CONDITIONS OF DIFFERENTIATION OF ORIGINAL MEMORY CELLS AND OF ENRICHED PRECURSORS OF SECONDARY CYTOTOXIC T LYMPHOCYTES SPECIFIC FOR THE CLASS I HISTOCOMPATIBILITY MOLECULE

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The conditions of maturation of memory cells (pCTL-2) into secondary cytotoxic effector T lymphocytes (CTL) are essentially simpler than those of differentiation of primary effector CTL from their naive precursors. Maturation of naive pCTL requires a high dose of antigen, presentation of this antigen to a living stimulator cell, expressing on its surface the Ia-molecule of the class II MHC (major histocompatibility complex) (see the review in [4]). An additional difference between donor and recipient for the class II MHC molecule also contributes to the formation of primary effector CTL specific for the class I MHC alloantigen [9, 11]. Conversely, pCTL-2 can mature into secondary CTL in the presence of a low dose of the corresponding antigen [10, 13], presented in the form of a purified molecule [14], and even in the case of identity of the class II MHC molecule between donor and recipient, under conditions leading to absence of primary CTL formation [6, 10, 12].

Since pCTL-2 are specifically adsorbed on a monolayer of macrophages (MPh) from the corresponding donor and can be physically separated from the other population of memory cells (secondary helper T cells), not adherent to the same monolayer [5], subsequent elution of the adherent pCTL leads to a marked increase in their relative number [4]. The aim of the present investigation was accordingly to study differences in the conditions of differentiation of the two categories of pCTL-2: original and enriched by elution from a donor monolayer.

EXPERIMENTAL METHOD

Mice of lines C57BL/6 (B6:$K^bI^bD^b$), B10.D2 (R10l)(R10l:$K^dI^dD^d$), B10.AKM(AKM:$K^kI^kD^q$), B10.MBR(MBR: $K^bI^kD^q$) were bred in the nursery of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. To induce pCTL-2 specific for the H-$2K^b$ molecule, two basic variants of immunization in vivo were used: R101 anti-B6 and AKM anti-MBR. In the first case the donor and recipient differed with respect to MHC molecules of both classes I + II (K^b + I^b), but in the second case they differed only with respect to the class I (K^b) MHC molecule. In both cases 2×10^7 donor's spleen cells (B6 cells were irradiated with 1500 rads) were injected into the hind foot pads (HFP) of the recipients or 2.5×10^7 thymoma EL-4 cells (obtained from B6) were injected intraperitoneally. In some experiments 2×10^7 irradiated spleen cells from B6 mice were injected into HFP of the recipients or 2.5×10^7 thymoma EL-4 cells were injected intraperitoneally. After 4 weeks, 5×10^6 immune spleen cells/ml were incubated for 4 days in 24-well plates ("Flow Laboratories," England) in mixed lymphocyte culture (MLC) with 5×10^6/ml of the corresponding stimulators (B6 and MBR), killed by heating to 45°C for 1 h. In the controls the same normal R101 and AKM lymphocytes were incubated with killed allogeneic stimulators (B6 and MBR, respectively) or immune lymphocytes were mixed with syngeneic stimulators. In some experiments from 1 to 200 units/ml of recombinant inter-

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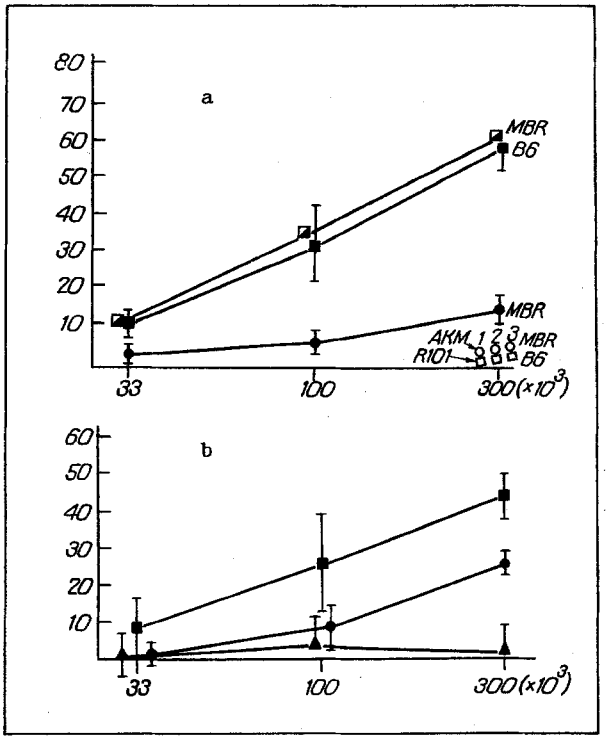

