PARALLEL FORMATION OF DELAYED-TYPE HYPERSENSITIVITY EFFECTOR CELLS AND T SUPPRESSORS AFTER MASSIVE INTRAPERITONEAL INJECTION OF SHEEP'S RED BLOOD CELLS

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The writers showed previously that injection of a massive dose of sheep's red blood cells (SRBC) into mice causes prolonged depression of the delayed-type hypersensitivity (DTH) reaction *in situ* [1]. On the 5th day after intraperitoneal injection of an antigen (AG), T suppressors (TS) accumulated in the spleen of the experimental animals and prevented sensitization of syngeneic recipients [2]. Preliminary injection of cyclophosphamide (CP) restored the ability of the experimental animals to form DTH and abolish the suppressor effect of TS in transfer [2]. It was postulated on the basis of these findings that absence of DTH *in situ* after massive injective of SRBC may be connected with the action of TS, whose precursors are sensitive to CP. However, it was still not known whether effectors of DTH (TE) are formed under these circumstances.

The aim of the present investigation was to study the ability of mice to form TE after receiving a massive injection of SRBC.

EXPERIMENTAL METHOD

Experiments were carried out on male C57BL/6 mice weighing 22-24 g. DTH was induced by intravenous injection of SRBC in a dose of 10^6 [6]. The level of DTH was estimated on the 4th day by means of skin tests [10]. An injection of 10^8 SRBC in 40 µl physiological saline was given into the hind foot pad of the mice. The thickness of both paws was measured 24 h later by means of an engineer's micrometer. The differences in thickness characterized the degree of edema and the intensity of DTH. TE formation was estimated by the local transfer method [4]. Different numbers of spleen cells were injected together with 10^8 SRBC in 40 µl of medium 199 directly into the paw. The reaction was read 22 h later by the skin test method. TS was induced by intraperitoneal injection of 6×10^9 SRBC. The suppressor activity of spleen cells (10^8) was estimated on the 1st-14th day in transfer to syngeneic recipients, which were sensitized immediately after transfer by 10^6 SRBC, and on the 4th day the level of sensitization was estimated by means of skin tests. The control in these experiments consisted of sensitized animals ("+control") and animals receiving only the reacting injection of AG ("− control"). Suppressor activity was expressed as the percentage suppression of DTH, which was determined by the formula:

$$\left(1 - \frac{\text{amount of edema in experimental group} - \text{amount of edema in "} - \text{control"}}{\text{amount of edema in "} + \text{control"} - \text{amount of edema in "} - \text{control"}}\right) \times 100\%.$$

Accumulation of TE also was tested in these same suspensions on the 5th day by the local transfer method. The number of T cells in the immune animals was increased by the method in [8]. For this purpose, 6 mg of rabbit antibodies (AB), obtained from antiserum against mouse immunoglobulins (Ig) with the aid of the immunosorbent Sepharose 4B with mouse Ig immobilized

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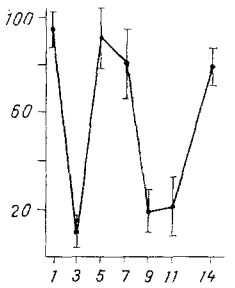

Fig. 1. Time course of suppressor activity of spleen cells from mice immunized with 6×10^9 SRBC. Abscissa, interval between immunization of donors and determination of suppressor activity in transfer to syngeneic recipients (in days); ordinate, percentage suppression of DTH.

