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Kinetics of Serum, Tear, and Corneal Antibody Responses in Resistant and Susceptible Mice Intracorneally Infected with *Pseudomonas aeruginosa*

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The studies described here are aimed at determining the kinetics of antibody responses specific to *Pseudomonas aeruginosa* ATCC 19660 in sera, tears, and corneas of naturally resistant DBA/2 mice and susceptible C57BL/6 mice after intracorneal infection. Immunoglobulin (IgG) and IgM responses in sera were significantly greater in DBA/2 mice for the first 2 weeks postinfection. Little or no IgA was detected in the sera of mice from either strain. IgG was the predominant immunoglobulin class present in the corneas of the infected eyes from both mouse strains. However, differences in both the magnitude and the kinetics of the corneal IgG responses were noted between mouse strains. The kinetics of the corneal IgG responses were more similar to those of the serum IgG response than to those of the tear IgG response. Tear antibody responses in DBA/2 mice differed from those of C57BL/6 mice in two ways. First, there was a sharp increase in tear IgG levels 2 weeks after infection in DBA/2 mice that was not present in C57BL/6 mice. Second, IgA levels present in tears from the infected eyes of C57BL/6 mice dropped to nearly preinfection levels after the first week, whereas in DBA/2 mice, IgA levels remained elevated in the infected eyes after the first week. Determination of *P. aeruginosa*-specific antibody responses in the uninfected, contralateral control eyes revealed that IgA was detectable in the tears but not in the corneas of DBA/2 mice. Very little IgA was detected in the tears of the uninfected eyes of C57BL/6 mice. IgG was the only immunoglobulin class present in the uninfected corneas in both mouse strains tested. These results suggest that ocular IgA was made locally, whereas most ocular IgG may have originated from the serum, with some possible local synthesis. These immunological results indicate that DBA/2 and C57BL/6 mice respond differently to corneal challenge with *P. aeruginosa*.

Pseudomonas aeruginosa is an opportunistic pathogen which causes severe corneal infections in humans and experimental animals (3, 6, 11, 32, 33). Previous experimental studies from our laboratory have indicated that DBA/2 mice can spontaneously restore corneal clarity within a few weeks after infection; they are therefore classified as naturally resistant (2, 12). Resistance was dependent, in part, on the presence of polymorphonuclear leukocytes, complement component C3, and the virulence of the infecting organism (7, 13, 16). On the other hand, C57BL/6 mice initially exhibit the same severity of ocular infection at 24 to 72 h postinfection that DBA/2 mice do but are unable to restore corneal clarity within a period of 4 weeks; they are therefore, classified as susceptible (2, 12). Studies of the inflammatory cell response during corneal infection in mice demonstrated that the polymorphonuclear leukocyte response was greater in DBA/2 mice than it was in C57BL/6 mice for the first 24 h of infection but was lower by 3 days postinfection (16a). Corneal infection in C57BL/6 mice usually leads to corneal perforation, phthisis bulbi (shrinkage), or both. We have recently described a correlation between the dissimilar corneal responses of the two mouse strains with the ability to mount a rapid and specific humoral response to *P. aeruginosa* before the development of permanent corneal damage (4). Thus, the susceptible C57BL/6 mice were initially hyporesponsive to infection. Recent substrate specificity studies that examined the serum antibody response to various exoenzymes and surface antigens of *P. aeruginosa* during corneal infection showed that susceptible C57BL/6 mice not only were capable of responding to flagella and the exoenzymes exotoxin A and phospholipase C during both primary and secondary infection but in some cases exceeded the response mounted by DBA/2 mice (29). Neither mouse strain produced antibody to elastase or alkaline protease after either primary or secondary infection (29). These data suggested that the hyporesponsiveness of C57BL/6 mice is restricted to as-yet-unidentified antigens present on the surfaces of *P. aeruginosa* cells. Previous studies using passive transfer of immune sera produced during corneal infection, monoclonal antibodies to outer membrane proteins of *P. aeruginosa*, and active immunization with lipopolysaccharide and elastase have all demonstrated a role for antibodies in corneal protection (4, 20, 22, 26). Since little is known about the nature and kinetics of the local antibody response during corneal infection by *P. aeruginosa*, the present report compares the local and systemic responses in two test strains of mice. In addition, these studies may provide us with further insight into the mechanisms that control the dissimilar corneal response to infection of these two mouse strains.