Fig. 1. Specific activation of pCTL-2 by alloantigen in MLC (a) and characteristics of phenotype of pCTL-2 (b) induced by class I H-2K^b molecule in vivo. Abscissa, dose of CTL per well; ordinate, CI (per cent). a: incubation of R1O1 anti-B6 pCTL-2 with B6 (filled and half-filled squares) or R1O1 (empty squares) stimulators and of AKM anti-MBR pCTL-2 with MBR (empty circle) or AKM (empty circle 2) stimulators. Incubation of naive R1O1 (empty square) or AKM (empty square) lymphocytes in MLC with B6 or MBR stimulators, respectively. CTL-2 were tested on B6 (filled squares), MBR (● 0, 2, and 3, ◪), R1O1 (□ 1), and AKM (○ 1) TC. Results of 12 experiments given for R1O1 anti-B6 and 7 experiments for AKM anti-MBR pCTL-2. b: AKM anti-B6 pCTL-2, purified from B cells, were treated with medium (●), or anti-L3T4 (■) or anti-Lyt-2 (▲) McAb and complement, followed by incubation in MLC with MBR stimulators in the presence of rIL-2 10-20 units/ml. CTL-2 were tested on MBR TC. Results of 4 experiments given.

leukin-2 (rIL-2) was added to MLC. Cells from MLC were washed, counted, and their cytotoxic index (CI) determined after incubation for 20 h with ^{51}Cr-labeled target cells (TC), namely MPh of the donor B6 or MBR mice (R1O1 or AKM respectively for the control) [8].

To assess the phenotype of the AKM anti-MBR pCTL-2, B cells were removed from a suspension of immune spleen cells by "panning" (adsorption on a plate covered with rabbit antibodies to mouse immunoglobulins (200 μg/ml) [7]), and the purified T cells were treated for 40 min at 4°C with monoclonal antibodies (McAb) to L3T4 (hybridoma GK1.5 or RL172.4) or Lyt-2 (hybridoma T1B150 or 3.168) antigens in the form of ascites fluid 1/50 or supernatant 1/4. The cells were then treated with nontoxic rabbit complement 1/20 at 37°C for 35 min. The T cells and two of their T-subclasses (L3T4 and Lyt-2) were washed off and incubated in MLC with MBR stimulators and with 10-20 units/ml of rIL-2. Addition of rIL-2 was necessary to preserve the function of the complement-treated pCTL-2 in the culture.

The pCTL-2 were enriched by adsorption on a monolayer of donor's MPh, treated with pronase (25 g/ml) for 2 h at 37°C. After removal of the nonadherent lymphocytes, the adsorbed lymphocytes were twice eluted with 25 and 100 μg/ml pronase ("Calbiochem," USA) in the presence of Viocase 1/40 [2]. The pronase was inactivated with 30% serum and the lympho-

