SELECTIVE REACTION OF EFFECTOR LYMPHOCYTES OF TRANSPLANTATION IMMUNITY TO PRIVATE SPECIFICITIES OF THE H-2K AND H-2D LOCI

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UDC 612.6.02.017.1:612.112.94

The use of three tests (the direct cytotoxic action of immune lymphocytes on target cells, the specific adsorption of lymphocytes on target cells, and the accelerated rejection of a skin graft) showed that the receptors of the effector lymphocytes do not identify general (public) specificities, but react only to special (private) H-2 specificities. Immunization with two private H-2 specificities induces two corresponding populations of effector lymphocytes in unequal proportions: the larger against the H-2K private specificity, the smaller against the H-2D private specificity. It is postulated that the private H-2 specificity is a configuration structure formed by the combination of the public H-2 determinants similar to the "carrier structure" or closely linked with it.

Effector lymphocytes of transplantation immunity react only to the whole complex of H-2 immunizing antigens determined by the H-2K or H-2D loci but, unlike antibodies, they do not identify it by its parts. These facts, established both by the reaction between lymphocytes and target cells in vitro [2-4] and during rejection of skin in vivo [5] have led to the conclusion that the receptors of the effector lymphocytes are complex in structure and that their precursors are polyspecific [6]. This conclusion was based on the assumption that all the H-2 specificities are equally important. However, it has recently been shown that they are divided into two categories: "private" – with one specificity for each of the H-2 loci (K and D) in a given H-2 haplotype (set), for which they are strictly characteristic, and "public" – which can be found in different haplotypes, are coded by both H-2 loci and, possibly, reflect crossed reactivity between the private specificities [13]. Despite the fact that both private and public H-2 specificities induce antibody formation, their role in the development of cellular immunity may not be identical.

The object of the present investigation was to study which H-2 specificities (private or public) are identified by effector lymphocytes of transplantation immunity, and to what extent this takes place.

EXPERIMENTAL METHOD

Mice of congeneic lines (C57BL/10ScSn(H-2^b) (abbreviated to B10) and C57BL/10-H-2^d (abbreviated to B10 · D2) were obtained from the nursery of the N. F. Gamaleya Institute of Epidemiology and Microbiology. Mice of congeneic lines with recombinant haplotypes H-2-B10 · A(H-2^a), B10 · D2(R107), abbreviated to R 107, (H-$2^{i\text{-}Eg}$), B10 · D2(R101), abbreviated to R101, and (H-$2^{g\text{-}Eg}$), B10 · A(2R), abbreviated to 2R, and (H-$2^{h\text{-}Eg}$) were reared in the Laboratory of Genetics of Tissue Compatibility, Institute of General Genetics. The H-2 genotypes of these lines are given in Table 1 in accordance with the data of Vedernikov and Egorov [7] and Klein [13]. The mice were used in the experiments at the age of 10-16 weeks.

Laboratory of Immunochemistry and Diagnosis of Tumors, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR. Laboratory of Genetics of Tissue Compatibility, Institute of General Genetics, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 77, No. 6, pp. 69-73, June, 1974. Original article submitted August 13, 1973.

TABLE 1. Cytotoxic Effect of B10 · D2 Anti-B10 Lymphocytes on Target Cells Containing Private or Public Specificities of H-2K and H-2D Loci

Source and genotype of H-2 target cells	Antigens*	No. of lympho-cytes ($\times 10^6$)	Cytotoxic effect (in %)‡			Mean†
			expt. No. 1	expt. No. 2	expt. No. 3	
B10 (K^bD^b)	2; 5; 33	8 4 2	91,0 68,9 30,6	83,8 77,6 NT	94,0 64,0 42,4	79,9 (100)
B10·D2 (K^dD^d)	None	8	NT	—1,5	4,7	1,6 (2,0)
B10·A(K^kD^d)	5	8 4	—31,5 —17,5	0,8 NT	—11,1 NT	—14,8 (0)
R107 (K^bD^d)	5; 33	8 4 2	64,2 27,5 NT	75,3 62,3 NT	77,3 38,7 19,7	57,6 (72,1)
R101 (K^dD^b)	2	8 4	23,5 2,1	34,5 5,7	46,9 12,8	20,9 (26,2)
R107 — R101 3:1	5; 33+2	8 4 2	95,0 NT NT	79,1 77,7 NT	90,6 66,0 41,1	81,7 (>100)
R2 (K^kD^b)	2; 5	8 4	NT NT	55,7 27,6	46,0 28,1	39,4 (49,3)

*H-2 specificities potentially capable of participating in the reaction: 2 and 33 – private specificities of genes H-2D^b and H-2K^b, respectively, 5 – public specificity.

†Results obtained with doses of lymphocytes of $8 \cdot 10^6$ and $4 \cdot 10^6$ were considered; relative cytotoxic activity of lymphocytes (in %) shown in parentheses.