MATERIALS AND METHODS

Bacterial cell cultures. Stock cultures of *P. aeruginosa* ATCC 19660 were stored at 25°C on tryptose agar slants (Difco Laboratories, Detroit, Mich.) and were used for the inoculation of 50 ml of broth medium containing 5% peptone

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(Difco) and 0.25% Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.). The culture was hemolytic, proteolytic, and lecithinolytic, and it produced exotoxin A. Cultures were grown on a rotary shaker at 37°C for 18 h, centrifuged at 6,000 × *g* for 10 min at 4°C, washed three times with saline, and suspended in 0.9% sterile nonpyrogenic saline (Travenol Laboratories, Deerfield, Ill.) to a concentration of 2×10^{10} CFU/ml by using a standard curve relating viable counts to the optical density at 660 nm.

Infection of animals. Mice from two inbred strains, DBA/2J and C57BL/6 (Jackson Laboratory, Bar Harbor, Maine) (weight, 18 to 22 g), were infected at 6 weeks of age. Before infection, they were lightly anesthetized with ether and placed beneath a stereoscopic microscope. The corneal surface of the left eye of each mouse was incised with three 1-mm-long incisions by using a sterile 26-gauge needle, taking care not to penetrate the anterior chamber or damage the sclera. A bacterial suspension (5 μl) containing 10^8 CFU was topically delivered onto the surfaces of the incised corneas by using a micropipette (Oxford Labs) with a sterile, disposable tip. The mice were examined 24 h later to determine that they were all infected.

Serum, tear, and cornea sample collection. A total of 75 mice of each strain were divided into 15 groups of five mice each. Three groups of five mice of each mouse strain were sampled prior to infection and each week for 4 weeks following infection. Each serum, tear, or cornea sample represents a pool of the five animals within each group. Tear and cornea samples were obtained from both the infected eye and uninfected, contralateral control eye of each mouse. Serum samples were obtained from blood collected via the tail vein. The animals were then lightly anesthetized with ether, and tear samples were carefully collected to minimize transudation of serum proteins from the corneas by gently touching the tear meniscus at the eyelid with a sterile wedge from a 5.5-cm Whatman no. 1 filter paper disk cut into 16 equal wedges. The wedge was then inserted into the conjunctival sac to collect the remaining tears. The collection procedure took between 5 and 10 s to complete, thus avoiding the possibility of artificially stimulating tear production. The volume of tears collected in this manner was approximately 0.5 to 1 μl per eye, determined by comparing the migration of the liquid front with that of known volumes of saline pipetted onto the filter paper wedges. The tips of the filter paper wedges containing the samples were then cut off and stored in Microfuge tubes at −70°C. Corneas were dissected away from the eyes and rinsed extensively in saline to remove contaminating blood and tears. The corneas were put into Microfuge tubes containing 1.1 ml of Tris-buffered saline–0.05% Tween 20, pH 7.4 (TBS-Tween). Samples from each group were kept frozen at −70°C until the end of the experiment, when antibody titers were determined. Prior to antibody and total protein measurement, 1.1 ml of TBS-Tween was added to the tear samples, and total protein was eluted from the tear and corneal samples by incubating them overnight at 4°C and then centrifuging them to remove filter paper and corneal tissue, respectively. Total protein levels of corneal and tear eluates were determined by using a commercial protein reagent (Bio-Rad Laboratories, Richmond, Calif.), with bovine serum albumin as the standard, to ensure that differences in sampling technique would not account for differences in antibody titers between the two mouse strains.

