MICROBIOLOGY AND IMMUNOLOGY

Effect of the Ly2 Molecule on the Function of Alloantigen-Specific Effector Cytotoxic T-Lymphocytes in Relationship to the Variation of T-cell Receptor Affinity

I. A. Popov, B. D. Brondz, N. G. Anosova, Yu. S. Krivoshein, and V. V. Kronin

UDC 612.017.1:576.314+576.5:616−097

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 5, pp. 492−494, May, 1993
Original article submitted January 21, 1993

Key Words: *Ly2 molecule; alloantigen-specific cytotoxic T-lymphocytes; anti-Ly2 monoclonal antibodies; T-cell receptors*

The Ly2 molecule (CD8) makes contact with a receptor in the cytoplasm of effector T-lymphocytes and is necessary both for the realization of function after antigen contact with the cytotoxic T-lymphocyte (CTL) receptor [10] and for the positive selection of CTL restricted by the major histocompatibility complex (MHC) class I antigens [8]. It is known that monoclonal antibodies (MAb) to the Ly2 molecule inhibit target cell lysis by CTL activated *in vitro*; however, these MAb do not abrogate the function of some CTL clones [9]. There are data providing evidence that the disappearance of CD8 molecules does not abrogate the cytolytic activity of CTL [11]. Nevertheless, it has been established that the CD8 molecule on the CTL surface directly interacts with the ligand sited in the α_3 domain of the MHC class I molecule; in the mouse system this is assigned to amino acid 227 (Glu) [6], and in the human system with amino acid 245 (Ala) [13]. It is thought that in the course of alloantigen recognition by CTL CD8 is required for an additional interaction only in the context of a low-affinity T-cell receptor [14].

Research Institute of Carcinogenesis, Cancer Research Center, Russian Academy of Medical Sciences, Moscow. (Presented by N. N. Trapeznikov, Member of the Russian Academy of Medical Sciences)

Earlier we showed that different fractions of effector CTL eluted from monolayers of macrophages (Mp) of different haplotype (donor K^b, mutant bmI, and unrelated K^k) are equally highly specific to the donor haplotype (K^b), and their activity to targets of mutant and/or unrelated haplotype is much lower, irrespective of the source of elution [2]. It seems that high-affinity CTL receptors recognize only a complex configuration of the MHC class I molecule ($\alpha_1+\alpha_2$ domains) independently of the CD8 molecule. This is confirmed by experiments with K^b-specific CTL clones [3].

In this work we studied the variations in the affinity of CTL receptors. For this purpose we analyzed the effect of anti-Ly2 MAb on the function of CTL of common specificity eluted from Mp monolayers of donor and unrelated haplotypes [5].

MATERIALS AND METHODS

Inbred mice of the following haplotypes were used: B10.D2(R101) (haplotype H-2$K^dI^dD^b$), C57Bl/6 (H-2$K^bI^bD^b$), B10.A(4R) (H-2$K^kI^{kb}D^b$), and B10.BR (H-2$K^kI^kD^k$). The animals were 4-6 months old. EL-4 thymoma induced in C57Bl/6 (B6) mice was maintained as an ascites tumor by weekly passages in

