EFFECT OF ANTISERUM AGAINST ISOLOGOUS AGGREGATED IMMUNOGLOBULINS ON DELAYED-TYPE HYPERSENSITIVITY REACTION IN MICE IMMUNIZED WITH SHEEP RED BLOOD CELLS

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Injection of mouse antiserum against isologous aggregated immunoglobulins (MAAS) into mice previously receiving 10^5 sheep red blood cells (SRBC) did not affect the intensity of the delayed-type hypersensitivity (HDT) reaction when tested at the peak of sensitization (4th day), but led to a marked increase in the intensity of the reaction in the later stages (6th day). MAAS completely abolished suppression of HDT observed after sensitization with $5 \cdot 10^7$ SRBC. Transfer experiments showed that under the influence of MAAS the number of suppressor cells of HDT in the spleen of the sensitized mice was reduced. MAAS had no effect on proliferation of antibody-forming cells or on the intensity of hemagglutinin production, but reduced by 70% the number of rosette-forming cells (RFC) detectable in the spleen at the peak of the primary immune response. The results may be evidence that RFC take part in the suppression of HDT.

KEY WORDS: hypersensitivity of delayed type; suppressor cells of HDT; rosette-forming cells.

Antigen, if injected in a dose leading to intensive antibody production, is known to prevent the development of the reaction of hypersensitivity of delayed type (HDT) to the same antigen [11]. Suppression of HDT reactions in this case is due more probably not simply to antibodies but to antigen—antibody complexes [6, 9]. It has been suggested that immune complexes have an inhibitory action on reactions of cellular immunity, including that of HDT, with the direct participation of lymphocytes [1]. In this connection that fact is worth noting that at the peak of the primary immune response lymphocytes with aggregates of antibodies adsorbed on their surface accumulate in large numbers in the spleen [3]. These cells have not been found in immunized animals receiving injections of antiserum against isologous aggregated immunoglobulins, despite the fact that this antiserum had no marked effect on antibody biosynthesis [4].

The object of this investigation was to study the mechanism of regulation of HDT reactions by the use of antiserum against isologous aggregated immunoglobulins.

METHODS

Male CBA mice aged 3 months, vaccinated with smallpox vaccine 5-6 weeks before the experiments, were used. Antiserum against isologous mouse immunoglobulins (MAAS) was obtained as described previously [5]. The action of MAAS on the HDT reaction was studied by the following method. The antiserum was injected intraperitoneally in doses of 0.1 ml over a period of 4-5 days into mice immunized with sheep's red blood cells (SRBC). The first injection of serum was given 2 h after injection of the antigen. Mice of the control group, immunized with SRBC, were injected with normal isologous serum (NMS) in the same doses and in accordance with the same scheme.

The animals were sensitized by intravenous injection of SRBC in two doses: 10^5 and $5 \cdot 10^7$ cells [11]. The intensity of the HDT reaction was determined by skin tests [10, 12, 15]. For this purpose, 10^8 SRBC in 40 µl sterile physiological saline was injected into the right hind footpad intradermally 4-6 days after sensitization. By means of the MK 025 engi-

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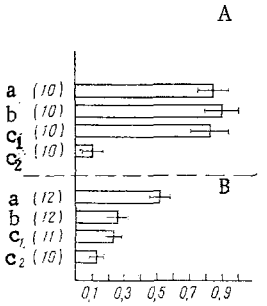

Fig. 1

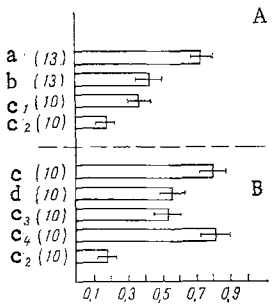

Fig. 2

Fig. 1. Effect of MAAS on HDT reaction in mice sensitized with 10^5 SRBC. Reacting intradermal injection of SRBC on 4th (A) and 6th (B) days. Abscissa, intensity of reaction (in mm); ordinate: a) reaction in sensitized mice receiving MAAS, b) reaction in sensitized mice receiving NMS; c_1) positive control — reaction in sensitized mice not receiving serum, c_2) negative control — reaction in intact mice to reacting intradermal injection of 10^8 SRBC. Number of animals in group shown in parentheses.

Fig. 2. Effect of MAAS on HDT reaction in mice sensitized with $5 \cdot 10^7$ SRBC. A) Reaction in experiments *in situ*, B) reaction in mice sensitized with 10^5 SRBC after receiving 10^8 spleen cells from donors sensitized with $5 \cdot 10^7$ SRBC. Reacting intradermal injection on 4th day. Abscissa, intensity of reaction (in mm); ordinate: in A) legend as in Fig. 1; in B) transfer of cells from sensitized donors receiving MAAS (c), NMS (d), not receiving serum (c_3); transfer of cells from intact syngeneic donors (c_4), c_2) negative control.

neer's micrometer the thickness of the footpads of both hind limbs was measured 24 h after the injection and the intensity of the reaction judged from the difference.

