MITOGENIC ACTION OF MOUSE ANTI-ISOLOGOUS AGGREGATED IMMUNOGLOBULIN SERUM FACTOR ON SPLEEN CELL PROLIFERATION

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The effect of mouse serum obtained on the 6th day after injection of isologous aggregated immunoglobulin (MAAS) on proliferative activity of spleen cells was studied in vitro. MAAS was shown to have a weak stimulating action on the cells in dilutions of 1:40-1:60, with maximal activity after 48 h; MAAS in these dilutions also potentiates the response of the cells to phytohemagglutinin (PHA) and concanavalin A (Con A). The potentiating effect of MAAS on Con A was stronger, indicating that it acts on cells carrying an Fc-receptor for immunoglobulin on its surface, on which the MAAS factor probably acts. The nature of the MAAS factor and its possible identity with the factor secreted during treatment of cells with aggregated immunoglobulin in vitro is discussed. The results agree with the hypothesis put forward previously that the effect of MAAS factor on antitumor transplantation immunity may be linked with its mitogenic action.

KEY WORDS: immunoglobulins; cell proliferation; isologous aggregated immunoglobulin.

Elucidation of the mechanisms of regulation of proliferative activity of lymphocytes, connected with the presence of receptors against antigen and lectins on their membrane [1, 9, 12], is an important task in immunology. In this connection it is interesting to study compounds which affect the antigen-binding receptors of lymphocytes and which exert a lectin-like action on them under these circumstances. It has been shown, for instance, that serum against heat-aggregated isologous mouse immunoglobulins (MAAS) has a marked action on antigen-binding receptors of rosette-forming cells (RFC) of the immune spleen and on transplantation immunity [3].

Considering earlier results, in the investigation described below the action of MAAS on proliferative activity of spleen cells in vitro and the nature of the factor contained in this serum were studied.

EXPERIMENTAL METHOD

The proliferative activity of spleen cells of BALB/c mice was investigated in the blast-transformation test (BTT) [5]. Cells in a concentration of 5×10^5 were cultured in wells in microdisks (Falcon Plastics No. 3040) at 37°C in an atmosphere containing 5% CO_2 with MAAS obtained as described previously [2], and with normal mouse serum (NMS) in dilutions of 1:15-1:60.

Phytohemagglutinin P (PHA, from Difco) and concanavalin A (Con A, from Sigma) in doses of 0.5 and 10 μg respectively to 1 ml culture medium, were used as mitogens. The mitogens were taken in optimal quantities after titration of their doses. Proliferative activity of the lymphocytes was estimated from incorporation of thymidine-^{3}H, added in a dose of 0.5 μCi per well 16 h before the end of culture as in the method described in [6].

EXPERIMENTAL RESULTS

To study the effect of MAAS on proliferative activity of spleen cells different doses of sera were added to them and incorporation of thymidine-^{3}H into the cells was determined after 24, 48, and 72 h. Dependence of

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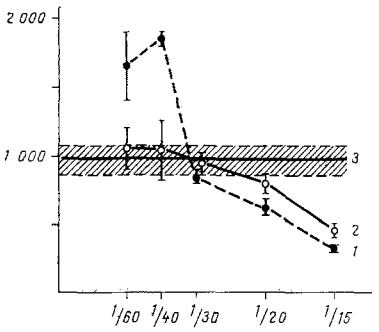

Fig. 1

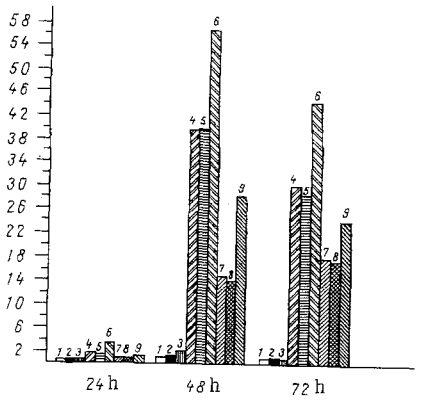

Fig. 2

Fig. 1. Action of MAAS on DNA synthesis in spleen cells during culture for 48 h. 1) MAAS; 2) NMS; 3) culture medium. Abscissa, dilution of sera; ordinate number of counts/min (CPM).

Fig. 2. Potentiating action of MAAS on DNA synthesis in spleen cells in response to PHA and con A. 1) Culture medium; 2) NMS (1 : 40); 3) MAAS (1: 40); 4) Con A; 5) Con A and NMS; 6) Con A and MAAS; 7) PHA; 8) PHA and NMS; 9) PHA and MAAS. Abscissa, duration of culture of cells (in h); ordinate, number of counts ($\times 10^{-3}$) per minute.

incorporation of the label into cell DNA after incubation for 48 h on the doses of sera tested showed (Fig. 1) that MAAS, in dilutions of 1 : 40-1 : 60, increased incorporation of thymidine-^{3}H into the lymphocytes. Low dilutions of MAAS (1 : 15-1 : 20) caused a statistically significant decrease in incorporation of thymidine-^{3}H into the cells compared with NMS. Under these circumstances NMS also depressed cell proliferation by comparison with the culture medium.

