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INTERACTION BETWEEN ANTI-K^b AND ANTI-D^d EFFECTOR LYMPHOCYTES AND TARGET CELLS OF MICE OF MUTANT HAPLOTYPES

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The cytotoxic action of anti-K^b (C57BL/6) and anti-D^d (B10.D2) immune lymphocytes on target cells of K^{ba} (Hz1) and K^{bd} (M505) mutants and also on D^{da} (M504) target cells was greatly reduced compared with their cytotoxic action on target cells of the original C57BL/6 and B10.D2 strains, respectively. The decrease in cytotoxic action was more marked on H(z1) and M504 than on M505. By adsorption on a monolayer of mutant target cells, monospecific anti-K^b and anti-D^d lymphocytes can be subdivided into subpopulations, one of which reacts only with target cells of the original strain, the other with target cells of both the original and mutant strains.

KEY WORDS: H-2 histocompatibility complex; mutations of the H-2 complex; alloantigenic specificities; receptors of effector lymphocytes.

The H-2K and H-2D loci of the basic histocompatibility complex of mice encode both special and general serologically determinable specificities (SDS) [15] and also determinants identifiable by the receptors of cytotoxic T lymphocytes (CTL), causing rejection of grafts *in vivo* and destruction of target cells *in vitro* [10]. It is not yet clear whether the SDS and the structures identified by the CTL are the same or different determinants. The writers showed previously that CTL receptors do not recognize the general but react either with the special SDS or with "CTL determinants" closely connected with them [6]. To study this problem further, it was decided to use mutants of the K^b allele, namely H(z1) (K^{ba}) and M505 (K^{bd}), derived from C57BL/6, and a mutant of the D^d allele, namely M504 (D^{da}), derived from B10.D2. In both cases reciprocal immunization between the original and mutant strains leads to graft rejection and induction of CTL [4, 9, 11, 12], despite the fact that special SDS of normal K^b (H = 2.33) and D^d (H = 2.4) haplotypes were preserved in the mutants, although evidently in somewhat reduced amounts [1, 17] or in a slightly modified form [8].

The object of this investigation was to study whether CTL directed against products of the normal K^b or D^d alleles can distinguish target cells of the original and mutant strains.

EXPERIMENTAL METHOD

Mice of strain C57BL/6 (K^bD^b) (abbreviation B6) and of the recombinant strain R101 (K^dD^b) and also of mutant strains M505 ($K^{bd}D^b$), H(z1) ($K^{ba}D^b$), and M504 (K^dD^{da}) were maintained in the Laboratory of Genetics of Tissue Compatibility, Institute of General Genetics. Mice of strain B10.D2(K^dD^d), abbreviation D2, and C57BL/10 (K^bD^b), abbreviation B10, were obtained from the nursery of the N. F. Gamaleya Institute of Epidemiology and Microbiology.

The ascites form of sarcoma MKh11 and the solid form of sarcoma MKh26, induced by methylcholanthrene in B10 and D2 mice, respectively, were maintained by regular passage.

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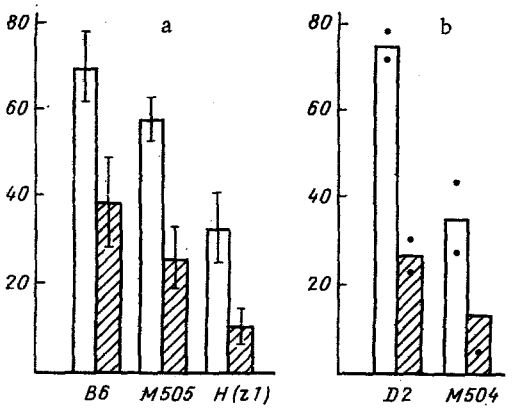

Fig. 1. Direct cytotoxic action of R101 anti-B6 (anti-K^b) lymphocytes (a) and R101 anti-D2 (anti-D^d) lymphocytes (b) on target cells of original and mutant strains. Unshaded columns represent dose of lymphocytes of $8 \cdot 10^6$ in macrotest and $2 \cdot 10^6$ in microtest; shaded columns represent dose of $4 \cdot 10^6$ in macro- and $1 \cdot 10^6$ in microtest. Vertical lines give confidence limits; points represent results of individual experiments. Ordinate, cytotoxic action (in %); abscissa, target cells of corresponding strains.

