CONCANAVALIN A-INDUCED SPLENOCYTE PROLIFERATION IN MICE OF DIFFERENT STRAINS AND INTERLEUKIN 2 PRODUCTION

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The height of the proliferative response to T-cell mitogens is a parameter of immunoreactivity. We know that lymphoid cells of different strains of mice differ in their ability to proliferate in vitro in response to the addition of concanavalin A (con A) to the culture. For instance, mice of strains BALB/c, C3H, CBA, AKR, and DBA/1 have high-responding genotypes, whereas strains C57BL/6 and C57BL/10 are low-responding [5]. The intensity of the proliferative responses to mitogens depends on various factors, one of which may be interleukin 2 (IL 2).

The aim of this investigation was to discover correlation between the height of the proliferative response to con A of splenocytes from mice of different genotypes and the intensity of IL 2 production by these cells.

EXPERIMENTAL METHOD

Male mice weighing 20-24 g were used in the experiments from the following inbred strains: BALB/cJLacSto, DBA/2JSto, CC57BR/MvRap, C57BL/6JSto. The method of performing the blast transformation reaction to con A was described by the writers previously [3]. Cells were cultured in medium RPMI-1640 ("Flow Laboratores," Great Britain), containing 10% inactivated horse serum, 2×10^{-3} HEPES, 2.8×10^{-6} 2-mercaptoethanol, and 20 μg/ml of gentamicin. To determine the intensity of IL 2 production, 5×10^6 spleen cells (in a volume of 1 ml) were introduced into wells of a 24-well panel ("Nunc," Denmark), con A ("Calbiochem," USA) was added in a concentration of 40 μg/ml, and the mixture was incubated for 3 h at 37°C in a humid atmosphere containing 5% CO_2. The panel was then centrifuged at 400 g for 10 min, the supernatant was removed, the wells of the panel were filled with fresh medium, and incubation was repeated for 18 h. The supernatants were then collected and kept at -70°C. The IL 2 concentration in the supernatant was determined on the basis of its ability to maintain growth of an IL 2-dependent cytotoxic cell line (CTLL). For this purpose, cells of the CTLL in a concentration of 4×10^4 cells/ml were cultured in flat-bottomed 96-well panels ("Nunc") for 24 h in the presence of different dilutions of supernatant. Recombinant IL 2 ("Biogen" Minsk Research and Production Combine) in concentrations of 0.08 to 5 IU/ml was used as the control. ^{3}H-Thymidine was added in a dose of 40 kBq/well 4 h before the end of culture. The cells were transferred by means of a semiautomatic cell harvester ("Flow Laboratories") to filters, the radioactivity of which was determined in a Mark III liquid scintillation counter ("Tracor Analytic," USA). The titration curves thus obtained (for the supernatant and recombinant IL 2) were analyzed by the probit method [2, 3], after which IL 2 activity contained in the whole supernatant was subtracted. The efficiency of IL 2 utilization was estimated by the ability of recombinant IL 2 to induce a state of committedness in thymocytes from mice of different strains cultured in the presence of phytohemagglutinin (PHA, from "Sigma," USA). Thymocytes (a cell pool from five animals) were cultured in flatbottomed 96-well panels in a concentration of 5×10^6 cells/ml in a volume of 200 μg in the presence of 2.5 μg/ml PHA. IL 2 was added in doses of between 5 and 90 IU/ml. The proliferative response was assessed on the basis of incorporation of ^{3}H-thymidine. To estimate the efficiency of IL 2 utilization the area under the titration curve was expressed in relative units, taking the value characteristic of the strain of mice with the minimal committedness-inducing action of IL 2 as one unit. Differences between the groups were evaluated by Wilcoxon's test for independent sets [1].

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TABLE 1. Proliferative Response of Splenocytes from Mice of Different Strains to Con A

H-2 haplotype	$H\text{-}2^d$		$H\text{-}2^b$	
Line	BALB/c[1]	DBA/2[2]	CC57BR[3]	C57BL/6[4]
Response to con A, cpm	133 158	114 341	98 147	109 326
	130 031	87 513	32 843	45 272
	154 000	89 230	35 268	51 549
	160 355	151 696	72 726	108 364
	152 534	100 163	64 304	85 528
Arithmetic mean	146 015	108 587	60 657	80 008
Conventional units	2,4	1,8	1	1,4

Legend. $p_{1.3}$ M 0.01; $p_{1.4} = 0.01$; $p_{2.3} = 0.05$; $p_{2.4} > 0.05$; $p_{db} < 0.01$.

