Anti-Macrophage Serum: Preparation and Some Properties

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The involvement of the macrophage in the induction of the immune response is well documented. Frei et al. (1965) showed that phagocytosis of antigen is an important preliminary step in the induction of the immune response. Askonas and Rhodes (1965) and Mitchison (1969) demonstrated that macrophage-associated antigen is more immunogenic than its native counterpart.

The precise nature of how the macrophage processes the antigen and renders it more immunogenic is unclear, but it is known that induction of antibody formation by lymphocyte requires their interaction with live and antigen-containing macrophages (Gallily & Feldman, 1967; Mosier, 1967).

It would thus appear that interference with the function of macrophages may lead to interference with the inductive process of the immune response. Indeed, such interference to both viral (Panigel & Cayeux, 1968) and sheep erythrocyte (Argyris & Plotkin, 1969) antigens has been induced by the use of antiserum specific to macrophages.

In the present investigation we present evidence that the use of antimacrophage serum (AMS) not only inhibits antibody formation to trachoma agent antigen, but its apparent immunosuppressive effect seems to be related to the time of its administration relative to that of antigen.

MATERIALS AND METHODS

Mice

Outbred white swiss mice of 12-14 weeks old were used throughout.

Macrophage preparation and immunization

Peritoneal exudate cells were obtained from mice that had been injected with 3 ml of thioglycollate fluid medium four days previously. The cells were harvested by washing the peritoneum with 3 ml of RPMI medium 1640 containing streptomycin and penicillin (Associated Biomedics Systems, Buffalo,
New York) and containing 2.5 units of heparin/ml. The peritoneal exudate cells were washed three times with the same medium and counted with the aid of a haemocytometer. Differential staining indicated the mixture of cells to consist of about 85-90% macrophages with the remainder lymphocytes and other mononuclear cells. For the preparation of the anti-macrophage serum, white New Zealand rabbits received a total of $4 \times 10^7$ macrophages each, given in three doses at two week intervals. The first dose consisted of cells emulsified in Freund's complete adjuvants (1:1) (Difco Laboratories, Detroit, Michigan, USA). Subsequent injections were given in physiologic saline. All injections were given in the footpads. Serum samples were collected from the marginal ear vein one week after the last injection. The serum was separated, decomplemented by incubation at $56^\circ$C for 30 min and stored at $-20^\circ$C until used. Mice were given either normal rabbit serum (NRS) or anti-macrophage serum (AMS) in three different doses consisting each of 0.25 ml of 20% dilution in physiologic saline via the intraperitoneal (i.p.) route and divided into four groups of at least 6 animals each:

Group A received NRS on days -1, 0, +1, Antigen on day 0
Group B received AMS 4 days before antigen
Group C received AMS 4 days after antigen
Group D received AMS on days -1, 0, +1 and antigen on day 0

The antigen used consisted of 0.1 ml of a 10% suspension of fluorocarbon-purified trachoma agent antigen strain Apache 2, given i.p.

Mice were bled at various times following antigen administration by retro-orbital bleedings.

Serologic procedures

The levels of antibody in the sera of pools of animals from the same group of at least 6 animals were measured by an indirect immunofluorescent antibody (IFA) method performed as described by Hanna and Bernkopf (1964). Fluorescein-labelled antisera to mouse globulins and prepared in the goat were purchased from Cappel Laboratories, Downingtown, Pa. Goat antisera specific to mouse IgM, IgA, IgG$_1$ (7S $\gamma_1$) and IgG$_2$ (7S $\gamma_2$) immunoglobulin fractions were purchased from Meloy Laboratories, Springfield, Va. These antisera were labelled separately with fluorescein iso-thiocyanate according to the method of Wood et al (1965).

RESULTS

The immunosuppressive effect of rabbit anti-mouse macrophage (AMS) serum on the production of serum antibodies to trachoma agent antigens was studied in Swiss Webster mice. AMS-treated mice showed significant reduction in their total antibody response when assayed by the indirect immunofluorescent
antibody (IFA) test and fluorescein-labelled anti-mouse globulin antisera. Figure 1 illustrates the results obtained on pools of sera taken at various times after antigen administration. Curve A represents data obtained from animals treated with normal rabbit (NRS) serum on days -1, 0, +1 and the antigen on day 0. These results are very similar to those obtained from control animals receiving no serum treatment, thus indicating that NRS contained no immunosuppressive activity.

The extent of immunosuppression produced following AMS treatment appears to be related to the time allowed between AMS administration and that of antigen. When AMS is given 4 days prior to or after antigen administration, the immune response elicited is normal (Figure 1, Curves B and C respectively). When AMS and antigen are administered simultaneously (AMS on days -1, 0, +1 and antigen on day 0) the antibody response is almost completely abolished (Figure 1, Curve D).

