thymus dependence during polymerization. Possibly the molecular weight of the polymer obtained in the present experiments is too low to change the thymus dependence of the antigen. The methods of polymerization used in this investigation did not allow stable polymers of the toxoid with higher molecular weight to be obtained. It is also possible that in order to change the thymus dependence of Cl. perfringens toxoid other "cross-linking" agents must be used rather than glutaraldehyde.

LITERATURE CITED

1. G. F. Shemanova, E. V. Vlasova, V. S. Tsvetkov, et al., Biokhimiya, 33, 130 (1968).
2. G. L. Ada, Nature, 199, 1257 (1963).
3. G. L. Ada, G. Nossal, J. Pye, et al., Aust. J. Exp. Biol. Med. Sci., 42, 267 (1964).
4. F. A. Anderer and H. D. Schlumberger, Immunochemistry, 6, 1 (1969).
5. L. Csismas, Proc. Soc. Exp. Biol. (New York), 103, 157 (1960).
6. M. Feldman and A. Basten, J. Exp. Med., 134, 109 (1971).
7. A. Ito, Jap. J. Med. Sci. Biol., 121, 379 (1968).
8. R. Patterson and J. Suszko, J. Immunol., 112, 1855 (1974).
9. R. Patterson, J. M. Suszko, and F. C. McIntire, J. Immunol., 110, 1402 (1973).
10. R. Patterson, J. M. Suszko, F. C. McIntire, et al., J. Immunol., 110, 1413 (1973).
11. J. W. Schrader, J. Exp. Med., 137, 844 (1973).

PREPARATION OF A MOUSE ANTISERUM AGAINST ISOLOGOUS AGGREGATED IMMUNOGLOBULINS AND THE STUDY OF ITS ACTION ON ROSETTE-FORMING CELLS

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UDC 615.373.3:612.112.94

A method of obtaining antiserum against isologous aggregated mouse immunoglobulins (MAAS) is described. This serum blocks the antigen-binding receptors of immune rosette-forming cells in vitro. MAAS was injected into mice immunized with sheep's red cells. By comparison with immunized mice receiving normal isologous serum, rosette-forming B cells were absent at the peak of the primary response in the spleen of the mice receiving MAAS. The number of antibody-forming cells was not reduced under the influence of MAAS.

KEY WORDS: rosette-forming cells; aggregated immunoglobulins; B cells; primary immune response

Data indicating that during the primary immune response changes take place in the supramolecular organization of the receptors of B lymphocytes were obtained previously. This effect was manifested in the fact that receptors of rosette-forming B cells (RFC) during the first days after immunization of mice with sheep's red cells could be blocked by means of rabbit antiserum against aggregated mouse immunoglobulins (RAAS). Subsequently the RFC were insensitive to the action of RAAS, which likewise did not affect the spontaneous RFC present in the spleen of the mice before immunization [1]. These results, pointing to the appearance of new antigenic determinants in the antibody-like receptors of the immune B lymphocytes, showed that, in principle, it is possible to use antibodies against aggregated immunoglobulins in order to block these immune B lymphocytes in vivo in the early stages of the immune response, so that an overall assessment could be made of the functional role of the immune RFC in various forms of immune response.

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(Presented by Academician P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 12, pp. 698-700, December, 1977. Original article submitted May 10, 1977.

TABLE 1. Effect of MAAS on RFC and PFC from Spleen of Mice Immunized with 5×10^8 Sheep's Red Cells (M ± m and 95% confidence interval)

Serum for treatment	Number of RFC per 10^3 lymphocytes	Log of number of PFC per spleen
MAAS	10,3±0,9 (8,2—12,4)	4,280±0,038 (4,204—4,356)
NMS	18,9±1,4 (15,7—32,1)	4,355±0,041 (4,273—4,437)

Legend. Mean results of 10 determinations given.

TABLE 2. Number of RFC and PFC in Spleen of Immune Mice Receiving MAAS (M ± m and 95% confidence interval)

Serum for passive immunization	Number of RFC per 10^3 lymphocytes	Log of number of PFC per spleen
MAAS	6,6±0,3 (6,03—7,17)	4,337±0,135 (4,087—4,587)
NMS	19,6±0,6 (18,5—20,7)	4,497±0,033 (4,435—4,559)

Legend. Mean data for 30 CBA mice immunized with 5×10^7 sheep's red cells given. Numbers of RFC and PFC determined on 5th day after immunization with sheep's red cells.

This paper gives data on the preparation of an antiserum against isologous aggregated mouse immunoglobulins and its effect on RFC in experiments in vitro and in vivo.

EXPERIMENTAL METHOD

All experiments were carried out on CBA and BALB/c mice.

Aggregated Mouse Immunoglobulin. The immunoglobulin fraction of mouse serum was precipitated by ammonium sulfate at 50% saturation. The preparation was stored in the frozen state at −20°C. To aggregate the immunoglobulins, a 1% solution of the protein in physiological saline, pH 7.5, was heated for 20 min to 63°C.

Mouse Antiserum against Isologous Aggregated Immunoglobulins (MAAS). The freshly prepared heated CBA mouse immunoglobulins were injected intravenously into mice of the same strain in a dose of 3 mg. The mice were killed on the 6th day after the injection. The serum was sterilized by passage through a GSWP Millipore filter and kept unfrozen at 4°C.