TABLE 2. IL 2 Production by Spleen Cells of Different Strains of Mice

H-2 haplotype	Line	IL 2 production in different experiments, IU/ml					Arithmetic mean	Relative units
		1	2	3	4	5		
$H\text{-}2^d$	BALB/c	40	28	53	59	37	43	4,3
	DBA/2	—	—	—	24	50	37	3,7
$H\text{-}2^b$	CC57BR	—	—	—	5,3	14	10	1,0
	C57BL/6	15	11	—	15	26	17	1,7

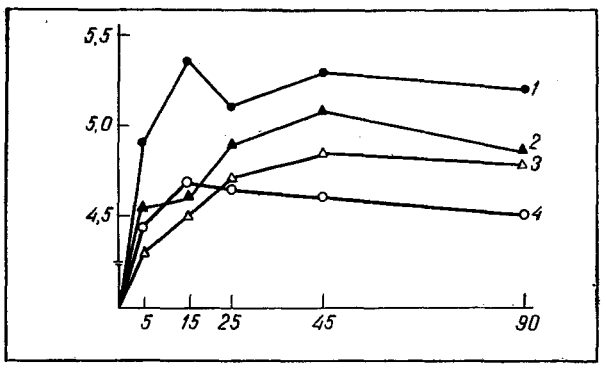

Fig. 1. Committedness inducing action of IL 2 on thymocytes from different strains of mice. Abscissa, doses of IL 2 (in IU/ml); ordinate, height of proliferative response of thymocytes (log cpm); 1) BALB/c; 2) C57BL/6; 3) DBA/2; 4) CC57BR.

EXPERIMENTAL RESULTS

Strains of mice can be arranged in the following order of increasing intensity of their proliferative response to the optimal dose of con A (40 μg/ml): CC57BR, C57BL/6, DBA, and BALB/c (Table 1). Despite the difference in height of the induced proliferative response, the level of spontaneous proliferation in the mice of these strains was virtually identical and did not exceed 500 cpm. The ability of all the strains tested to give a proliferative response to con A can be expressed in conventional units. The proliferative activity of the lowest responders is taken to be 1 unit (Table 1). The intensity of the proliferative response in BALB/c mice was statistically significantly higher than in

TABLE 3. Comparison of Height of Proliferative Response with Production and Efficiency of Utiliziation of IL 2 by Mice of Difference Strains

H-2 haplotype	Line	IL 2 production, relative units	Efficiency of IL 2 utilization, relative units	IL 2 production + efficiency of IL 2 utilization	Proliferative response to con A, relative units
$H\text{-}2^d$	BALB/c	4,3	3,1	7,4 (3,5)	2,4
	DBA/2	3,7	1,1	4,8 (2,3)	1,8
$H\text{-}2^b$	CC57BR	1,0	1,1	2,1 (1,0)	1,0
	C57BL/6	1,7	1,7	3,4 (1,6)	1,4

Legend. Relative units obtained after addition taking minimal value obtained as 1, given in parentheses.

CC57BR and C57BL/6 mice. The height of the proliferative response in DBA/2 mice differed significantly only from that in CC57BR mice. If the results obtained for CC57BR and C57BL/6 mice ($H\text{-}2^b$ haplotype) are added together and compared with the added data for BALB/c and DBA/2 mice ($H\text{-}2^d$ haplotype), the differences are highly significant ($p < 0.01$). Thus within the range of inbred lines which we investigated, animals with the $H\text{-}2^d$ haplotype can be classed as high responders, those with the $H\text{-}2^b$ haplotype as low responders.

The study of IL 2 production in mice of different strains showed that spleen cells of BALB/c and DBA/2 mice ($H\text{-}2^d$ haplotype) are good IL 2 producers, whereas spleen cells of C57BL/6 and CC57BR mice ($H\text{-}2^b$ haplotype) produce significantly smaller amounts of IL 2 (Table 2). Under these circumstances IL 2 production by cells of mice with the $H\text{-}2^d$ haplotype was statistically significantly higher than in mice with the $H\text{-}2^b$ haplotype ($p < 0.01$).

Induction of the proliferative response by mitogens is connected not only with stimulation of IL 2 production, but also with synthesis of the corresponding membrane receptors, whose interaction with IL 2, together with membrane and intracellular processes arising as a result, also evidently make a definite contribution to genetically determined responsiveness to T-cell mitogens. This whole group of processes we conventionally defined as the "efficiency of IL 2 utilization" and investigated it on a model of committedness-inducing action of IL 2 on thymocytes of mice of the same four strains. The results of one of two analogous experiments are given in Fig. 1. They show that the efficiency of IL 2 utilization was greatest in the case of BALB/c mice, whereas in mice of the other strains (especially DBA/2 and CC57BR), these processes were much weaker.

When the results are analyzed it will be clear that the highest-responding mouse strain (BALB/c) is distinguished by the highest values both of IL 2 production and of the efficiency of its utilization. In mice of the lowest responding strain (CC57BR) these parameters were correspondingly the lowest. In DBA/2 mice, distinguished by relatively high production and low efficiency of utilization of IL 2, the intensity of the proliferative response to con A occupied an intermediate position. C57BL/6 mice, in which relatively weak IL 2 production was found, together with relatively low efficiency of its utilization (both parameters, however, were higher than in CC57BR mice), may also be classed as low responders, although their ability to give a proliferative response to the mitogen is higher than that of CC57BR mice. These arguments are illustrated in Table 3, in which parameters characterizing production and efficiency of utilization of IL 2 are added together.

The results described above show that the height of the response to T-cell mitogens of different strains of mice is largely connected with the intensity of IL 2 production. Meanwhile, there exists another group of factors, which we conventionally describe as the efficiency of IL 2 utilization. These factors also make a contribution of their own to the intensity of the proliferative response.

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