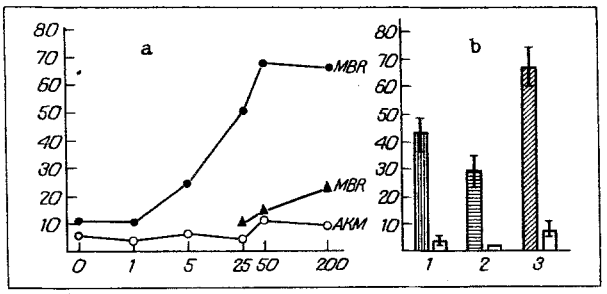

Fig. 2. Synergic effects of alloantigen and rIL-2 on activation of AKM anti-MBR (a) and R1O1 anti-B6 pCTL-2 (b), induced in vivo, in MLC. a: incubation of anti-MBR pCTL-2 in MLC with MBR (●; ▲) or AKM (▲) stimulators. CTL-2 were tested on MBR (●; ○) or AKM (○) TC. Abscissa, dose of rIL-2 (units/ml); ordinate, CI (per cent). b: incubation of R1O1 anti-B6 pCTL in MLC with B6 (1, 3) or R1O1 (2) stimulators, in the absence (1) or presence of rIL-2 200 units/ml (2, 3). CTL-2 were tested on B6 (shaded columns) or R1O1 (unshaded columns) TC. Ordinate, CI (per cent).

cytes were washed off and incubated in MLC, under varying conditions: 1-4 days with or without stimulators, and with or without the addition of rIL-2.

EXPERIMENTAL RESULTS

Although pCTL-2 are activated much more effectively when the B6 donor and R1O1 recipient differed with respect to K^b + I^b than when they differed only for K^b in the AKM anti-MBR system (Fig. 1a), it will be noted that in both cases the progenies of pCTL-2, namely secondary CTL, were specific for the same K^b molecule: R1O1 anti-B6 CTL lysed B6 and MBR TC, identical only for K^b, equally. Under these circumstances neither type of CTL lysed syngeneic TC or was activated in MLC by syngeneic stimulators, and naive R1O1 and AKM lymphocytes matured minimally into CTL on incubation in MLC with killed allogeneic stimulators. Thus both differentiation of pCTL-2 and the lytic activity of their progenies were specific for the K^b antigen which induced them in vivo.

It will be clear from Fig. 1b that anti-K^b pCTL-2 have the $L3T4^-$ $Lyt\text{-}2^+$ phenotype, for their activity rises sharply or disappears after removal of $L3T4^+$ or $Lyt\text{-}2^-$ cells, respectively.

The data in Fig. 2a indicate that the sharp rise in the intensity of differentiation of anti-K^b pCTL-2 during their reaction on not only a K^b molecule, but also a class II MHC molecule (I^b), is connected with the function of IL-2. This was directly confirmed by the marked synergism of the K^b antigen (MBR stimulators) and the small dose of rIL-2 (5-50 units/ml) on differentiation of AKM anti-MBR pCTL-2 Fig. 2a). Similar synergism of alloantigens and rIL-2 also was produced for R1O1 anti-B6 pCTL when a high dose of rIL-2 (200 units/ml; Fig. 2b) was used. Moreover, rIL-2 itself, but only in a high dose, can cause weak differentiation of pCTL-2 in the absence of the corresponding alloantigen, in agreement with earlier data [15].

IL-2 in a small dose can promote maturation of pCTL-2 cells activated by class I MHC alloantigen, on account of additional proliferation or differentiation of CTL. Since proliferation is usually facilitated by helper T cells, the pCTL-2 were separated from helper T cells by adsorption on a monolayer of donor's MPh. After elution of the pCTL-2 from this monolayer they were cultured under different conditions. This showed that unlike intact immune spleen cells maturing into CTL in the course of 4 days only in the presence of the corresponding allogeneic stimulators (MLC), the eluted pCTL-2 completed differentiation spontaneously in 3 days, i.e., irrespective of the presence of the alloantigen, and without rIL-2 (Fig. 3a, b). Addition of rIL-2 to the monoculture led to a further increase in activity of CTL, while preserving their specificity. These data suggest that pCTL-2 themselves secrete a factor inducing their differentiation. It can be tentatively suggested that the additional contribution of rIL-2 to maturation of eluted pCTL-2 in monoculture is due, not to proliferation of the cells (in the absence of helper T cells and of alloantigen), but to an increase in secretion of CTL differentiation factor. This is a promising model for the study of this factor.