Maximal incorporation of thymidine-^{3}H into DNA of the lymphocytes in the presence of MAAS (1 : 40) was observed during their culture for 48 h (Fig. 2). This difference between the experimental and control could no longer be observed after 72 h. Consequently, MAAS in dilutions of 1 : 40-1 : 60, like PHA and Con A, has a mitogenic action on mouse spleen cells.

To study whether MAAS may have a synergic action on lymphocyte stimulation under the influence of T-cell mitogens, as has been observed in the case of the weak mitogen phorbol myristate acetate [11], in the next experiments the test sera were added together with PHA and Con A. The results indicate that proliferation of the cells in response to PHA and Con A was statistically significantly increased in the presence of MAAS in dilutions of 1 : 40-1 : 60 at all times of culture (Fig. 2). Low dilutions of the sera (1 : 15-1 : 20) inhibited BTT, and in response to PHA, MAAS had a greater blocking effect than NMS. The experiments with Con A also agree with the view that cells carrying an Fc-receptor for immunoglobulin on their surface [12], on which the MAAS factor probably acts, are essential for the response to this mitogen. The hypothesis that MAAS may act on cells carrying the Fc-recptor is also supported by the results of experiments in which Ehrlich's ascites carcinoma and leukemia EL4 cells, which do not possess this receptor [10], were treated in vitro by MAAS and then transplanted into animals or injected into mice inoculated with tumor cells. In that case no increase in proliferation of the ascites cells and no increase in the mortality of the animals were observed compared with the control groups.

The results of this investigation thus indicate that MAAS contains a factor which stimulates proliferation of spleen cells and potentiates their response to PHA and Con A. Since mainly T-cells react in the BTT to these mitogens at these times, it is logical to suppose that the MAAS factor acts on T-lymphocytes, at least as regards the potentiating effect.

When the mechanism of action of this factor on cells is studied, attention must be paid to its nature. As was stated, the factor appears in the serum after injection of aggregated immunoglobulins. It blocks antigen-binding receptors of immune RFC in the spleen in experiments both in vitro and in vivo [2, 3]. This factor may be either an antibody against the antigenic determinants of immunoglobulins, modified after heat aggregation [4], or a factor of a different nature. In this connection its affinity for immune complexes, namely sheep's red blood cells (SRBC), sensitized by subagglutinating doses of antierythrocytic antibodies [9] of mouse IgM

or IgG, was investigated. The results showed that adsorption of MAAS with SRBC sensitized by IgG_γ-antibodies abolished its blocking action on receptors of immune RFC. Meanwhile, adsorption by immune complexes with IgM-antibodies did not reduce the blocking action of MAAS on receptors of RFC.

To rule out completely the idea that the MAAS factor could be an IgM antibody, the test sera were treated with 0.1 M 2-mercaptoethanol, which destroys IgM-antibodies [4]. This substance was then removed from the serum by dialysis against Hanks' solution for 48 h. The MAAS, after this treatment, was found not to have lost its blocking action on immune RFC, which was the same as that of the native serum. These facts are evidence against the view that MAAS belongs to the IgM-antibodies which might be formed in mice at these times after injection of isologous aggregated immunoglobulin (IAG).

It is difficult to imagine that this factor belongs to the IgG-antibodies, for they usually appear much later after injection of the protein. Further evidence in support of this view is given by the decrease in activity of MAAS after keeping for 2 weeks at 4°C, whereas antibodies in sera preserve their activity for longer. The MAAS factor also evidently appears as early as 2-3 days after injection of IAG, as is shown by the marked leukocyte-agglutinating action of MAAS on spleen cells. Fifteen days after injection of IAG into mice, when mainly IgG-antibodies ought to be appearing, the serum contained virtually no leukoagglutinins and had no significant action on RFC receptors. The affinity of the MAAS factor for immune complexes formed with the aid of IgG-antibodies thus cannot be regarded as evidence that this factor consists of antibodies against IAG. In this connection it is interesting to note that when cells were treated with aggregated immunoglobulin or with antigen–antibody complexes in vitro [7, 8, 10] factors with affinity for aggregated IgG and for immune complexes were secreted into the culture medium. These factors [13, 14], like the MAAS factor, were identical in their action in certain immunologic tests. It is perfectly probable that the injected IAG interacts with the surface of lymphocytes and leads to the secretion of analogous factors into the serum by these cells.

Consequently, the results of this investigation are evidence that a biologically active factor, probably of nonimmunoglobulin nature, which blocks the antigen-binding receptors of immune splenic RFC, stimulates cell proliferation, and potentiates the mitogenic action of PHA and Con A on T-lymphocytes, may appear in the serum of animals after injection of IAG. These results are further confirmation of the previous hypothesis that the influence of MAAS factor on transplantation immunity is linked with its mitogen-like action.

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