GENETIC CONTROL OF INTERLEUKIN 2 IN INBRED MICE

A. L. Pukhal'skii and A. P. Toptygina

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The writers showed previously that inbred BALB/*c* and C57BL/6 mice differ significantly both in the height of the proliferative response of their spleen cells to concanavalin A (con A) and in the intensity of interleukin 2 (IL 2) production. Similar differences were found between DBA/2 and CC57BR mice. In this case, BALB/*c* and DBA/2 mice (H-2^d haplotype) were high producers, but C57BL/6 and CC57BR mice (H-2^b haplotype) were low IL 2 producers. In the light of these data it was considered important to study the connection between IL 2 production and the H-2 haplotype and also to study the problem of genetic determination of the intensity of IL 2 production.

EXPERIMENTAL METHOD

Inbred BALB/cZLacSto (H-2^d) and C57BL/6ZSTo (H-2^b) mice and their F_1 and F_2 hybrids, obtained in the animal house of the Institute of Medical Genetics, Academy of Medical Sciences of the USSR, were used. Altogether 50 F_2 hybrids were used in the experiments. The intensity of IL 2 production by lymphoid cells was determined by the method described previously [5]. Mouse spleen cells, after stimulation by con A, were incubated for 3 h at 37°C, after which they were washed to remove the mitogen and reincubated for 18 h. The supernatant was collected and tested for its IL-2 content by studying their ability to maintain growth of an IL 2-dependent cytotoxic cell line. Various concentrations of recombinant IL 2 ("Biogen" Moscow Research and Production Combine) were used as the control. Titration curves for the supernatant and recombinant IL 2 were analyzed by the probit method [2, 3], followed by calculation of IL 2 activity contained in the whole supernatant. The committogenic action of the supernatant (CAS) also was tested on mouse thymocytes cultivated in the presence of a low dose of phytohemagglutinin (PHA), which itself does not induce a proliferative response. For this purpose, thymocytes of CBA/CaLacSto mice were incubated for 96 h in a concentration of $5 \cdot 10^6$ cells in 1 ml in a volume of 200 μl in the presence of 2.5 μg/ml of PHA ("Sigma," USA) and with the addition of 100 μl of the test supernatant. The intensity of the proliferative response was estimated by the incorporation of ^{3}H-thymidine. The results were expressed in relative units, the proliferative response characteristic of a specimen of supernatant with minimal committogenic action being taken as 1 unit. The H-2 haptotype in F_2 hybrids was determined by the microlymphocytotoxic test [8]. Typing sera against H-2 antigens were obtained by 5-times repeated cross immunization with spleen cells of BALB/*c* and C57BL/6 mice. Serum against H-2^b antigens, obtained by the use of mice of congeneic lines (B10 and B10D2), was also used. Differences between the groups were estimated by Wilcoxon's test for independent populations [1]. Segregation analysis was undertaken by the λ^2 test [4]. The method of Mather and Jinks [7] was used for genetic analysis of variability of IL 2 production in different generations.

EXPERIMENTAL RESULTS

The experiments showed direct correlation to be present between the IL-2 and CAS content in the supernatants obtained from mice of the test lines, and also their F_1 hybrids (Table 1). The F_1 hybrids in this case occupied an intermediate position between the parental lines. The intensity of IL 2 and CAS production, determined individually in the F_2 hybrids, lay within the interval between values characteristic of BALB/*c* and C57BL/6 mice. Under these circumstances, the intensity of

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TABLE 1. Committogenic Action of Supernatant (CAS) and IL 2 Production by Spleen Cells of Mice of different Genotypes

Line of mice	Intensity of production of	
	CAS, conventional units	IL 2, IU
BALB/c	5,4	43 (3,9)
C57BL/6	2,1	17 (1,5)
(C57BL/6× BALB/c) F_1	3,2	22 (2,0)

Legend. Intensity of IL 2 production shown in parentheses in relative units.

