CORRELATION BETWEEN CYTOTOXIC T LYMPHOCYTES AND SUPPRESSORS BLOCKING ACTIVATION OF DNA SYNTHESIS IN MIXED LYMPHOCYTE CULTURES

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A parallel study was made of cytotoxic T lymphocytes (CTL) and suppressors induced by immunization in the H-2 system and inhibiting the activation of DNA synthesis in mixed cultures. Unlike the CTL, the suppressors do not adhere specifically to a monolayer of target cells, are not inactivated by treatment with anti-θ serum and complement, and their action is nonspecific: They inhibit the activation of DNA synthesis induced by any stimulator cells whatsoever and also by phytohemagglutinin and concanavalin A. CTL and suppressors are different populations of effector cells which can be separated from each other.

KEY WORDS: mixed lymphocyte cultures; suppressors; activation of DNA synthesis; cytotoxic T lymphocytes.

Immunization of mice with allogeneic spleen cells [14, 17] or tumor cells [5] induces not only cytotoxic T lymphocytes (CTL), but also suppressor cells which block reactions of cellular immunity. One such reaction, highly sensitive to the inhibitory action of suppressors, is unidirectional blast transformation in a mixed lymphocyte culture (MLC), a model of immunologic identification in vitro. However, it is not yet clear whether the blocking of blast transformation is connected with the cytotoxic action of CTL on stimulators of blast transformation [12] or with the activity of a population of suppressor cells that differ from CTL in a number of features and have no cytotoxic action on target cells (TC). Data on differences in the dynamics of formation of CTL and suppressors during immunization in vivo [5, 14, 15] and in vitro [13] and in their localization in the lymphoid organs are indirect evidence that the activity of the suppressors is not connected with CTL.

In the investigation described below the properties of CTL were compared with those of suppressors found by the writers previously and induced during immunization of mice in the same H-2 system [5]. The well-studied properties of CTL, namely their ability to adhere specifically to a TC monolayer [1, 2], high specificity of lysis [4], and sensitivity of the CTL to anti-θ antibodies [10], including in the system now used [3], were chosen for this purpose.

EXPERIMENTAL METHOD

B10.D2(H-2^d) or BALB/c(H-2^d) mice were immunized by single subcutaneous injections, at five separate points, of ascites cells of a sarcoma MCh-11, induced and maintained in C57BL/10 (H-2^b) mice (abbreviated to B10). Lymphocytes were obtained from the regional lymph nodes 8 days after immunization.

To set up the MLC equal volumes of reacting cells of lymph nodes and stimulating cells of allogenic spleen, irradiated in a dose of 1500 rad, were mixed. In some experiments DNA synthesis was activated by mitogens: phytohemagglutinin (PHA-P from Difco) or concanavalin A (Con A from Calbiochem). The doses of the cells, the culture medium, the conditions of culture in wells in microdisks, and the method of determination of DNA synthesis were all described previously [5]. Samples were applied to filters after culture for 96-120 h in MLC or incubation for 72 h with mitogens 16 h after the addition of 1 μCi [^{3}H]thymidine (1 Ci/mmole). The incorporation of [^{3}H]thymidine was measured in a scintillation β-spectrometer (Mark II from Nuclear Chicago, USA).

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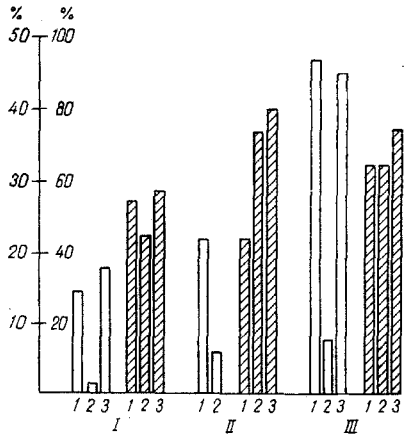