0007-4888/93/0005-0536$12.50

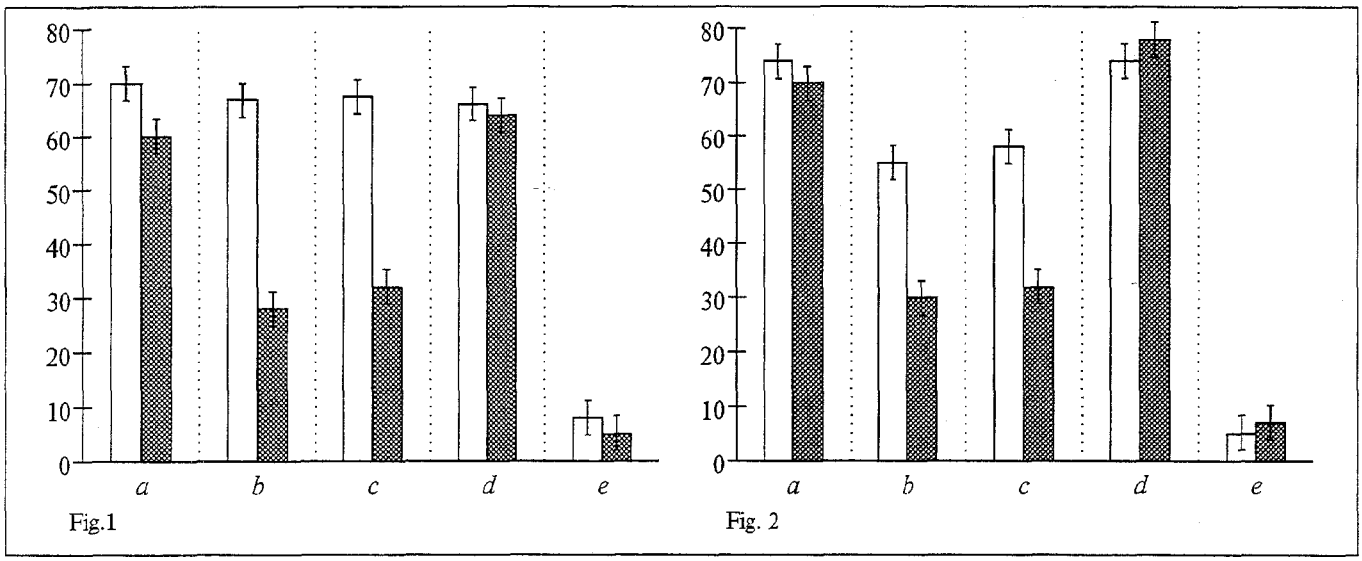

Fig. 1. Differences in the sensitivity of CTL−1 fractions to anti−Ly2 MAb. Ordinate: CI (%) of CTL eluted from B6 (*a*), 4R (*b*), and BR (*c*) Mp monolayers, unattached to BR Mp and further eluted from B6 Mp (*d*), and eluted from R101 monolayer (*e*; control). CTL were treated with medium (closed bars) or anti−Ly2 MAb (open bars) before performance of cytotoxicity test. Effector: target ratio 90:1. The results of 7 experiments are presented.

Fig. 2. Sensitivity of different CTL−2 fractions to anti−Ly2 MAb The results of 5 experiments are persented. For details see legend to Fig. 1.

syngeneic mice. In order to induce H-2K^b-specific primary effector CTL (CTL-1), R101 mice were immunized intraperitoneally with 2.5×10^7 EL-4 cells. Immune splenocytes were obtained 11 days later. Secondary CTL (CTL-2) were obtained from the spleen of mice that had been immunized intraperitoneally with EL-4 cells twice at a 2-month interval; the spleen cells were used in the experiment 6 days after the last immunization. Mouse fibroblasts (L-cells) Ltk$^-$ of H-2^k haplotype and their transfected variants 1-4 expressing the K^b molecule were kindly provided by Dr. Allen [4]. CTL enrichment and separation of the CTL-1 and CTL-2 fractions were performed by means of adsorption on monolayers of B6 Mp, Mp of third-party haplotype (4R, BR), and R101 (recipient cells, control experiment). After the removal of nonadherent lymphocytes the adsorbed cells were eluted twice using a mixture of pronase (Calbiochem) and pancreatin (Sigma) solution (each enzyme taken in a final concentration of 25 and 100 μg/ml [1]. The action of pronase was arrested by the addition of 30% bovine serum. The lymphocytes were washed three times and treated with 1:100 diluted ascitic fluid of an anti-Ly2 hybridoma [12] for 2 hours at 37°C in an atmosphere of 5% CO_2. The cytotoxic test (CTT) was set up as follows: ^{51}Cr-labeled L-cells were placed in the wells of 96-well flat-bottom tissue culture plates (Linbro), 10^4 cells per well, and washed twice. Various fractions of immune lymphocytes were added to the wells and the plates were incubated for 16 hours at 37°C in an atmosphere of 5% CO_2. The incubation medium was RPMI-1640 supplemented with 100 U/ml gentamicin, 5% heated bovine embryonic serum, 2 mM L-glutamine, and 10 mM HEPES. The cytotoxic index (CI) was calculated according to the formula: CI=(a-c)/(b-c)× ×100%, where a, *b*, and *c* are the experimental, total (maximum, in the presence of 1% Triton X-100), and spontaneous ^{51}Cr release (cpm), respectively [7].

