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Genetic Control of the Murine Corneal Response to *Pseudomonas aeruginosa*

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Inbred mouse strains differ in susceptibility to intracorneal challenge with *Pseudomonas aeruginosa*. Genetic studies indicate that resistance to corneal infection is dominant over susceptibility and is controlled by autosomal genes, at least one of which is located outside of the *H-2* locus. On the basis of genetic complementation studies, the susceptible strains BALB/c and C57BL/6 each bear one resistance gene, since the F_1 hybrid (BALB/c × C57BL/6) was uniformly resistant to infection.

Recent studies in our laboratories have shown that the corneal response of an outbred mouse strain (Swiss Webster) was strikingly different from that of an inbred strain (BALB/c) (7). The infection in Swiss Webster mice remained localized and spontaneously healed, whereas the infected eyes of BALB/c mice underwent necrosis and became shrunken (phthisical) within 12 to 15 days after infection. These results suggested a relationship between the natural immune status of the eye and genetic background. Although extensive immunological studies on the genetic control of mice to chemically defined antigens have been carried out (1), the literature on genetic control of natural immunity to bacterial infections is sparse (2, 4, 5, 6, 8-10), whereas virtually nothing is known with regard to genetic control of infections of the eye. The present study describes the dissimilar response of inbred strains of mice to corneal infection by *Pseudomonas aeruginosa*, with results suggesting that resistance is under multigenic control.

Stock cultures of *P. aeruginosa*, ATCC strain 19660, stored at 25°C on tryptose agar slants (Difco Laboratories, Detroit, Mich.) were used for inoculation of 50 ml of broth medium containing 5% peptone (Difco) and 0.25% Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.). Cultures were grown on a rotary shaker at 37°C for 18 h, centrifuged at 27,000 × *g* for 20 min (4°C), and suspended in 0.9% sterile nonpyrogenic saline (Travenol Laboratories, Inc., Deerfield, Ill.) to a concentration of 5×10^{10} colony-forming units (CFU)/ml, using a standard curve relating viable counts to optical density at 440 nm.

Inbred strains of female mice weighing 15 to 20 g were obtained from Cumberland View Farms, Clinton, Tenn., from Jackson Laboratories, Bar Harbor, Maine, and from Flow Research Laboratories, Inc., Dublin, Va. The following inbred strains were employed in this study: BALB/c, C57BL/6, C3H/HeJ, DBA/1, and DBA/2. CD_2F_1 mice (BALB/c × DBA/2) were raised in our animal facility. In all experimental studies involving either F_1 or backcross animals, both male and female progeny were used in approximately equal numbers. Mice were infected at 4 to 6 weeks of age. Before infection, they were anesthetized with ether, and under a stereoscopic microscope, the surface of the left eye (upper right quadrant) was incised with a sterile, 26-gauge needle, taking care not to penetrate the anterior chamber. A bacterial cell suspension, (10 μl) containing 5×10^8 CFU, was delivered onto the incised corneas, using a micropipette (Oxford Laboratories, Foster City, Calif.) with a sterile disposable tip. Control animals received a similar wounding and 10 μl of sterile saline. All experimental data represent the results of two or more independently performed experiments.

Mice were examined macroscopically daily for 3 to 6 weeks by two investigators independently and were also intermittently monitored for the presence of bacteria by gently swabbing the cornea with a sterile cotton swab and subsequently inoculating tryptose agar plates. The plates were incubated at 37°C for 24 to 48 h. All plates showing growth contained pure cultures of *P. aeruginosa*.

