INFECTION AND IMMUNITY, June 1993, p. 2713–2716
0019-9567/93/062713-04$02.00/0

Vol. 61, No. 6

Kinetics of Serum and Ocular Antibody Responses in Susceptible Mice That Received a Secondary Corneal Infection with *Pseudomonas aeruginosa*

MICHAEL J. PRESTON,† KAREN KERNACK, AND RICHARD S. BERK*

Department of Immunology/Microbiology, Wayne State University School of Medicine, Detroit, Michigan 48201

Received 26 October 1992/Accepted 18 March 1993

When susceptible C57BL/6J mice were infected with *Pseudomonas aeruginosa* in one eye and then reinfected in the previously uninfected contralateral control eye either 4 or 8 weeks after the primary infection, approximately 20 to 30% of the mice receiving a 4-week reinfection regimen restored corneal clarity within 4 weeks, while almost all of the 8-week-reinfected mice restored corneal clarity within 3 to 6 days postinfection. However, the rate of bacterial clearance was the same in both sets of mice despite the presence of opsonophagocytic antibodies only in the 8-week-reinfected mice. As determined by enzyme-linked immunosorbent assay, immunoglobulin G was the major immunoglobulin in both serum and ocular tissue of both mouse sets, and the immunoglobulin G level was two- to fourfold higher after the 8-week secondary infection than in the 4-week-reinfected mice.

Pseudomonas aeruginosa is an opportunistic pathogen which causes severe corneal infections in humans. Many of the clinical features of the infection can be reproduced in a variety of animal models (3, 4, 6–8, 15, 16). Previous experimental studies from our laboratory have demonstrated that susceptible C57BL/6J mice were unable to restore corneal clarity upon primary infection and eventually underwent corneal perforation, phthisis bulbi (shrinkage), or both (3–5). When the C57BL/6J mice were given a secondary infection in the previously uninfected, contralateral control eye 30 days after the primary infection, approximately 20 to 30% of the mice restored corneal clarity within 4 weeks, while others showed some improvement over that seen in mice receiving only the primary infection (5). However, when the secondary infection was given 60 days after the primary infection, most of the animals restored corneal clarity within 3 to 6 days, and the remainder recovered within 14 days. According to Allansmith et al., the eye provides a very useful model for the study of general immunologic principles (1). Also, we need to know more about the relationship between serum antibodies, their transfer through ocular structures, and their appearance in intra- and extravascular spaces. Therefore, since little is known about the nature and kinetics of the immune response of the eye, the purpose of the present report is to characterize the local and systemic response in susceptible C57BL/6J mice given a secondary ocular infection at either 30 or 60 days after the primary corneal infection.

C57BL/6J mice were administered a primary corneal infection in the left eye. Either 4 or 8 weeks after the primary infection the mice were administered a secondary corneal infection in the previously uninfected contralateral control eye. Serum, tear, and cornea samples were collected from 15 mice either 4 or 8 weeks after the primary infection for determining presecondary antibody levels. Samples from five mice were pooled to give three experimental pools per time point. Samples were then collected each week for 4 weeks after the secondary infections and pooled in the same way for enzyme-linked immunosorbent assay (ELISA) as previously described (12). It should be noted that serum, tear, and cornea ELISA units should not be compared with each other because of the different collection methods and dilution factors. Almost all of the 8-week-reinfected mice restored corneal clarity within 7 days and remained so for the length of the experiment. On the other hand, none of the 30-day-reinfected mice exhibited corneal clarity during the first 3 weeks of the infection. Finally, by week 4, 25% of the mice restored corneal clarity, while the remainder still expressed the susceptibility phenotype.