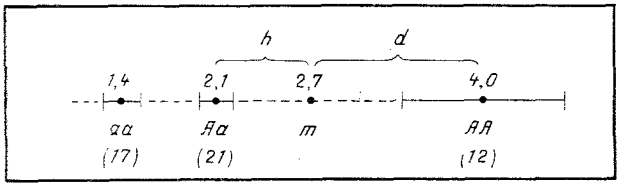

Fig. 1. Scale of distribution of F_2 hybrids by intensity of IL 2 production. *AA*) Homozygotes with high IL 2 production, *aa*) homozygotes with low IL 2 production; *Aa*) heterozygotes, *m*) mean; *h*) distance from mean to heterozygotes; *d*) distance from mean to homozygotes. Numbers above scale correspond to intensity of IL 2 production (in relative units). Continuous line — confidence intervals at $p < 0.05$. Numbers in parentheses correspond to number of F_2 hybrids.

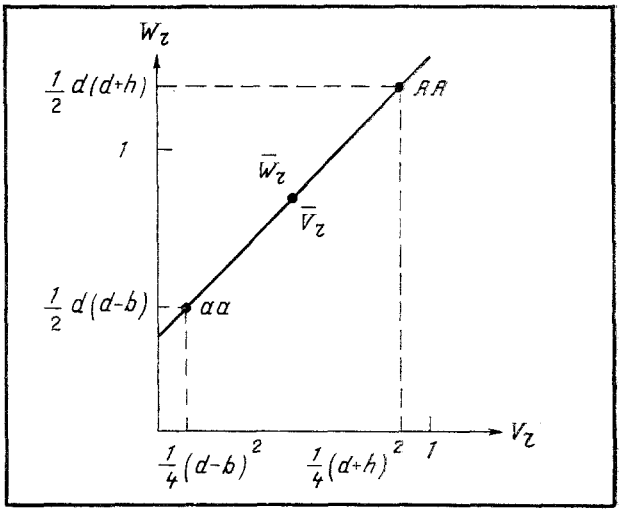

Fig. 2. Graph of correlations between variance (*Vr*) and covariance (*Wr*) for F_2 hybrids. Position of *aa* on regression line lower than that of *AA* indicates that aa is the dominant allele.

IL 2 and CAS production in the same animal did not always coincide, although when the hypothesis of the independent distribution of these traits was tested by the chi-square method, statistically significant correlation could be found between them ($0.01 < p < 0.05$). The distribution of H-2^b, H-$2^{b/d}$, and H-2^d haplotypes and also of such traits as CAS and the intensity of IL 2 production (high, average, low) among the F_2 hybrids lay within the limits of the ratio 1:2:1 (Fig. 1). Testing

the initial hypothesis on connection of the H-2 haplotype with different degrees of intensity of IL 2 and CAS production showed that these traits segregate independently in F_2 hybrids. Diallelic analysis, conducted by the method of Mather and links [7], showed that the F_2 hybrids could be subdivided into three significantly differing groups on the basis of the intensity of their IL 2 production (Fig. 1). The results obtained for groups of F_2 hybrids with low and high IL 2 production were virtually identical with the corresponding results for high- and low-responding parental lines (Table 1; Fig. 1). The results are in agreement with the hypothesis that a single gene controls the intensity of IL 2 production. Construction of a graph of correlation between variance (*Vr*) and covariance (*Wr*), calculated by the method of Mather and Jinks [7], showed that the dominant trait is a low level of IL 2 production (Fig. 2).

The use of methods of genetic analysis thus showed that CAS is largely connected with the presence of IL 2 in the supernatant. The data given above are in agreement with the hypothesis that a single gene controls the intensity of IL 2 production, and is also in agreement with similar results obtained on inbred rats [6], in which the regulator gene which was found likewise was not connected with the RT-1 system. Meanwhile in rats, unlike in mice, high IL 2 production is the dominant trait.

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