The inbred mouse strains, BALB/c, C3H/HeJ, C57BL/6, DBA/1, and DBA/2, were challenged intracorneally with *P. aeruginosa* (Table 1). Corneal opacity developed in all strains within 18 to 24 h and remained localized to the experimentally infected eye. The infected eyes were "shrunken" or phthisical. No spontaneous

recovery occurred in the susceptible strains during the 4- to 6-week holding period. In contrast to the behavior of these susceptible strains, DBA/1 and DBA/2 showed spontaneous recovery with clearing of corneal opacity within 3 to 4 weeks postinfection and near-normal-appearing ocular histology. F_1 animals from the cross between the susceptible BALB/c and either of the resistant strains, DBA/1 (33 females and 40 males) or DBA/2 (25 females and 29 males) were uniformly resistant.

The backcross between resistant CD_2F_1 males to susceptible BALB/c females and resistant CD_2F_1 females to resistant DBA/2 males was examined. The results (Table 2) are essentially identical with the expected theoretical frequencies for models of either one or two autosomal genes determining resistance. However, the latter model is supported by the observation that when the two susceptible strains, BALB/c and C57BL/6, were crossed the F_1 animals (32 females and 28 males) were uniformly resistant. In preliminary experiments, the F_1 progeny from a mating of BALB/c × C3H/HeJ were uniformly susceptible, indicating that C3H/HeJ mice either bear the same resistance gene as BALB/c or no resistance gene.

The data presented permit four conclusions concerning the genetic control of the murine response to *P. aeruginosa* corneal infections. First, resistance must be controlled, at least in part, by a gene or genes outside of the *H-2* locus. This conclusion is established by the different responses of two strains with the same H/2 haplotype, $H\text{-}2^d$, namely, BALB/c (susceptible) and DBA/2 (resistant). Earlier studies of resistance to systemic infection of mice by *Salmonella typhimurium* (9, 10), *Leishmania donovani* (2, 3), and *Rickettsia tsutsugamushi* (5) showed no relationship between resistance and *H-2* haplotype. Second, resistance to *P. aeruginosa* infection is dominant over susceptibility. This is based on the fact that F_1 generation obtained from crossing DBA/1 or DBA/2 (both resistant) with BALB/c (susceptible) were uniformly resistant. The dominance of resistance over susceptibility has been the general pattern in reports of genetic control of nonophthalmic bacterial or rickettsial infections (5, 10). Third, the response to infection of the eye by *P. aeruginosa* appears to be under multigenic control. The F_1 hybrids obtained by crossing the two susceptible strains, BALB/c and C57BL/6, were uniformly resistant. This genetic complementation fits a model where each susceptible parent strain contributes at least one gene responsible for resistance. At least one of these genes must be outside of the *H-2* locus as noted above. This model differs from those reported for systemic infection by *S. typhimurium*, where resistance is controlled by a single gene or a closely linked cluster of genes (9, 10), and from those reported for infection with *R. tsutsugamushi* (5), where progeny of crosses between susceptible strains were uniformly susceptible. Finally, the uniformity of responses of both males and females in the backcrosses (Table 2) as well as the F_1 hybrids (see above) demonstrates that resistance is governed by autosomal genes.

TABLE 1. *Response of inbred mice to intracorneal challenge with P. aeruginosa*[a]

Mouse strain	Response[b]
BALB/c ($H\text{-}2^d$)	Susceptible
C3H/HeJ ($H\text{-}2^k$)	Susceptible
C57BL/6 ($H\text{-}2^b$)	Susceptible
DBA/1 ($H\text{-}2^q$)	Resistant
DBA/2 ($H\text{-}2^d$)	Resistant

[a] All mice received a topical application of 5×10^8 CFU of *P. aeruginosa*.

[b] Susceptibility or resistance in each case was 100%.

TABLE 2. *Responses of backcrosses to intracorneal challenge with P. aeruginosa*

Backcross[a]	Resistant (%)		Susceptible (%)	
	Experimental	Theoretical	Experimental	Theoretical
CD_2F_1 × DBA/2	97.2 (68/70)	100	2.8 (2/70)	0
BALB/c × CD_2F_1	52.3 (34/65)	50	47.7 (31/65)	50

[a] Female × male, respectively.

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