BASIC DIFFERENCE BETWEEN RECEPTOR REPERTOIRE OF EFFECTOR CYTOTOXIC T CELLS AND THEIR SECONDARY PRECURSORS (MEMORY CELLS)

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The conditions of activation of the lytic function of effector cytotoxic T cells (CTL) and differentiation of their secondary precursors (pCTL-2) differ significantly. By contrast with realization of the function of CTL, incubation of pCTL-2 with a low dose of antigen, namely virus [12] or hapten [10, 13] (as a complex with syngeneic cells), with a pure allogeneic Class I MHC (major histocompatibility complex) molecule, incorporated into a liposome [14], or even without antigen – with a low dose of recombinanat interleukin-2 [10, 15] – is sufficient for their differentiation from memory cells (MC). Accordingly, pCTL-2 can be induced without the formation of primary effector CTL – not only in culture [7], but also in vivo [10, 11], given the presence of Class II MHC identity. Detection of the marker of the small fraction of immature thymocytes, absent on effector CTL [9], on pCTL-2 indicates nonidentity of origin of the pCTL-2 and primary CTL, despite their common Thy-1^+, Lyt-2^+, L3T4^- phenotypes. Also, pCTL-2 differ from CTL which cross-react to foreign antigen in their stricter specificity: pCTL-2 are sensitive only to the antigen which induced them beforehand [12, 13]. Strictness of specificity of pCTL-2, induced by wild-type H-$2K^b$ alloantigen, is manifested also by the fact that if they are secondarily activated by a mutant K^{bm1} molecule, their CTL-progeny will lyse target cells (TC) of the bm1 mutant and wild type equally, which is completely uncharacteristic of the same CTL, if their precursors are secondarily activated by wild-type antigen [4].

The study of epitopes recognized by receptors of pCTL-2 and of effector CTL, specific for the same antigen, has become possible as a result of separation of pCTL-2 from another MC population, namely secondary helper T cells, on account of a difference in the conditions of detection of their receptors by adsorption on a monolayer of macrophages (Mph) and subsequent elution of the pCTL-2 adherent to such a monolayer [5].

EXPERIMENTAL METHODS

Mice of lines C57Bl/6 (B6; $K^bI^bD^b$), B10·D2 (R101) (R101:$K^dI^dD^b$), B10.A (4R) (4R:$K^kI^{k/b}D^b$) were obtained from the nursery of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, and mice of mutant line B6. C-H-2^{bm1} (bm1:$K^{bm1}I^bD^b$) were obtained from the Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR. To induce anti-H-$2K^b$ MC, R101 mice were immunized with irradiated (1500 rads) B6 mouse spleen cells. Trials of different versions of immunization (dose of cells, site of injection, source of MC, times of secondary immunization) showed that optimal conditions for induction and detection of MC were injection of $2\cdot10^7$ cells into each hind footpad, followed after an interval of 4 weeks by incubation of $5\cdot10^6$ immune spleen cells, in mixed lymphocyte culture (MLC) with an equal number of stimulating cells from a B6 donor, killed by heating to 45°C for 1 h. The conditions of incubation in medium RPMI-1640 with additives in FB-16-24-TC panels (Flow Laboratories, England) were described in detail previously [1]. In the controls, the same immune lymphocytes were incubated with syngeneic R101 stimulators, and normal R101 lymphocytes were incubated with heated B6 cells. In the latter case, lytic activity of the primary CTL did not exceed 5-7%.

Cells obtained from MLC were washed and counted and their cytotoxic index (CTI) was determined after they had been incubated for 16 h with ^{51}Cr-labeled TC, namely Mph from B6

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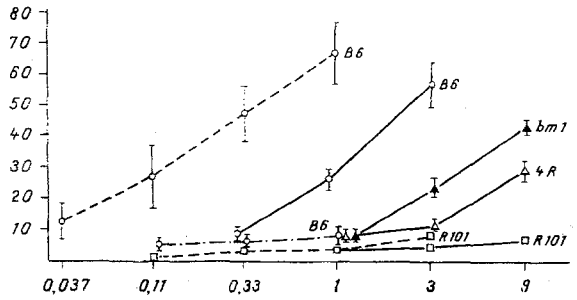

Fig. 1. Cross-reactivity of secondary anti-K^b CTL and specificity of enrichment of anti-K^b pCTL-2 by elution from monolayer of donor's Mph. Continuous line) intact pCTL-2; broken line) pCTL-2 eluted from B6 Mph monolayer; line of dots and dashes represents pCTL-2 eluted from monolayer of R101 Mph. TC: B6 (empty circles), R101 (empty squares), bml (filled triangles). Abscissa, dose of secondary CTL ($\times 10^5$), ordinate CTI (in %). Results of 4-7 experiments shown.

mice (R101 in the control), cultured beforehand in 96-well panels (Linbro, England) in a concentration of $6 \cdot 10^4$ cells per well [8].

