Consequently, the distant effect observed in the experiments described above can only be dependent on low-molecular-weight shortliving metabolites or on certain physical factors.

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EFFECT OF MOUSE ANTISERUM AGAINST ISOLOGOUS AGGREGATED IMMUNOGLOBULINS ON ACCUMULATION OF ROSETTE-FORMING AND ANTIBODY-FORMING CELLS IN MICE IMMUNIZED WITH SHEEP'S RED BLOOD CELLS

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Data are given on the effect of mouse antiserum against isologous aggregated immunoglobulins (MAAS) on the kinetics of rosette-forming (RFC) and antibody-forming cells (AFC) in mice immunized with sheep's red blood cells (SRBC). The effect of MAAS in the experiments *in vivo* was assessed by injecting this serum for 5 days into CBA mice, the first injecting being combined with injection of 5.10^7 SRBC. Injection of MAAS into mice immunized with SRBC was shown to cause a marked decrease in the number of RFC in the spleen on the 5th and 9th days after immunization. MAAS has no appreciable effect at these same times on proliferation of AFC producing IgM hemagglutinins. Meanwhile MAAS intensified proliferation of IgG-AFC during the period when the number of these cells of the spleen in the immunized mice was maximal. After adsorption of MAAS with immune complexes formed by mouse IgG antibodies this serum was shown to lose much of its ability to block RFC *in vivo*. It is postulated on the basis of these results that the property of MAAS of influencing the accumulation of RFC and AFC producing IgG hemagglutinins is due to a factor which reacts with the immune complex formed by mouse IgG antibodies. This factor may perhaps be antibodies against aggregated immunoglobulins of this class.

KEY WORDS: *Humoral immune response; rosette-forming and antibody-forming cells.*

The mechanism of specific inhibition of the humoral immune response by antibodies has not yet been explained [1]. It was recently suggested that an important role in this mechanism is played by antigen–antibody complexes, which are fixed to the FC receptors of B lymphocytes and depress their antigen-dependent proliferation and transformation into antibody-producing cells [7]. This hypothesis is of considerable interest in the light of the previously established fact that aggregated antibodies are present on rosette-forming B cells which appear in the spleen of mice after antigenic stimulation [2, 4]. These cells lost their ability to interact with antigen after treatment with rabbit antibodies specifically

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recognizing aggregated mouse immunoglobulins. Mouse antiserum against isologous aggregated immunoglobulins (MAAS) possessed a similar property. If the serum was injected into mice at the same time as the antigen, no accumulation of rosette-forming B cells took place [3]. This could mean that MAAS either prevents binding of immune complexes by B cells or that it facilitates their screening and rapid elimination from the cell surface. Regardless of the concrete mechanism of action of this antiserum, it was decided to make use of it to determine the effect of B cells carrying aggregated antibodies on the proliferation and differentiation of precursors of antibody-forming cells (AFC). The effect of MAAS on the kinetics of rosette-forming cells (RFC) and of AFC was studied in mice immunized with sheep's red blood cells (SRBC).

EXPERIMENTAL METHODS

Experiments were carried out on male CBA mice. The preparation of the mouse antiserum against isologous immunoglobulins was described previously [3]. A hemagglutinating serum containing mainly IgM antibodies was obtained on the third day after immunization of mice with $5 \cdot 10^8$ SRBC. The serum of mice repeatedly immunized with the above dose of SRBC served as the source of IgG hemagglutinins.

RFC were determined by the method of Biozzi et al. [5] and AFC by the method of Jerne et al. [6]. Rabbit antiserum against mouse IgG, generously provided by S. P. Pershin, was used to determine IgG-AFC.

The effect of MAAS in the experiments *in vivo* was assessed by injecting this serum in a dose of 0.1 ml into mice immunized with 5.10^7 SRBC. The first injection of serum was given 2 h after immunization. Injections of MAAS continued at intervals of 24 h during the next 4 days. Mice of the control group, immunized with SRBC, were injected with normal isologous serum (NMS) in the same volumes and according to the same scheme instead of MAAS.

