INFECTION AND IMMUNITY, Feb. 1988, p. 532–533
0019-9567/88/020532-02$02.00/0

Vol. 56, No. 2

Multigenic Control of Resistance to *Yersinia enterocolitica* in Inbred Strains of Mice

GERALD E. HANCOCK,†* RUSSELL W. SCHAEDLER, AND THOMAS T. MACDONALD‡
Department of Microbiology, Jefferson Medical College, Philadelphia, Pennsylvania 19107

Received 27 July 1987/Accepted 11 November 1987

By using recombinant inbred mice derived from strains genetically resistant or susceptible to *Yersinia enterocolitica*, we demonstrated a tentative linkage of resistance with the *Es-1* locus on murine chromosome 8. No correlation with resistance and genes associated with immune regulation was evident. In addition, resistance appeared to be multigenic.

Inbred mouse strains differ markedly in their resistance to a variety of infectious agents, and the identification of the genetic loci responsible for this resistance is an area of current interest. In our previous report (4), we demonstrated that BALB/c mice are susceptible (50% lethal dose [LD_{50}], 3×10^2 organisms intraveneously [i.v.]) to *Yersinia enterocolitica*, whereas C57BL/6 mice are resistant (LD_{50}, 2×10^5 organisms i.v.). All other strains studied are susceptible. On the other hand, (BALB/c × C57BL/6)F_1 hybrid mice are intermediate (LD_{50}, 3×10^4 organisms i.v.) in their resistance to *Y. enterocolitica*, suggesting that although resistance is a dominant trait, gene interactions or dosage effects occur in hybrid mice. Our results (4) further indicate that resistance is determined by genes that control acquired immunity. Resistance to *Y. enterocolitica* is thymus dependent and not associated with the *Ity* locus (4), an important locus in early nonspecific resistance to a variety of facultative intracellular pathogens (5). With C57BL/6 and BALB/c mice as the respective prototype resistant and susceptible strains, we examined the genetic control of resistance to *Y. enterocolitica* as reported here. We report a tentative linkage of resistance with the *Es-1* locus located on murine chromosome 8, and in addition, we suggest that resistance is controlled by at least two dominant genes.

The autoagglutination-positive *Y. enterocolitica* WA strain (serotype O:8, biotype 2, Vwa^+) originally isolated from human infection by Carter et al. (1) was used in all studies. Virulence of the organism was maintained by periodic oral passage in susceptible strains followed by isolation from infected ileal Peyer's patches (4). Animal infection and bacterial enumeration were performed as previously described (4). All animals were housed in an American Association for Accreditation of Laboratory Animal Care-accredited facility and received food and water ad libitum.

CXB recombinant inbred (RI) strains and their susceptible (BALB/cBy) C and resistant (C57BL/6By) B progenitor strains (Jackson Laboratory, Bar Harbor, Maine.) were used to investigate the association of genes that encode resistance to *Y. enterocolitica* with other mapped genes. Lethal studies of CXB RI strains demonstrated a wide range of resistance to the organism (Table 1). On the basis of these LD_{50} data, CXBD, CXBH, and CXBK strains appear to be as susceptible to *Y. enterocolitica* as is their C progenitor strain. No CXB RI strains are as resistant as the B progenitor strain. To further determine the CXB RI strain distribution pattern of resistance, CXB RI mice and their progenitors were examined again for their resistance to an i.v. infection with 10^4 organisms. Mice were defined as resistant if they survived the infection. All deaths occurred between 2 to 10 weeks postinfection. No deaths occurred after 10 weeks in either the CXB RI set or the progenitor controls. Surviving mice were sacrificed 4 to 6 months after infection. Percent survival 10 weeks after i.v. infection (Table 1) demonstrates that CXBE and CXBI strains are the only strains as resistant as the B progenitor strain. All other CXB RI strains are as susceptible as the C progenitor strain. These data, along with the LD_{50} data presented in Table 1, suggest a strain distribution pattern for resistance to *Y. enterocolitica* that is fully concordant with the *Es-1* (esterase-1) locus on mouse chromosome 8 (8).