Fig. 1

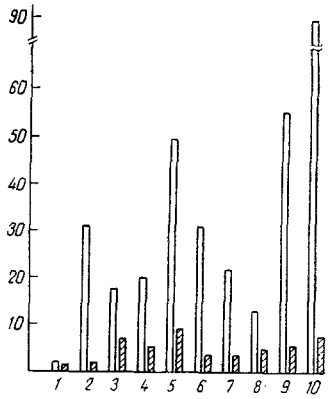

Fig. 2

Fig. 1. Adsorption of B10.D2 anti-B10 lymphocytes on TC: 1) original immune lymphocytes; 2) lymphocytes adsorbed on B10 TC; 3) the same, on B10.D2 TC. I, II, III) No. of experiment. Ordinate: left) cytotoxic effect relative to B10 TC (unshaded columns), right) decrease in activation of DNA synthesis (B10 cells as stimulators) compared with normal lymphocytes (shaded columns).

Fig. 2. Activation of DNA synthesis by stimulators: B10.D2 (1), B10 (2), B10.A (3), A.CA (4), DBA/1 (5), DBA/2 (6), R107 (7), 2R (8), PHA 2.5 μl/ml (9), Con A 10 μg/ml (10). Unshaded columns denote normal, shaded columns immune B10.D2 anti-B10 lymphocytes. Ordinate, IS.

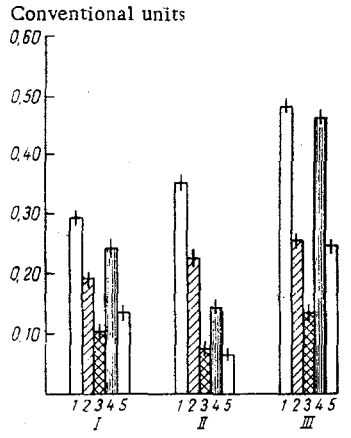

Fig. 3. Effect of anti-θ antibodies on blocking of activation of DNA synthesis by immune lymphocytes in MLC. B10 cells were stimulators. 2) Control treatment with culture medium; 3) with normal mouse serum 1/3 + complement; 4) with anti-θ serum 1/3 + inactivated complement; 5) with anti-θ serum 1/3 + complement: I, II, III) No. of experiment. Ordinate, IS of normal BALB/c lymphocytes (1) and mixture of them (10:1) with immune BALB/c anti-B10 lymphocytes (2-5).

As a control, either a mixture of reacting and syngeneic stimulating lymphocytes or twice the volume of stimulating and reacting cells separately (monocultures) were incubated in MLC. The index of stimulation (IS) of DNA synthesis was assessed as the ratio between incorporation of [^{3}H]thymidine (in cpm) into allogeneic MLC and incorporation into syngeneic MLC or half the combined incorporation into the monocultures.

For the cytotoxic test, based on liberation of ^{51}Cr from labeled macrophages, the microvariant described in [5] of the method [6] was used.

Adsorption of the immune lymphocytes on the monolayer of macrophages was carried out for 3-4 h by the method described previously [2]. Nonadherent lymphocytes were collected and counted, and the cytotoxic activity and ability to react in the MLC were determined at the same time.

Anti-θ serum obtained by the method described in [16] was used to treat the lymphocytes at the rate of 1 ml serum to $2 \cdot 10^7$ cells. After incubation of 30 min at 20°C the lymphocytes were centrifuged and the residue of cells was incubated in 1 ml nontoxic guinea pig complement (1:3) for 1 h at 37°C, followed by washing three times. Treatment with culture medium, with normal mouse serum and complement, and also with anti-θ serum and inactivated complement were used as the controls. After treatment all suspensions were counted and equalized for concentration of living cells. Under these conditions the anti-θ serum in dilutions of up to 1:27 killed up to 20-25% of lymph node cells of BALB/c mice.

EXPERIMENTAL RESULTS

After adsorption of the CTL by the corresponding allogeneic TC the cytotoxic activity of the nonadherent lymphocytes fell by 75-85%. This decrease in the cytotoxic index was specific, for it was not observed during analogous adsorption with syngeneic TC (Fig. 1). It will be clear from Fig. 1 that in the same experiments the ability of the immune lymphocytes to inhibit activation of DNA synthesis in MLC was not reduced after adsorption either by allogeneic or by syngeneic TC. Thus unlike CTL, the suppressors do not adhere to TC under the experimental conditions used.

