Macrophage Cytophilic Antibody in Mice: Mechanism of Action of Bacterial Lipopolysaccharide on the Uptake of Immunoglobulins by Mouse Peritoneal Cells

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The action of injected bacterial lipopolysaccharide upon the uptake of cytophilic antibodies by macrophages was found to be unrelated either to changes in surface charge or to the rate of anaerobic glycolysis of these cells. It is apparently mediated by alterations in the levels of material in the serum which enhances the uptake of cytophilic antibody by macrophages. This enhancing material does not appear to be an immunoglobulin nor can it be generated by the action of endotoxin on either whole blood or serum.

Endotoxins produce a very wide range of physiological and pathological effects in susceptible animals. Although many of these effects are biphasic in nature, very few of them have been shown to be related. The response of the macrophage receptor for cytophilic antibodies to bacterial lipopolysaccharide is no exception. It stands as an isolated phenomenon with no apparent relationship to any of the other responses induced by extracts of cell walls of gram-negative bacteria.

It was found that lipopolysaccharide from *Escherichia coli* when injected into normal mice produced changes in the surface properties of the peritoneal macrophages of these animals, so that their ability to adsorb macrophage cytophilic antibodies was altered (12). After a single intravenous injection of this lipopolysaccharide, a triphasic sequence of changes in macrophage-adsorptive capacity occurred. Enhancement of antibody uptake took place 5 min after injection; however, this enhancement occurred for only a few minutes and was followed by a period of depressed uptake. Maximum depression occurred approximately 30 min after injection and preceded a prolonged phase of enhanced adsorption which reached a maximum 24 hr after injection before declining slowly. This second phase of enhanced adsorption was found to be inhibited or reversed either by multiple injections of lipopolysaccharide or by prior immunization of mice with dead *E. coli*.

The investigations reported in this paper were designed to identify the mechanism of altered uptake of cytophilic antibody by macrophages in the hope of relating these changes to other effects of lipopolysaccharide upon reticuloendothelial function (3) and also to provide information on the factors which influence the cellular fixation of immunoglobulins.

MATERIALS AND METHODS

Animals. White Swiss mice of both sexes, weighing between 20 and 25 g and reared in this laboratory, were used.

Antigens and antisera. Sheep erythrocytes were obtained at weekly intervals from the defibrinated blood of a female Blackface sheep and stored at 4 C. Antisera were raised as described previously (12).

Bacterial lipopolysaccharide. Difco *E. coli* 026:B6 prepared by the method of Westphal and Luderitz (13) was stored as a stock solution of 200 μg/ml in phosphate-buffered saline (pH 7.2).

Preparation of cell monolayers and titration of cytophilic antibodies. These procedures were performed as described previously (12).

Electrophoresis of peritoneal cells. Electrophoresis was performed in a cylindrical, all-glass, electrophoresis chamber by the method of Bangham et al. (1). All solutions in contact with the electrophoresis chamber were made up in doubly glass-distilled water which had not been exposed to ion-exchange resin.

Anaerobic glycolysis of peritoneal cells. The release

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of carbon dioxide from peritoneal cells was measured in a Warburg constant-volume respirometer under an atmosphere of 94.71% nitrogen and 5.29% carbon dioxide (Canox).

**Measurement of immunoglobulin G levels in serum.** The levels of total immunoglobulin G (IgG) in mouse sera were measured by radial immunodiffusion by the method of Mancini, Carbonara, and Heremans (7).

**RESULTS**

Two of the changes reported to occur in cells after administration of endotoxin were considered to be capable of influencing immunoglobulin adsorption. These changes were in the surface potential of the cell as reflected in its electrophoretic mobility (2) and in the metabolic activity of the cell as reflected in its rate of carbon dioxide production under anaerobic conditions (14).

**Electrophoretic mobility of lipopolysaccharide-treated mouse macrophages.** Bergmann et al. (2) demonstrated that the electrophoretic mobility of rabbit polymorphonuclear neutrophils underwent a biphasic change after treatment of the animal with bacterial lipopolysaccharide. These cells showed a drop in electrophoretic mobility which reached a minimum value 2 hr after injection of lipopolysaccharide but subsequently increased rapidly to reach its maximum value 8 hr after injection, from which it slowly declined. It was considered improbable that similar changes in macrophage electrophoretic mobility would directly affect immunoglobulin adsorption especially at a physiological pH. Nevertheless, changes in the degree of adsorption of more highly charged serum proteins could be expected to alter the amount of cell surface available for immunoglobulin uptake. The electrophoretic mobility of peritoneal macrophages from normal and lipopolysaccharide-treated mice was therefore determined (Table 1 and Fig. 1). No significant difference could be detected between normal macrophages and cells examined 30 min and 24 hr after administration of lipopolysaccharide.

