INDUCTION OF T SUPPRESSORS OF DELAYED TYPE HYPERSENSITIVITY DURING KEY EFFECTOR FORMATION

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It was shown previously that injection of 10 syngeneic spleen cells modified by trinitrobenzenesulfonic acid (TNB-SC) or by azobenzarsenate (ABA-SC) into mice induces, on the 3rd-4th day, the accumulation of T effector cells of delayed-type hypersensitivity (DTH) in the spleen of the experimental animals [1]. Epicutaneous application of trinitrochlorobenzene (TNCB) at these times caused skin reactions which developed not earlier than after 22 h [1]. Sensitization was specific, transient in character, and could no longer be detected on the 7th day after injection of TNB-SC [1]. This type of course of DTH could be attributed to the formation of suppressor cells which, as we know, are easily activated in response to intravenous injection of an antigen [2-4, 6, 7].

In the investigation described below the possibility of formation of DTH suppressors on the 6th-7th day after injection of TNB-SC was studied, i.e., at a time when the level of sensitization in the experimental animals had fallen considerably.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA and BALB/c mice weighing 18-20 g. Modification of the cells by haptens was carried out as described previously [1]. The formation of DHT suppressors after intravenous immunization with 10^5 TNB-SC or ABA-SC was determined on the 6th-7th day. For this purpose different numbers of unseparated spleen cells, enriched with a population of splenic T cells or of T cells capable of adsorption on plastic dishes covered with a conjugate of TNB with bovine serum albumin (TNB-BSA), was injected intravenously into intact syngeneic recipients, which, immediately after transfer, were sensitized by epicutaneous applications of 150 µl of a 7% alcoholic solution of TNCB [1, 8] or subcutaneous injection of 3×10^7 ABA-SC, which were injected beneath the dorsal skin at two points [1, 7]. The intensity of the DTH reaction was estimated on the 6th day by skin tests [1]. For this purpose, in the case of sensitization by TNCB, 25 µl of a 1% solution of TNCB in olive oil was applied to the skin of the ear, whereas in the case of sensitization with ABA-SC intradermally, 50 µg of ABA in 12 µl of Hanks' solution, pH 8.2, was injected intradermally into a footpad of the hind limb. The reaction was read after 24 h by means of a type MKO-25 engineer's micrometer. The difference in thickness of the ears or paws reflected the intensity of the reaction.

In some experiments suppressor activity of the spleen cells of mice immunized with 10^5 TNB-SC was tested in the phase of expression of the DTH reaction. For this purpose, spleen cells of immune animals were transplanted into sensitized recipients before performance of the skin tests. The formation of DTH suppressors also was investigated in mice treated with cyclophosphamide (CP) in a dose of 50 mg/kg, which was injected 2 days before intravenous immunization.

In all experiments mice sensitized with TNCB or ABA (positive control) and intact mice on which only skin tests were carried out (negative control) were used as the controls.

The pressor activity of the spleen cells was estimated from the degree of suppression of the DTH reaction (in %), calculated as the ratio:

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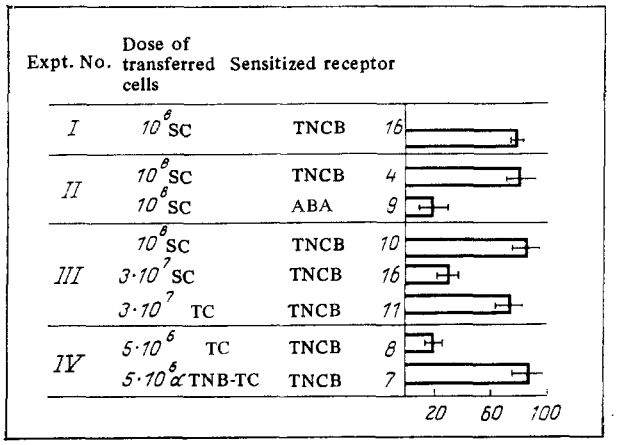

Fig. 1. Effect of different doses of unfractionated spleen cells, enriched population of splenic T cells (TC), and T cells isolated on TNB-BSA (TNB-TC) of CBA (series I) and BALB/c (series II-IV) mice sensitized with 5×10^4-5×10^5 of 10 mM TNB-SC (series I) or 10^5 of 1 mM TNB-SC (series II-IV) on development of sensitization in syngeneic recipients to TNCB or ABA. Horizontally — suppression of DTH in %. On left — experimental conditions. Numbers indicate number of recipients. Level of sensitization of positive control in series I was 0.23 ± 0.018, of negative control 0.045 ± 0.001; in series II: TNCB — 0.36 ± 0.02 and 0.06 ± 0.001, ABA — 0.92 ± 0.08 and 0.12 ± 0.03; in series III 0.32 ± 0.02 and 0.03 0.002; in IV 0.36 ± 0.023 and 0.04 ± 0.001 respectively. SC) Spleen cells, TC) T cells.

$$\frac{\text{Size of edema in experiment} - \text{size of edema in negative control}}{\text{Size of edema in positive control} - \text{size of edema in negative control}} \times 100.$$