‡NT – not tested.

The tumor, an ascites form of sarcoma MCh11, induced by methylcholanthrene in B10 mice, was maintained by weekly passage.

The B10 · D2 mice were immunized with washed cells of sarcoma MCh11 by single injections at six points ($40 \cdot 10^6$–$50 \cdot 10^6$ cells per mouse). Cells of the regional (normal in the control) lymph glands were obtained 8 days after immunization. Peritoneal macrophages grown for 2 days as an incomplete monolayer served as the source of target cells. The methods of obtaining the cell suspensions, of determining their viability, and of growing the target cells and the experimental conditions for studying the cytotoxic action of the lymphocytes and for counting them were fully described previously [1-3]. Adsorption of the lymphocytes by target cells was carried out twice, for 3 h at 30°C each time [2, 4]. The adsorption index (AI) was calculated by the equation

$$AI = \frac{a - b}{a} \times 100,$$

where a is the cytotoxic action of the intact lymphocytes and b is that of the lymphocytes unfixed on adsorption. The time for rejection of skin grafts from the tail of four donors on one recipient [9] was determined macroscopically.

Plan of the Experiments. B10 · D2 anti-B10 lymphocytes, theoretically directed against three H-2 specificities – two private, coded by the H-2K^b locus (specificity 33) and the H-2D^b locus (specificity 2), and one public locus (specificity 5)* – were brought into contact with various target cells containing the following specificities: 5 (line B10 · A), 2 (line R101), 5 and 33 (line R107), and 2 and 5 (line 2R), and also with a mixture of R107 and R101 target cells (Table 1). The reaction with target cells of the donor (B10)

*Public specificity 39, also found in B10 mice but absent in B10 · D2 mice, was not taken into account in these experiments for it is weak and is found irregularly [12].

TABLE 2. Rejection of Skin Grafts from Mice of Different Genotypes on B10 · D2 Mice Immunized with B10 Spleen Cells*

Donor of graft	Recipients	No. of B10 · D2 recipients	Mean survival time of graft, in days (M ± m)	P
B10	Normal Immune	9 10	9,7±0,33 6,0±0	<0,001
B10·A	Normal Immune	8 8	9,6±0,42 8,5±0,5	>0,05
R107	Normal Immune	8 10	9,3±0,6 6,7±0,3	<0,001
R101	Normal Immune	8 10	12,4±0,56 7,9±0,66	<0,001

*$5 \cdot 10^6$ cells injected intraperitoneally 7 days before skin grafting.

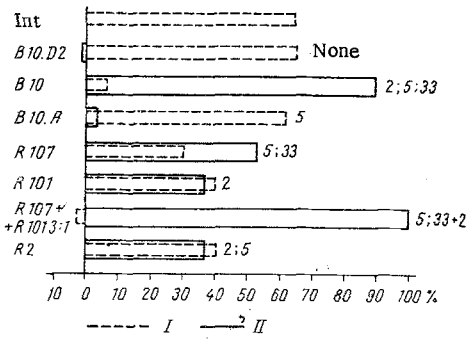

Fig. 1. Adsorption of B10 · D2 anti-B10 lymphocytes by target cells containing private (2 or 33) or public (5) specificities of H-2K^b and H-2D^b genes. Abscissa, cytotoxic action on B10 macrophages (I) and adsorption index (II) of target cells of these lines of mice. Int) Intact immune lymphocytes. Number of lymphocytes during adsorption $3 \cdot 10^7$, in the cytotoxic action test $4 \cdot 10^6$. Numbers on the right show H-2 specificities theoretically participating in the reaction (Table 1).

and recipient (B10 · D2) was used as the positive and negative control, respectively. In the experiments in vivo, skin from mice of 4 lines (B10, B10 · A, R107, and R101) was grafted on to B10 · D2 mice immunized with B10 spleen cells (Table 2).

EXPERIMENTAL RESULTS

In all the doses used (8, 4, and $2 \cdot 10^6$; Table 1) the immune lymphocytes destroyed the target cells of the corresponding donor (B10) but did not affect the target cells of the recipient (B10 · D2) and of a third line B10 · A (reaction on account of specificity 5 only) in agreement with previous observations [2]. However, the same lymphocytes in the same experiments had a partial cytotoxic action on target cells of recombinant lines R107, R101, and 2R. It is clear from Table 1 that if the magnitude of the cytotoxic action on B10 target cells is taken as 100%, the cytotoxic action on R107 and R101 target cells was about 75 and 25%, respectively, i.e., in the ratio of 3 : 1. Since specificity 5 does not take part in the reaction it must be assumed that 75% of the activity of B10 · D2 anti-B10 lymphocytes is directed against private specificity 33 and 25% against private specificity 2. This was confirmed by the fact that the cytotoxic action of these same lymphocytes on a mixture of R107 and R 101 target cells taken in the ratio of 3 : 1 also exceeded 100%. The 2R target cells were destroyed in rather larger numbers than the R101, on account of the greater activity of a small dose of lymphocytes.

