GENETIC REGULATION OF THE HOST'S RESPONSE TO INJECTION OF *Mycobacterium bovic* (BCG) AND *Mycobacterium tuberculosis* H37RV

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Depending on the level of response to intravenous injection of low doses of *M. bovis* (BCG) inbred lines of mice were divided into two groups: senstive, in whose organs intensive multiplication of mycobacteria takes place, so that by the 3rd week their number in the spleen is 100 times greater than the number injected; mice of resistant lines inhibit growth of injected mycobacteria [8]. Genetic analysis has shown that this trait (natural resistance is controlled by a gene located in chromosome 1 of the mouse, and is designated Bcg, and it exists in the form of two allelic states Bcg^s and Bcg^r [9]. Phenotypically the action of the Bcg gene is evidently realized at the macrophagal level [6].

Besides natural resistance there are other aspects of the response to BCG, for example, the formation of protective immunity against subsequent infection of mice with virulent strains of *M. tuberculosis.* There is evidence that the level of protection created by high doses of BCG is dependent on genes of the H-2 complex [3]. Other data on genetic control on the protective effect of BCG virtually do not exist, nor has the question of the role of the Bcg gene in the formation of protective immunity during vaccination by BCG and in the susceptibility of mice to infection with virulent strain *M. tuberculosis* H37Rv, has likewise not yet been investigated. The present investigation was devoted to the study of these problems.

EXPERIMENTAL METHOD

Experiments were carried out on inbred lines of mice obtained from the "Stolbovaya" Laboratory Animals Nursery, Academy of Medical Sciences of the USSR, and the Jacksonian Laboratory, USA, and maintained in the nursery of the Tuberculosis Research Institute: CBA/Sto, AKR/Y, BALB/sSto, C57BL/10ScSnYCit, C3H/Sn, C3H/Hel, CBA/N. (B10-fz^y) mice were obtained from the "Svetlye Gory" nursery, Academy of Medical Sciences of the USSR. (AKR × B10-fz^y)F_1 hybrids and progenies from back crossing BC_1(F_1 × B10-fz^y) were bred in the nursery of the Tuberculosis Research Institute. The mice were immunized by subcutaneous injection of a living 3-week culture of BCG, obtained from the museum of our Institute, in a dose of 500 μg per mouse (the mice used weight 18 g).

The mice were infected by intravenous injection of 25 μg/mouse of a living 3-week culture of *M. tuberculois* H37Rv.

Tuberculin tests were carried out by the method described previously [1].

EXPERIMENTAL RESULTS

In the first part of the investigation we studied the problem of whether genetic control of the protective effect of vaccination, different from control of resistance detected by primary infection of mice with virulent strains of *M. tuberculosis*, really exists. An affirmative answer to this question could be given by the existence of at least two lines of mice with identical levels of resistance after primary infection with tuberculosis, but differing in their level of resistance after BCG vaccination. In this case, by the term resistance, we emply the survival rate of infected mice, which is determined by nonimmunological factors (natural resistance) and by factors of antituberculosis immunity, developing in the course of infection. Curves reflecting death of BCG-vaccinated and unvaccinated mice of various

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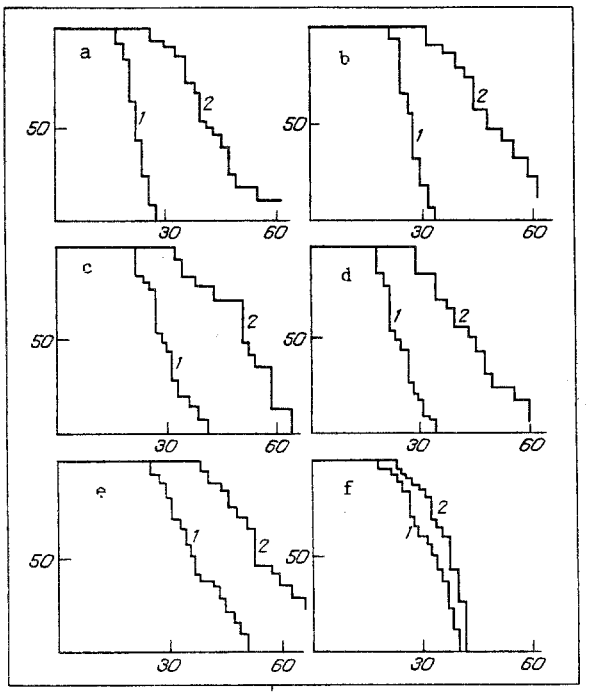