Enzyme-linked immunosorbent assay. Nunc immunoplates (Nunc, Thousand Oaks, Calif.) were coated with 100 μl of heat-killed *P. aeruginosa* per well as previously described (4). The pooled mouse serum was diluted for testing in TBS-Tween. The following dilutions were used: IgG, 1:200; IgM, 1:200; and IgA, 1:25. Tear and cornea samples were tested undiluted. The plates were washed three times with TBS-Tween before the addition of serum. The samples (100 μl) were then added in triplicate to the inner wells and incubated at 22°C for 90 min. After incubation, the plates were washed three times with TBS-Tween, and a 1:1,000 dilution of alkaline phosphatase-conjugated goat anti-mouse IgG (γ-chain specific), IgM (μ-chain specific), or IgA (α-chain specific) (Sigma Chemical Co., St. Louis, Mo.) conjugate was added. The plates were incubated again for 90 min at 22°C and washed, and 100 μl of the enzyme substrate was added. The enzyme substrate consisted of 10% diethanolamine solution, pH 9.7, with 0.01% $MgCl_2$ and 0.02% sodium azide and with 1 mg of *p*-nitrophenolphosphate per ml. The enzyme substrate reaction was allowed to proceed at room temperature until a 1:1,600 dilution of a positive control reference serum pool from C.D2.Idh/Pep-3 mice with high titers of IgG and IgM specific to whole *P. aeruginosa* cells reached an A_{405} of 0.55 to ensure reproducibility between assays. Enzyme activity was inhibited by the addition of 50 μl of 3 N NaOH to each well. Substrate conversion was measured spectrophotometrically at 405 nm with a plate reader (model EL307; Biotek, Burlington, Vt.). Serum enzyme-linked immunosorbent assay units were determined by multiplying the A_{405} by the dilution of the test serum and subtracting the background values for preinfection mouse serum. Values for tear and cornea antibody levels are reported as the A_{405} obtained by the experimental samples when the positive control serum reached 0.55.

Quantitation of viable *P. aeruginosa* in infected and uninfected eyes. At various times after infection, the mice were sacrificed and both eyes were enucleated. The eyes were ground in a sterile tissue grinder containing 1.0 ml of sterile 0.15 M sodium chloride–0.25% bovine serum albumin. The samples were diluted appropriately in the same solution and plated in triplicate on Pseudomonas Isolation Agar (Difco). The uninfected control eyes were always sterile.

Statistical analysis. Analysis of variance was used to determine the statistical differences between continuous sets of data, such as the tear or corneal protein levels over 4 weeks. The statistical differences at individual time points were determined by using an unpaired, two-tailed, Student's *t* test. Statistical analysis was done by using the Statview statistical package (Abacus Concepts, Berkeley, Calif.) on a Macintosh SE/30 computer.

RESULTS

These experiments were designed to examine the kinetics of specific immunoglobulin appearance in both serum and ocular tissues during a primary corneal infection with *P. aeruginosa*. Serum, tear, and corneal samples were pooled from five mice of each strain. IgG, IgA, and IgM antibodies specific to whole heat-killed *P. aeruginosa* were measured by enzyme-linked immunosorbent assay for both resistant DBA/2 and susceptible C57BL/6 mice prior to infection and weekly for 4 weeks following infection. The data represent the means and standard deviations from three groups of five animals each at each time point. Antibody levels were measured in tears and corneas of both the infected eyes and the uninfected control eyes.

Serum antibody levels. The serum antibody response in both mouse strains consisted of IgG and IgM. There was little or no detectable serum IgA response. Serum IgG first