In transfer experiments spleen cells of donors immunized with $5 \cdot 10^7$ SRBC in combination with MAAS and NMS were washed once in medium 199, resuspended in the same medium, and injected in a dose of 10^8 into intact syngeneic recipients. The recipients were sensitized 1 h after injection of the cells by intravenous injection of 10^5 SRBC. The intensity of the HDT reaction was estimated, as described above, on the 4th day after sensitization.

The number of rosette-forming cells (RFC) in the spleen of the immunized mice was determined by Biozzi's method [4]. The number of antibody-forming cells (AFC) was determined by Jerne's direct method. The antibody titer was determined by the hemagglutination test.

RESULTS

After sensitization of the mice with 10^5 SRBC a second intradermal injection of the same antigen on the 4th day led to the development of an intensive HDT reaction. If the reacting injection was given 6-7 days after sensitization, the intensity of the HDT reaction was much less (Fig. 1) These results are in full agreement with those described previously [11]. Injection of MAAS into mice receiving 10^5 SRBC did not affect the intensity of the HDT reaction when tested at the peak of sensitization (the 4th day), but greatly increased the intensity of the reaction at later times (6th day).

The potentiating effect of MAAS on the HDT reaction was particularly marked after sensitization with the large dose of SRBC ($5 \cdot 10^7$). In this case the intensity of the reaction tested on the 4th day after sensitization was much lower than after sensitization with 10^5 SRBC (Fig. 2). After injection of MAAS the suppressing effect of the large dose of antigen was not observed: during testing on the 4th day the intensity of the reaction in these animals ($M \pm m = 0.74 \pm 0.046$ mm) was indistinguishable from that in animals sensitized with 10^5 SRBC (0.8 ± 0.047). The high level of sensitization after injection of $5 \cdot 10^7$ SRBC combined with MAAS still remains when the reaction was tested on the 5th day (0.72 ± 0.043 mm), compared

with 0.29 ± 0.034 and 0.30 ± 0.063 respectively in the control (sensitization with a large dose of antigen combined with injections of physiological saline or NMS).

Abolition of suppression of HDT due to the large dose of antigen by means of MAAS may take place as a result of elimination of suppressor cells from the spleen. This hypothesis was tested in transfer experiments. These were carried out in accordance with the scheme used previously to detect HDT suppressor cells [13]. In our experiments mice were given an injection of 10^5 SRBC and 10^8 spleen cells of syngeneic donors immunized with $5 \cdot 10^7$ SRBC. Compared with the animals of the control group, receiving spleen cells of intact donors, the HDT reaction in the animals of the experimental group was significantly reduced (0.81 ± 0.043 and 0.58 ± 0.051 respectively).

In the next series of experiments mice receiving MAAS and NMS, in addition to $5 \cdot 10^7$ SRBC, in accordance with the adopted scheme were used as donors. As Fig. 2 shows, only spleen cells from donors receiving MAAS were unable to suppress the HDT reaction in sensitized recipients. It must be remembered that suspensions of transplanted cells from immunized donors receiving MAAS and NMS contained equal numbers of AFC against SRBC (log of number of AFC per spleen 4.68 ± 0.03 and 4.65 ± 0.02 respectively). The hemagglutinin titer in donors receiving MAAS and NMS also was equal at the time of transplantation of the cells ($\log_2$ of titer 5.2 ± 0.24 and 5.4 ± 0.22). Meanwhile spleen cells of immune donors receiving MAAS and NMS differed significantly in the number of lymphocytes interacting specifically with antigen. It was shown by the rosette formation test that the number of RFC in mice receiving MAAS was reduced approximately by 70%.

As was demonstrated previously, injection of MAAS into mice immunized with SRBC leads to elimination of rosette-forming B cells with aggregates of antibodies, probably immune complexes, adsorbed on their surface [3, 4]. In turn, the spleen of sensitized animals is known to contain B cells which perform the function of HDT suppressors [7, 14]. If data showing the essential role of immune complexes in HDT suppression also are taken into account, it can be tentatively suggested that the rosette-forming B cells which carry aggregated antibodies on their surface, and which can be eliminated *in vivo* with the aid of MAAS, are among the HDT suppressor cells.

A definite similarity must be noted between the action of MAAS and that of an immunodepressant such as cyclophosphamide on the HDT reaction. By means of cyclophosphamide, in particular, rapidly dividing HDT suppressor cells can be eliminated [8]. The essential point is that immune lymphocytes, carrying aggregated antibodies on their surface, also have a high mitotic index [2].

LITERATURE CITED

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