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Dominant Susceptibility Effect on the Murine Corneal Response to *Pseudomonas aeruginosa* (41592)

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Abstract. Natural host resistance to *Pseudomonas aeruginosa* corneal infection is regulated by two complementing dominant genes *PsCR1* and *PsCR2* (RS Berk, MA Leon, LD Hazlett. Infect Immun 26:1221–1223, 1979). In this study we have demonstrated a third dominant gene, which determines susceptibility to *P. aeruginosa*-induced eye damage. This gene was designated as *PsCS*. The F_1 progeny from matings between the resistant DBA/2J strain and the susceptible strain C57BL/6J and C3H/HeJ displayed the susceptibility phenotype. Backcross and F_2 studies using the C3H/HeJ and DBA/2J strains suggested the presence of two linked *PsCS* loci.

A number of studies recently have been performed in our laboratory to examine the response of the murine cornea to challenge with *Pseudomonas aeruginosa* (1–6). We have established that the Swiss–Webster, DBA/1J, and DBA/2J strains are naturally resistant to intracorneal infection and that the infection remains localized and spontaneously heals within 3 to 4 weeks (3, 4). On the other hand, the C57BL/6J, BALB/cJ, NZB, and C3H/HeJ strains are classed as susceptible, since the infection results in blindness 21 to 30 days after infection (2–4). An intermediate ocular response also has been observed in the A mouse congenic lines. Further studies suggested that at least two autosomal dominant nonlinked genes control natural resistance to intracorneal infection by *P. aeruginosa* and that they both reside outside the *H-2* complex (2, 3). We have provisionally designated the C57BL/6J resistance gene as *PsCR1* and the BALB/cJ resistance gene as *PsCR2* (3). However, current studies with C3H/HeJ mice suggest that the present view of the multigenic control of natural resistance to pseudomonas eye injury is more complex than expected. The purpose of this study is to establish the presence of two linked genes which are expressed as dominant susceptibility genes (*PsCS*).

Materials and Methods. *Bacterial cell cultures.* Stock cultures of *P. aeruginosa,* strain 19660 ATCC, stored at 25°C on tryptose agar slants (Difco, Detroit, Mich.) were used for inoculation of 50 ml of broth medium containing 5% peptone (Difco) and 0.25% trypticase soy broth (Baltimore Biological Laboratories, Cockeysville, Md.). The culture was hemolytic, proteolytic, and produced lecithinase and exotoxin A. Cultures were grown on a rotary shaker at 37°C for 18 hr, centrifuged at 27,000*g* for 20 min (4°C), washed with saline, and resuspended in 0.9% sterile nonpyrogenic saline (Travenol Laboratories, Inc., Deerfield, Ill.) to a concentration of 2.5×10^{10} colony forming units (CFU)/ml using a standard curve relating viable counts to optical density of 440 nm.

Infection of animals. Inbred lines of all F_1, F_2, and backcross matings were bred in our own animal facilities or purchased from Jackson Laboratories (Bar Harbor, Maine). In all experimental studies involving either F_1 or F_2 animals, both male and female progeny were used in approximately equal numbers. Mice were infected at 5–6 weeks of age (18–22 g). Prior to infection, they were lightly anesthetized with ether and placed beneath a stereoscopic microscope. The corneal surface of the left eye was incised (three 1-mm-long incisions) with a sterile 26-gauge needle, taking care not to penetrate the anterior chamber or damage the sclera. A bacterial cell suspension (5 μl) containing a final concentration of 1.25×10^8 CFU, was topically delivered onto the surface of incised corneas using a micropipete (Oxford Laboratories, Foster City, Calif.) with a sterile disposable tip. Control animals received a similar wounding and 5 μl of sterile saline. All experimental data represent the re-

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sults of two or more independently performed experiments. In order to ensure objectivity, each animal evaluation was performed by one of the investigators without knowing the genetic background of the control and infected animals.