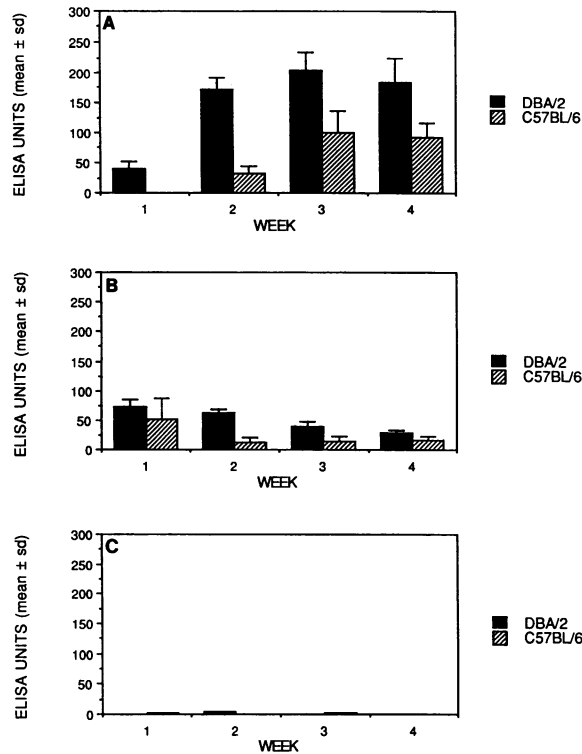

FIG. 1. Serum IgG (A), IgM (B), and IgA (C) responses to heat-killed *P. aeruginosa* after corneal infection. Data were determined from sera obtained from three groups of five animals each at each time point. The sera from the five animals within each group were pooled. ELISA, enzyme-linked immunosorbent assay.

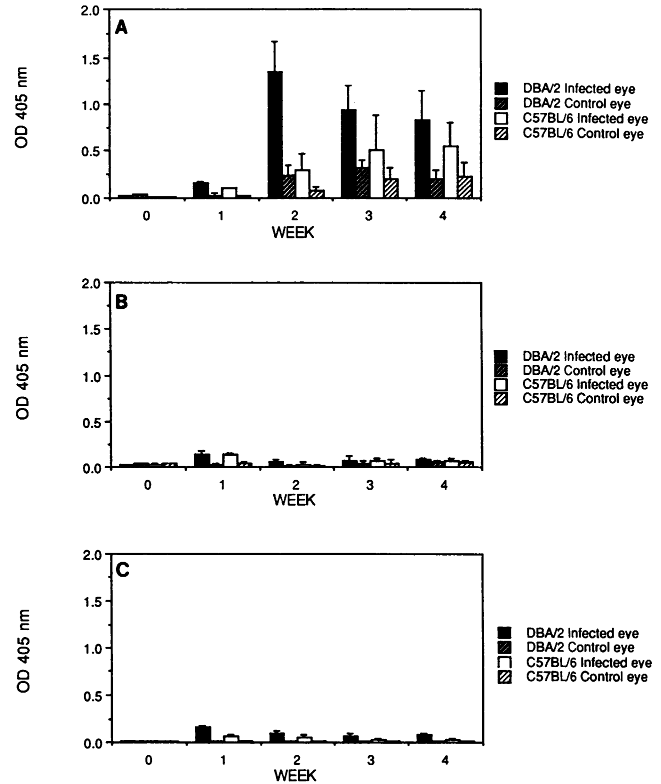

FIG. 2. Cornea IgG (A), IgM (B), and IgA (C) responses to heat-killed *P. aeruginosa* in both infected eyes and uninfected control eyes. Data were determined as described in the legend to Fig. 1, except that the infected and uninfected corneas were pooled separately. OD, optical density.

appeared in DBA/2 mice 1 week after infection, while detectable serum IgG did not appear in C57BL/6 mice until 2 weeks after infection at the dilution tested (1:200) (Fig. 1). Specific IgG has been previously detected in C57BL/6 mice at a 1:25 dilution (4). However, for the purpose of these experiments, the dilution tested allowed comparison between the two mouse strains at all time points at the same dilution. Beyond the first week of infection, there were statistical differences in IgG and IgM levels at two weeks after infection ($P = 0.0091$ and 0.0093, respectively), but not thereafter. Serum IgG levels appeared to peak at 3 weeks after infection in both mouse strains, with no significant increases after this time. IgM was the predominant immunoglobulin class present in the sera of both DBA/2 and C57BL/6 mice 1 week after infection. There was no significant difference between the two mouse strains in the serum IgM levels at 1 week after infection. However, there was a much more rapid decline in IgM levels in C57BL/6 mice between weeks 1 and 2 than there was in DBA/2 mice.