To separate pCTL-2 from other MC, spleen cells of immunized R101 anti-B6 mice were adsorbed on a pronase-treated [6] Mph monolayer from a B6 donor, R101 recipient, and "foreign" mutant bml and 4R lines, at the rate of $12 \cdot 10^7$ and $30 \cdot 10^7$ lymphocytes per flask (Nunclo, Denmark), with an area of 75 and 175 cm^2 respectively. Nonadherent lymphocytes were harvested twice after two periods of incubation, each for 1.5-2 h at 37°C, on the same Mph monolayer. Lymphocytes adherent to the monolayer were eluted with pronase twice in concentrations of 25 and 100 μg/mol in the presence of Viokase 1/40 [8, 3]. After neutralization of the pronase the cells were washed and used in MLC as reacting lymphocytes.

EXPERIMENTAL RESULTS

Secondary anti-K^b CTL appearing in MLC after activation of R101 anti-B6 MC by B6 donor's cells effectively lysed B6 TC (Fig. 1). Crossed lysis of cells containing the mutant K^{bm1} molecule or allelic K^k, was 3-4 and 9 times respectively less than lysis of the donor's TC, whereas lysis of TC of syngeneic line R101 was absent or minimal (Fig. 1). Activity of CTL was just as minimal if the same MC were incubated in MLC with syngeneic R101 stimulators (data not shown).

To develop a test for anti-R^b pCTL-2 receptors they were eluted from a monolayer of Mph of a B6 donor (the fraction of eluted lymphocytes amounted to 5-7% of the cells) and incubated in MLC with B6 stimulator. It will be clear from Fig. 1 that activity of the eluted pCTL-2 was 8-9 times higher than that of the original pCTL-2, to judge by the decrease in the dose of secondary CTL causing the same degree of lysis of B6 TC. The same highly active CTL progeny did not lyse R101 TC. Since immune spleen cells, eluted from a monolayer of syngeneic R101 Mph do not contain pCTL-2 (do not generate CTL; see Fig. 1), selective enrichment of pCTL-2 by elution from a monolayer of donor's Mph is evidently associated with an increase in the fraction of pCTL-2 whose antigen-binding receptors make contact specifically with K^b antigen. The specificity of this contact also follows from the results of adsorption of pCTL-2; activity of MC nonadherent to the R101 Mph monolayer was unchanged (Fig. 2a, 2; b, 2) compared with that of intact MC (Fig. 2a, 1; b, 1), whereas after adsorption on a B6 Mph monolayer their MC activity was sharply reduced (by 60-75%) when secondary CTL were tested to both B6 (Fig. 2a, 3) and bml (fig. 2b, 3) TC. It is important to note that anti-K^b pCTL-2, whose progenies lyse TC of a B6 donor, do not adhere to an Mph monolayer of the bml mutant (Fig. 2a, 4), whereas the small proportion of pCTL-2 whose progenies lyse TC of the bml mutant adhere effectively to a monolayer of the same mutant: lytic activity was reduced by 50% (Fig. 2b, 4). The results show that pCTL-2 differ from effector CTL in the diversity and strict specificity of their receptors [4].

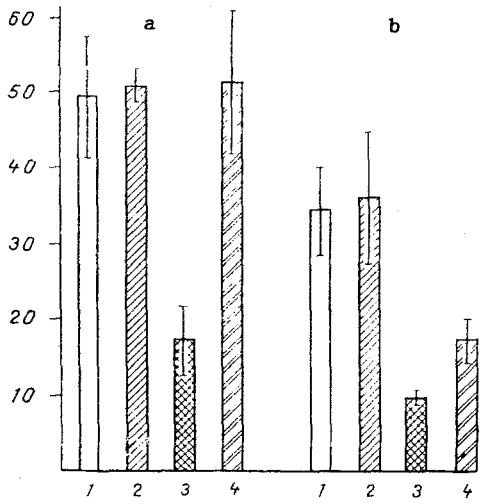

Fig. 2. Detection of specific adherence of anti-K^b pCTL-2 to Mph monolayer of bml mutant during testing of secondary CTL only to bml TC, but not to TC of the B6 donor. TC: B6 (a) and bml (b). 1) Intact pCTL-2; 2) pCTL-2 not adherent to R101 Mph monolayer; 3) the same, to B6 Mph monolayer; 4) the same to bml Mph monolayer. Ordinate, CTI (in %). Results of three to four experiments are given.

