CROSS REACTIONS IN THE H-2 SYSTEM OF CYTOTOXIC T-LYMPHOCYTES CONCENTRATED BY ADSORPTION-ELUTION ON A TARGET CELL MONOLAYER

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UDC 612.112.94.017.1

Cytotoxic T-lymphocytes (CTL) obtained by immunization in vivo were concentrated by adsorption-elution on a monolayer of corresponding allogeneic target cells (TC). C57BL anti-A^k (anti-K^kD^d) lymphocytes, after separation into anti-K^k and anti-D^d populations, had a crossed cytotoxic effect on H-2^d and H-2^k TC respectively. B10.D2 anti-B10 (anti-K^bD^b) lymphocytes cross-reacted with TC of the H-2^a and H-2^g haplotypes. The results are discussed in terms of views regarding the heterogeneity of clones of CTL or their receptors.

KEY WORDS: cytotoxic T-lymphocytes; H-2 histocompatibility complex; receptors of T-lymphocytes.

Cytotoxic T-lymphocytes (CTL), like antibodies against H-2 antigens, react mainly with gene products of two regions (K and D) of the principal histocompatibility complex [8]. The problem of whether the determinants of these products identified by CTL (CTL-determinants) are identical with serologically determined H-2 specificities is not yet clear. Each H-2K (or H-2D) molecule contains a set of serologically determined specificities (one partial and several general) [10]. By contrast with antibodies, CTL induced by an allograft in vivo have been shown to cause lysis of target cells (TC) containing only partial specificities, but not to cause lysis of TC containing general specificities of the H-2 immunizing complex [4, 9]. By adsorption on a TC monolayer, strict selectivity of the receptors of each CTL population toward the partial specificity H-2K (or H-2D) or toward the determinant closely linked with it, coded by the gene of the same K (or D) region [4], has been established. However, lymphocytes containing high proportions of CTL induced in vivo [11] or in mixed cultures in vitro [12] caused weak but significant lysis of foreign TC not carrying special specificities of the H-2 immunizing complex.

In this work, we studied the transferred specificity of immunized lymphocytes using enriched anti-K^k, anti-D^d, and anti-K^bD^b CTL with the aid of their adsorption-elution on a monolayer of various TC.

EXPERIMENTAL METHOD

Mice of strains C57BL/10, abbreviated to B10 (K^bD^b), A/f (K^kD^d), C3H (K^kD^k), and B10.D2, abbreviated to D2 (K^dD^d), were obtained from the nursery of the N.F. Gamaleya Institute of Epidemiology and Microbiology. Mice of strains B10.A (K^kD^d), DBA/1 (K^qD^q), and recombinant strains R101 (K^dD^b) and R107 (K^bD^d) were maintained in the Laboratory of Genetics of Tissue Compatibility, Institute of General Genetics. Ascites forms of sarcomas Sal and MKh11, induced in mice of strains A and B10 respectively, were maintained by regular passages. CTL were obtained by a single immunization in vivo by the method described in [2]. The cytotoxic test was set up in the microvariant [6] of the corresponding method [7] using peritoneal macrophages labeled with ^{51}Cr as TC. Adsorption of CTL on a monolayer of macrophages and subsequent elution of the CTL with pronase were carried out by the method described in [5].

EXPERIMENTAL RESULTS

When using a B10 anti-A (anti-K^kD^d) system the basic assumption was that during immunization through the whole H-2 barrier two independent and, evidently, nonoverlapping populations of anti-K^k and anti-D^d CTL [3], which could be separated from one another by adsorption-elution on the corresponding TC, and then tested

Laboratory of Immunochemistry and Diagnosis of Tumors, Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 2, pp. 176-179, February, 1979. Original article submitted March 29, 1978.

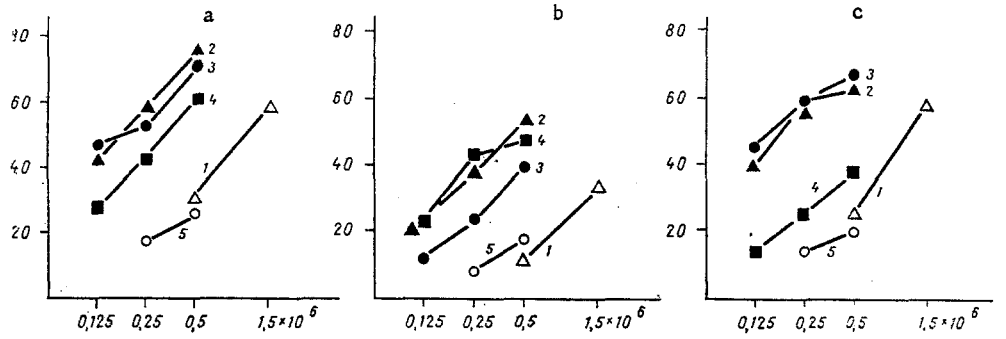

Fig. 1. Cytotoxic effect of eluted B10 anti-A lymphocytes on A (a), D2 (b), and C3H (c) target cells. 1) Original CTL; CTL eluted from TC; 2) A; 3) C3H; 4) D2; 5) B10. Ordinate, cytotoxic effect, %; abscissa, dose of CTL.