The ocular response was grossly examined at 24 hr after bacterial challenge and then at weekly intervals over a 3 to 6-week time period and compared with the contralateral eye as well as the saline control eyes. In addition, the eyes were monitored intermittently for the presence of bacteria by gently swabbing the cornea with a sterile cotton swab and inoculating tryptose agar plates. The plates were incubated at 37°C for 24–48 hr. All plates showing growth contained pure cultures of *P. aeruginosa.*

Results. Previous investigations have suggested that at least two dominant autosomal genes control natural resistance to intracorneal infection by *P. aeruginosa* (1–3). In order to determine the exact number of genes which confer resistance, F_1 studies were initiated to screen for an inbred mouse strain which lacked $PsCR1^R$ and $PsCR2^R$ genes. Once this $PsCR1^{r/r}$ $PsCR^{r/r}$ strain was found, genetic analysis could be done using F_1, F_2, and backcross matings with the resistant DBA/1 or DBA/2 strains. These studies would determine the number of genes conferring resistance in DBA/1 and DBA/2. In our initial screening we found that progeny of C3H/HeJ mated with either BALB/c or C57BL/6J animals were all susceptible to eye infection (Table I). From these data, we reasoned that the genotype of C3H/HeJ was $PsCR1^{r/r}$ $PsCR2^{r/r}$. When (C3H/HeJ × DBA/2)F_1 were infected, an unexpected result was obtained. All these F_1 animals were susceptible to corneal infection and exhibited permanent eye damage. A dominant susceptibility effect, apparently contributed by the C3H parent, was the most likely explanation for this result. Furthermore, since C3H/HeJ appears to carry a dominant susceptibility effect, there is no longer sufficient data to support the proposed $PsCR1^{r/r}$ $PsCR2^{r/r}$ genotype of C3H/HeJ.

TABLE I. RESPONSE OF F_1 HYBRIDS TO INTRACORNEAL CHALLENGE WITH *P. aeruginosa*[a]

Strains	Response	Resistant/ total
Parental strains:		
DBA/2	Resistant	75/75
C57BL/6	Susceptible	0/24
BALB/c	Susceptible	0/115
C3H/HeJ	Susceptible	0/71
F_1 hybrids:[b]		
(C57BL/6 × BALB/c)F_1	Resistant	60/60
(C3H × C57BL/6)F_1	Susceptible	0/40
(C3H × BALB/c)F_1	Susceptible	0/23
(C3H × DBA/2)F_1	Susceptible	0/46

[a] All mice received a topical application of 1.25×10^8 CFU *P. aeruginosa.*
[b] ♀ × ♂.

In order to demonstrate that the C3H susceptibility effect was under genetic control, (C3H × DBA/2)F_2 animals and progeny of (C3H × DBA/2)F_1 ♀ backcrossed with DBA/2 ♂ were examined for their susceptibility to *P. aeruginosa* infection. In the backcross progeny 33/94 (35.1%) recovered from the corneal infection, whereas 61/94 (64.9%) were permanently blinded (Table II). Since two nonlinked resistant genes, *PsCR1* and *PsCR2,* have previously been established (5), we proposed that a dominant susceptibility gene(s), provisionally designated as *PsCS* is involved. These backcross data were then statistically analyzed to determine if one or more dominant susceptibility genes were present. Both one gene ($X^2 = 8.32$, $P < 0.01$) and two nonlinked genes ($X^2 = 4.53$, $P < 0.05$) were rejected. The expected percentages of resistant backcross offspring are 50% and 25% for the one gene and the two genes hypotheses, respectively. Since the observed frequency was 35.1% resistant offspring, alternatives to simple Mendelian genetics were considered. One possibility is that two linked dominant susceptibility genes of C3H origin existed. Progeny carrying the recombinant genotypes would be phenotypically susceptible. Furthermore, all the resistant offspring would represent animals which lacked the C3H/HeJ dominant susceptibility alleles. Since the frequency of the resistant offspring (35.1%) represents half of the expected nonrecombinant DBA/2J parental genotype, the frequency of animals carrying the parental genotype would be 70.2%. Thus, the crossover frequency would equal the frequency of recombinant individuals, which is 29.8%. In order to properly assess the F_2 results using this theory, the number

TABLE II. SEGREGATION ANALYSIS OF THE C3H SUSCEPTIBILITY GENE

Cross[a]	Response	Experimental (%)	Theoretical[b]
1. (C3H × DBA/2)F_1 × DBA/2	Resistant (33/94)	35.1	—
	Susceptible (61/94)	64.9	—
2. (C3H × DBA/2)F_1 × (C3H × DBA/2)F_1	Resistant (9/58)	15.5	12.3
	Susceptible (49/58)	84.5	87.7

[a] ♀ × ♂.

