SUPPRESSION OF DELAYED TYPE HYPERSENSITIVITY IN MICE RECEIVING MASSIVE DOSES OF XENOGENEIC RED BLOOD CELLS

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Injection of 6×10^9 sheep's red blood cells into mice led to suppression of hypersensitivity of delayed type (HDT) in situ and caused activation of spleen cells suppressing sensitization of the recipients. Preliminary thymectomy on the donors and treatment of the cell suspensions with anti-T-globulin abolished the suppressor effect. Preliminary injection of small doses of cyclophosphamide potentiated both the response of antibody formation and the formation of HDT. With an increase in the dose of cyclophosphamide antibody formation was depressed and the HDT response further intensified. The results suggest that suppression of HDT is due to short-living, intensively proliferating cells of thymus origin and also, perhaps, B-cells.

KEY WORDS: hypersensitivity of delayed type; antibody formation; suppressor cells.

It was shown previously that injection of a massive dose of antigen (sheep's red blood cells – SRBC) into mice leads to suppression of hypersensitivity of delayed type (HDT) [2, 4, 6, 9, 11]. Spleen cells of these animals acquire the ability to specifically suppress the formation and realization of HDT in syngeneic recipients [2, 5, 7, 14]. Different workers have ascribed suppression of HDT to the activity of B-cells [6, 8], T-helpers [11], or a specialized subpopulation of T-suppressors [5, 7, 14]. Cyclophosphamide (CP), an inhibitor of proliferating cells, is known to prevent suppression of HDT but the mechanism of this effect is not clear [4, 6, 8, 12].

The object of the present investigation was to study the nature of suppressor cells by the parallel use of different techniques (thymectomy, injection of large doses of CP, treatment of the cells with anti-T-globulin).

EXPERIMENTAL METHOD

(CBA × C57BL/6)F_1 mice weighing 22-24 g were used. To study suppression of HDT in situ the mice were sensitized by intravenous injection of 6×10^9 SRBC. In the cell transfer experiments the same dose of SRBC was injected intraperitoneally into the donors. Five days later, 1×10^8 donors' spleen cells were injected intravenously into intact syngeneic recipients. The recipients were sensitized 1 h later by intravenous injection of 1×10^5 SRBC. In some experiments the suspension of spleen cells (in a concentration of 2×10^7/ml) was first incubated for 45 min at 37°C with rabbit anti-T-globulin in a final dilution of 1:20 and with rabbit complement in a final dilution of 1:5. The anti-T-globulin was obtained in the writers' laboratory by the method described previously [1].

The level of sensitization was determined on the 4th day with the aid of skin tests [2, 6]. The reacting injection (1×10^8 SRBC in 40 μl physiological saline) was given into a hind footpad. The intensity of the HDT reaction was read after 24 h. For this purpose, the thickness of the footpads of both hind limbs was measured with an MK 0-25 micrometer. The difference in thickness reflected the presence of edema and the degree of sensitization.

CP was injected intraperitoneally into the mice in doses of between 20 and 200 mg/kg. The thymus was removed by Miller's method [9] using the OKh-V1 surgical suction system. The number of antibody-forming cells (AFC) in the spleen was determined by Jerne's method on the 4th day after injection of SRBC.

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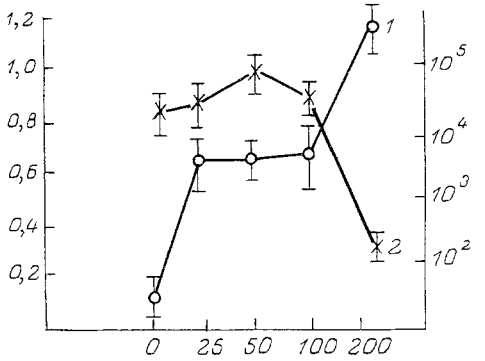

Fig. 1. Effect of preliminary injection (2 days beforehand) of CP on HDT level (1) and number of AFC (2) in spleen of animals immunized by intravenous injection of 6×10^9 SRBC. Abscissa, dose of CP (in mg/kg); ordinate: left – intensity of skin reaction (in mm), on right – number of AFC in spleen.