Fig. 1. Trend of mortality of BCG-vaccinated and unvaccinated mice as a result of infection by M. tuberculosis H37Rv. Aggregated results of three or four experiments given, and in each case 40-50 mice were studied. Abscissa, days after infection ordinate, % of surviving mice. 1) Unvaccinated mice; 2) vaccinated with BCG; a) B6; b) BALB/c; c) C3H/Sn; d) C3H/HeJ; e) CBA; f) CBA/N.

lines after infection with M. tuberculosis are given in Fig. 1. B10 and BALB/c mice have low initial resistance and, as is clear from Fig. 1a, b, BCG has an equal protective action on mice of these lines. C3H/HeJ mice differ from C3H/Sn in an inherited defect of the immune response to the lipopolysaccharide. As Fig. 1c, d shows the primary resistance of C3H/HeJ mice is only very slightly lower than that of C3H/Sn, and the protective effect of BCG vaccination is identical. In CBA mice a similar picture is observed except that this line is more resistant, and curves of death of the mice are shifted to the right (Fig. 1e). So far as CBA/N mice are concerned, in this case the effect of vaccination is virtually absent (Fig. 1f), whereas the survival rate of unvaccinated CBA/N mice is almost indistinguishable from CBA, and actually exceeds the survival rate of mice of other lines. CBA/N mice are carriers of the xid mutation (immunodeficiency linked with the X-chromosome), and in these mice differentiation of B cells is disturbed and, in particular, the subpopulation Lyb-5^+ of lymphocytes is absent. CBA mice have the normal alternative xid allele. Thus these results indicate the existence of genetically determined differences in the effect of BCG vaccination accompanied by identical primary resistance of these lines of mice to tuberculosis. The xid gene, while leaving primary resistance virtually unchanged, completely abolished the effect of BCG vaccination. Consequently, it can be postulated that its normal allele in CBA mice regulates this effect of BCG vaccination.

Regulation of the response to injection of BCG by the Bcg gene is not restricted purely to early (natural) resistance. The Bcg gene also has an indirect regulating effect of proliferation of immune T lymphocytes to PPD [5], and directly regulates the level of expression of Ia-antigens on macrophagens and the ability of macrophages to undergo antigenic presentation [7]. Thus the Bcg gene is a pleiotropic gene. Hence the great importance of the question of involvement of the Bcg gene in the control of formation of protective immunity against tuberculosis following BCG vaccination. To solve this problem, in the present investigation, mice with alternative alleles of the Bcg gene were investigated by the approach described above: B10-fz^y (Bcg^s), AKR (Bcg^r), F_1 ($Bcg^{r/s}$) hybrids, and progenies of back crossing (F_1 × B10-fz^y)BC_1, ($Bcg^{r/s}$ and $Bcg^{s/s}$). Instead of mice of line B10 (Bcg^s, +) we used B10-fz^y (Bcg^s, fz^y) mice which, unlike B10, carry the recessive mutation fz^y in chromosome 1, leading to a readily identified disturbance of the hair cover in the homozygote. We used this mutation as marker of chromosome 1 and of the Bcg^s gene. Survival rate of B10-fz^y, AKR, F_1, and BC_1 (F_1 × B10-fz^y) mice infected with M. tuberculosis is shown in Fig. 2. The BC_1 genotype was judged by its phenotype: the fz phenotype corresponds to the $Bcg^{s/s}$ genotype, whereas the + phenotype corresponds to the $Bcg^{r/s}$ genotype. The survival rate of AKR mice was significantly higher than that of B10-fz^y mice. The F_1 hybrids were ore resistant still. The survival rate of the BC_1 mice (altogether in two experiments 50 mice were used), as Fig. 2 shows, was independent of the fz^y phenotype. Progenies of BC_1 (fz^y) and BC_1 (+) have virtually identical distributions with respect to survival. Consequently, the survival rate of

