COMPARATIVE STUDY OF CONDITIONS FOR DEMONSTRATING RECEPTORS OF MEMORY T CELLS AND OTHER T LYMPHOCYTE SUBPOPULATIONS IMMUNE TO ANTIGENS OF THE H-2 COMPLEX

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The presence of antigen-binding receptors on the surface of cytotoxic T lymphocytes (CTL) [2] and of specific T suppressor cells (STS) [4] has been demonstrated by adsorption of lymphoid cells on a cellular immunosorbent. CTL and STS immune to a given antigen of the H-2 complex can be selectively removed from a cell population by causing their adhesion to corresponding target cells (TC), and they can then be concentrated by elution of lymphocytes attached to the monolayer [2, 4]. Attempts to demonstrate receptors on the surface of primary [9, 13, 14] and secondary [10-12] precursors of CTL (memory cells — MC) have given contradictory results when lymphoid cells were used as the adsorbing monolayer.

This paper describes a study of the conditions of formation of antigen-binding receptors on the surface of MC and primary precursors of CTL by means of their adsorption on monolayers of macrophages; their ability to be adsorbed on monolayers of macrophages fixed with glutaraldehyde (GA) was compared with that of CTL and STS.

EXPERIMENTAL METHOD

Mice of lines B10.D2 ($H\text{-}2^d$), abbreviated to D2, and C57BL/6 ($H\text{-}2^b$), abbreviated to B6, were obtained from the nursery of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR.

During induction of CTL and MC *in vitro*, D2 mice were immunized by a single intraperitoneal injection of $2.5 \cdot 10^7$ EL-4 ascites leukemia cells from B6 mice. To induce STS, $9 \cdot 10^7$ B6 spleen cells irradiated with γ-rays in a dose of 1500 rads (^{137}Cs, 740 rads/min), were injected [4]. CTL also were induced *in vitro* in a one-way mixed lymphocyte culture (MLC), as described previously [6], using B6 lymphocytes irradiated with γ-rays as the stimulating cells. Immune spleen cells obtained 10-12 days after immunization *in vivo* for 5 days after immunization *in vitro* were used as primary CTL. To obtain secondary CTL, MC induced *in vivo* 1-3 months before the experiments were stimulated in MLC by B6 lymphocytes, killed by heating for 1 h at 45°C, for 4 days [6]. The resulting CTL were washed, counted, and their cytotoxic index (CI) was determined for action on TC (peritoneal macrophages), labeled with ^{51}Cr and cultured for 2 days, using a microversion [7] of the test described previously [8].

To determine activity of STS, D2 mouse spleen cells obtained 3-4 days after immunization (normal D2 spleen cells in the control) were treated with mitomycin C, washed, and added to a one-way D2 anti-B6 MLC, in the ratio of 1:1.5 to the reacting cells [4]. Suppressor activity was calculated by means of the index of inhibition (II) of DNA synthesis.

Adsorption of lymphocytes on monolayers of macrophages in large (No. 3024) and small (No. 3012) plastic flasks (Falcon Plastics, USA) was carried out by the method described previously [4]. In some experiments the monolayer of macrophages, washed to remove serum, was treated beforehand with pronase, 25 μg/ml, for 30 min at 37°C, after which the pronase was neutralized by an excess of bovine serum [4], or they were fixed in different concentrations of GA for 5 min for 30 sec [3], then washed 4 times, and free GA groups were blocked with

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TABLE 1. Comparison of Adsorption of Primary Precursors of CTL and MC on Macrophage Monolayer in Different Flasks (mean result of four experiments, M ± m)

Lymphocyte fraction	Type of flasks	CI (in %) of CTL ($3 \cdot 10^5$) induced in vitro	
		from MC	from primary precursors of CTL
Unfractionated	—	68,0±3,3	52,0±5,7
Not adherent to B6 TC	№ 3012	12,0±3,4 (82)	31,0±5,9 (40)
	№ 3024	10,0±4,2 (85)	47,0±4,9 (10)
Not adherent to D2 TC	№ 3012	33,0±7,1 (51)	46,0±6,0 (12)
	№ 3024	57,0±4,7 (16)	51,0±5,2 (2)

Legend. Here and in Table 2, adsorption index of lymphocytes.

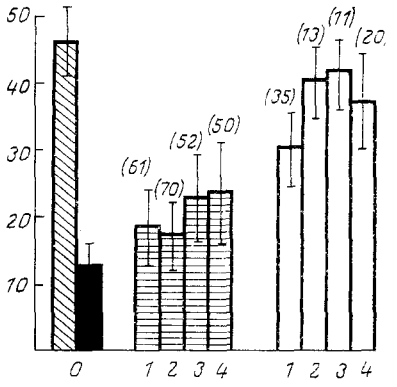

Fig. 1. Dependence of adsorption of D2 anti-B6 MC on duration of adsorption on macrophage monolayer. Abscissa, time of adsorption of MC (in h); ordinate, CI (in %) during incubation of secondary CTL, obtained from MC, with B6 TC: unfractionated (obliquely shaded) or nonadherent to B6 (horizontal shading) or D2 (unshaded columns) macrophage monolayer. In control normal D2 lymphocytes were stimulated by treated B6 cells (black columns). AI) (in %) shown in parentheses. Each column represents M ± m for 6-10 experiments.