Serum antibody levels. The serum antibody response specific to *P. aeruginosa* cells after secondary corneal infections was composed mainly of immunoglobulin G (IgG). Very little IgM or IgA antibody was detected (≤50 ELISA units each; data not shown). The preinfection IgG levels, measured either 4 or 8 weeks after the primary infections, were not significantly different (Fig. 1). However, by the first week after the secondary infections, the mice that received the 8-week secondary infection produced a more rapid and heightened serum IgG response than mice that received the 4-week secondary infection. The IgG levels after 8-week secondary infection were two- to fourfold higher than those after the 4-week secondary infection.

Corneal antibody levels. A pattern similar to that observed for serum IgG response was also seen in the corneal IgG response. Very little IgM or IgA was measured in the corneal eluates (≤0.2 ELISA unit each; data not shown). Cornea IgG levels were also two- to fourfold higher after the 8-week secondary infection than after the 4-week secondary infection (Fig. 2). The preinfection values were not significantly different. When IgG was measured in the eyes that received the primary infection only, the values were nearly identical to those of the eyes that received the secondary infections.

Tear antibody levels. IgG was also the predominant antibody class present in the tears of the eyes that received the secondary infections. IgG levels in the tears were approxi-

* Corresponding author.
† Present address: Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115.

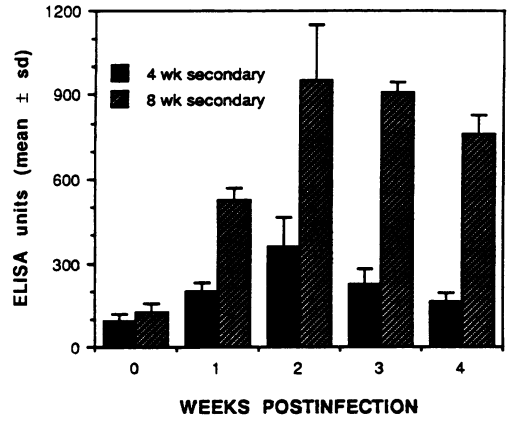

FIG. 1. Serum IgG levels after secondary corneal infections administered 4 or 8 weeks after a primary corneal infection. Sera were pooled from blood collected from five mice. Data are means ± standard deviations (sd) from three pools at each time point. An ELISA unit is defined as the reciprocal of the serum dilution that gave an optical density at 405 nm (OD_{405}) within the linear range of the assay multiplied by the OD_{405}. Week 0 values are the pre-secondary-infection values taken at either 4 or 8 weeks, versus 4-week secondary infections in the infected eyes. *P* values for 8-week versus 4-week secondary infections in the infected eyes: week 1, 0.0004; week 2, 0.0046; week 3, 0.0001; week 4, 0.0001.

mately 10-fold higher than either IgA or IgM levels in the reinfected eyes (Fig. 3 and 4; also data not shown for IgM). Tear IgG levels in the 4- and 8-week-reinfected eyes were not significant one week after infection but were significant thereafter. Tear IgG was not detected during weeks 3 and 4 after the 4-week secondary infection. Although tear IgG was present at weeks 3 and 4 after the 8-week secondary infection, the levels dropped more rapidly than the serum or cornea IgG levels at the same time points. During the first 2 weeks after infection, tear IgG levels remained low in the eye that received the primary infection. This is in contrast to the cornea IgG levels in the eyes that received the primary

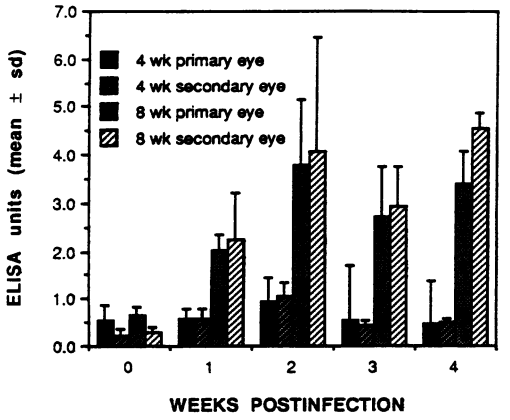

FIG. 2. Cornea IgG levels after secondary corneal infections administered 4 or 8 weeks after a primary corneal infection. The data were determined as described in the legend to Fig. 1. Cornea IgG levels in the eyes that received the primary corneal infection and in the previously uninfected eyes that received the secondary corneal infection were measured. *P* values for 8-week versus 4-week secondary infections in the infected eyes: week 1, 0.0459; week 2, 0.050; week 3, 0.0076; week 4, 0.0001.