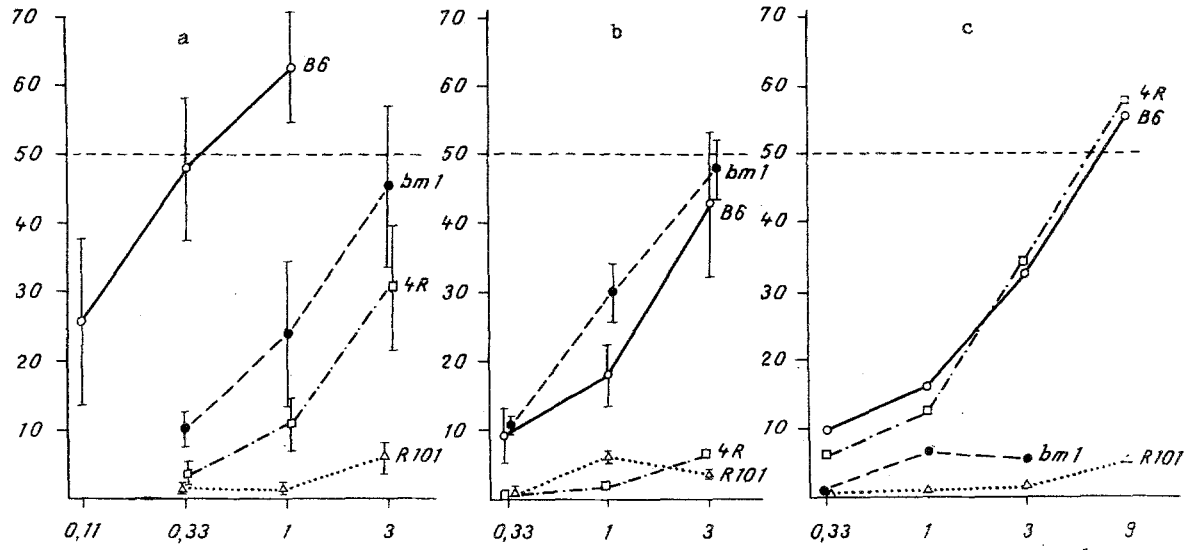

Fig. 3. Separation of anti-K^b pCTL-2 into fractions specific for mutant (K^{bm1}) and allelic (K^k) molecule. a) pCTL-2 eluted from monolayer of B6 Mph; b) the same, from monolayer of bml Mph; c) the same from monolayer of 4R Mph. Sources of TC for secondary CTL: empty circles) B6; filled circles) bml; empty squares) 4R; empty triangles) R101. Abscissa) doses of CTL per well ($\times 10^5$); ordinate) CTI (in %). Mean results of four experiments (a and b) and of two experiments (c) shown.

To analyze the fine specifity of anti-K^b pCTL-2 receptors, they were eluted in the same experiments from a monolayer of Mph of the B6 donor and two third-party lines (mutant bml and allelic 4R), then activated in MLC by stimulators of the B6 donor, and each of the secondary CTL progenies thus obtained was tested on four TC. It will be clear from Fig. 3a that pCTL-2 progenies, enriched by elution from a monolayer of B6 MPh, lysed bml and 4R TC 9 and 27 times less strongly respectively than B6. If, however, pCTL-2 were eluted from the monolayer of a third-party line, their pCTL-progenies differed in principle from those of secondary CTL whose progenitors were eluted from a monolayer of donor's cells.

These differences apply to at least three parameters. First, lysis of third-party TC from which the pCTL-2 were eluted – bml (Fig. 3b) and 4R (Fig. 3c) – was not lower (and in some experiments it was higher) in intensity than lysis of the donor's TC. Second, if pCTL-2 were eluted from a bml or 4R monolayer, their progenies were without cross reactivity: they did not lyse 4R (Fig. 3b) for bml (Fig. 3c) TC respectively. Third, after elution of pCTL-2 from a bml (Fig. 3b) and 4R (Fig. 3c) monolayer, activity of the secondary CTL was 6 and 12 times lower respectively than after elution of the same pCTL from the B6 monolayer (Fig. 3a), to judge from the dose of CTL producing 50% lysis. In every case the function of CTL was specific: they did not lyse syngeneic R101 TC (Fig. 3).