EXPERIMENTAL RESULTS

Injection of MAAS into mice immunized with SRBC led to a marked decrease in the number of RFC in the spleen (Table 1). This effect was most marked at the peak of the immune response, i.e., on the 5th day after immunization. Nine days after injection of the antigen and, consequently, 4 days after the end of the injections of MAAS, the number of RFC in the mice of the experimental group was significantly smaller than in the animals of the control group receiving NMS. By the 14th day after immunization, as a result of the more rapid decrease in the number of RFC in the mice of the control group, the differences between the mice of the groups compared ceased to be significant with respect to this parameter.

As the results in Table 2 show MAAS had no appreciable effect on proliferation of AFC producing IgM hemagglutinins. No significant differences with respect to this parameter were observed between the animals of the experimental and control groups throughout the period of observation. Meanwhile, MAAS had a definite effect on proliferation of AFC producing IgG hemagglutinins (Table 2). On the 9th day after immunization, i.e., when the number of these cells in the spleen was maximal, their number in the mice receiving MAAS was significantly greater than in the mice receiving NMS. On the 14th day after injection of the antigen the number of IgG-AFC in the mice of the experimental group was also significantly greater than their number in the control animals. Consequently, MAAS led to an increase in the antigen-dependent proliferation of cells producing IgG antibodies while at the same time it depressed the accumulation of RFC.

TABLE 1. Kinetics of RFC in Spleen of Mice after Injection of $5 \cdot 10^7$ SRBC and MAAS (M±m and 95% confidence intervals)

Serum	Number of RFC in spleen after immunization		
	5-th day	9- th day	14-th day
MAAS	6,2±0,6 (4,6—7,9)	7,1±0,3 (6,4—7,9)	6,5±1,5 (2,7—10,3)
NMS	17,8±1,4 (14,2—21,5)	12,7±1,0 (10,4—15,3)	5,9±0,7 (4,2—7,7)

TABLE 2. Kinetics of AFC Producing IgM and IgG Heamgglutinins in Spleen of Mice after Injection of $5 \cdot 10^7$ SRBC and MAAS (log. of number of AFC per spleen)

Serum	IgM-AFC			IgG-AFC	
	4-th day	9-th day	14-th day	9-th day	14-th day
MAAS	4,969±0,067 (4,783–5,155)	3,272±0,140 (2,911–3,634)	1,922±0,079 (1,740–2,104)	4,009±0,108 (3,731–4,287)	2,737±0,073 (2,569–2,905)
NMS	4,814±0,062 (4,642–4,986)	3,087±0,197 (2,581–3,593)	2,055±0,087 (1,854–2,256)	3,526±0,093 (3,287–3,765)	2,512±0,047 (2,404–2,620)

TABLE 3. Effect of MAAS Adsorbed by Immune Complexes on Accumulation of RFC in Mice Immunized with SRBC

Serum	Adsorbent	Number of RFC per 10^3 lymphocytes	Log. of number of IgM-AFC per spleen
MAAS	—	8,8±1,4 (5,7–11,9)	4,25±0,06 (4,11–4,39)
	SRBC	6,2±1,0 (3,9–8,5)	4,27±0,06 (4,13–4,41)
	SRBC with IgM antibodies	8,8±1,3 (5,6–11,9)	4,32±0,07 (4,16–4,48)
	SRBC with IgG antibodies	14,6±0,9 (12,6–16,6)	4,31±0,07 (4,15–4,47)
NMS	—	20,0±2,6 (14,0–25,8)	4,65±0,12 (4,37–4,93)
	SRBC	19,9±2,4 (14,4–25,4)	4,57±0,05 (4,45–4,71)
	SRBC with IgM antibodies	23,2±1,5 (19,8–26,6)	4,83±0,11 (4,57–5,09)
	SRBC with IgM antibodies	20,0±0,8 (18,1–21,8)	4,27±0,02 (4,22–4,32)

Note Data given on 5th day after immunization; each preparation was tested on 5 CBA mice; 95% confidence interval shown in parentheses.

