GENETIC ANALYSIS OF FACTORS DETERMINING SUSCEPTIBILITY TO TUBERCULOSIS

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UDC 616-002.5-055.5/.7-092.9

KEY WORDS: susceptibility; resistance; genetic control.

Recent studies of genetic control of susceptibility and resistance to various infectious diseases in experimental animals have led in some cases to conclusions regarding the character of inheritance of susceptibility factors [3, 5, 7], and for some infections the number of genes determining sensitivity of the animals and their linking with known markers have actually been established [4, 8]. Meanwhile such data have been obtained for only a few diseases, and so far as an important infectious disease such as tuberculosis is concerned only the first reports have been published on genetic aspects of BCG vaccination of mice [6], and investigations of virulent strains of *Mycobacterium tuberculosis* in genetic experiments are only just beginning. The writers previously [2] demonstrated the existence of two lines of mice (I/st and A2G) which differed sharply in sensitivity to intravenous infection with virulent strain H37Rv of *M. tuberculosis* and in the character of the tuberculin tests (primary sensitivity to tuberculosis infection — PSTI) in infected animals. In this paper we analyze the inheritance of factors determining differences in survival of mice of these lines and discuss the question of differences in genetic control of susceptibility and resistance to tuberculosis.

MATERIALS AND METHODS

Progenitors of mice of line I/st (H-2^Y) and A2G (H-2^a) were generously provided by Z. K. Blandova (Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR). Experimental inbred mice, F_1 hybrids, and progenies of back-crosses (F_1 × I/st) and (F_1 × A2G) were obtained in the Nursery of the Central Tuberculosis Research Institute. Animals of both sexes were used in the experiments at the age of 3-5 months. Data for males and females were pooled for analysis of the results because no significant differences were found between them.

Infection of the animals, the tuberculin tests, and statistical analysis were all fully described previously [1, 2].

EXPERIMENTAL RESULTS AND DISCUSSION

Data on survival of I/st, A2G, (A2G × I/st)F_1, (F_1 × A2G)BC_1, and (F_1 × I/st)BC_1 mice are given in Table 1. F_1 hybrids between the two lines inherited the A2G trait of resistance. This trait is consequently dominant relative to sensitivity. The somewhat longer survival of F_1 hybrids than of the original resistant A2G line can probably be explained by genetic complementation for unknown factors favoring survival, taking place on account of the heterozygosity of the animals. Analysis of these factors is currently in progress, but even on the basis of the first results it can be postulated that resistance to tuberculosis is a complex trait. The same conclusion can be drawn from an examination of the results for the progenies of the F_1 × A2G back cross. These results, first, confirm the dominant character of the resistance trait (all animals were resistant to infection) and, second, they indicate the need for study of the character of control of this resistance, for the longer survival of the (F_1 × A2G)BC_1 mice than of the A2G line and, more especially, of the F_1 hybrids is still unexplained and requires complementation analysis.

Central Tuberculosis Research Institute, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR B. V. Bogush.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 12, pp. 83-85, December, 1982. Original article submitted December 1, 1981.

 0007-4888/82/9412-1720$07.50

TABLE 1. Survival of Mice after Intravenous Infection with *M. tuberculosis*

Mice	Day of death	Mean survival period, days
I/st	16, 17, 20, 21, 21, 22, 22, 22, 22, 22, 23, 23, 23rd	21,08±1,38
A2G	46, 54, 57, 62, 64, 65, 68, 68, 68, 69, 69	62±73±4,95
(I/st × A2G)	72, 75, 80, 82, 83-	77,83±4,78
(F_1 × A2G) BC_1	94, 97, 97, 100, 100, 112, 117, 118th	104,38±8,42*
(F_1 × I/st) BC_1:		
Group 1	18, 23, 23, 24, 24, 24, 25, 25, 26, 27, 28, 30th	24,75±1,98
Group 2	46, 52, 54, 55, 55, 67, 71, 71, 75, 76, 89, 90th	66,75±15,34*

Legend. Asterisk indicates that the mean survival time does not give a reliable idea of the group because of the wide scatter (see text).

TABLE 2. Values of Tuberculin Tests after Intravenous Infection of Mice with *M. tuberculosis**

Mice	I/st	A2G	F_1	(F_1×I/st) BC_1	
				group 1	group 2
Tuberculin test (mean increase, mm)	0,100±0,052	0,324±0,049	0,343±0,054	0,055±0,032	0,330±0,064

*Increase in thickness of paw exceeding 0.15 mm is considered significant.

The most informative experiments, as might be expected, were those with back crossing of F_1 hybrids with the sensitive I/st line. The progeny from this cross was divided into two groups. Group 1 (sensitive mice) included mice which died during the first 18-30 days after infection; for most animals the deviation from the mean survival time was very small. Group 2 (resistant mice) were those which did not begin to die until the 46th day after infection and the last of which died on the 90th day, i.e., considerable scatter was observed. The ratio between the numbers of animals in the groups was 12:12, i.e., it corresponded exactly to the hypothesis of monogenic control of sensitivity of infection. Mice of the I/st line carry a recessive gene, responsible for rapid death of the animals in the homozygous state in progenies of back crossing. Meanwhile, in the group of resistant animals, different degrees of resistance to infection were observed, probably reflecting the polygenic character of control. Susceptibility is thus controlled by a "sensitivity gene" and several "resistance genes." Irrespective of the combination of alleles of "resistance genes" in a given mouse, they are not exhibited if the animal is homozygous for the recessive allele of the "sensitivity gene." This is demonstrated by the fact that the mice of group 1 died at roughly the same time. The recessive allele of the "sensitivity genes" is not exhibited in homozygotes against the background of the action of this allele. In the mice of group 2 the defect was blocked by the dominant normal allele of this gene (it can be called normal because only mice of the I/st line died so early), and against this background the time of death depends on a combination of alleles of the "resistant genes" in the given mouse. The number of these genes cannot be determined from our data because a much larger number of segregating progenies of back crossing would have to be investigated for this purpose.

Table 2 gives values of the tuberculin tests of the various animals to PPD. The picture observed agrees well with the survival data. F_1 hybrids reacted in the same way as the resistant A2G line whereas the back cross progenies segregated according to the character of the PSTI reaction into two groups in the ratio of almost 1:1 (10:11 in the experimental group, the tuberculin tests were not carried out on three mice); mice of the sensitive, early-dying group differed statistically significantly ($P < 0.02$) from mice of the resistant, late-dying group.

Anergy in the PSTI test, like the trait of sensitivity to infection, is thus controlled by the recessive allele of one gene, which may perhaps also be the "sensitivity gene" of I/st mice or may be closely linked with it; whatever the case, among all the mice tested there was not a single recombinant with a high reading of the tuberculin test but which died quickly. As regards linking of genes determining the different degrees of resistance to infection in mice carrying the normal allele of the "defective gene" with the gene controlling the level of the PSTI reaction, no conclusions can be drawn without further research.

According to our preliminary observations, the genetic factor of susceptibility of mice to tuberculosis and, naturally, the factor of a low level of PSTI reaction either are not linked with the H-2 complex (the H-2 haplotype in line I) or they are separated from it by a distance of at least 25 morgans.

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