The times of appearance of a cross-reacting antigen characteristic of stratum basale cells of the cutaneous epithelia of human and animal ectodermal origin were thus established by the indirect immunofluorescence method. The appearance of the CRA in the neonatal period determines the formation of natural immunologic tolerance to this antigen. This conclusion is supported by the results of investigations by other workers [8], indicating that no antibodies against antigens of the stratum basale cells of cutaneous epithelium can be found in the serum of clinically healthy persons. Antibodies against antigens of the subject's own body tissues appear only in the case of development of a pathological state or of experimental surmounting of immunologic tolerance.

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EFFECT OF ANTISERUM AGAINST ISOLOGOUS AGGREGATED IMMUNOGLOBULINS ON SYNTHESIS OF NONSPECIFIC IMMUNOGLOBULINS IN MICE IMMUNIZED WITH SHEEP'S RED BLOOD CELLS

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KEY WORDS: nonspecific immunoglobulins; aggregated immunoglobulins; rosette-forming cells.

As has been demonstrated in experiments in vitro, aggregated immunoglobulins activate mouse B lymphocytes and induce polyclonal antibody formation [6]. A similar situation might be created in vivo on account of aggregated antibodies formed in the course of the immune response. After immunization of mice, B lymphocytes with aggregated antibodies adsorbed on their surface are known to accumulate in the spleen [1]; it might be suggested that such cells, detected by the rosette-formation test with specific antigen, after activation by aggregates of antibodies, take part in the production of nonspecific immunoglobulins (NIG).

The object of the present investigation was to test this hypothesis by a study of NIG synthesis in mice receiving antiserum against isologous segregated immunoglobulins; as previous investigations [5] showed, such an antiserum can eliminate rosette-forming cells (RFC) carrying aggregated antibodies in vivo, without having any significant effect on antibody-forming cells (AFC).

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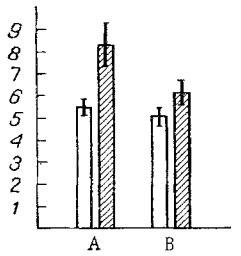

Fig. 1. Intensity of synthesis of NIG by spleen cells of mice immunized with 5×10^7 SRBC, after injection of NMS (A) and MAAS (B). Unshaded columns represent increase in radioactivity on immunosorbent with ovalbumin; shaded columns represent increase in radioactivity on immunosorbent with rabbit antibodies against mouse globulins. Ordinate, level of radioactivity (in cpm/sample $\times 10^{-3}$).

EXPERIMENTAL METHOD

Experiments were carried out on CBA mice. For 4 days the animals were given 0.1 ml of isologous antiserum against aggregated mouse immunoglobulins (MAAS) or 0.1 ml of normal mouse serum (NMS) daily, intraperitoneally. An intravenous injection of 5×10^7 sheep's red blood cells (SRBC) was given to the mice 2 h before the first intraperitoneal injection [4, 5]. On the 4th day the mice were killed, the spleens were removed, and cell suspensions were prepared and used for determination of the number of RFC and AFC and the quantity of antibodies synthesized against SRBC and NIG. The number of RFC was determined by Biozzi's method [7] and the number of AFC by Jerne's method [8]. The method suggested by Sidorova et al. [2, 3] was used to assess the synthesis of antibodies against SRBC and antigen-dependent NIG. For this purpose spleen cells (5×10^7 per sample) were incubated in penicillin flasks at 37°C in 2 ml of Eagle's medium containing 10 μCi glycine-^{14}C for 16-20 h. Immunoglobulins were removed from the supernatant with the aid of immunosorbents. Antibodies against SRBC were adsorbed on aminocellulose conjugated with water-soluble SRBC antigen (WSSA) [9] and antigen-dependent NIG were adsorbed on a sandwich-adsorbent containing rabbit antibodies against mouse immunoglobulin. A conjugate of aminocellulose with ovalbumin was used as the nonspecific immunosorbent. Radioactivity was determined on the Intertechnique SL-40 counter (France).

EXPERIMENTAL RESULTS

In agreement with previous findings, the number of RFC in the mice receiving MAAS was reduced by 70%. The number of RFC per thousand lymphocytes was 5.6 ± 0.5 in the experimental group and 17.2 ± 0.8 in the control group. No reduction in the number of AFC, estimated by Jerne's direct method, was observed. The number of AFC per million spleen cells was 962 ± 72 in the experimental group and 903 ± 40 in the control group. Injection of the antigen was accompanied by intensification of NIG synthesis in animals receiving both NMS and MAAS. However, the increase in NIG synthesis in the group of animals receiving MAAS was on average 20% less. The differences observed were significant. These results were obtained on 40 mice in four experiments. The results of one of the experiments are illustrated in Fig. 1.

It is important to note that the specific increase in radioactivity on WSSA was not observed, just as previously [3] on sheep's red cells. Evidently quantitatively few antibodies against SRBC were synthesized and the sensitivity of the method used was insufficient to detect them.

When the results are assessed it should be borne in mind, first, that after injection of MAAS into mice immunized with SRBC in no case was a decrease in the number of AFC or a decrease in hemagglutinin production observed [4]. Consequently, MAAS had the opposite action on synthesis of antibodies and NIG. Since a decrease in the number of RFC was observed at the same time under the influence of MAAS, RFC evidently cannot be regarded as the precursors of the antibody-producing cells. The problem of whether these cells act as precursors of the cells responsible for antigen-dependent NIG synthesis cannot be finally settled on the basis

of the results of the present investigation. Most likely the greater part of the antigen-dependent NIG is produced by certain other cells. The fact that the effect of MAAS on antigen-dependent NIG synthesis was less marked than its effect on accumulation of RFC in immunized animals suggests that some of the B lymphocytes, which carry aggregated immunoglobulins on their surface, can be activated by these aggregates and can participate in the synthesis of nonspecific immunoglobulins.

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VALUE OF THE SPONTANEOUS ROSETTE FORMATION TEST WITH MOUSE ERYTHROCYTES IN MULTIPLE ASSAY OF HUMAN B LYMPHOCYTES

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KEY WORDS: lymphocytes; T and B systems of immunity; receptors; immunologic insufficiency; leukemia.

Identification of lymphocyte subpopulations is one of the foremost problems in human immunology. To detect T lymphocytes spontaneous rosette formation with sheep's erythrocytes (SE-RFC) is used. To detect B lymphocytes several variants of rosette formation are used, based on detection of receptors for the C'_3 component of complement (EAC-RFC) or of receptors for the Fc fragment of IgG (EA-RFC).

The phemenon of spontaneous rosette formation of human lymphocytes with mouse erythrocytes (ME-RFC) was described in 1974 [10]. It has been shown that B lymphocytes have the property of binding mouse erythrocytes. Some particular features of the phenomenon have been studied and the ME-RFC level has been determined in certain pathological processes [6-8].

In the present investigation ME-RFC were investigated along with other tests for detecting human T and B lymphocytes. The role of these markers in the reaction of rosette formation between B lymphocytes and mouse erythrocytes was studied by the method of inhibition of surface receptor structures.

EXPERIMENTAL METHOD

Lymphocytes were isolated from the blood of patients and healthy blood donors by centrifugation in a Ficoll-Hypaque density gradient by the method of Böyum [5]. The pure suspension of lymphocytes was diluted with Hanks' solution to a concentration of 2×10^6 cells/ml.

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