The *Es-1* locus on mouse chromosome 8 encodes an esterase found in serum and erythrocytes (3). The esterase is also found in kidney, but it is not clear whether this is from serum contamination. At present, there are two known alleles for the *Es-1* locus. The $Es\text{-}1^a$ allele is present in the C57BL family of strains, and the $Es\text{-}1^b$ allele is found in the BALB/c strain (3). No correlation with other mapped loci (*H-2* and *Igh*) important in the development of acquired immunity is evident.

To investigate the number of genes that encode resistance to *Y. enterocolitica*, male C57BL/6 and BALB/c parental strains (Jackson Laboratory) and male and female mice derived from (BALB/c × C57BL/6)F_1 × parental backcrosses and $F_1 \times F_1$ intercrosses (Thomas Jefferson University) were infected i.v. with 10^5 organisms. At 10 days after infection, the number of bacteria in their tissues was determined and each animal was classified as resistant ($\log_{10}$ geometric mean = <6.0) or susceptible (4). Whereas 9 of 10 F_1 hybrid (90%) and 20 of 20 (100%) C57BL/6 mice were resistant ($\log_{10}$ geometric mean = 3.2 and 1.8 organisms, respectively), 17 of 20 (85%) BALB/c mice appeared susceptible ($\log_{10}$ geometric mean = 5.9 organisms). However, strong environmental influences resulted in clear segregation within the genetically homogeneous BALB/c and F_1 hybrid populations and therefore interpretation of F_1 × parental backcross resistance became difficult. However, only 25 of 59 (42%) F_2 mice appeared resistant to *Y. enterocolitica*, significantly different (chi-square = 33.49, $P < 0.001$) from the 44 of 59 (75%) resistant mice expected if one gene

* Corresponding author.
† Present address: Laboratory of Cellular Physiology and Immunology, The Rockefeller University, New York, NY 10021.
‡ Present address: Department of Pediatric Gastroenterology, St. Bartholomew's Center for Research, St. Bartholomew's Hospital, London, EC1A 7BE, England.

TABLE 1. Strain distribution pattern of resistance to *Y. enterocolitica* in the CXB recombinant inbred and progenitor strains

Strain	LD_{50}[a]	% Survival[b]	Phenotype[c]	Progenitor strain concordant with locus:		
				Es-1	*H-2*	*Igh*
C	3×10^2	10	S	C	C	C
B	10^5	70	R	B	B	B
D	10^2	0	S	C	C	B
E	3×10^4	70	R	B	B	B
G	3×10^3	18	S	C	B	C
H	6×10^1	0	S	C	C	B
I	10^4	60	R	B	B	B
J	3×10^3	18	S	C	B	C
K	9×10^2	27	S	C	B	B

[a] The LD_{50} 10 weeks after i.v. infection was determined as previously described (4) and calculated by the method of Reed and Muench (7).

[b] Percent survival was determined 10 weeks after i.v. infection with 10^4 organisms. No deaths occurred after 10 weeks. There were 10 or 11 mice per strain.

[c] R, Resistant to *Y. enterocolitica*; S, susceptible.

[d] CXB RI strain *Es-1*, *H-2*, and *Igh* haplotype classifications were taken from reference 8.

encoded resistance. An estimate of the number of genes (n) that control resistance to the organism was calculated from the following equation: $n = R^2/8[V_{F2} - V_{F1}]$, where R is the range between mean values of the parental strains, V_{F2} is the variance of F_2, and V_{F1} is the variance of the F_1 hybrid mice (2). Substitution of the following $\log_{10}$ geometric means and variances into the equation resulted in the following equations: $n = 2.3$; C57BL/6 mean = 1.8; BALB/c mean = 5.9; F_2 variance = 6.4; F_1 variance = 5.5. Thus, resistance to *Y. enterocolitica* appears to be multigenic and encoded by at least two genes.

