DISTINGUISHING FEATURES OF HUMORAL IMMUNITY IN BALB/c MICE WITH RAUSCHER LEUKEMIA

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Following injection of a relatively small dose of Rauscher virus into BALB/c mice highly sensitive to this virus an increase in the number of B-lymphocytes was found in association with stimulation of synthesis of nonspecific immunoglobulins. After injection of the virus together with Freund's complete adjuvant, normal cells synthesizing antibodies against surface antigen of Rauscher leukemia and leukemic cells with blocking antibodies adsorbed on their surface were present simultaneously in the spleen of the mice in the early stages of development of the disease.
KEY WORDS: *Rauscher leukemia; antitumor antibodies.*

In BALB/c mice highly sensitive to Rauscher virus, antileukemic antibodies appear only in the presence of added stimulation of their immunologic system by Freund's complete adjuvant (FCA). Work in the writers' laboratory revealed cytotoxic antibodies in the early period of development of the disease [1, 7]. Stimulation of the leukemic process observed under those conditions was connected with the appearance of blocking antibodies in the blood serum. Antibodies were found on the surface of the spleen cells of these animals [6].

The problem of whether antibodies fixed on the surface of spleen cells are serum antibodies of blocking type, adsorbed on spleen cells, or whether antibody synthesis takes place in the spleen cells remains unsolved.

The object of this investigation was to study the distinctive features of antibody formation in BALB/c mice. An original method of short-term cultivation of previously trypsinized and intact cells was used, by means of which protein-synthesizing cells could be distinguished from cells adsorbing protein on their surface [5]. Another object of the investigation was to elute immunoglubulins fixed on the cell surface and to study their functional characteristics.

EXPERIMENTAL METHOD

BALB/c mice from the Stolbovaya nursery, Academy of Medical Sciences of the USSR, were used. Plasma of BALB/c mice with a developed Rauscher leukemia was used as the virus-containing material. The virus was injected intraperitoneally in a volume of 0.1 ml. In some experiments FCA was injected intraperitoneally into the animals in a dose of 0.1 ml.

Immunoglobulins on the cell surface were tested by the immunofluorescence (IF) method on living cells [3] with the use of antiserum against mouse globulins conjugated with fluorescein isothiocyanate (from the N. F. Gamaleya Institute of Epidemiology and Microbiology) and monospecific antisera against mouse G- and M-immunoglobulins (personally prepared). Antibodies against surface antigens of the leukemic cell were detected by the indirect IF method and by their cytotoxicity (CT), using guinea pig serum as complement [4]. Immunoglobulins fixed to the cell surface were eluted by dissociation of the antigen–antibody complex in an acid medium (pH 3.8) [5].

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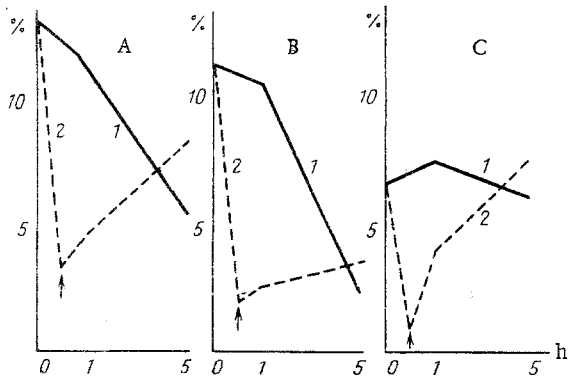

Fig. 1. Results of experiments with trypsin and short-term cultivation of spleen cells of BALB/c mice receiving Rauscher virus together with Freund's complete adjuvant (3rd day after injection of virus): A) using antisera against mouse immunoglobulins; B) using monospecific antiserum against IgG; C) using monospecific antiserum against IgM. Abscissa, time of cultivation (in h); ordinate, percentage of fluorescent cells. 1) Intact cells; 2) cells treated with trypsin. Arrow indicates time of treatment with trypsin.

To study whether immunoglobulins fixed to the cell surface are synthesized by the cells or whether they are adsorbed antibodies of the blocking type, a method of short-term cultivation of intact and previously trypsinized cells [5] was used.