Cornea antibody levels. At 1 week postinfection, IgG, IgM, and IgA were found at approximately the same levels in the corneas of the infected eyes of DBA/2 mice (Fig. 2). In C57BL/6 mice, IgG and IgM levels were similar, while IgA levels were lower. However, DBA/2 mice had significantly higher IgG levels for the first 2 weeks after infection ($P =$ 0.0158 and 0.0076, respectively). IgG was the predominant immunoglobulin class present in the infected corneas of both mouse strains during the remainder of the 4 weeks tested. IgM and IgA levels did not increase after the first week after infection. Specific IgG was also detected in the uninfected contralateral eyes of both mouse strains by 2 weeks after infection. No increases in specific IgM or IgA were detected in the uninfected control corneas. The kinetics of IgG appearance in the infected eyes of the two mouse strains were also different. In C57BL/6 mice, there was a slow, steady increase over the 4 weeks tested. In DBA/2 mice, there was a very rapid increase between weeks 1 and 2, with a slight decline at weeks 3 and 4.

Tear antibody levels. As was observed with cornea antibody levels, IgG, IgM, and IgA levels in tears from infected eyes were approximately the same 1 week after infection, with no significant differences between the mouse strains in IgG and IgM levels (Fig. 3). Beyond the first week of infection, tear IgG levels in C57BL/6 mice declined and remained low during the 4 weeks tested. However, IgG levels in DBA/2 mice peaked sharply 2 weeks after infection and declined at weeks 3 and 4. Although the standard deviation at week 2 is somewhat large and the difference in IgG levels was not significant between the mouse strains, this same pattern of a sharp IgG peak at 2 weeks postinfection in DBA/2 mice has been observed in two other separate experiments, with the decline between weeks 2 and 3 being even greater. IgA levels in tears from the infected eyes of DBA/2 mice were significantly higher at all time points than they were in C57BL/6 mice. In contrast to the uninfected corneas, little or no specific IgG was detected in tears from the uninfected eyes of either mouse strain. However, IgA levels in tears from the uninfected control eyes of DBA/2 mice increased to equal those in tears from the infected eyes

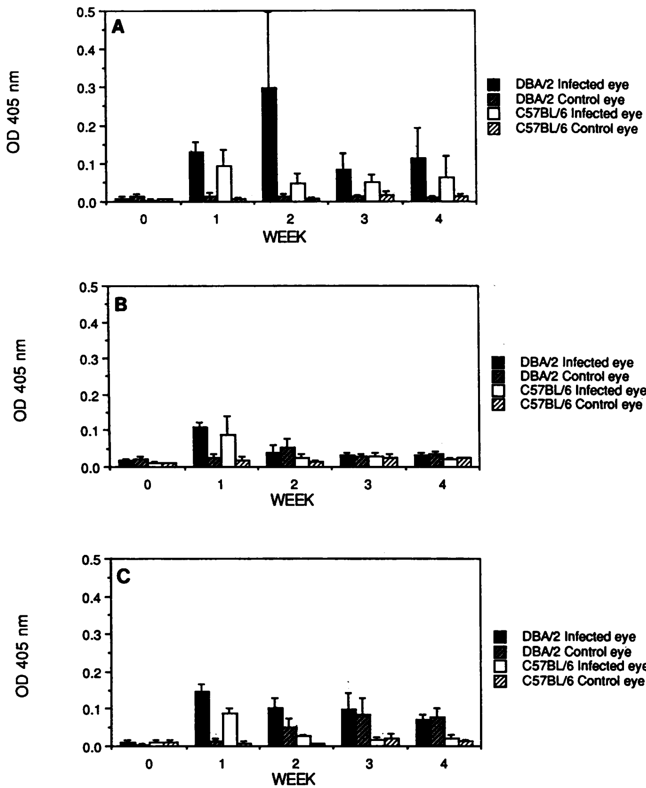

FIG. 3. Tear IgG (A), IgM (B), and IgA (C) responses to heat-killed *P. aeruginosa* in both infected eyes and uninfected, contralateral control eyes. Data were determined as described in the legend to Fig. 1, except that tears from infected and uninfected eyes were pooled separately. OD, optical density.