As was shown previously, highly specific CTL react with "foreign" TC when crossed with immunizing TC only with respect to special H-2 specificities [4]. As Fig. 2 shows, immune B10.D2 anti-B10 lymphocytes synthesize much less DNA than normal lymphocytes following contact with stimulators not only of the B10, but also of other lines with a crossing-over with B10 for both special (R107 and 2R) and for separate general H-2 specificities (B10.A, A.CA, and DBA/1) [7]. It is also clear from Fig. 2 that immune lymphocytes react in MLC to a lesser degree than normal, even on DBA/2 cells, which are identical with the reacting B10.D2 cells with respect to the H-2 system but differ from them in their M locus [11]. In all these cases not only was IS reduced, but the absolute incorporation of [^{3}H]thymidine into the immune lymphocytes also was reduced, although by a lesser degree, compared with normal. IS also was substantially lower in the immune than in normal lymphocytes in the reaction to PHA and Con A (Fig. 2).

Unlike the highly specific CTL, the suppressors contained in the same suspension of immune lymphocytes are thus nonspecific: They inhibit activation of DNA synthesis in T cells induced by any of the stimulators used.

Activation of DNA synthesis in MLC is blocked by the addition of only 10% of immune lymphocytes to normal lymphocytes [5]. If immune BALB/c lymphocytes with anti-B10 were treated with anti-θ serum and complement, and then added to normal syngeneic lymphocytes, their blocking activity was completely preserved (Fig. 3). In the same experiments, activity of CTL was inhibited by anti-θ serum by 90-95% (control treatment did not change the cytotoxic effect). By contrast with CTL, the suppressors are thus not inactivated by anti-θ serum.

These results indicate that inhibition of the activation of DNA synthesis by suppressors in MLC is not due to their cytotoxic action on the TC inducing this activation. Moreover, the suppressors can be separated from CTL contained in the same suspension of immune lymphocytes both by adsorption on a TC monolayer and by treatment with anti-θ serum. The absence of specificity of action of the suppressors agrees with data obtained with other models [13, 14] and may be due to polyclonal activation of precursors of the suppressors during immunization with H-2 antigens. Since anti-θ serum does not inactivate the suppressors, this suggests that they either contain little θ antigen or that they are not T lymphocytes at all. Suppressors thus differ both from CTL and from the T cells that produce mediators of hypersensitivity of delayed type, which are highly sensitive to anti-θ antibodies [9] and which act specifically, although less selectively than CTL [8]. This means that H-2 antigens induce a spectrum of subpopulations of effector lymphocytes, the histogenetic relations and interaction between which are of considerable interest.

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EFFECT OF PRELIMINARY INJECTION OF ALLOGENEIC CELLS ON TRANSPLANTATION IMMUNITY IN MICE RECEIVING CYCLOPHOSPHAMIDE

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Injection of 1×10^8 C57BL/6 mouse spleen cells into CBA mice 1 day before treatment with cyclophosphamide (CP) was shown to promote survival of 2×10^7 allogeneic or semiallogeneic cells injected later (3-6 h after CP). The criterion of survival was the ability of the donor cells to produce antibodies against sheep's red blood cells in recipients tolerant to this antigen. Injection of 1×10^8 allogeneic cells 2 days before CP treatment had no protective effect. After intravenous immunization with allogeneic cells, killer cells began to appear in the recipient's spleen as early as on the 2nd day, and their number reached a maximum on the 5th day. The results suggest that CP eliminates the recipient's lymphocytes responding to transplantation antigens, but the killer cells already formed are resistant to the action of CP.

KEY WORDS: transplantation immunity; tolerance; killer cells, cyclophosphamide.

The writers showed previously that by combined injections of nonlethal doses of cyclophosphamide (CP) and spleen cells of $(CBA\times C57BL/6)F_1$ hybrid mice, a long-lasting (up to 12 days) semiallogeneic chimerism of the lymphoid tissue can be induced in adult CBA mice [1, 2]. This chimerism is accompanied by tolerance to the donors' tissues, which differ from those of the recipient in their strong H-2 antigens. It has been shown that a transplanted allogeneic heart will survive for a long time, for 5 months or more, in such mice [4]. The

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