[b] Theoretical recombination values were calculated on the basis of the backcross data and thus are only presented for the F_2 cross.

of resistance genes in DBA/2 had to be determined. Previously, only a single gene controlling resistance was indicated by the segregation studies between DBA/2 and BALB/c (2). Since BALB/c carries the *PsCR2* gene, the segregating DBA/2 gene must be of another locus and is presumably *PsCR1*. If DBA/2 also carries the *PsCR2* gene (i.e., similar to BALB/c), then (DBA/2 × C57BL/6)F_1 animals should be resistant. Completed studies indicate that the progeny of DBA/2 × C57BL/6 matings were all susceptible (manuscript in preparation). It is apparent that the DBA/2 *PsCR2* allele is unlike that of BALB/c. The phenotypic expression of the DBA/2 *PsCR2* gene still remains to be elucidated. These experiments do suggest that DBA/2 has one gene conferring resistance and that this gene can be masked by susceptible gene(s) from C3H and quite probably from C57BL/6, too. In the F_2 offspring, the expected frequency of resistant animals can be calculated by squaring the frequency of resistant animals (35.1%) obtained in the backcross experiment. Using this information, one could predict that 12.3% of the F_2 animals should be resistant. F_2 progeny from the C3H × DBA/2)F_1 × (C3H × DBA/2)F_1 matings were then examined for resistance to *P. aeruginosa* infection (Table II). Of 58 animals infected, 9 (15.5%) were resistant and 49 (84.5%) were susceptible. The supposition of two linked *PsCS* genes was accepted as indicated by the X^2 value of 0.291 ($0.7 > P > 0.5$). Other possibilities have been considered using both allelic and epistatic interactions to explain both the backcross and F_2 data. So far, only the theory presented herein is consistent with previously published data and hypotheses.

Discussion. In general, the genetic control of natural resistance to bacterial infections has been described to be under dominant autosomal resistance gene(s). Infectivity of *Corynebacterium kutscheri* (7, 8), *Mycobacterium lepraemurium* (9–11), *M. tuberculosis* (12), *Listeria monocytogenes* (13–18), *Rickettsia tsutsugamushi* (19), *R. akari* (20), *Salmonella typhimurium* (21–27), and *P. aeruginosa* (1–3) has been demonstrated to be under this type of host regulation. This communication presents a unique finding where two linked dominant susceptibility genes also regulate the natural resistance to *P. aeruginosa* infection. Offspring of the susceptible strains C3H mated with the resistant DBA/2 strains incurred eye shrinkage (phthisis bulbi) as a result of infection. Data from (C3H × DBA/2)F_2 and (C3H × DBA/2)F_1 × DBA/2 backcross studies suggested the presence of two linked *PsCS* loci. If the *PsCS* loci were sex-linked, then only some of the F_2 males would have exhibited the resistance phenotype. However, this was not the case. Thus, the C3H *PsCS* genes are autosomal dominant alleles. The *PsCS* loci do not appear to be linked to the *PsCR1* locus. The relationship of these two linked *PsCS* genes with *PsCR2* cannot presently be assessed.