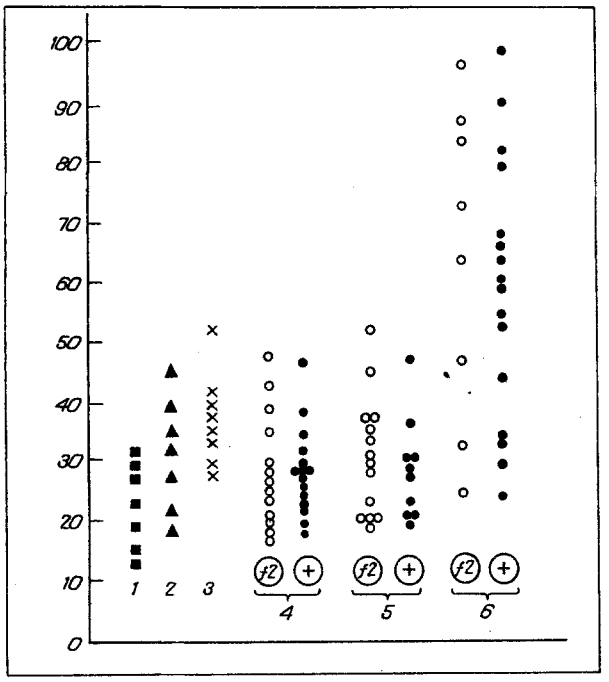

Fig. 2

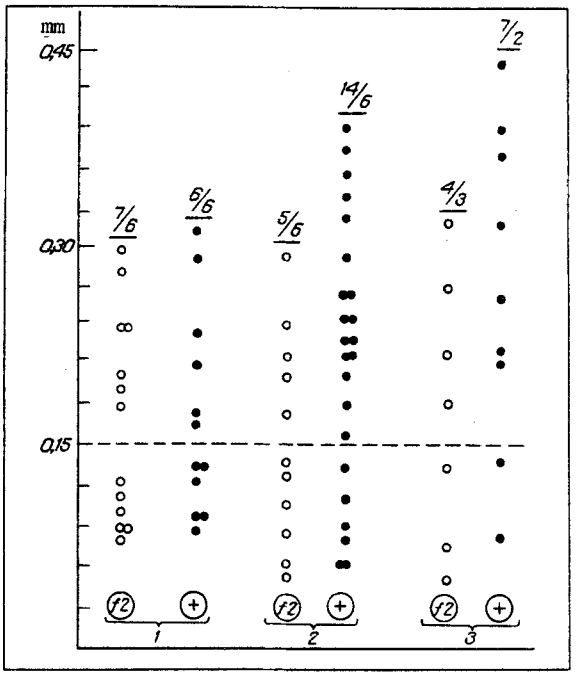

Fig. 3

Fig. 2. Survival rate of B10-fz^y, AKR, F_1, and BC_1 (F_1 × B10-fz) mice infected with M. tuberculosis H37Rv. Ordinate, days: 1) B10-fz^y; 2) AKR; 3) F_1; 4, 5) BC_1(F_1 × B10-fz^y); 6) survival rate of BC_1 mice vaccinated previous with BCG. Here and in Fig. 3, empty circles denote BC_1 with fz^y phenotype, shaded circles − + phenotype. Significance of differences between 1 and 2: $p < 0.05$.

Fig. 3. Distribution of BC(F_1 × B10-fz^y) mice according to values of delayed-type hypersensitivity test to tuberculin. Ordinate, value of test (in mm). 1) Mice infected with M. tuberculois H37Rv; 2 and 3) mice vaccinated with BCG. 1) $x^2 = 0.17$; 2) $x^2 = 5.6$; $p < 0.025$; 3) $x^2 = 3.6$, $p < 0.1$.

mice infected with M. tuberculosis H37Rv is not under the control of the Bcg gene.