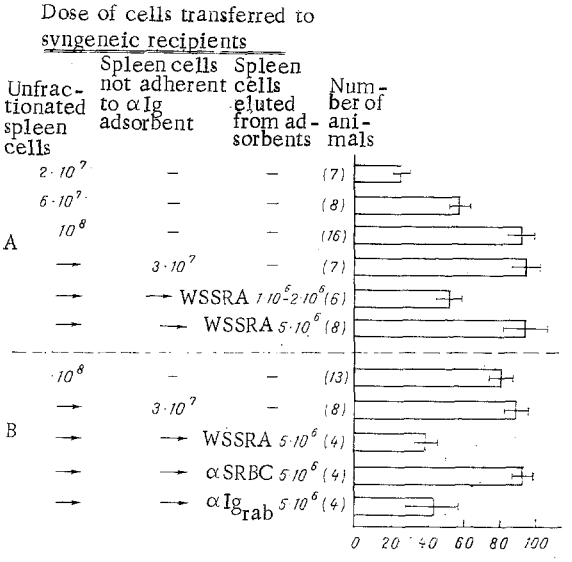

Fig. 2. Suppressor activity of unfractionated spleen cells from mice immunized with 6×10^9 SRBC, obtained on the 5th day (A) and 14th day (B) after immunization, and also of an enriched population of T cells (Ig^- cells not adherent to αIg adsorbent) and of T cells eluted from WSSRA adsorbent, αSRBC adsorbent, and adsorbent of antibodies against rabbit Ig (αIg_{rab}). Abscissa, percentage suppression of DTH.

on it, in 6 ml buffered physiological saline, was applied to plastic Petri dishes (Leningrad Medical Polymers Factory, diameter 100 mm), pretreated for 18 h with buffered physiological saline, pH 7.2. After incubation for 2 h at room temperature, and after thorough washing, 80-120 million spleen cells in 6 ml RPMI-1640 medium with 5% embryonic calf serum and 10 mM HEPES were applied to the dishes and incubated for 1 h at room temperature. The enriched T cells, which did not adhere to the dishes, were washed off with medium 199 and used for further work. The number of Ig^- cells, determined by the indirect rosette formation method [7] and the indirect immunofluorescence method, in the nonadherent fraction was 92-95% (the donkey AB against rabbit Ig, labeled with fluorescein isothiocyanate were generously provided by T. A. Danilova). Preparations of $F(ab')_2$ fragments of rabbit AB against mouse Ig, prepared by the method described in [11], were used in both tests. The preparations of $F(ab')_2$ fragments of rabbit AB against mouse Ig were generously provided by I. A. Tarkhanova.

To isolate AG-specific TS, enriched T cells numbering 75 million in 6 ml of RPMI-1640 medium with additives were applied to dishes 100 mm in diameter, preincubated for 2 h at room temperature with 30 mg water-soluble SRBC antigen (WSSRA), obtained by the method in [12], in 6 ml buffered physiological saline. Isolation of TS on AB was carried out by the method in [8] on dishes 40 mm in diameter, covered with 600 μg AB against SRBC in 2.25 ml buffered physiological saline. The number of cells destined for separation was 20 million.

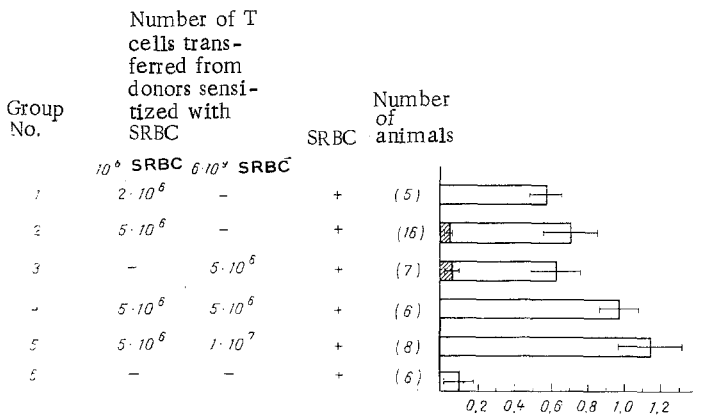

Fig. 3. Quantity of edema in recipient after local injection of splenic T cells from mice immunized 4 days previously with 10^6 SRBC or 5 days previously with 6×10^9 SRBC. Abscissa, amount of edema (in mm). Shaded parts of column indicate amount of edema after transfer of corresponding number of cells without SRBC.

AB aginst SRBC were obtained on adsorbent consisting of Sepharose 4B-WSSRA from hyperimmune serum from C57BL/6 mice immunized with six weekly intraperitoneal injections each of 2×10^8 SRBC. Dishes covered with goat AB against rabbit Ig (from Calbiochem, USA) served as the control. In both cases, after incubation for 1 h at room temperature, the nonadherent cells were removed and the dishes washed with a fresh portion of RPMI-1640 medium with additives, and incubated for 15-20 min at 0-4°C. Cells eluted from the dishes were washed twice with medium 199 with additives, after which their suppressor activity was investigated in transfer.