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1. Berk RS, Leon MA, Hazlett LD. Genetic control of the murine corneal response to *P. aeruginosa.* Infect Immun **26**:1221–1223, 1979.
2. Berk RS, Beisel K, Hazlett LD. Genetic studies on the murine corneal response to *Pseudomonas aeruginosa.* Infect Immun **34**:1–5, 1981.
3. Berk RS, Leon MA, Hazlett LD. Further studies on the genetic control of murine corneal response to *P. aeruginosa.* Rev Infect Dis, in press, 1983.
4. Hazlett LD, Rosen DD, Berk RS. Experimental eye infections caused by *Pseudomonas aeruginosa.* Ophthalmic Res **8**:311–318, 1976.
5. Hazlett LD, Rosen DD, Berk RS. *Pseudomonas* eye infections in cyclophosphamide-treated mice. Invest Ophthalmol Vis Sci **16**:649–652, 1977.
6. Hazlett LD, Berk RS. Heightened resistance of athymic, nude (nu/nu) mice to experimental *Pseudomonas aeruginosa* ocular infection. Infect Immun **22**:926–933, 1978.
7. Hirst RG, Wallace ME. Inherited resistance to *Corynebacterium kutscheri* in mice. Infect Immun **14**:475–482, 1976.
8. Pierce-Chase CH, Fauve RM, Dubos R. Corynebacterial pseudotuberculosis in mice. I. Comparative susceptibility of mouse strains to experimental infection with *Corynebacterium kutscheri.* J Exp Med **120**:267–284, 1964.
9. Closs O, Haugen OA. Experimental murine leprosy. 1. Clinical and histological evidence for varying susceptibility of mice to infection with *Mycobacterium lepraemurium.* Acta Pathol Microbiol Scand Sect A **81**:401–410, 1973.
10. Closs O, Haugen OA. Experimental murine leprosy. 2. Further evidence for varying susceptibility of outbred mice and evaluation of the response of 5 inbred mouse strains to infection with *Mycobacterium lepraemurium.* Acta Pathol Microbiol Scand Sect A **82**:459–474, 1974.
11. Lefford MJ, Patel PJ, Poulter LW, Mackaness GB. Induction of cell-mediated immunity to *Mycobacterium lepraemurium* in susceptible mice. Infect Immun **18**:654–659, 1977.
12. Allen EM, Moore VL, Stevens JO. Strain variation in BCG-induced chronic pulmonary inflammation in mice. I. Basic model and possible genetic control by non-*H-2* genes. J Immunol **119**:343–347, 1977.
13. Cheers C, McKenzie IFC. Resistance and susceptibility of mice to bacterial infection: genetics of listeriosis. Infect Immun **19**:755–762, 1978.
14. Cheers C, McKenzie IFC, Pavlov H, Waid C, York J. Resistance and susceptibility of mice to bacterial infection: Course of listeriosis in resistant or susceptible mice. Infect Immun **19**:763–770, 1978.
15. Farr AG, Kiely JM, Unanue ER. Macrophage–T cell interactions involving *Listeria monocytogenes*—role of the *H-2* gene complex. J Immunol **122**:2395–2404, 1979.
16. Skamene E, Kongshavn PAL, Sachs DH. Resistance to *Listeria monocytogenes* in mice: genetic control by genes that are not linked to the *H-2* complex. J Infect Dis **139**:228–231, 1979.
17. Zinkernagel RM. Restriction by *H-2* gene complex of the transfer of cell-mediated immunity to *Listeria monocytogenes.* Nature (London) **251**:230–233, 1974.
18. Zinkernagel RM, Althage A, Adler B, Blanden RV, Davidson WF, Kees U, Dunlop MBC, Shreffler DC. *H-2* restriction of cell-mediated immunity to an intracellular bacterium. Effector T cells are specific for *Listeria* antigen in association with *H-2I* region-coded self-markers. J Exp Med **145**:1353–1367, 1977.
19. Groves MG, Osterman JV. Host defenses in experimental scrub typhus: genetics of natural resistance to infection. Infect Immun **19**:583–588, 1978.
20. Anderson Jr GW, Osterman JV. Host defenses in experimental Rickettsialpox: Genetics of natural resistance to infection. Infect Immun **28**:132–136, 1980.
21. Gowen JW, Calhoun ML. Factors affecting genetic resistance of mice to mouse typhoid. J Infect Dis **73**:40–56, 1943.
22. Misfeldt ML, Johnson W. Variability of protection in inbred mice induced by a ribosomal vaccine prepared from *Salmonella typhimurium.* Infect Immun **14**:652–659, 1976.
23. Oakberg EF. Constitution of liver and spleen as a physical basis for genetic resistance to mouse typhoid. J Infect Dis **78**:79–98, 1946.
24. Plant J, Glynn AA. Natural resistance to *Salmonella* infection, delayed hypersensitivity and Ir genes in different strains of mice. Nature (London) **248**:345–347, 1974.
25. Plant J, Glynn AA. Genetics of resistance to infection with *Salmonella typhimurium* in mice. J Infect Dis **133**:72–78, 1976.
26. Plant J, Glynn AA. Locating salmonella resistance gene on mouse chromosome 1. Clin Exp Immunol **37**:1–6, 1979.
27. Robson HG, Vas SI. Resistance of inbred mice to *Salmonella typhimurium.* J Infect Dis **126**:378–386, 1972.

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