for cross-reactivity with H-2D and H-2K antigens. It will be clear from Fig. 1 that activity of the B10 anti-A CTL, eluted from A, C3H, and D2 TC (anti-K^kD^d, anti-K^k, and anti-D^d CTL respectively) was 6-7 times stronger than the activity of the original CTL when tested on the TC from which they had been eluted, if lysis and the shift of the lysis curves into the region of lower values of doses of immune lymphocytes are judged to be 30% or 50%. The increase in activity of the eluted CTL was specific, for on elution from the monolayer of TC of the B10 recipient no increase in activity took place (Fig. 1a, c) or the increase was very small (Fig. 1b).

If CTL populations uncrossed for specificity were formed against products of the K^k and D^d values, only anti-K^k and anti-D^d CTL populations respectively ought to be adherent to and subsequently eluted from the monolayer. However, in reality, anti-K^k CTL isolated by elution from a C3H monolayer were found to have a cytotoxic action on D2 TC (Fig. 1b), which was about three times stronger than the lytic action of CTL eluted from B10 TC (control), although it was significantly weaker than the action of CTL eluted from A and D2 TC. In the same way anti-D^d CTL, isolated by elution from a D2 TC monolayer, destroyed C3H TC (Fig. 1c), although to a much weaker degree than CTL eluted from C3H and A TC. It will be clear from Table 1 that the magnitude of the direct cytotoxic effect of the eluted CTL on those TC (C3H and D2) from which the CTL had been eluted was about twice as strong as cross-lysis of TC caused by the same eluted CTL. This cross-lysis was not due to nonspecific adsorption of CTL on the TC monolayers; it was 2.1-2.5 times stronger than the cytotoxic activity of lymphocytes eluted from syngeneic B10 TC (Table 1).

Similar results were obtained by the use of D2 anti-B10 (anti-K^bD^b) CTL. It will be clear from Fig. 2 that these CTL caused lysis, besides of B10 TC, only of R107 TC, carrying the same K^b allele as the B10 strain. Conversely, B10.A and DBA/1 TC, carrying foreign H-2 alleles, were not destroyed by the original CTL. After concentration of the CTL by adsorption-elution on a B10 TC monolayer their activity was increased fourfold against B10 and R107 TC, and also against R101, carrying the D^b allele of strain B10. The eluted CTL were found to cause lysis also of B10.A and DBA/1 TC, although to a much weaker degree than B10 and R107 TC. This cross lysis was specific, for the same eluted CTL did not cause lysis of syngeneic D2 TC (Fig. 2).

CTL isolated by adsorption-elution, specific for the products of one H-2 allele, or concentrated CTl against products of both H-2 alleles thus caused lysis of foreign TC, differing in their H-2 alleles from the immunizing cells. Cross lysis may be due to clonal heterogeneity of the CTL: Although most CTL carry receptors selectively reacting with the partial H-2 specificity or the determinant linked with it (see the introductory remarks), a minority, possessing receptors against common H-2 specificities, was detected only after concentration of the CTL. For instance, eluted D2 anti-B10 CTL (Fig. 2), after contact with A TC, could react with the H-2.5 specificity, whereas after contact with DBA/1 TC they could react with H-2.5, 54, and 56 specificities. However, when CTL immunized in vitro were used, the degree of cross lysis did not correlate with the number of identical (common) H-2 specificities potentially capable of participating in the reaction with the given foreign TC, and this lysis took place even when no such identity was present whatsoever [12]. Meanwhile serologically "silent" determinants could be the object identified by CTL, for each H-2 K/D gene may control a set of partially overlapping CTL-determinants in the products of different H-2 alleles. In this case the clonal heterogeneity of CTL may be associated with the multiplicity of CTL-determinants, and their cross reactivity may be linked with a cross between H-2 alleles with respect to sets of CTL-determinants.

TABLE 1. Cross Cytotoxic Effect of B10 Anti-A (anti-K^kD^d) Lymphocytes Fractionated by Adsorption-Elution into Anti-K^k and Anti-D^d Populations

Lymphocytes eluted from TC	Dose of cells $\times 10^6$	Cytotoxic effect on TC (M ± m for 3 experiments)			
		A	C3H	D2	C3H/D2
A	0,25	44±6	40±10 (4,0)	42±10 (2,8)	0,95
	0,5	61±6	59±4 (5,4)	61±11 (3,8)	0,97
C3H	0,25	32±12	41±14 (4,1)	19±2 (1,3)	2,16
	0,5	45±12	49±19 (4,5)	34±6 (2,1)	1,44
D2	0,25	24±9	14±5 (1,4)	38±3 (2,5)	0,37
	0,5	47±10	27±6 (2,5)	56±4 (3,5)	0,48
B10	0,25	9±4	10±6	15±3	0,67
	0,5	17±5	11±5	16±1	0,68
Not fractionated	1,6	39±11	36±14	28±3	1,29

Note. Enrichment index of cytotoxic lymphocytes – ratio of cytotoxic effects of lymphocytes eluted from TC of specified strain compared with those eluted from B10 TC – shown in parentheses.