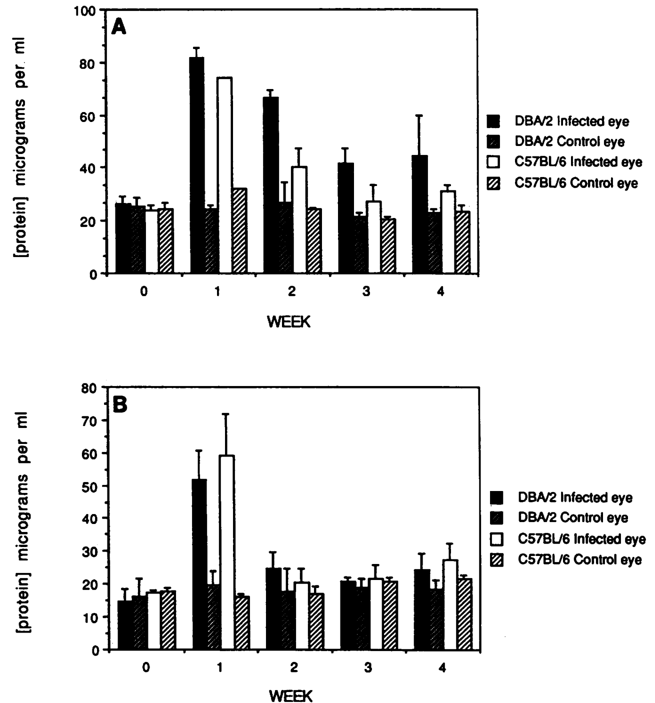

FIG. 4. Protein levels of the corneal (A) and tear (B) eluates from infected and uninfected, contralateral control eyes. Data were determined as described in the legends to the other figures.

by weeks 3 and 4. The only significant increase in IgA observed in the tears from the uninfected control eyes of C57BL/6 mice was at 3 weeks after infection ($P = 0.0311$).

Cornea and tear protein levels. To determine whether sampling errors contributed to differences in the antibody levels between the two mouse strains, total protein was measured in the cornea and tear eluates. Corneal and tear eluate protein levels in the uninfected control eyes of the two mouse strains were not significantly different and showed no increase with time (Fig. 4). However, protein levels in the corneas of DBA/2 mice were consistently higher after infection than they were in corneas of C57BL/6 mice ($P \leq 0.0001$) when all time points were analyzed together by analysis of variance, indicating that there were physiological differences between the mouse strains as a result of infection. Peak protein levels were found in infected corneas by 1 week after infection, when the inflammatory response is greatest when measured weekly (4, 16a). Protein levels in tears also peaked 1 week after infection but returned to nearly preinfection levels thereafter (Fig. 4). There was no significant difference between the two mouse strains in tear protein levels.

Quantitation of viable *P. aeruginosa* in the infected eyes. Three mice of each strain were sacrificed at 3, 6, 9, and 12 days after infection, and both the infected and uninfected control eyes were enucleated for viable bacterial counts. A representative experiment is shown in Table 1. The uninfected, contralateral control eyes were routinely free of viable *P. aeruginosa* (data not shown). DBA/2 mice consistently yielded higher viable counts in the infected eyes than did C57BL/6 mice. However, no difference was noted in the rate of clearance of the organisms from the eyes of the two mouse strains. Viable bacteria were present in the infected corneas of both mouse strains for up to 12 days after infection, but not after 12 days (data not shown). These results have been reproduced in the control mice of two other experiments that examined different treatment effects on clearance of bacteria from the eye.

TABLE 1. Determination of viable *P. aeruginosa* in infected eyes[a]

Mouse strain and animal no.	CFU in infected eye at postinfection day:			
	3	6	9	12
DBA/2				
1	1.2×10^6	1.2×10^7	4.5×10^5	8.0×10^2
2	1.7×10^6	1.0×10^7	6.1×10^5	1.1×10^3
3	8.0×10^5	3.6×10^6	2.1×10^5	8.0×10^2
C57BL/6				
1	2.0×10^5	6.2×10^5	1.8×10^3	0
2	7.0×10^5	4.5×10^6	4.1×10^3	0
3	1.5×10^5	9.5×10^5	1.2×10^3	1.0×10^2
P value	0.0482	0.0823	0.0236	0.0012

[a] Both the infected and uninfected eyes of three individual mice of each mouse strain were enucleated, and total viable *P. aeruginosa* was determined for the individual eyes as described in Materials and Methods. The uninfected eyes did not yield viable *P. aeruginosa*. An unpaired, two-tailed, Student's *t* test was used to evaluate the differences observed between the two mouse strains.