Other Antisera. Rabbit antiserum against aggregated mouse immunoglobulins (RAAS) was obtained by the method described above [1] and anti-θ-serum by repeated intravenous immunization of AKR mice with spleen cells of C3H mice (titer in cytotoxic test 1 : 600). Rabbit serum against mouse thymocytes was provided by N. A. Kraskina.

Immunological Methods. RFC in mouse spleens were determined by the method of Biozzi et al. [2]. All procedures were carried out at 4°C. The experiments to study inhibition of rosette formation with the aid of MAAS were carried out at 4°C as described above [1]. To eliminate cells containing θ-antigen, 5×10^6 spleen cells were incubated with anti-θ-serum (final dilution 1 : 400) and with rabbit complement (final dilution 1 : 10) for 45 min at 37°C in a final volume of 1 ml. Thymus-dependent spleen cells were eliminated in the same way by means of an anti-T-serum, which was used in a final dilution of 1 : 64.

The number of plaque-forming cells was determined by the method of Jerne et al. To assess the effect of MAAS on the immune response, this antiserum was injected into CBA mice daily for 5 days in a dose of 0.1 ml. On the first day the mice were given an intravenous injection of 5×10^7 sheep's red cells followed, 2 h later, by a similar injection of MAAS. On the next 4 days MAAS was injected intraperitoneally. On the 5th day after injection of the sheep's red cells the number of RFC and PFC in the spleen was determined. Mice of the control group, immunized with sheep's red cells, received injections of normal CBA mouse serum (NMS) in the same doses and by the same scheme.

EXPERIMENTAL RESULTS

Treatment of the spleen cells obtained from mice on the 5th day after injection of 5×10^8 sheep's red cells with MAAS (final dilution 1 : 40) led to a decrease in the number of RFC detected by about 40%. Under the same conditions MAAS had no effect on the PFC (Table 1). These results indicate the similarity of the properties of MAAS and heterologous antiserum against aggregated mouse immunoglobulins [1].

Altogether nine batches of MAAS were obtained in CBA mice and two batches of this antiserum in BALB/c mice. In all cases RFC of mice of strains CBA and BALB/c, immunized with sheep's red cells, were blocked in vitro equally effectively. Normal serum, used in the same concentration as MAAS, did not block the immune RFC. No cytotoxic action of the MAAS on spleen cells could be observed.

In the subsequent experiments in vivo the effect of MAAS on accumulation of RFC in the spleen was investigated in mice immunized with sheep's red cells. If MAAS was injected in the course of immunization of the mice by the scheme described above, the number of RFC in the spleen on the fifth day after injection of the antigen was about 70% less than in the spleen of mice receiving NMS instead of MAAS (Table 2). When different batches of MAAS were tested, the results were similar. In no case were significant differences found in the number of nucleated cells in the spleen of the mice of the control and experimental groups. Consequently, the reduction in the number of RFC in the mice receiving MAAS was not due to the nonspecific cytotoxic action of that serum.

Immune RFC belonging to the series of T and B lymphocytes were unequally sensitive to MAAS. Spleen cells of mice of the experimental and control groups obtained on the 5th day after immunization were treated, in the presence of complement, with allogeneic anti-θ-serum or with rabbit antiserum against mouse thymocytes. The results showed that after elimination of the T lymphocytes, RFC could no longer be found in the spleen of the mice receiving MAAS. After similar treatment of the cells of immune mice receiving normal serum, the number of RFC was reduced by not more than 30%. This means that if MAAS was injected in the first phase of the immune response, this antiserum acted selectively, evidently only on RFC belonging to the class of B lymphocytes.

Despite the fact that the first contact with the antigen coincided with the beginning of passive immunization of mice by means of MAAS, this did not affect proliferation of the 19S PFC in the period of exponential growth in the number of these cells in the spleen (Table 2).

The results of these experiments are evidence that it is possible to obtain a mouse antiserum against isologous aggregated immunoglobulins, capable of blocking immune RFC in vitro and of preventing the accumulation of rosette-forming B cells in the spleen of mice immunized with sheep's red cells. Evidence was given previously to show that aggregated immunoglobulins performing the role of antigen-binding receptors can be found on the surface of rosette-forming B cells at the peak of the primary response [1]. It is therefore logical to suggest that the number of RFC in immunized mice receiving antiserum against isologous aggregated immunoglobulins is reduced on account of cells carrying aggregated immunoglobulins. At the peak of the immune response in mice receiving MAAS, no RFC inactivated in vitro by RAAS could in fact be found. In the mice receiving normal serum, the number of RFC blocked by heterologous antiserum against aggregated immunoglobulins reaches 70% [1]. In accordance with the preliminary results, after adsorption of MAAS by immune complexes, it loses its property of depressing the accumulation of RFC in immunized mice.

LITERATURE CITED

1. A. Ya. Kul'berg, V. V. Khorobrykh, I. A. Tarkhanova, et al., Byull. Éksp. Biol. Med., No. 6, 73 (1974).
2. G. Biozzi, C. Stiffel, D. Mouton, et al., Immunology, 14, 7 (1968).