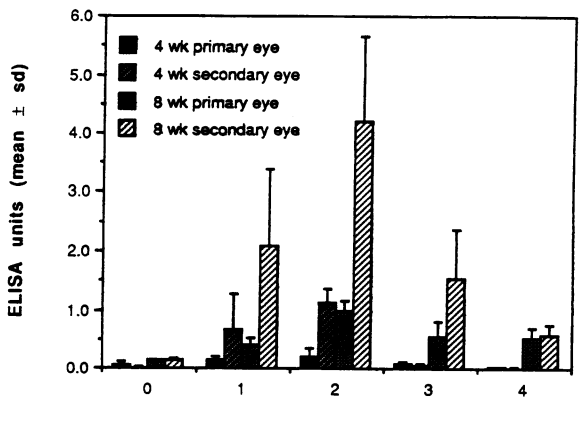

FIG. 3. Tear IgG levels after secondary corneal infections administered 4 or 8 weeks after a primary corneal infection. The data were determined as described in the legends to Fig. 1 and 2. *P* values for 8-week versus 4-week secondary infections in the infected eyes: week 1, 0.1586; week 2, 0.002; week 3, 0.0402; week 4, 0.010.

infection, which were nearly identical to levels in those that received the secondary infection.

Determination of opsonic-antibody concentrations in serum. It is clear that C57BL/6J mice mount a significantly higher IgG response in the serum, tears, and cornea after the 8-week secondary infection than in the 4-week secondary infection. However, this does not give information about the biological function of these antibodies. Therefore, we examined the ability of the serum antibodies to opsonize the bacteria for killing by polymorphonuclear leukocytes as previously described (2). Serum samples were collected from five mice 1 week after either the primary corneal infection or the 4- or 8-week secondary corneal infection. More than 50% killing of the bacteria in the assay mixture is considered biologically significant. As seen in Table 1, C57BL/6 mice produced opsonic antibodies to *P. aeruginosa* only after the 8-week secondary infection.

Determination of viable *P. aeruginosa* in the infected cor-

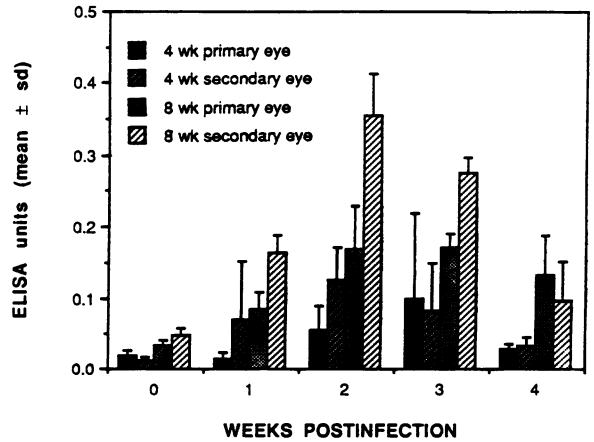

FIG. 4. Tear IgA levels after secondary corneal infections administered 4 or 8 weeks after a primary corneal infection. The data were determined as described in the legends to Fig. 1 and 2. *P* values for 8-week versus 4-week secondary infections in the infected eyes: week 1, 0.1187; week 2, 0.0251; week 3, 0.0148; week 4, 0.0407.