DISCUSSION

In these studies, we have observed that the *Pseudomonas*-specific serum antibody response consisted of IgG and IgM, while little or no IgA was measured during primary infection of the cornea. The inability to detect specific IgA in the serum of either mouse strain is consistent with data previously reported by our laboratory (4) and by others utilizing ocular immunization regimens (24, 25). Clearly, C57BL/6 mice are hyporesponsive compared with DBA/2 mice with respect to serum and corneal antibody response against *P. aeruginosa*. However, it is not known whether this is because there are fewer viable organisms in the eye, thus resulting in a dosage effect, or whether there is a unique regulatory environment in the ocular tissues of C57BL/6 mice. The latter possibility is suggested by the studies of Kielty et al., in which DBA/2 and C57BL/6 mice differed in their ability to develop a vigorous delayed-hypersensitivity (DH) response when herpes simplex virus type 1 was inoculated into the anterior chamber of the eye (18). This phenomenon is known as anterior chamber-associated immune deviation (ACAID) (31). In those studies, C57BL/6 mice were resistant to the development of ACAID and developed a DH response similar to that of animals primed subcutaneously, while DBA/2 mice developed ACAID and the DH response was suppressed. At present, the role of DH in the murine *P. aeruginosa* corneal infection model is not known. Current, unpublished observations in our laboratory suggested that DBA/2 and C57BL/6 mice produce similar levels of *P. aeruginosa*-specific IgG when immunized intraperitoneally with equal numbers of heat-killed *P. aeruginosa*. These data suggest that the hyporesponsiveness of C57BL/6 mice may be explained, at least in part, by the observation that there are fewer organisms present in the infected eyes of these mice.

Besides measuring serum antibodies directed toward *P. aeruginosa*, we also measured antibodies eluted from whole corneas dissected away from both the infected and uninfected eyes. Allansmith et al. determined that 40, 45, and 48% of rabbit IgG, IgA, and IgM, respectively, may be recovered in this manner when they are injected into human corneas, which makes it difficult to compare directly serum and corneal antibody levels (1). In both mouse strains tested, IgG was the predominant immunoglobulin present in the corneas of both the infected and uninfected control eyes. In support of these observations, IgG is also the predominant immunoglobulin class found in normal human corneas, although IgA and IgM have been detected in small amounts (1). The relative amounts of IgG detected in the corneas of DBA/2 and C57BL/6 mice seemed to mirror the relative amounts found in serum, except at 1 week after infection. This is consistent with the concept that corneal antibodies were derived by transudation from the limbal blood vessels (1, 34). However, IgG was present in the corneas of C57BL/6 mice 1 week after infection at levels which indicated that there may have been some local synthesis soon after infection. Interestingly, specific IgG, but not IgA or IgM, started to appear in the uninfected corneas by 2 weeks after infection. Diffusion of IgG from the blood to the cornea has been demonstrated to occur naturally in the normal, uninflamed cornea (1, 34). Since IgA was detected in the corneas of infected eyes, one of two possible explanations is suggested. Either some local synthesis of IgA occurred within the corneal stroma, since IgA is not found in the serum, or the cornea was contaminated by IgA synthesized in the lacrimal gland, which is intimately associated with the mucous glycoproteins at the corneal surface (15).