RESULTS

The experiments were devoted to the study of the effect of MAb to the Ly2 (CD8) molecule on the CTL-1 and CTL-2 fractions, which differed in the affinity of T-cell receptors. In the course of the affinity-based subdivision of the effector T-lymphocytes specific to the MHC class I H-2K^b molecule, we used not only donor-derived (B6) and recipient-derived (R101, control) Mp monolayers, but also those of third-party strains, 4R and BR. CTL-1 fractions enriched on third-party Mp appeared to be highly sensitive to anti-Ly2 MAb: CI was decreased by 65 and 56%, respectively (Fig. 1, *b* and *c*). On the other hand, the same CTL-1 enriched on donor B6 Mp exhibited just a slight reduction of CI (12%) following the treatment with anti-Ly2 MAb (Fig. 1, *a*). Moreover, when these CTL were enriched in advance on the third-party Mp, then both unattached CTL and CTL further enriched on donor Mp (i.e., adsorbed and eluted from B6 Mp) were totally resistant to the given MAb (Fig. 1, *d*).

A similar effect was observed in the case of the CTL-2 fractions. Anti-Ly2 MAb inhibited the CI of

CTL-2 eluted from the third-party Mp (Fig. 2, *b* and *c*) by 49 and 43%, respectively. Moreover, CTL-2 enriched on the monolayer of donor B6 Mp were totally resistant to the effect of the antibodies, both after exhaustion on the BR monolayer (Fig. 2, *d*) and in the absence of this step (Fig. 2, *a*).

Thus, it was established that effector CTL-1 and CTL-2 fractions eluted from Mp monolayers of third-party 4R and BR strains are highly sensitive to the inhibitory effect of anti-Ly2 MAb, which is explained by the low affinity of the T-cell receptors of those CTL. On the other hand, antibodies produced minimal or no effect on the CI of high-affinity effector CTL-1 and CTL-2 enriched on donor B6 Mp. Although CTL-1 activity dropped slightly under these conditions, still after removal of a fraction adherent to the third-party Mp monolayer the remaining CTL-1 were totally resistant to anti-Ly2 MAb.

The results obtained may prove that the Ly2 (CD8) molecule plays an appreciable role in CTL-MHC class I alloantigen interaction only for a subpopulation of CTL characterized by a low affinity of the T-cell receptor. The insignificant differences in the sensitivity to anti-Ly2 antibodies between high-affinity fractions of CTL-1 and CTL-2 may be related to the low frequency of low-affinity receptor-bearing CTL-2 as compared to CTL-1 and/or the slightly increased affinity of CTL-2 T-cell receptors.

REFERENCES

1. B. D. Brondz, A. A. Pimenov, Z. K. Blandova, *et al.*, *Molek. Biol.*, **16**, 481 (1982).
2. B. D. Brondz and T. V. Osipova, *Byull. Eksp. Biol.*, **105**, № 6, 694 (1988).
3. P. Ajitkumar, S. Geier, K. Kesari, *et al.*, *Cell*, **54**, 47 (1988).
4. H. Allen, J. Fraser, D. Flayer, *et al.*, *Proc. Nat. Acad. Sci. USA*, **83**, 7447 (1986).
5. B. Brondz, I. Egorov, and G. Drizlikh, *J. Exp. Med.*, **141**, 11 (1975).
6. J. Connolly, T. Potter, E. Wormstall, *et al.*, *Ibid.*, **168**, 325 (1988).
7. G. Drizlikh, A. Andreyev, I. Kotomina, *et al.*, *J. Immunol. Meth.*, **8**, 383 (1975).
8. W. Fung-Leung, M. Schilham, A. Rahemtulla, *et al.*, *Cell*, **65**, 443 (1991).
9. A. Glasebrook, A. Kelso, and H. McDonald, *J. Immunol.*, **130**, 1545 (1983).
10. J. Klein, *Natural History of the MHC*, New York (1986).
11. H. McDonald, A. Glasebrook, and T. Cerottini, *J. Exp. Med.*, **156**, 1711 (1982).
12. M. Sarmiento, A. Glasebrook. and F. Fitch, *J. Immunol.*, **125**, 2665 (1980).
13. R. Salter, A. Norment, B. Chen, *et al.*, *Nature*, **338**, 345 (1989).
14. A. Townsend, *Immunol. Today*, **6**, 68 (1985).