# GENETIC RESTRICTION OF INTERACTION BETWEEN DELAYED TYPE HYPERSENSITIVITY EFFECTS AND SUPPRESSOR CELLS

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It was shown previously that injection of a massive dose of sheep's red blood cells (SRBC) into mice causes the accumulation in the spleen of an independent population of T suppressor cells, capable of preventing sensitization of syngeneic recipients [4, 11]. Preliminary results showed that transfer of suppressors of delayed type hypersensitivity (DTH) to allogeneic recipients is ineffective.

The causes of absence of this suppressor effect in an allogeneic system were studied in the investigation described below.

## EXPERIMENTAL METHOD

Experiments were carried out on CBA (haplotype H-$2^k$), C57BL/6 (H-$2^b$), and hybrid (CBA × C57BL/6)$F_1$ (H-$2^{k/b}$) mice and also on A/Sn and B10.A mice, with the identical H-$2^a$ haplotype. To induce DTH suppressors 6 × $10^9$ SRBC were injected intraperitoneally into the mice. After 5 days the spleen cells of these animals were washed with medium No. 199, resuspended in the same medium, and transferred in a dose of $10^8$ into syngeneic or allogeneic recipients. The recipients were sensitized immediately after transfer of the suppressor cells by intravenous injection of 2 × $10^5$ SRBC. The C57BL/6 (N6) mice were an exception, for they were sensitized with $10^6$ SRBC. The DTH level was determined on the 4th day by skin tests [7]. For this purpose, $10^8$ SRBC in 40 μl sterile physiological saline were injected subcutaneously into the hind foot pad. The difference in thickness of the hind paws was a measure of the intensity of edema and, consequently, of the level of the reaction. Depending on the aims of the experiment the animals were sensitized 1, 2, and 4 days before transfer of the suppressor cells. To determine the duration of survival of the lymphoid cells in the allogeneic recipient, double passage of the cells was used. Spleen cells of CBA mice, sensitized 7 days before the experiment with $10^6$ SRBC, were injected intravenously in a dose of $10^8$ into intact B6 mice. At various intervals later (2-4 days) the spleen cells of the intermediate recipients were injected in a dose of 1.5 × $10^8$ intravenously into CBA mice which had been immunized 1 and 5 days before the experiments with B6 spleen cells (2.5 × $10^7$ cells, intraperitoneally), and irradiated 1 day before transfer in a dose of 500 R on a cobalt source. The final recipients were given an intravenous injection of 2 × $10^8$ SRBC and the number of antibody-forming cells (AFC) in the spleen was determined 5 days later by the local hemolysis in gel method. In the control experiments the intermediate recipients were (CBA × C57BL/6)$F_1$ mice (positive control). CBA mice not receiving cells of the primary or intermediate donor, irradiated in a dose of 500 R, served as the negative control. The line to which the AFC belonged was determined by the method of discriminative analysis with antilinear sera [1]. For this purpose, 0.3 ml of a cell suspension containing 3 × $10^6$ cells was incubated with 0.1 ml of antilinear CBA anti-C57BL/6 serum and with 0.1 ml of fresh rabbit complement at 37°C for 45 min. A parallel cell sample was treated with normal mouse serum.

To study the dynamics of killer cell formation, CBA mice were given an intravenous injection of $10^8$ B6 mouse spleen cells. The cytotoxic activity of CBA mouse spleen cells against B6 macrophages was determined with effect from the first day [5, 6].

## EXPERIMENTAL RESULTS

The results of experiments in which suppressor cells were transplanted into allogeneic recipients are given in Fig. 1. They show that transfer of spleen cells of animals subjected to a massive dose of SRBC led to disturbance of DTH formation in syngeneic recipients but had no effect on DTH formation in allogeneic recipients. The absence of a suppressor effect in the allogeneic system could be due to several factors and, first of all, to rejection of the allogeneic cells. To test this

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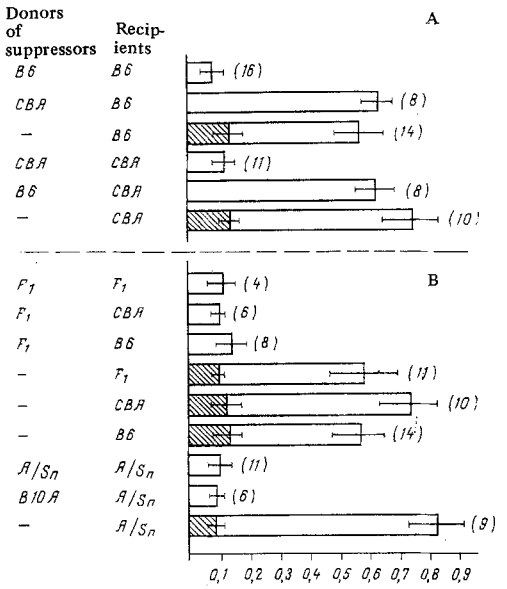


Fig. 1. Role of identity of H-2 haplotype in effectiveness of interaction between DTH suppressors and effectors. Abscissa, intensity of skin reaction (in mm); shaded part of columns indicates intensity of reaction of negative control (skin-tested intact animals). Number of animals given in parentheses.

hypothesis the first essential was to determine the time in which transplantation of DTH suppressors was effective. The results of these experiments (Fig. 2) showed that the suppressor effect of spleen cells of animals receiving a massive dose of SRBC was observed only if the cells were injected not later than 1 day after intravenous immunization of the recipients. Suppression of DTH was not observed when the cells were transplanted 2 days after immunization, and near the time of the reacting injection of antigen. These results suggest that the suppressors in this particular model act on the early stages of differentiation of precursors of DTH effectors and that they are ineffective against formed effectors.