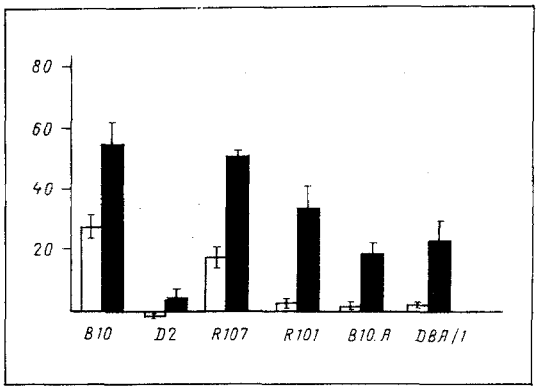

Fig. 2. Cytotoxic effect of D2 anti-B10 (d anti-b) lymphocytes on TC of various H-2 haplotypes. Unshaded columns: original immune lymphocytes in dose of 10^6; shaded columns: eluted immune lymphocytes in doses of $5 \cdot 10^5$ for B10, D2, R107, and R101 TC or 10^6 for B10.A and DBA/1 TC. Ordinate, cytotoxic effect, %; abscissa, strain of mice (source of TC).

The alternative possibility is that cross reactivity of CTL is connected with heterogeneity, not of clones, but of cell receptors of the same clone, for example, differences between them as regards affinity. This suggestion is based on the fact that for each H-2 allele there is one corresponding CTL-determinant, inducing one clone of CTL that is homogeneous for specificity but heterogeneous for the affinity of its receptors. Heterogeneity for affinity may be the reason why only a small proportion of CTL, directed against the product of the normal K^b allele, reacts with the product of the mutant allele K^{ab} [1,13]. This alternative is being investigated by the writers by the adsorption-elution method.

The authors are grateful to I. K. Egorov for providing mice of the recombinant strains and to G. N. Vornakova for expert assistance. The work was partly subsidized by the World Health Organization.

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REPLACEMENT OF THE HELPER FUNCTION OF T-CELLS BY RNA-CONTAINING ANTIGEN-SPECIFIC LYSIS FACTOR

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UDC 612.112.94.017.1

The supernatant obtained after centrifugation of a suspension of viable lymph node cells from immunized mice was chromatographed on Sephadex G-200 and the fractions were deproteinized. The third fraction (molecular weight 30,000 daltons) specifically stimulated antibody formation in intact mice immunized with sheep's red cells and restored ability to form antibodies in lethally irradiated intact mice protected with syngeneic bone marrow. The activity of this fraction disappeared after treatment with RNase but not with DNase or trypsin. The first and second deproteinized fractions of supernatant of the suspension of viable lymph node cells from immunized animals nonspecifically inhibited antibody formation in intact mice immunized with sheep's red cells.

KEY WORDS: helper T-lymphocytes; antigen-specific RNA-containing factor.

During immunogenesis substances containing antigen-specific information and capable of influencing the development of the immune response in intact animals appear in the lymphoid tissue. RNA preparations capable of inducing antibody synthesis both in vitro and in vivo have been isolated from extracts of lymphoid tissue of immunized animals [8-10,14]. On the other hand, antigen-specific [13,19] and antigen-nonspecific [15,20] factors replacing the helper function of T-cells in antibody formation have been isolated from the supernatant of lymphocyte cultures.

The writers showed previously [1,2,6] that the supernatant obtained after centrifugation of a suspension of viable lymph node cells from immunized animals contains an RNA-containing factor capable of inducing sensitivity to lysis by specific antigen in the lymph node and thymus cells of intact animals. The object of the present investigation was to study the effect of this factor and of other deproteinized preparations from such a supernatant on the primary immune response of mice to sheep's red cells.

EXPERIMENTAL METHOD

CBA mice weighing 16-18 g were immunized subcutaneously in the inguinal region with sheep's red cells in a dose of 200-300 million cells or with bovine serum albumin (BSA) in a dose of 0.5-1.0 mg per mouse. On the 8th day after immunization, when a considerable quantity of RNA-containing factor had accumulated in the lymph nodes [1,2], the regional lymph nodes were removed and a cell suspension ($6-9 \cdot 10^8$ cells/ml) prepared from them in Hanks' solution (90-95% of the cells were viable). The suspension was centrifuged at 6000g for 15 min and the resulting supernatant dialyzed at 4°C against 0.0175 M Na-phosphate buffer, pH 6.5. The

Department of Morphology and Immunology, Central Research Laboratory, Vitebsk Medical Institute.
(Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 2, pp. 179-182, February, 1979. Original article submitted March 30, 1978.

0007-4888/79/8702-0164$07.50