It follows from the data given above that pCTL-2 specific for one molecule are heterogeneous: they can be divided into fractions, the receptors of each of which are strictly specific for a separate fragment of this molecule and they do not cross-react to a different fragment of the same molecule. The opposite result was obtained with effector CTL specific for the same K^b molecule; irrespective of the monolayer from which they were eluted, whether the B6 donor, bml mutant [8], or allelic variant [3], in all cases they lyzed predominantly B6 TC, and the third-party molecule from which they were eluted significantly less strongly, cross-reacting under these circumstances to the second third-party antigen. The results point to the cardinal difference between the receptor repertoires of effector CTL and pCTL-2. Unlike CTL receptors, which are homogeneous in specificity, they recognize the immunodominant complex epitope of the Class I MHC molecule both as donor and as third-party molecule (on account of the difference in structure of receptors of the same specificity with respect to the degree of labile complementarity toward the donor antigen), the receptors of each fraction of pCTL-2 are strictly specific for the separate fragments of the same complex epitopes. Suppressor T cells specific for the MHC molecule differ from effector CTL and resemble pCTL-2 with respect both to the narrow specificity of their receptors [2] and to their ability to adhere specifically to a monolayer of not only native, but also of fixed Mph [6]. Nevertheless, the frequency of each fraction of eluted suppressor T cells [2] is significantly lower (1.5%) than the frequency of the pCTL fractions eluted from the monolayer of the mutant and allelic variants (16 and 8% respectively relative to pCTL-2 eluted from the donor monolayer; Fig. 3).

On the basis of this difference the idea grew that epitopes for the active center of receptors of these two subclasses also are nonidentical: suppressor T cells react to many simple serologically demonstrative determinants, whereas pCTL-2 respond to a limited number of fragments of the complex mosaic recognized by effector CTL as a single epitope. The results show that the structure of the active center of antigen-binding receptors changes in the course of differentiation of pCTL-2 into CTL. In order to prove the hypotheses described above, contact of receptors of each of T subclasses with the antigen of the Mph monolayer must be inhibited with the aid of a set of synthetic peptides of the K^b molecule and monoclonal antibodies obtained to the pCTL-2 receptor.

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SUPPRESSORS OF THE GRAFT VERSUS GRAFT REACTION IN TOLERANCE TO ALLOANTIGENS

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The study of the active mechanisms of creation and maintenance of tolerance has led to the discovery of suppressor cells in some forms of transplantation tolerance [9, 10, 13]. However, the role of suppressors in tolerance induced with cyclophosphamide (CP) has so far received only little study. On the one hand, antigen-specific suppressor T cells have been demonstrated in tolerance induced to sheep red blood cells (SRBC) with the aid of CP [2, 14], and activation of antigen-nonspecific suppressor cells has been found after administration of CP [11, 15]. On the other hand, many investigations have revealed high sensitivity of precursors of suppressor T cells and their inducers to CP [8].

The writers showed previously that after thymectomy and injection of a massive dose of allogeneic splenocytes and CP, lasting (at least 8 months) tolerance to an allogeneic heart graft arises in the majority of adult CBA mice [3, 5]. Lymphocytes of tolerant animals were specifically areactive to the donor's alloantigens in mixed lymphocyte culture (MLC) [5] and in the graft versus graft reaction (GVGR) [6].

The aim of this investigation was to study the possible role of suppressor mechanisms in the observed form of tolerance.

EXPERIMENTAL METHODS

Adult male CBA ($H\text{-}2^k$), C57Bl/6 ($H\text{-}2^b$), and BALB/c ($H\text{-}2^d$) mice and (CBA × C57Bl/6)F_1 hybrids, obtained from the Stolbovaya nursery, Academy of Medical Sciences of the USSR, were used in the experiments. The (CBA × BALB/c)F_1 hybrids were specially bred by the writers.

Adult CBA mice were thymectomized by the method described previously [3, 5] and, 3-4 weeks later, they were given an intravenous injection of a massive dose (10^8) of spleen cells of C57Bl/6 mice, followed (after 18-24 h) by a single intraperitoneal injection of 200 mg/kg CP. The reactivity of cells of the tolerant mice was studied 1-2 months after the induction of tolerance.

To carry out the GVGR, the ability of spleen cells of intact CBA mice to prevent the immune response to SRBC of spleen cells from (CBA × C57Bl/6)F_1 hybrid mice, presensitized to SRBC, during combined adoptive transfer into CBA mice irradiated in a dose of 9 Gy, was used. Simultaneously with transfer of the splenocytes, the recipients received an intravenous injection of $5 \cdot 10^8$ SRBC. The immune response of the (CBA × C57Bl/6)F_1 hybrids to SRBC was determined in the spleen of irradiated recipients 5 days after adoptive transfer, using the local hemolysis in gel method. For the GVGR either 10^7 splenocytes of intact CBA mice or 10^6 splenocytes of CBA mice sensitized 5 days before the experiments by intravenous injection of 10^8 spleen cells of C57Bl/6 mice, or 10^7 splenocytes of CBA donor mice tolerant

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