In the light of these results it is a most interesting fact that after adsorption of the MAAS by immune complexes formed by mouse IgG antibodies this serum lost most of its activity *in vivo*. SRBC sensitized with agglutinating doses of mouse IgM or IgG antibodies were used as immune complexes. The agglutinated masses were carefully washed with physiological saline and added to MAAS, previously heated to 56°C, at the rate of 1 ml of residue of sensitized SRBC to 1 ml serum. The samples were incubated for 1 h at room temperature, with periodic mixing. The SRBC were then sedimented by centrifugation at 1500 rpm in the cold for 10 min. The supernatant was separated and sterilized by passage through a Millipore GSWP-0.25 membrane. Samples of normal serum were treated in the same way. Samples of NAAS and normal serum, adsorbed by the same doses of unsensitized SRBC, also were prepared. Each of the sera thus obtained was injected into different groups of mice immunized with SRBC in accordance with the scheme described in the section "Experimental Method." On the 5th day after immunization the number of RFC and AFC in the spleen was determined. As Table 3 shows, compared with MAAS adsorbed by unsensitized SRBC, the same serum when adsorbed with sensitized SRBC blocked RFC less effectively. The greatest effect was given by adsorption of MAAS by agglutinated masses containing IgG antibodies. The number of RFC discovered in this case was closer to their number in the mice receiving normal serum previously treated with unsensitized or sensitized SRBC. In the course of adsorption no contamination of the serum by erythrocytic antigen or by immune complex capable of influencing the immune response took place. As Table 3 shows, none of the sera used had any effect on proliferation of AFC producing IgM antibodies.

It can be concluded from the results described in this paper that the property of MAAS of influencing the accumulation of RFC and AFC producing IgG hemagglutinins is due to a factor which reacts with immune complex formed by mouse IgG antibodies. This factor may perhaps be antibodies against aggregated immunoglobulins of this class. The concrete mechanism where-

by antiserum against isologous immunoglobulins regulates the proliferation *in vivo* of AFC producing IgG antibodies is not yet known. Since this antiserum interacts *in vitro* with immune rosette-forming B cells, containing aggregated antibodies on their surface [3], and since it eliminates such RFC *in vivo* (Table 1), this suggests that in the course of the immune response it is the RFC, on the surface of which immune complexes are adsorbed, which inhibit the antigen-dependent activation of those immunocompetent B lymphocytes which are the precursors of the plasma cells producing IgG antibodies.

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ULTRASTRUCTURE OF CONJUGATES OF CYTOLYTIC T LYMPHOCYTES AND TARGET CELLS

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Conjugates of target cells and cytolytic T lymphocytes, isolated on the 11th day after alloimmunization, were investigated. Conjugates were formed by small and medium-sized lymphocytes, in the cytoplasm of which mature secretory granules, crystalloid structures, and lipids were found. The lymphocyte was spherical in shape and its area of contact with the target cell did not exceed 5-15%. Cytolysis of the target cells was observed after incubation for 30-60 min. The lymphocyte became flatter, its nucleus became oval in shape, and the area of its contact with the target cell increased. Meanwhile, hypertrophy and a change in the orientation of the Golgi complex were found in the zone of contact with the target cell, fusion of the secretory granules with the lipids and crystalloid structures took place, and immature secretory granules and vacuolar degeneration of the mitochondria appeared. "Peeling" of the lymphocyte membrane was observed, and structures connected with it and called "membranosomes" are described. It is suggested that secretory processes are activated in the cytoplasm of cytolytic T lymphocytes during their interaction with target cells.

KEY WORDS: *cytolytic T lymphocytes; Golgi complex; secretory granules.*

The study of the mechanisms of action of cytolytic T lymphocytes lies at the basis of the solution of the problem of transplantation and antitumor immunity. Immune T lymphocytes can be specifically adsorbed *in vitro* on target cells (TC) carrying stimulating antigen on their surface and can cause their lysis [7]. This model provides wide opportunities for the study of the mechanisms of cytolysis and the causes of death of the TC.

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