Next we studied the survival rate of BC_1 mice infected with M. tuberculosis H37Rv after BCG vaccination (Fig. 2). It will be clear from Fig. 2 that survival rate (which means resistance) of these mice was considerably higher than in the unvaccinated BC_1, but in this case, survival rate was independent of the origin of chromosome 1 and, consequently, of the Bcg gene. Thus the protective effect of vaccination, manifested as an increase in the length of survival of the infected mice, is evidently not controlled by the Bcg gene, as is the case with the xid gene.

In the next stage of the work we studied the role of the Bcg gene in a simpler anti-tuberculosis phenomenon that survival, namely delayed-type hypersensitivity (DTH) to tuberculin, tested in mice infected with M. tuberculosis H37Bv and mice vaccinated with BCG.

The distribution of BC_1 mice by level of DTH (the pincushion test), carried out 2 weeks after infection with M. tuberculosis H37Rv, is shown in Fig. 3. Data on reactivity of the original lines are not given in Table 3, and the value of DTH in AKR mice was 0.26 ± 0.02 and in B10 mice 0.18 ± 0.03. Clearly BC_1(fz^y) and BC_1(+) mice have identical distributions for reactivity in DTH (in this test, a value of under 0.15 mm for the reaction is considered not to be significant). The ratio of the numbers of reactive (≥ 0.15 mm) and nonreactive (<0.15 mm) for BC_1(fz^y) was 7/6, whereas for BC_1(+) it was 6/6. Thus reactivity in DTH to tuberculin during infection with M. tuberculosis H37Rv is independent of the Bcg gene.

Results of determination of the valeu of the DTH test to tuberculin in BC_1 mice vaccinated with BCG (but not infected with H37Rv) are also given in Fig. 3. The results of two experiments showed that in this case reactivity of BC_1(+) mice was significantly higher than in BC_1(fz^y) (altogether 48BC_1 mice were tested). The ratio of DTH ≥ 0.15 and <0.15 for BC_1 × (fy^z) was 6/5 and 4/3, whereas for BC_1(+) it was 16/6 and 7/2. In this case therefore, the value of the reaction depends on the origin of chromosome 1 and also, evidently, of the Bcg

gene. It must be pointed out here that the pattern obtained evidently reflects involvement of the Bcg gene in the control of DTH inaccurately, for recombinations on the fz^y–Bcg region detract from the clarity of the results to a certain extent.

On the basis of the character of distribution of the mice by level of DTH, as shown in Fig. 3, it can be postulated that this trait is controlled by more than one gene. Besides the Bcg gene, this is evidently the H-2 genes, but it was not part of the aim of the present investigation to study this matter, nor likewise to determine the number of genes segretating in this particlar model.

The results of this section of the work are evidence that the Bcg gene is not involved in the regulation of processes caused by infection with M. tuberculosis H37Rv; even the protective action of BCG vaccination, in the case of subsequent infection, does not depend on the allele of the Bcg gene. The action of the gene is manifested only in the development of the immune response (DTH) to tuberculin in mice vaccinated with BCG, but not infected with M. tuberculosis H37Rv. Thus these results are yet another example of the action of the Bcg gene, which, as in cases already known [7], may perhaps be linked with the different antigen-presenting ability of Bcg^r and Bcg^s macrophages.

On the basis of the results of this investigation, it can be concluded that there are significant differences in genetic regulation of the response of the host to injection of Bcg and of M. tuberculosis H37Rv.

The response to BCG is regulated by the Bcg gene (natural resistance, antigenic presentation), by genes regulating the formation of protective immunity, manifested as strengthening of the resistance of animals to infection with tuberculosis (these are the xid gene and its normal allele), and by the H-2 complex [3].

The course of infection caused by virulent M. tuberculosis H37R strains, which differs from that of infection with BCG in a chain of dramatic events, culminating in death, on the other hand, is under the control of other genetic systems, not yet properly studied. They include the H-2 genes [6], the Tbc-1 gene [2], and other genes segregating during genetic analysis of inbred lines of mice [4]. There is reason to suppose that H-2 genes do not play the most important role in this case. The action of the Bcg gene, however, as our results indicate, during the development of a pathological tuberculus process, caused by M. tuberculosis H37Rv, is not manifested even after preliminary BCG vaccination.

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