The pattern of specific antibody responses in the tears appeared to differ from those of the serum and corneas and also differed between mouse strains. IgA was present in significant amounts in the tears, while it was not found in the serum and was found only minimally in the corneas after the first week. It was interesting that specific IgA was measured in the tears of both the infected and the uninfected contralateral control eyes of DBA/2 mice. These data are supported by evidence for a common mucosal immune system, in which IgA-committed B cells are preferentially retained in mucosal tissues (5, 21, 23). IgA-committed B cells stimulated by antigen in the infected eye may migrate to other mucosal sites, including the lacrimal gland of the uninfected contralateral eye, and differentiate into IgA-secreting plasma cells without further antigenic stimulation. Alternatively, bacteria in the eye may drain from the nasolacrimal duct to the gut, where they may stimulate IgA-committed B cells in the Peyer's patches that would then migrate to the lacrimal gland of the uninfected eye. Transfer of bacteria from the infected eye to the uninfected eye as the stimulus for the appearance of antibody in the uninfected eye is unlikely, since viable bacteria were not detected in the uninfected control eyes. Peppard et al. have demonstrated that gastrointestinal administration of antigen results in the appearance of antigen-specific IgA but not IgG in the tears of these animals (27). This possibility is further supported by the fact that we did not detect IgG in the tears of the uninfected eyes.

When total protein levels were measured, there was no difference between the two mouse strains in the total protein measured in the tears from either eye or in the corneas of the uninfected eyes. However, C57BL/6 mice had significantly less protein present in the infected corneas than did DBA/2 mice. This was especially notable at weeks 2 and 3 after infection. These data are consistent with those of Epstein and Stulting, who demonstrated that there are genetic differences between mouse strains in their abilities to vascularize the corneas, a phenomenon associated with activated T cells and their products (8, 9). The kinetics of protein levels in the corneas appeared to follow those of corneal inflammation, which is most severe during the first 10 days after infection and declines thereafter (4). However, the kinetics of protein levels in tears appeared to follow those of viable bacteria in the eye, which were present at 1 week but absent by 2 weeks after infection.

At 1 week postinfection, DBA/2 mice had antibody levels in ocular tissue that were similar to or higher than those of C57BL/6 mice. However, DBA/2 mice had consistently higher levels of viable *P. aeruginosa* in the infected eyes. This phenomenon has been observed for *Mycobacterium avium* infections of susceptible mice, where increased synthesis of antibacterial antibodies correlated with increased numbers of bacterial CFU in the liver and spleen (10). Pier and Ames have suggested that nonfunctional, blocking antibodies may inhibit complement-mediated killing of *P. aeruginosa* (28). The ability of the antibodies synthesized by the two mouse strains in the present study to initiate complement-mediated killing of *P. aeruginosa* needs to be determined in light of the present results. However, the difference in the observed corneal responses may not be accounted for by excessive bacterium-related damage in C57BL/6 mice, but rather may be the result of immune response-related damage. A number of studies have implicated host factors in causing ocular damage during infection (14, 17, 19, 30, 32). In particular, Steuhl et al. suggested that at 48 h postinfec-

tion of rabbits, lysosomal enzymes and oxidative substances produced by polymorphonuclear leukocytes, keratocytes, and damaged corneal epithelial cells may be more important than damage caused by *P. aeruginosa* (30). The exact mechanism(s) that each mouse strain utilizes to clear the organism from the eye are unknown. The two mouse strains used in our study may use different combinations of both humoral (antibody, complement, lactoferrin, etc.) and cellular factors to eliminate the bacteria from the eye. In addition, it remains to be determined whether there are differences in the ability of the organism to adhere to and colonize the eyes of these two mouse strains.

In summary, DBA/2 and C57BL/6 mice respond differently to ocular challenge with *P. aeruginosa*, both immunologically and ocularly. We found that resistant DBA/2 mice demonstrated generally higher antibody levels in serum and ocular tissues than did susceptible C57BL/6 mice. However, C57BL/6 mice had fewer viable organisms in their infected eyes throughout the course of infection. These data indicate that the host-parasite relationship resulting in perforation of the cornea and/or phthisis of the infected eye in susceptible mice is a complex interaction between bacterial and host factors. In addition, the data suggest that local synthesis of antibodies may occur prior to systemic synthesis in the infected corneas, although antibodies synthesized systemically seem to account for the major proportion of the antibodies produced during infection. To our knowledge, this is the only report to simultaneously examine the kinetics of antibody responses in both infected and uninfected eyes during a corneal infection.

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