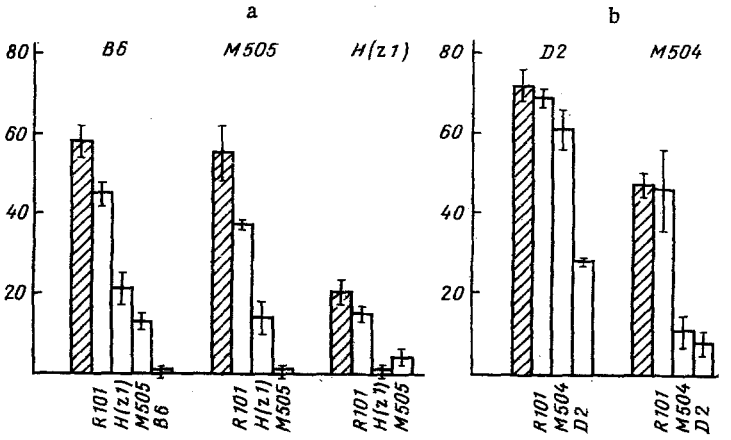

Fig. 2. Adsorption of anti-K^b (a) and anti-D^d (b) lymphocytes on target cells of original and mutant strains. Shaded columns represent intact immune lymphocytes. Ordinate, cytotoxic action (in %) of nonadherent lymphocytes in a dose of $2 \cdot 10^6$ on target cells of strains indicated on top of figure; abscissa, target strains taken for adsorption.

Cytotoxic T lymphocytes were obtained by immunization *in vivo* or *in vitro*. In the first case, R101 mice were immunized by a single injection of either sarcoma MKh11 (anti-K^b reaction) or sarcoma MKh26 (anti-D^d reaction) by the method described earlier [2]. To induce anti-K^b CTL *in vitro* a mixture of reacting R101 lymph node cells and stimulating irradiated (^{60}Co, 1000 rad) B6 spleen cells was incubated for 5 days [17]. Peritoneal macrophages labeled with ^{51}Cr and cultivated for 2 days as a discontinuous monolayer in Leighton's tubes (Gallenkamp, England) or in wells on microplates (Microplate II, No. 3040, Falcon Plastics, USA) were used as target cells in the cytotoxic test. In the first case the macrophages were seeded in a dose of $3 \cdot 10^5$–$4 \cdot 10^5$ in a volume of 1 ml (macrotest), in the second case $5 \cdot 10^4$ in a volume of 200 µl (microtest). The cytotoxic action (CA) was determined by the equation:

$$CA = \frac{R_{im} - R_{norm}}{R_{max} - R_{norm}} \times 100,$$

where R_{im}, R_{norm}, and R_{max} represent the radioactivity liberated from target cells after incubation for 15-20 h with immune and normal lymphocytes and with a 2% solution of sodium dodecylsulfate, respectively. Adsorption of CTL on a complete layer of macrophages was carried out once or twice, for 3 h each time, at 30°C [5].

EXPERIMENTAL RESULTS

Target cells of M505 and H(z1) mutants were much less sensitive to the cytotoxic action of R101 anti-B6 lymphocytes than target cells of the B6 strain (Fig. 1a). The difference in cytotoxic action was greater in the test on H(z1) target cells than on M505, and it was reproduced in all six experiments with immunization both *in vivo* and *in vitro* and with testing the cytotoxic action by both the macro- and the microtest. The same result was obtained in the D2-M504 combination: anti-D^d lymphocytes had a much weaker cytotoxic action on target cells of the M504 mutant than of the D2 strain (Fig. 1b). Unlike humoral antibodies, CTL, theoretically aimed against special SDS H-2.33(K^b) or H-2.4(D^d), clearly distinguish between target cells of the mutants and the original line and between the mutants themselves in their direct cytotoxic action, despite the fact that the corresponding special SDS in the mutants were preserved. Similar results obtained with H(z1) and HTG anti-K^b lymphocytes were published recently [18].