TABLE 2. Adsorption of Different Subpopulations of D Anti-B T-Cells on Monolayers of Intact and Fixed Macrophages*

Lymphocyte fraction	CI (in %) of CTL†, induced in secondary MLC (1)	in primary MLC (2)	in vivo (3)	II (in %) of DNA synthesis by suppressors (4)
Unfractionated	68,0±3,3 (n=10)	52,0±5,7 (n=8)	54,0±1,5 (n=12)	56,0±3,2 (n=4)
Not adherent to B6 TC				
intact	12,0±3,4 (82)	31,0±5,9 (40)	6,0±3,0 (89)	18,0±5,8 (67)
fixed with GA	25,0±4,5 (63)	49,0±6,2 (6)	35,0±6,0 (35)	23,0±5,4 (59)
Not adherent to D2 TC				
intact	33,0±7,1 (51)	46,0±6,0 (12)	55,0±2,0 (0)	45,0±2,1 (20)
fixed with GA	54,0±6,5 (20)	52,0±5,7 (0)	54,0±3,0 (0)	51,0±4,8 (9)

*Macrophages fixed with 0.05% GA solution (30 sec).

†Doses of CTL $3\cdot10^5$ and $9\cdot10^5$ after immunization *in vitro* and *in vivo* respectively. MC (1), normal spleen cells (CTL precursors) (2), CTL (3), and STS (4) adsorbed. n) Number of experiments.

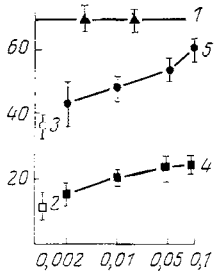

Fig. 2. Adsorption of MC on macrophages fixed with GA. Abscissa, concentration of GA (in %); ordinate, CI (in %) of secondary CTL. 1) Unfractionated MC. 2, 3) MC adsorbed on monolayer of intact B6 and D2 macrophages respectively. 4, 5) The same on a monolayer of B6 and D2 macrophages, fixed with GA, respectively.

0.1 M glycine buffer, pH 7.4. To determine the adsorption index (AI) of the CTL or STS the ratio of CI or II of lymphocytes not adhering to the monolayer to those of intact lymphocytes was calculated in percent.

EXPERIMENTAL RESULTS

Stimulation of D2 anti-B6 MC in MLC with B6 cells led to the formation of secondary CTL, which destroyed B6 TC to the extent of 46% (Fig. 1), whereas normal spleen cells under similar conditions formed hardly any CTL. The ability of secondary CTL to destroy B6 TC was reduced by 70% after adsorption of the MC for 2 h on a monolayer of B6 cells, but only by 13% after their adsorption on a monolayer of syngeneic D2 macrophages (Fig. 1). It will be clear from Fig. 1 that 2 h was the optimal incubation time for adhesion of MC to cells of the monolayer; a decrease or increase in this time led to a decrease in adsorption on the B6 monolayer or to an increase in nonspecific adsorption on the monolayer of D2 cells. Preliminary treatment of cells of the monolayer with pronase led to a decrease in nonspecific adhesion of MC, so that the ratio of adsorption indices on allogeneic and syngeneic monolayers increased by 1.6 times (data not given). Similar results were obtained during adsorption of STS [4] and CTL [2]. In the next experiments all the cells studied were adsorbed for 2 h. Unlike CTL and STS, MC were found to be completely and specifically adsorbed only at 37°C, and at 30°C adsorption was reduced by half and was completely nonspecific (data not given).

To study the possibility that MC are adsorbed on killed TC, the macrophage monolayer was fixed with GA. The results of one experiment to study dependence of the degree of adsorption of MC on the concentration of GA solution which fixed the TC for 30 sec (fixation of TC for 5 min in all cases reduced adsorption of the cells considerably), are given in Fig.

2. It will be clear from Fig. 2 that treatment of TC with GA reduced adsorption of MC on B6 macrophages only slightly, but considerably reduced nonspecific adsorption of the cells on a syngeneic monolayer. In the next experiments GA was used in a concentration of 0.05%, which reliably stabilizes the TC monolayer, whereas a reduced concentration of GA fixes the monolayer inadequately, and this leads to partial detachment of macrophages from the plastic.

By contrast with MC, primary precursors of CTL were adsorbed to a much lesser degree on a monolayer of B6 macrophages growing in a small plastic flask, and were not adsorbed at all on the monolayer of B6 macrophages in a large flask, differing from the small flask only in its large surface area, whereas MC were adsorbed on the monolayer in the large flask just as well as in the small flask (Table 1). Even greater differences between T-cell subpopulations were found during their adsorption on TC fixed with GA. It will be clear from Table 2 that fixation of TC completely abolishes adsorption of CTL precursors in the small flasks, reduces adsorption of CTL by more than half, but reduces adsorption of MC and STS only very slightly.

Specificity of secondary CTL formed from MC immune to H-2 antigens is thus connected with the presence of receptors for H-2 antigens on the surface of MC, which enables these cells to be selectively attached to the monolayer of the corresponding TC. MC that are precursors of secondary CTL differ from primary precursors of CTL in their greater ability to be adsorbed on a monolayer of the corresponding TC and, in particular, after preliminary fixation of the cells on the monolayer. In the latter case, qualitative differences appeared between precursors of primary and secondary CTL, on the basis of which they can be separated. It can be tentatively suggested that primary immunization leads to changes in the MC receptor. The advantage of this suggestion is evidenced by the data we obtained about the variety of specificities of MC and primary CTL [5].

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