TABLE 1. Determination of the presence or absence of opsonic antibodies in serum after a primary or secondary corneal infection

Time of serum sample	Mean no. of bacteria present after 90 min (10^6 CFU)[a]	% Killing[b]
Preinfection	2.75 ± 2.06	
1 wk after primary infection	2.75 ± 0.95	0
1 wk after 4-wk secondary infection	2.75 ± 1.71	0
1 wk after 8-wk secondary infection	0.77 ± 0.33	71.8[c]

[a] Data are means of duplicates from two separate assay tubes (total, four samples). Serum samples were collected from five mice 1 week after the indicated primary or secondary infection and were pooled (100 μl of each) for the opsonophagocytic assays.

[b] More than 50% killing by the experimental samples is considered significant compared with preinfection values.

[c] Opsonic antibodies were present.

neas. For determination of viable-bacterium counts in the infected corneas, four mice were sacrificed from each group at the time points indicated in Fig. 5, and the whole eyes were enucleated for evaluation as previously described (12). There were no statistical differences in the mean viable counts between the mice that received the 4- and 8-week secondary corneal infections. Both sets of mice receiving a secondary infection showed few or no viable bacteria by 7 days postinfection, as opposed to 9 to 12 days for primary cornea-infected mice (12), as seen in Fig. 5. These results suggest that there is a correlation between the ability to restore corneal clarity and the production of opsonic antibody, especially since resistant DBA/2J mice also produce opsonins (unpublished observations). However, since there appears to be no direct relationship between these antibodies and the rate of bacterial clearance in these and previous studies (12), their role, if any, is currently unknown.

Although these and other studies demonstrate a correlation between serum and ocular antibody levels and corneal clarity, they also suggest that the ability to restore corneal clarity and bacterial clearance rates are not totally dependent on each other. In addition, recent clinical findings by Irvine et al. are consistent with the present findings, in that *P. aeruginosa* was associated with a poor visual prognosis, since only one of twelve patients recovered 20/400 or better visual acuity despite rapid sterilization of the eye with antibiotic treatments (9). Possibly, the continued opacity observed in treated patients may be due to release of toxic bacterial factors as the result of both infection and antibiotic treatment, as well as to continued production of tissue-damaging inflammatory factors as described by Steuhl et al. (13, 14). On the other hand, both active and passive immunization prior to corneal infection resulted in restoration of corneal clarity and/or reduced corneal opacity (5, 10, 11). Results of these types suggest that protective antibodies must be present as early as possible and in high enough concentrations in ocular tissues in order to protect the eyes from ocular damage. In conclusion, the present studies suggest that the interaction between host and bacteria during ocular infection is indeed complex and that restoration of corneal clarity may be the result of a delicate temporal balance between bacterial numbers, inflammatory and immune responses, angiogenesis, and wound healing of the corneal matrix.

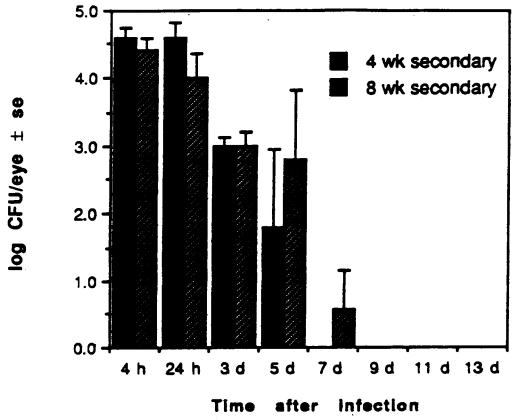

FIG. 5. Quantitative microbiology of corneas of mice receiving a secondary corneal infection 4 or 8 weeks after a primary infection. Data are means ± standard errors of the log of the viable CFU per eye obtained from four eyes at each time point.

These studies were supported in part by grants from the National Eye Institute, the National Institutes of Health (RO1EYO1935 and T32EYO7093).

We thank Julianna M. Berk for her technical assistance and Gerald B. Pier, Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, for his opsonic-antibody studies.