Similar results were obtained in experiments in which the same lymphocytes were adsorbed by the same target cells. It will be clear from Fig. 1 (the results of a typical experiment are given) that the cytotoxic action, amounting to 64%, was almost completely suppressed (AI 90%) by adsorption with B10 target cells, was not reduced after adsorption with B10 · D2 target cells, and was reduced by only 3.4% after adsorption with B10 · A target cells (difference between adsorbing activities of B10 · D2 and B10 · A target cells not significant: $P > 0.1$). Conversely, after adsorption of the lymphocytes by R107, R101, and 2R target cells the cytotoxic action was reduced by 53, 37.2, and 36.9% ($P < 0.01$ in all cases), corresponding to 60, 40, and 40% of the AI of the B10 target cells. Adsorption with a mixture of R107 and R101 target cells completely suppressed the activity of the lymphocytes (AI = 100%).

These results show that the receptors of the effector lymphocytes responsible for their specific attachment to the target cells do not identify the public specificities but react only with the private H-2 specificities; each of the lymphocyte populations, moreover, identifies only one of these specificities – either H-2K or H-2D. Immunization with two private H-2 specificities induces two corresponding lymphocyte populations in unequal proportions: the larger number directed against private specificity H-2K, the

smaller number against private specifity H-2D. This interpretation agrees fully with the results obtained previously in a different system of lines of mice [4].

The importance of this conclusion is confirmed by experiments to study skin grafting in vivo on B10·D2 mice previously immunized with a small dose of B10 spleen cells. Immunization in this way induces accelerated rejection of skin grafts not only of the donor's line (B10), but also of the recombinant lines R107 and R101, carrying single private specificities – H-2K^b and H-2D^b, respectively. Conversely, acceleration of rejection of a B10·A skin graft, with only public specificity H-2, was not significant (Table 2). Similar results were obtained previously by skin grafting within another system of lines [5].

The selective direction of cellular immunity against private H-2 specificities may be connected with the fact that these specificities constitute a configuration structure formed on the surface of the target cell by a particular combination of public H-2 determinants and performing the function of a "carrier structure," which is identified by the receptors of the T-lymphocytes [14]. Evidence in support of this possibility is given by the results of a study of mutant H-2 haplotypes [8]. Meanwhile the possibility cannot be ruled out that the receptors of the effector lymphocytes identify, not the private H-2 specificity, but a chemically distinguishable "carrier structure" linked with it that does not induce antibody formation [15]. This structure can be coded either by H-2 genes (and forms part of the H-2K and H-2D molecule) [8] or by another gene closely linked with H-2K [10]. In the last two cases cellular immunity may arise in the absence of antibody formation given complete identity of the hapten determinants coded by the H-2K and H-2D loci [11, 16].

The authors are grateful to Professor G. I. Abelev and to G. I. Drizlikh for their critical discussion of the research, to G. N. Vornakova for specialized technical assistance, and to N. N. Medvedev for providing the B10 and B10·D2 mice. The work was partly subsidized by the World Health Organization.

LITERATURE CITED

1. B. D. Brondz, Vopr. Onkol., No. 8, 64 (1964).
2. B. D. Brondz, Folia Biol. (Prague), 14, 115 (1968).
3. B. D. Brondz, N. E. Gol'dberg, and G. N. Vornakova, Tsitologiya, No. 11, 1411 (1969).
4. B. D. Brondz and A. E. Snegireva, Immunology, 20, 457 (1971).
5. B. D. Brondz, Byull. Éksperim. Biol. i Med., No. 12, 61 (1972).
6. B. D. Brondz, Transplant Rev., 10, 112 (1972).
7. A. A. Vedernikov and I. K. Egorov, Genetika, No. 2, 60 (1973).
8. I. K. Egorov, Immunogenetics, 1, 415 (1974).
9. L. A. Zil'ber, O. M. Lezhneva, and B. D. Brondz, Byull. Éksperim. Biol. i Med., No. 11, 91 (1964).
10. F. Bach, M. B. Widmer, M. Segall, et al., Science, 176, 1024 (1972).
11. G. Berke and D. B. Amos, Nature New Biol., 242, 237 (1973).
12. P. Demant, M. Cherry, and G. D. Snell, Transplantation, 11, 238 (1971).
13. J. Klein, Transplant. Proc., 5, 11 (1973).
14. N. A. Mitchison, Europ. J. Immunol., 1, 18 (1971).
15. G. Senyk, D. Nitecki, and J. W. Goodman, Science, 171, 407 (1971).
16. M. B. Widmer, B. J. Alter, F. H. Bach, et al., Nature New Biol., 242, 239 (1973).