md.

We thank Ronald Barrett for his excellent technical support.

REFERENCES

1. **Babu, J. S., S. Kanangat, and B. T. Rouse.** 1995. Predominance of Th1 cells in ocular tissues during herpetic stromal keratitis. J. Immunol. **149:**3035–3039.
2. **Berk, R. S., L. D. Hazlett, and K. W. Beisel.** 1987. Genetic studies on resistance and susceptibility genes controlling the mouse corneal infection by *Pseudomonas aeruginosa*. Infect. Immun. **39:**83–91.
3. **Callard, R. E., and A. J. H. Gearing.** 1994. The cytokine facts book. Academic Press, Inc., San Diego, Calif.
4. **Chomczynski, P., and M. Sacchi.** 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162:**156–159.
5. **Chu, E. B., M. V. Hobbs, C. B. Wilson, C. G. Romball, P. S. Linsley, and W. O. Weigle.** 1996. Intervention of $CD4^+$ cell subset shifts and autoimmunity in the BXSB mouse by murine CTLA4 Ig. J. Immunol. **156:**1262–1268.
6. **Cubitt, C. L., R. N. Lausch, and J. E. Oakes.** 1994. Differential regulation of granulocyte-macrophage colony stimulating factor gene expression in human corneal cells by pro-inflammatory cytokines. J. Immunol. **153:**232–240.
7. **Hazlett, L. D., F. B. Kreindler, R. S. Berk, and R. Barrett.** 1990. Age alters the phagocytic capability of inflammatory cells induced into the cornea. Curr. Eye Res. **9:**129–138.
8. **Hazlett, L. D., M. Zucker, and R. S. Berk.** 1992. Distribution and kinetics of the inflammatory cell response to ocular challenge with *Pseudomonas aeruginosa* in susceptible and resistant mice. Ophthalmic Res. **24:**32–39.
9. **Heinzel, F. P., R. M. Rerko, F. Ahmed, and E. Pearlman.** 1995. Endogenous IL-12 is required for control of Th2 cytokine responses capable of exacerbating leishmaniasis in normally resistant mice. J. Immunol. **155:**730–739.
10. **Hobden, J. A., S. A. Masinick, R. P. Barrett, and L. D. Hazlett.** 1995. Aged mice fail to upregulate ICAM-1 after *Pseudomonas aeruginosa* corneal infection. Investig. Ophthalmol. Vis. Sci. **36:**1107–1114.
11. **Hobden, J. A., S. A. Masinick, R. P. Barrett, and L. D. Hazlett.** 1997. Proinflammatory cytokine deficiency and pathogenesis of *Pseudomonas aeruginosa* keratitis in aged mice. Infect. Immun. **65:**2754–2758.
12. **Kennedy, M. C., J. T. Rosenbaum, J. Brown, S. R. Planck, X. Huang, C. A. Armstrong, and J. C. Ansel.** 1995. Novel production of interleukin-1 receptor antagonist peptides in normal human cornea. Investig. Ophthalmol. Vis. Sci. **95:**82–88.
13. **Kernacki, K. A., and R. S. Berk.** 1994. Characterization of the inflammatory response induced by corneal infection with *Pseudomonas aeruginosa*. J. Ocul. Pharmacol. **10:**281–288.
14. **Kernacki, K. A., R. Fridman, L. D. Hazlett, M. A. Lande, and R. S. Berk.** 1997. *In vivo* characterization of host and bacterial protease expression in naive and immunized mice. Curr. Eye Res. **16:**289–297.
15. **Kwon, B., and L. D. Hazlett.** 1996. Characterization of *P. aeruginosa* ocular infection using T-cell-depleted and $\beta 2m^{\circ}$ mice. ARVO abstracts. Investig. Ophthalmol. Vis. Sci. **37:**B814.
16. **Kwon, B., and L. D. Hazlett.** Association of $CD4^+$ T-cell-dependent keratitis with genetic susceptibility to *P. aeruginosa* ocular infection. J. Immunol., in press.
17. **Laibson, P. R.** 1990. *Pseudomonas aeruginosa*, p. 35–37. *In* F. T. Fraunfelder and F. H. Roy (ed.), Current ocular therapy. The W. B. Saunders Co., Philadelphia, Pa.
18. **Lausch, R. N., S. H. Chen, T. M. Tumpey, Y. H. Su, and J. E. Oakes.** 1996. Early cytokine synthesis in the excised mouse cornea. J. Interferon Cytokine Res. **16:**35–40.
19. **Nakamura, S., T. Yamakawa, M. Sugita, M. Kijima, M. Ishioka, S.-I. Tanaka, and S. Ohno.** 1994. The role of tumor necrosis factor-alpha in the induction of experimental autoimmune uveoretinitis in mice. Investig. Ophthalmol Vis. Sci. **35:**3884–3889.
20. **Niemialtowski, M. G., and B. T. Rouse.** 1992. Predominance of Th1 cells in ocular tissues during herpetic stromal keratitis. J. Immunol. **149:**3035–3039.
21. **Planck, S. R., X. N. Huang, J. E. Robertson, and J. T. Rosenbaum.** 1994. Cytokine mRNA levels in rat ocular tissues after systemic endotoxin treatment. Investig. Ophthalmol. Vis. Sci. **35:**9324–9330.
22. **Schall, T. J.** 1991. Biology of the RANTES/SIS cytokine family. Cytokine **3:**165–183.
23. **Steuhl, K.-P., G. Doring, A. Henni, H.-J. Thiel, and K. Botzenhart.** 1987. Relevance of host-derived and bacterial factors in *Pseudomonas aeruginosa* corneal infections. Investig. Ophthalmol. Vis. Sci. **28:**1559–1568.
24. **Thiel, H.-J., K.-P. Steuhl, and G. Doring.** 1987. Therapy of *Pseudomonas aeruginosa* eye infections. Antibiot. Chemother. **39:**92–102.
25. **Torres, P. F., A. F. DeVos, R. van der Gaag, B. Martins, and A. Kijlstra.** 1996. Cytokine mRNA expression during corneal allograft rejection. Exp. Eye Res. **63:**453–461.
26. **Tran, M. T., M. Tellaetxe-Isusi, V. Elner, R. M. Streiter, R. N. Lausch, and J. E. Oakes.** 1996. Proinflammatory cytokines induce RANTES and MCP-1 synthesis in human corneal keratocytes but not corneal epithelial cells. Beta chemokine synthesis in corneal cells. Investig. Ophthalmol. Vis. Sci. **37:**987–996.
27. **Walley, K. R., N. W. Lukacs, T. J. Standiford, R. M. Streiter, and S. L. Kunkel.** 1996. Balance of inflammatory cytokines related to severity and mortality of murine sepsis. Infect. Immun. **64:**4733–4738.
28. **Young, J. A., and J. Trowsdale.** 1985. A processed pseudogene in an intron of the HLA-DP beta 1 chain is a member of the ribosomal protein L32 gene family. Nucleic Acids Res. **13:**8883–8891.

Editor: R. E. McCallum