The suppressor action of the spleen cells in a syngeneic system is thus realized during the first day. Are the allogeneic cells rejected during this time? Special experiments in which the dynamics of killer cell formation was studied after intravenous injection of $10^8$ allogeneic cells showed that some killer activity of the spleen cells of the immune animals was observed only toward the 2nd or 3rd day, but not 1 day after immunization. However, it was important to determine the duration of persistence of allogeneic lymphocytes in more direct experiments. This was done by passage of lymphocytes through allogeneic and semiallogeneic animals at different times. The secondary syngeneic recipients were treated so as to reject the cells of the intermediate donors and so as not to produce antibodies against the test antigen (SRBC). The presence of AFC in the spleen of the secondary recipients was evidence of survival of the primary donor's lymphocytes in the spleen of the intermediate allogeneic recipient during the time which elapsed between the two successive cell transfers. The donor nature of the AFC was additionally verified with the aid of antilinear sera. As Fig. 3 shows, animals irradiated in a dose of 500 R and not receiving donors' lymphocytes were unable to form AFC after immunization with SRBC. Conversely, after passage of the lymphocytes through a semisyngeneic recipient, a fairly high level of response was observed (in this case the number of AFC was 28,000). In the case of passage of the lymphocytes through allogeneic intermediate recipients, AFC of donor origin also were found in the final recipient, although their number was substantially less than in the case of passage through an $F_1$ intermediate recipient.

Lymphocytes injected intravenously in a dose of $10^8$, according to the results of these experiments, can thus be detected in allogeneic recipients for not less than 4 days. Meanwhile the action of the stressors was not exhibited in the allogeneic system, although contact with the precursors of the DTH effectors for 1 day was necessary for their action. It is evident that the absence of the suppressor effect in an allogeneic system was due not to rejection of the suppressor cells, but to some other mechanism – genetic restriction of interaction between DTH suppressors and effectors. Further experiments were carried out to test this hypothesis. Data on transfer of suppressor cells, generated in $F_1$ mice, into mice of the parental lines are given in Fig. 1. $F_1$ cells effectively suppress DTH formation both in syngeneic recipients and in the recipients of parental lines, although $F_1$ cells present in the latter ought to be rejected just like allogeneic cells. Data on transfer of allogeneic suppressor cells (B10·A) into recipients of the same H-2 haplotype (A/Sn) also are given in Fig. 1. They show that effective interaction between suppressors and effectors in an allogeneic system is possible if their H-2

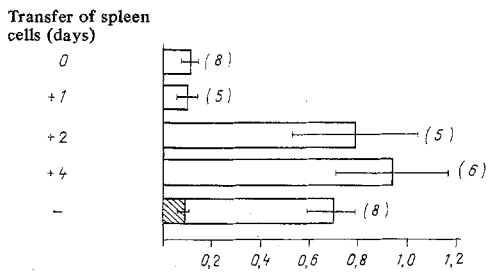


Fig. 2

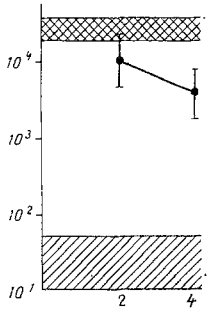


Fig. 3

Fig. 2. Effectiveness of transfer of suppressor cells at various times after sensitization of recipients. Legend as to Fig. 1.

Fig. 3. Periods of persistence of spleen cells in allogeneic recipient. Abscissa, periods of persistence of CBA lymphocytes in intermediate recipients (in days): ordinate, number of AFC against SRBC in spleen of secondary recipients irradiated in dose of 500 R and presensitized with C57BL/6 mouse cells. Filled circles denote level of response of secondary recipients in the case of passage of CBA lymphocytes through C57BL/6 intermediate recipients (experimental groups). Cross-hatching denotes level of response of secondary recipients after passage of CBA lymphocytes through (C57BL/6 × CBA) $F_1$ recipients (positive control); oblique shading represents level of response of secondary CBA recipients, not receiving donors' lymphocytes (negative control).

complex is identical. Disturbance of DTH formation could not be due to competition between the alloantigen of the donors' cells and SRBC, for transfer of cells from donors differing in the structure of their H-2 complex (Fig. 1) did not affect the development of sensitization in the recipients.

All in all the results are evidence that interaction between DTH suppressors and effectors, when recipients are sensitized with the optimal dose of ARBC, just as in the case of the action of suppressor factors regulating the intensity of DTH against haptens [9, 10], is restricted by the main histocompatibility complex.

Liew and Gill [8] found no genetic restriction during the action of suppressor factor on expression of DTH against SRBC. This may be due to technical factors arising from their experiments. In particular, these workers induced suppressors by intravenous injection of the antigen, and during sensitization of the recipients they injected cyclophosphamide (CP) [8]. However, injection of CP is known to cause elimination of a certain proportion of precursors of DTH effectors [2, 3], precursors of T suppressors [13], and also their amplifiers [12, 13]. Restriction of the suppressor effect may perhaps be regulated by cells sensitive to CP. The possibility likewise cannot be ruled out that the DTH effectors induced after injection of CP differ from ordinary effectors in their ability to interact with nonsyngeneic T suppressors. These two hypotheses require experimental verification.

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