The critical events that occur which effectively restrict the growth of *Y. enterocolitica* in C57BL/6 mice appear to take place during the later stage of infection. This suggests that the genes that control resistance are those genes which regulate the development of acquired immunity. In this report and our previous work (4), we have been unable to correlate resistance with mapped genes (*H-2* and *Igh*) that regulate immune mechanisms. Thus, we are unable to determine where the putative lesion occurs in the development of specific immunity and BALB/c mice. However, we have demonstrated (4) that the thymus appears to be important in resistance to *Y. enterocolitica*. In addition, preliminary experiments (data not shown) fail to demonstrate a strain difference in the temporal development of anti-yersinia agglutinins. In fact, the data suggest that BALB/c anti-yersinia agglutinins are quite protective in passive immunity against i.v. inoculated organisms. Thus, it appears that BALB/c susceptibility is a result of defective cell-mediated immune mechanisms. Recent studies of cutaneous leishmaniasis in BALB/c and C57BL/6 mice suggest that the defect in BALB/c mice may reside in an inability to mount the appropriate local T-cell response (6).

The findings in this report also suggest that the genetic regulation of resistance to *Y. enterocolitica* is multigenic. Our mathematical analysis indicates that at least two autosomal dominant genes are involved in C57BL/6 resistance. However, because of strong environmental influences, these data must be interpreted with caution.

It is questionable what role, if any, the *Es-1* locus has in resistance to *Y. enterocolitica*. There are only seven strains in the CXB RI set. Thus, there is a 1-in-128 probability that the association with resistance and the *Es-1* locus may occur by chance alone. However, it is interesting that no association with genes of the major histocompatibility complex located on chromosome 17 and with strain differences in resistance to Y. enterocolitica is evident. This suggests that in addition to immune-response genes of the major histocompatibility complex, other non-major histocompatibility complex-linked immune-response genes are important in resistance. The data also suggest that these hypothetical genes are located on chromosome 8 associated with the *Es-1* locus.

We thank Philip B. Carter for his helpful discussions in the preparation of the manuscript. We also acknowledge Kathleen Givens for her excellent secretarial assistance.

LITERATURE CITED

1. **Carter, P. B., C. F. Varga, and E. E. Keet.** 1973. New strain of *Yersinia enterocolitica* pathogenic of rodents. Appl. Microbiol. **26**:1016–1018.
2. **Falconer, D. S.** 1963. Quantitative inheritance, p. 193–216. *In* W. J. Burdette (ed.), Methods in mammalian genetics. Holden-Day, San Francisco.
3. **Green, M. C.** 1981. Catalog of mutant genes and polymorphic loci, p. 75. *In* M. C. Green (ed.), Genetic variants and strains of the laboratory mouse. Fischer Verlag, Stuttgart, Federal Republic of West Germany.
4. **Hancock, G. E., R. W. Schaedler, and T. T. MacDonald.** 1986. *Yersinia enterocolitica* infection in resistant and susceptible strains of mice. Infect. Immun. **53**:26–31.
5. **Lissner, C. R., R. N. Swanson, and A. D. O'Brien.** 1983. Genetic control of the innate resistance of mice to *Salmonella typhimurium*: expression of the Ity gene to peritoneal and splenic macrophages isolated *in vitro*. J. Immunol. **131**:3006–3013.
6. **McElrath, M. J., G. Kaplan, A. Nusrat, and Z. A. Cohn.** 1987. Cutaneous leishmaniasis: the defect in T cell influx in BALB/c mice. J. Exp. Med. **165**:546–559.
7. **Reed, L. J., and H. Muench.** 1938. A simple method of estimating fifty percent endpoints. Am. J. Hyg. **27**:493–497.
8. **Taylor, B. A.** 1981. Recombinant inbred strains, p. 397–407. *In* M. C. Green (ed.), Genetic variants and strains of the laboratory mouse. Fischer Verlag, Stuttgart, Federal Republic of West Germany.