The difference in cytotoxic action on target cells of the original and mutant strains may be connected with the fact that the anti-K^b and anti-D^d CTL populations were heterogeneous and consisted of several subpopulations, one of which, for some reason or other, reacted less well with target cells of the mutants than with those of the original strain.

To test this hypothesis, the CTL were adsorbed on a monolayer of target cells of the original and mutant strains, and also of strain R101 (negative control). The results of one typical experiment (Fig. 2a) show that the cytotoxic action of anti-K^b lymphocytes on target cells of strains B6, M505, and H(z1) disappeared almost completely after adsorption by the corresponding target cells, but was reduced only very slightly after adsorption by R101 target cells, thus demonstrating that the conditions of adsorption were adequate. Cytotoxic T lymphocytes adsorbed on a monolayer of target cells of each of the mutants preserved some of their original cytotoxic activity against target cells of both the original B6 strain and of the other mutant (Fig. 2a). A similar result again was obtained in the D2-M505 combination: The anti-D^d subpopulation of CTL, not adherent to M504 target cells, lost its activity against the same mutant but preserved its activity against the original D2 strain (Fig. 2b).

It follows from analysis of the combined results of six experiments to study the adsorption of anti-K^b CTL and to test the cytotoxic action on B6 target cells that the adsorbing power of H(z1) target cells was only one-third that of the B6 target cells, and the M505 target cells occupied an intermediate position (Fig. 3, curve 1). Adsorption of CTL by a monolayer of target cells of each of the mutants also preserved 20-30% of the cytotoxic action against the other mutant (Fig. 3, curves 2 and 3). By contrast with humoral antibodies, CTL directed against the products of the alleles under investigation can thus be separated, with the aid of adsorption on target cells of the mutants, into two subpopulations, one of which, not adherent to target cells of the mutants, destroys only target cells of the original strain in the doses used. The second subpopulation, which is adherent to target cells of the mutants, can destroy target cells of both the mutant and the original strain.

The results are in harmony with the view that CTL receptors highly sensitive to changes in the configuration of the structure to be identified [3], react with a special SDS, constituting a conformational determinant [6]. The very slight change in this configuration resulting from a point mutation is difficult to identify by antibodies, for CTL receptors with high affinity for the normal special SDS inevitably distinguish its mutant configuration and react with it differently from the original strain. This same principle may be connected with the reaction of CTL against the special SDS modified by viruses [14] or haptens [19].

An alternative possibility is that the product of the normal K^b and D^d alleles contains not only SDS, but also several "CTL determinants" [10, 13], against which the CTL subpopulations discovered in these experiments are directed; one of them reacts with the "CTL determinant" preserved in the mutant, but the other, directed against the "CTL determinant" lost during mutation, reacts with target cells of the original strain but not of the mutant.

Another possibility is that several subpopulations of CTL heterogeneous for affinity are formed against the natural "CTL determinant" indistinguishable from SDS, and only one of them

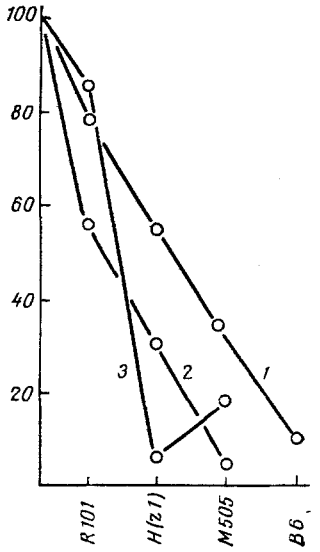

Fig. 3. Combined results of experiments to study adsorption of anti-K^b lymphocytes on target cells of mutants derived from B6. Ordinate, relative cytotoxic action of nonadherent lymphocytes (in % of cytotoxic action of intact lymphocytes) on B6 (1), M505 (2), and H(z1) (3) target cells; abscissa, target cells taken for adsorption.

can react with the "CTL determinant" modified as a result of mutation. Further investigations into the isolation of discrete CTL subpopulations by means of an adsorption–elution technique [7] must show whether they differ in the specificity or affinity of their receptors, and they may perhaps be helpful in obtaining new evidence on the structure of products of the K and D loci identified by CTL receptors.

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