REFERENCES

1. **Allansmith, M. R., C. R. Whitney, B. H. McLellan, and L. P. Newman.** 1973. Immunoglobulins in the human eye. Arch. Ophthalmol. **89**:36–45.
2. **Ames, P., D. DesJardins, and G. B. Pier.** 1985. Opsonophagocytic killing activity of rabbit antibody to *Pseudomonas aeruginosa* mucoid exopolysaccharide. Infect. Immun. **49**:281–285.
3. **Berk, R. S., and L. D. Hazlett.** 1983. Further studies on the genetic control of murine corneal response to *Pseudomonas aeruginosa*. Rev. Infect. Dis. Suppl. **5**:S936–S940.
4. **Berk, R. S., M. A. Leon, and L. D. Hazlett.** 1979. Genetic control of the murine corneal response to *Pseudomonas aeruginosa*. Infect. Immun. **26**:1221–1223.
5. **Berk, R. S., I. N. Montgomery, and L. D. Hazlett.** 1988. Serum antibody and ocular responses to murine corneal infection caused by *Pseudomonas aeruginosa*. Infect. Immun. **56**:3076–3080.
6. **Chusid, M. J., and S. D. Davis.** 1979. Experimental bacterial keratitis in neutropenic guinea pigs: polymorphonuclear leukocytes in corneal host defense. Infect. Immun. **24**:948–952.
7. **Gerke, J. R., and M. V. Magliocco.** 1971. Experimental *Pseudomonas aeruginosa* infection of the mouse cornea. Infect. Immun. **3**:209–216.
8. **Hazlett, L. D., D. D. Rosen, and R. S. Berk.** 1976. Experimental eye infections caused by *Pseudomonas aeruginosa*. Ophthalmic Res. **8**:311–318.
9. **Irvine, W. D., H. W. Flynn, Jr., D. Miller, and S. Pflugfelder.** 1992. Endophthalmitis caused by gram negative organisms. Invest. Ophthalmol. Visual Sci. **33**:745. (Abstract.)
10. **Kreger, A. S., D. M. Lyerly, L. D. Hazlett, and R. S. Berk.** 1986. Immunization against experimental *Pseudomonas aeruginosa* and *Serratia marcescens* keratitis: vaccination with lipopolysaccharide endotoxins and proteases. Invest. Ophthalmol. Visual Sci. **27**:932–939.
11. **Moon, M. M., L. D. Hazlett, R. E. W. Hancock, R. S. Berk, and R. Barrett.** 1988. Monoclonal antibodies provide protection against ocular *Pseudomonas aeruginosa* infection. Invest. Ophthalmol. Visual Sci. **29**:1277–1284.
12. **Preston, M. J., K. A. Kernacki, J. M. Berk, L. D. Hazlett, and R. S. Berk.** 1992. Kinetics of serum, tear, and corneal antibody responses in resistant and susceptible mice intracorneally infected with *Pseudomonas aeruginosa*. Infect. Immun. **60**:885–891.
13. **Steuhl, K.-P., G. Döring, A. Henni, H.-J. Thiel, and K. Botzen-**

hart. 1987. Relevance of host-derived and bacterial factors in *Pseudomonas aeruginosa* corneal infections. Invest. Ophthalmol. Visual Sci. **28:**1559–1568.

14. **Steuhl, K.-P., G. Döring, and H.-J. Thiel.** 1989. The significance of bacterial and host factors in corneal infections caused by *Pseudomonas aeruginosa*. Fortschr. Ophthalmol. **86:**283–286.
15. **Twining, S. S., K. M. Lohr, and J. M. Moulder.** 1986. The immune system in experimental Pseudomonas keratitis: model and early effects. Invest. Ophthalmol. Visual Sci. **27:**507–515.
16. **Van Horn, D. L., S. D. Davis, R. A. Hyndiuk, and H. J. Pedersen.** 1981. Experimental pseudomonas keratitis in the rabbit: bacteriological, clinical and microscopic observation. Invest. Ophthalmol. Visual Sci. **20:**213–221.