EXPERIMENTAL RESULTS

Data on suppressor activity of mouse spleen cells at different times after injection of SRBC in a dose of 6×10^9, tested in transfer to syngeneic recipients, are given in Fig. 1. They show that the curve of accumulation of TS had two peaks: the first on the 5th day, the second on the 14th day after injection of AG. Spleen cells taken on the 9th-11th day had no suppressor activity. Suppression of DTH observed after transfer of cells taken on the first day was evidently due to the presence of erythrocytic AG in the cell suspensions.

According to the hypothesis of Germain and Benacerraf [5], if supraoptimal doses of hapten-modified syngeneic cells are injected into mice, the experimental animals accumulate Lyl^+ $I\text{-}I^+$ cells, carrying the idiotype and capable of adsorption on AG. These "efferent T suppressors" induce Ly_2^+, $I\text{-}I^+$ cells, carrying the anti-idiotype and capable of adsorption on AB to the given AG, in syngeneic recipients. In turn, the secondary suppressors interact with TS_3 and cause suppression of DTH.

It will be clear from Fig. 2 that cells of the enriched T fraction obtained on the 5th day after injection of a massive dose of AG were capable of being adsorbed on dishes covered with WSSRA, and they perhaps carried the idiotype. T cells eluted from dishes covered with WSSRA possessed suppressor activity. Cells incapable of being adsorbed on AG did not possess suppressor activity. The suppressor activity of T cells capable of adsorption on WSSRA was dose-dependent. For instance, 1-2 million AG-specific TS caused 50% suppression of DTH, whereas if transferred in a dose of 5 million, the same suppression of DTH was observed as after transfer of 30 million cells of the enriched T fraction or 100 million unfractionated spleen cells.

Investigation of the ability of suppressor cells taken on the 14th day after injection of 6×10^9 SRBC to be adsorbed on dishes covered with WSSRA or AB against SRBC showed that these suppressors could be adsorbed on the AB and possibly were anti-idiotypic in nature. Some degree of suppressor activity was observed after transfer of T cells capable of adsorption on dishes covered with WSSRA (Fig. 2), and during testing of T cells eluted from dishes covered with AB against rabbit Ig. This last finding is evidently connected with adsorption of T cells possessing Fc receptors.

In the next series of experiments ability to form TE was investigated after immunization with a massive dose of AG, i.e., under conditions when TS are formed. The results of these experiments are shown in Fig. 3.

As Fig. 3 shows, local transfers of splenic T cells from mice immunized with 6×10^9 SRBC led to the development of DTH in syngeneic recipients. Under these circumstances combined injection of splenic T cells from mice sensitized with 10^6 SRBC and from mice immunized with 6×10^9 SRBC caused potentiation of DTH in the recipients, i.e., suppression of DTH was not observed.

These experiments showed convincingly that if a massive dose of AG is injected into mice, both TS, whose activity can be tested in transfer to syngeneic recipients before sensitization of the latter, and TE, which can be detected by the local transfer method simultaneously with the reacting injection of AG, are formed. The absence of DTH reactions in the early period after injection of a massive dose of AG is not connected with the action of TS capable of adsorption on AG, but is evidently due, just as after intravenous immunization with 10^8-10^9 SRBC, to the action of B cells, carrying immune complexes and preventing the release of TE from the spleen into recirculation [9]. Later, after 14 days, absence of DTH may also be due to the formation of TS capable of adsorption on AB against specific AG. A similar situation is evidently found on transfer of TS obtained from mouse spleen on the 5th day after injection of a massive dose of SRBC [2] into recipients sensitized with a small dose of AG. In this case, just as in the experiments of Sy et al. [13], who studied the mechanism of suppression of DTH to syngeneic cells modified by azobenzarsonate, TS transferred in the phase of DTH induction do not themselves cause suppression, but they induce accumulation of TS with properties identical to those of suppressors obtained on the 14th day after injection of a massive dose of AG. On transfer of these same cells in the phase of expression of DTH, i.e., toward the time of the reacting injection of AG [3], or in response to combined local injection of preformed effectors and suppressors (Fig. 3), suppression of the reaction does not take place. However, in the case of sensitization of the recipients to AG against the background of preliminary injection of CP, TS obtained on the 5th day after injection of a massive dose of AG can also suppress the phase of DTH expression [3]. The causes of these differences are not yet known, although it may be that injection of CP causes elimination of precursors of certain regulatory cells that prevent the action of this particular type of suppressors in the phase of DTH expression.

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