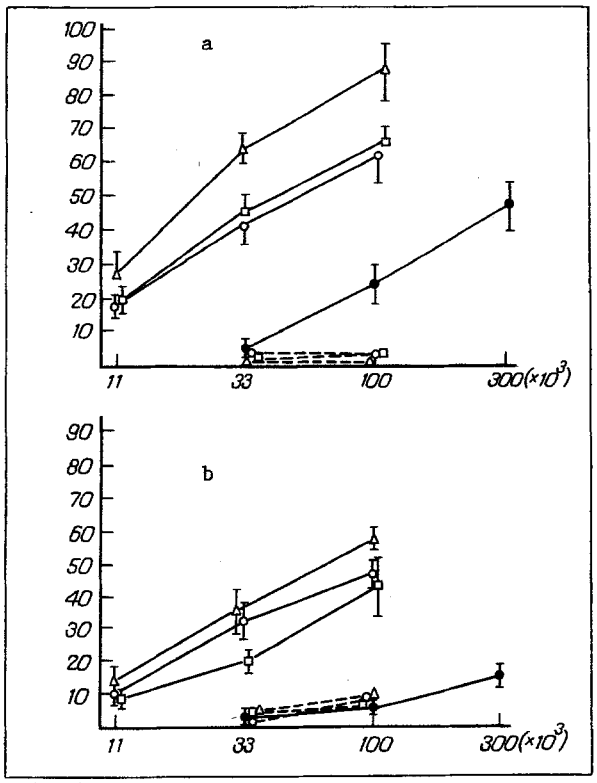

Fig. 3. Conditions of differentiation of R1O1 anti-B6 (a) and AKM anti-MBR (b) pCTL-2, enriched by elution from monolayer of donor's MPh. Original pCTL-2 were incubated in MLC for 4 days with donor's stimulators (●). Eluted pCTL-2 were incubated for 3 days with donor's stimulators (○), without stimulators (□ △), and in the presence of rIL-2 200 units/ml ( ). CTL-2 were tested on donor's (——) or recipient's (— — —) TC. Abscissa, dose of CTL per well; ordinate, CI (per cent). Results of 3-5 experiments given.

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LITERATURE CITED

1. B. D. Brondz, A. P. Suslov, A. V. Chervonskii, and A. A. Pimenov, Byull. Éksp. Biol. Med., No. 10, 426 (1979).
2. B. D. Brondz, A. A. Pimenov, Z. K. Blandova, and G. N. Vornakova, Mol. Biol., **16**, No. 3, 481 (1982).
3. B. D. Brondz, T Lymphocytes and Their Receptors in Immunologic Recognition [in Russian], Moscow (1987).
4. B. D. Brondz and T. V. Osipova, Byull. Éksp. Biol. Med., No. 6, 694 (1988).
5. A. A. Pimenov and B. D. Brondz, Byull. Éksp. Biol. Med., No. 11, 79 (1983).
6. F. Bach, B. J. Alter, B. Dunlap, et al., Fed. Proc., **40**, 1466 (1981).
7. K. Kitamura, H. Kiyono, K. Fujihashi, et al., J. Immunol., **139**, 3251 (1987).
8. B. D. Brondz, A. V. Andreev, S. G. Egorov, and G. I. Drizlikh, Scand. J. Immunol., **10**, 195 (1979).
9. H. Cantor and E. A. Boyse, J. Exp. Med., **141**, 1390 (1975).
10. M. Hurme, K. Varkila, and M. Sihvola, J. Immunol., **137**, 1782 (1986).
11. H. Iwasaki, M. Tuniguchi, and N. Shinohara, J. Immunol., **134**, 3592 (1985).
12. K. Mizoguchi, L. Nakashima, K.-I. Isobe, et al., Eur. J. Immunol., **15**, 487 (1985).
13. A. M. Schmitt-Verhulst, F. Albert, A. Guimezanes, et al., J. Supramol. Struct., **16**, 359 (1981).
14. O. Weinberger, S. H. Herrmann, J. L. Greenstein, et al., Eur. J. Immunol., **15**, 1013 (1985).
15. R. Yoshimoto, N. Kashima, K. Okada, et al., Eur. J. Immunol., **15**, 325 (1985).