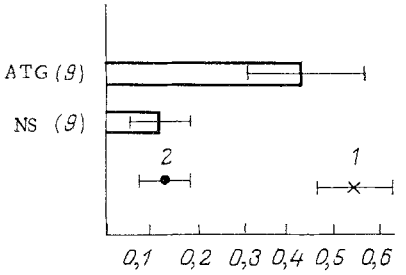

Fig. 2

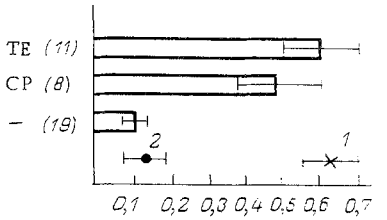

Fig. 3

Fig. 2. Effect of treatment with anti-T-globulin (ATG) and normal serum (NS) on suppressor activity of spleen cells of animals treated with massive doses of SRBC. 1) Level of positive control reaction; 2) intensity of edema of negative control. Number of animals in parentheses. Abscissa, intensity of skin reaction (in mm).

Fig. 3. Effect of thymectomy (TE), performed 2 weeks before injection of massive dose of SRBC, and of CP injected 1 day before immunization with SRBC in a dose of 200 mg/kg, on suppressor effect of spleen cells. Legend as to Fig. 2.

EXPERIMENTAL RESULTS

The results of experiments in which the effect of different doses of CP on the HDT level and on the number of AFC was studied are given in Fig. 1. They show that injection of a massive dose of SRBC caused intensive antibody formation, against the background of which HDT formation was weakened. Injection of CP 48 h before the antigen in doses of 25 to 100 mg/kg restored the ability of the experimental animals to form HDT. The number of AFC in the spleens of the animals treated with CP not only was not reduced, but after CP in a dose of 50 mg/kg it was actually increased. Injection of CP in a dose of 200 mg/kg caused considerable intensification of the skin reactions against the background of total suppression of AFC formation.

In the next two series of experiments suppressor activity was investigated during transfer of lymphocytes from animals receiving a massive dose of SRBC into intact recipients. The results of experiments with treatment of the cells with anti-T-globulin are illustrated in Fig. 2. As Fig. 2 shows, cells treated with serum from intact animals preserved their ability to suppress HDT formation. Treatment of the cells with anti-T-globulin abolished the suppressor effect. Similar results were obtained also when the donors underwent preliminary thymectomy or were injected with CP in a dose of 200 mg/kg (Fig. 3).

The results are evidence that inhibition of HDT formation as a result of injection of a massive dose of antigen is due to active suppression of this process and not to redistribution of HDT effectors between the blood and the spleen, as some workers have claimed [8]. The mechanisms of this suppression are many and varied. Abolition of suppressor activity as a result of previous thymectomy or after treatment of the cell suspension with anti-T-globulin points to the existence of a T-cell component of suppression. The experiments with thymectomy and preliminary injection of small doses of CP, when antibody formation and the function of the B-cells are preserved, showed that the precursors of T-suppressors of HDT or specific "amplifiers" of suppressors [12], unlike T-helpers, are a short-living (not more than 2 weeks), actively proliferating subpopulation. These observations agree with those published in the literature [3-5]. On the other hand, the further intensification of HDT under the influence of increased doses of CP, inhibiting antibody formation, suggest that the intensity of HDT is regulated by B-cells also. In the writers' view, the long familiar antagonism between antibody formation and HDT reactions can be explained by the influence of B-cells [11].

The small increase in antibody formation after injection of small doses of CP may be due to elimination of cells suppressing antibody production. These cells, as Whisler and Stobo [13] and Yamaguchi et al. [14] found, appear in the spleen of animals exposed to the action of a massive dose of antigen, and they differ in certain features from HDT suppressors.

Disturbance of HDT formation as a result of injection of a massive dose of SRBC is thus due, in the writers' opinion, to the combined action of T-suppressors and antibody-forming B-cells.

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