Enrichment of the T-cell population was carried out on plastic dishes covered with antibodies against mouse immunoglobulins by the method in [5]. For this purpose, 6.5 ml of a solution of rabbit antibodies against mouse immunoglobulins in buffered physiological saline, pH 7.2, in a concentration of 400 μg/ml was applied to polystyrene dishes (manufactured by the Leningrad Medical Polymers Factory), 100 mm in diameter (some of the antibodies were generously provided by O. P. Terekhov). The rabbit antibodies were isolated by affinity chromatography from the serum of rabbits repeatedly immunized either with the total mouse globulin fraction or with mouse γ-globulin. Tissues with antibodies were incubated for 18-20 h in a refrigerator, after which they were washed 5 times with buffered physiological saline, once with Hanks' solution, and incubated for 15 min with Hanks' solution containing 10% embryonic calf serum and 10 mM HEPES. Next, 5×10^7 spleen cells in 6.5 ml of RPMI-1640 medium with additives were applied to the dishes. After incubation for 1 h at room temperature the nonadherent cells were removed and washed twice with medium 199 with additives, and the suppressor activity of these cells was tested by transplantation as described above. With this method [2] of enrichment of T cells the efficiency of purification from B lymphocytes, determined by the local hemolysis method, was 90-95%. Antigen-specific suppressor cells were isolated from plastic dishes covered with TNB-BSA in a concentration of 1 mg/ml. After incubation with antigen for 18 h at 4°C the dishes were washed just as during enrichment of the T cells, and 5×10^7-7.5×10^7 of the enriched population of splenic T cells in 6 ml of medium RPMI-1640 with additives were applied to the dishes. After incubation for 1 h at room temperature the nonadherent cells were removed, the dishes were washed, fresh medium was poured into them, and incubation continued for 30 min at 0-4°C. After incubation

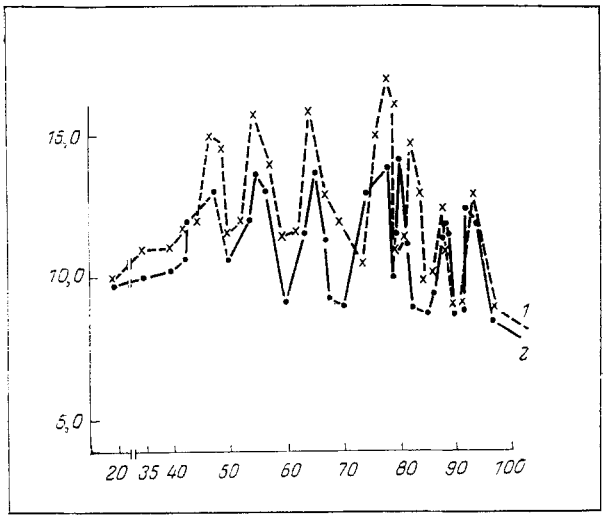

Fig. 2. Suppressor activity of spleen cells of BALB/c mice immunized with 10^5 of 1 mM TNB-SC (alone or together with CP) in the phase of DTH induction or expression. Level of sensitization of positive control 0.26 ± 0.008, of negative control 0.03 ± 0.002. Remainder of legend the same in Fig. 1.

the cells were washed off by pipeting. Suppressor activity of the adherent cells was tested by the transfer method.

EXPERIMENTAL RESULTS

The results of experiments to study suppressor activity of spleen cells of mice immunized with TNB-SC are illustrated in Fig. 1. It will be clear from Fig. 1 that spleen cells of CBA or BALB/c mice suppressed the phase of DTH induction practically completely in recipients sensitized epicutaneously with TNCB (series I and III). Suppressor activity was dose-dependent, and transfer of 3×10^7 unseparated spleen cells caused 30% suppression of DTH. After injection of the same number of the enriched population of T cells suppression of DTH was largely restored (series III). A suppressor effect analogous to that of 10^8 unfractionated spleen cells could be obtained by transplanting 3×10^6-5×10^6 T cells capable of being adsorbed on TNB-BSA, i.e., by adsorption on specific antigen the suppressor population could be enriched by 20-30 times (series IV). Counting the number of cells capable of adsorption on TNB-BSA from immune mice showed that it is about 8%. Nonspecific adsorption of spleen cells of intact animals under these same conditions did not exceed 2%. The results of the experiments of series II showed that the action of the suppressors was specific, so that suppressors obtained by immunization with TNB-SC depressed sensitization to ABA only very little. Under these same conditions suppressors induced by ABA-SC suppressed the development of the DTH reaction to ABA [7].

The data in Fig. 2 show that suppressors induced by injection of 10^5 modified spleen cells suppress the phase of DTH induction but do not suppress the phase of DTH expression, and their precursors are sensitive to the action of small doses of CP, which do not inhibit antibody synthesis [3].

The results are evidence that, just as after injection of 3×10^7 modified spleen cells [4, 6, 7], immunization with 10^5 TNB-SC induces T suppressor formation. However, whereas after injection of 3×10^7 modified cells, T suppressor formation induces absence of the DTH reaction in experimental animals, after injection of 10^5 TNB-SC, T suppressor formation is superposed on induction of T effectors [1].

The suppressor effect in the DTH induction phase is due to the presence of idiotype-positive suppressors capable of adsorption on antigen, whose precursors are sensitive to the action of small doses of CP (Tc1). The action of suppressors in the expression phase of DTH is connected with activation of anti-idiotypic suppressors (Tc2) [3, 6, 7]. After intravenous injection of 3×10^7 modified spleen cells, both populations of suppressor cells

are formed [3, 6]. In that case donors' spleen cells suppress both stages of DTH. In the present experiments suppression was observed only after transfer of spleen cells in the induction phase of DTH. These results suggest that after injection of 10^5 TNB-SC or ABA-SC suppressor cells of only one population were formed on the 6th day after immunization: In their positive action (action on the DTH induction phase, ability to be adsorbed on antigen, sensitivity of their precursors to CP) they were similar to Tcl described in other systems. However, the possibility cannot be ruled out that under the present experimental conditions a third population of suppressor cells with the same properties, but differing in Ly-phenotype (Tc3) also may be generated. Such suppressors are formed in lymph nodes in response to induction of DTH by epicutaneous application of a contact allergen or subcutaneous injection of modified spleen cells [3, 6].

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