EXPERIMENTAL RESULTS

Mice of strain BALB/c, highly sensitive to Rauscher virus, did not produce antibodies against Rauscher leukemia antigens. Antibodies likewise could not be found in these mice by either the IF or the CT method. The number of spleen cells carrying immunoglobulins on their surface in this series of experiments varied from one experiment to the next. Accordingly a special comparative study was carried out to study the relationship between the number of spleen cells carrying immunoglobulins on their surface and the dose of virus. BALB/c mice were given the virus in different dilutions — 1:10, 1:100, and 1:1000 — on the same day. On the 18th day the animals were killed and the number of cells with immunoglobulins on their surface was determined by the IF method. The number of these cells, calculated per spleen was $12 \cdot 10^6$-$15 \cdot 10^6$ for animals receiving the virus in a dilution of 1:10, $48 \cdot 10^6$ $89 \cdot 10^6$ (1:100), and $133 \cdot 10^6$-$135 \cdot 10^6$ (1:1000) respectively. The decrease in the number of fluorescent cells after injection of the large dose of virus may have been due to the immunodepressive activity of the virus, for after administration of the immunodepressant cyclophosphamide to intact animals, cells carrying immunoglubulins on their surface virtually disappeared by the 2nd or 3rd days of the experiment. The increase in the number of cells with immunoglobulins on their surface ought to reflect stimulation of the immunological system of the animal. For instance, after injection of bovine serum albumin, sheep's red cells, or FCA into the mice an increase in the number of spleen cells with immunolobulins on their surface was regularly observed. To test the specificity of this stimulation in mice with Rauscher leukemia eluates from spleen cells were investigated. Even after twelvefold concentration of the eluate as protein, no antibodies could be found by either the IF or the CT method, although experiments with Pujman's leukemia showed that this is possible.

Consequently, an increase in the number of spleen cells carrying immunoglobulins on their surface (B-lymphocytes [9]) in this case was unconnected with the production of specific antibodies and it could be interpreted as a sign of minimal stimulation of the immunological system — an increase in the synthesis of nonspecific immunoglobulins.

An increase in the synthesis of nonspecific immunoglobulins accompanies any immunological response [2, 8]. Even in the tolerant organism, injection of an antigen which does not induce the appearance of antibodies does lead to an increase in the synthesis of nonspecific immunoglobulins and an increase in the number of cells carrying immunoglobulins on their surface [10].

In the next series of experiments humoral immunity was studied in BALB/c mice during stimulation of their immunological system with FCA. Rauscher virus was injected in a dilution of 1:50 and FCA was given 3 days before the virus. Antibodies against surface antigen of Rauscher leukemia were found in the sera of the mice in this series of experiments on the 3rd-4th day. The IF index of whole serum reached 0.36 when luminescent serum against mouse globulins was used, and 0.32 with anti-IgM and 0.28 with anti-IgG sera. The same sera gave positive CT indices (up to 0.30). An increase in the number of cells with both classes of immunoglobulins on their surface was observed at the same times. IgG and IgM

antibodies against surface antigen of Rauscher leukemia were found by the IF method in eluates from these cells. After eightfold concentration of the eluates the IF index relative to that of the normal eluate concentrated in the same way was 0.34 for IgG and 0.23 for IgM. The same eluate was inactive in the CT test, but the simultaneous presence of antibodies of both classes suggests neutralization of the CT activity of the IgM antibodies by the blocking power of the IgG antibodies. Experiments with trypsinization and short-term cultivation were carried out with mouse spleen cells on the 3rd day after injection of the virus. The decrease in the number of fluorescent cells during simple cultivation obtained by means of antiserum against mouse globulins (Fig. 1A) cannot be combined with an increase in the number of fluorescent cells in the case of cultivation after treatment of the cells with trypsin. Experiments were carried out with separate testing of cells carrying different classes of immunoglobulins on their surface (Fig. 1B, C). When the untreated suspension was cultivated the number of cells carrying IgG on their surface was reduced by 3.5 times but the number carrying IgM was virtually unchanged. Nearly all cells lost immunoglobulins from their surface during treatment of the suspension with trypsin, but only the IgM level returned to its initial value during subsequent cultivation. The results of these experiments indicate that normal cells synthesizing IgM antibodies were present in the spleen of BALB/c mice receiving FCA and Rauscher virus on the 3rd day after injection of the virus, and that the leukemia cells which adsorbed IgG antibodies on their surface were most probably antibodies of the blocking type. The distinctive pattern of manifestation of the humoral immunological response to antigens of developing leukemia discovered in these experiments were possibly connected with the form of the disease induced by the Rauscher virus: Rauscher leukemia has no exact analog among the forms of leukemia widespread in man.

It is perhaps the presence of blocking antibodies at the period of development of the disease when the number of leukemic cells is very small and the immunological response can arrest the leukemia which leads to the paradoxical existence of leukemia in an immunologically active organism. Later the normal cells are surplanted, at least from the spleen, so that complete freedom is given to the developing leukemia. The special nature of humoral immunity in BALB/c mice with Rauscher leukemia indicates that the possible effect of antibodies on leukemia depends not so much on the presence or intensity of immunity as on the relationship between factors stimulating and inhibiting tumor growth.

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