In an effort to determine the class of immunoglobulin(s) produced in the mouse following stimulation with trachoma agent antigens and to find out whether AMS selectively inhibits the formation of any one immunoglobulin class specifically, the sera from groups A and D were analysed by IFA. For this purpose whole mouse anti-trachoma antisera from both groups were examined for the presence of IgM, IgA, IgG$_1$ (7S $\delta_1$) and IgG$_2$ (7S $\delta_2$) antibodies with specificity to trachoma agent. Table I depicts the results of these studies and shows that IgM, IgA and IgG$_2$ (7S $\delta_2$) were produced in high levels in the
Table I. Distribution of IFA-reactive antibodies to trachoma agent antigens in NRS- and AMS-treated animals at various times following antigen administration

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Weeks after immunization</th>
<th>IgM</th>
<th>IgA</th>
<th>IgG*1</th>
<th>IgG*2</th>
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<tr>
<td>A</td>
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* = normal rabbit serum given on days -1, 0, +1, Antigen on day 0
b = serum negative when tested undiluted
c = anti-macrophage serum given on days -1, 0, +1, Antigen on day 0

NRS-treated animals (group A). These antibodies were detected as early as 4 days, reached peak levels at 1 week and persisted at elevated levels for at least three weeks following antigen stimulation. The IgG*1 (7S γ*1) antibody response was consistently low throughout the experimental period. Treatment with AMS, however, caused a significant reduction in total antibody as well as in the IgM, IgA and IgG*2 (7S γ*2) antibody response.

DISCUSSION

The results presented in this investigation indicate that the macrophage may play an important role in the induction of the primary antibody response to trachoma agent antigens. Treatment with macrophage specific antisera interfered with the macrophage function and resulted in the impairment of the antibody response.

The finding in the present investigation that administration of AMS simultaneously with antigen (Figure 1D) is more effective in suppressing the antibody response than when given 4 days prior to or after antigen administration strongly suggests that the macrophage is involved in a very early step during the induction of the immune response.

The manner in which AMS acts on the macrophage is not known. Monaco et al (1966) reported that antilymphocyte serum (ALS) exerts its immunosuppressive effect due to the interaction of the lymphocyte with its specific
antibody. More recently, Pisano et al (1969) and Maclaurin and Humm (1970) reported that ALS exerts its immunosuppressive effect by virtue of possessing antibodies that are specific to the macrophage.

The observation by immunofluorescence in the present study that AMS coats the surface of peritoneal macrophages may indicate that interaction between the macrophage and its specific antibody may interfere with its function.

Whether AMS interferes with the macrophage function by causing immune cytolyis of the macrophage in the presence of complement or by blocking the antigen-recognition receptor sites on its surface could not be concluded from these experiments. Although the AMS used in the present studies caused clumping of peritoneal macrophages and not of spleen lymphocytes, it could not be said with confidence that AMS acts primarily on the macrophage.

Little is known about the involvement of the macrophage in the rejection of the allogeneic tissue grafts. Gillette and Lance (1970) observed that the number of macrophages in the vicinity of allogeneic skin grafts was significantly higher than in normal skin during the time of rejection and that AMS treatment caused a delay in the rejection time of allogeneic skin graft (Dyminski & Argyris, 1969).

Different classes of antibodies produce different effects in the transplantation of tumour allografts. While the IgG₂ (7S δ₂) antibody of the mouse possesses enhancement activity (Irvin et al, 1967) the IgM antibody lacks this activity (Kaliss, 1970). The recent studies of Ting and Herberm (1970) on the appearance of antibodies from different immunoglobulin classes following skin allografts in the mouse showed an increase of IgM which was then followed by an increase of IgG₁ (7S δ₁) and IgG₂ (7S δ₂) antibody levels following the onset of graft rejection. If these classes of antibodies play a role in the rejection of the skin allografts, it would follow that modification of the rejection reaction may be brought about by inhibiting the production of these antibodies. The finding in the present investigation that AMS treatment inhibited the production of IgM, IgA, IgG₁ and IgG₂ antibodies point to the possible use of AMS as an immunosuppressive agent in solid tissue and organ transplantation. This possibility is under investigation in our laboratory at present.

ACKNOWLEDGMENTS

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OPEN DISCUSSION

R SELLS (Liverpool): Can I ask you whether you absorbed this antiserum against lymphocytes?

ISA: What you are asking is whether this antiserum is specific for macrophages or not? The answer to your question is no, it has not been absorbed, but it is specific. If we absorb it with lymphocytes or with spleen cells there is no substantial reduction in its activity.

SELLS: So you have absorbed against lymphocytes?

ISA: Yes we did — against spleen cells — but spleen does contain some macrophages too. If we purify the macrophages by taking advantage of their property of adhering to glass surfaces and thus get rid of the lymphocytes, we get the same effect. So therefore we can assume it's really more specific.

SELLS: Well, I disagree with you, because you said that 80% of your cells were macrophages.

ISA: About 90% actually.
SELLS: All right, 90%; even so, if you prepare antimacrophage serum with 99.5% macrophages, you still get a high proportion of antilymphocyte activity. You really cannot say this is specific anti-macrophage activity until you have absorbed out the antilymphocyte activity.

ISA: In fact we can say so, because even if you get an extremely pure antimacrophage serum, you still have some reactivity with lymphocytes and other leucocytes which share antigens. Our evidence indicates that our antisem did not interfere with lymphocytes because there was no lymphopaenia. The antiserum did not agglutinate lymphocytes from lymph nodes, but it did agglutinate peritoneal macrophages. There was no difference between the results presented and the results obtained using macrophages that had been purified by adherence to glass.

SELLS: Can I just make one more comment? This doesn't agree with the work of Shapanich in Boston (Shapanich, T. et al (1970) Surgical Forum, 21, 275), where they found that if you increased the purity and specificity of your antimacrophage serum you did not get a proper immunosuppressive effect on skin grafts, unless you also added antithymocyte serum. There is definitely some synergistic effect with antimacrophage serum. I think there are several other workers who would agree with that.

ISA: Well I have in turn to refer you to papers by Gallily and Feldman and Argyris and Plotkin (see references, Ed) and a number of others, who seem to agree with what we found.