**Anaerobic glycolysis of mouse peritoneal cells from lipopolysaccharide-treated mice.** Woods et al. (14) demonstrated that mouse peritoneal cells underwent a triphasic series of changes in their rate of anaerobic glycolysis in response to a single injection of either Salmonella enteritidis or Shigella dysenteriae endotoxins. Although the time course of this response appeared to be unrelated to the changes occurring in cytoplasmic antibody uptake, experiments were performed to determine whether there is any relationship between these alterations in metabolic activity and changes in cytoplasmic antibody uptake.

**Table 1. Electrophoretic mobility of peritoneal macrophages derived from normal and lipopolysaccharide-treated mice**

<table>
<thead>
<tr>
<th>Cells</th>
<th>No. of cells examined</th>
<th>Mobility (μL per sec per v per cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal macrophages</td>
<td>100</td>
<td>1.49 ± 0.25 SD</td>
</tr>
<tr>
<td>Macrophages 30 min after lipopoly-</td>
<td>25</td>
<td>1.36 ± 0.24 SD</td>
</tr>
<tr>
<td>saccharide treatment...</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrophages 24 hr after lipopoly-</td>
<td>100</td>
<td>1.36 ± 0.22 SD</td>
</tr>
<tr>
<td>saccharide treatment...</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Electrodes, reversible Ag/AgCl/KCl; Potential difference, 50 V; current, 0.017 ma. Diluents, 0.145 m NaCl containing 3 × 10⁻⁴ m NaHCO₃. Electrical tube length, 126.5 mm; diameter, 2.014 mm. Distance timed, 40.3 m. Dose of lipopolysaccharide, 1.0 μg per g of body weight. SD, standard deviation.*

20 male Swiss mice of comparable age and size was examined at intervals after each had received a single intravenous dose of E. coli lipopolysaccharide which ranged from 0.5 to 2 μg per g of body weight. The release of carbon dioxide was measured as described above. Under these conditions, no relationship could be detected between macrophage metabolic activity as measured in this way and the degree of enhancement or inhibition of cytoplasmic antibody uptake expressed as a percentage of the normal value (Fig. 2). The correlation coefficient r between these two factors was 0.0004.

The average rate of CO₂ production under anaerobic conditions for normal mouse peritoneal cells was found to be 1.65 μliters of CO₂ per 10⁶ cells per hr.

**Changes in the properties of mouse serum accounting for alterations in the capacity of mouse peritoneal cells to adsorb cytoplasmic antibodies.** The ability of a macrophage to adsorb cytoplasmic antibodies when these antibodies are in relative excess depends upon the number of available receptors upon the cell surface. The number of these free receptors will in turn be influenced by exposure to cytoplasmic immunoglobulins in vivo. Cells derived from an environment with a high concentration of cytoplasmic antibodies will have fewer available receptors when tested in vitro.

Investigations were therefore performed to determine whether injected lipopolysaccharide influences cytoplasmic antibody uptake in vitro by its capacity to alter the serum levels of non-specific cytoplasmic material or material which influences the uptake of cytoplasmic antibody by macrophages.

The levels of such material were estimated by
FIG. 1. Electrophoretic mobility of peritoneal cells from normal and lipopolysaccharide-treated mice. For details, see Table 1.

FIG. 2. Relationship between carbon dioxide production of lipopolysaccharide-treated peritoneal cells under anaerobic conditions and their capacity to adsorb cytophilic antibodies. (Expressed as a percentage of the ability of normal cells to take up these antibodies).

the capacity of the material to inhibit or enhance the uptake of cytophilic antibodies of known specificity.

The serum to be tested for "nonspecific cytophilic" activity was adsorbed once with sheep erythrocytes and diluted 1:5 in Hanks basic salt solution. The capacity of this unknown serum to inhibit the absorption of cytophilic antibody to macrophages was then tested in a system in which a standard anti-sheep erythrocyte serum containing cytophilic antibodies was used. The degree of inhibition or enhancement of cytophilic antibody uptake was judged by alterations in the cytophilic antibody titer of the standard serum.

It was found that serum taken from mice 30 min after injection of lipopolysaccharide markedly inhibited cytophilic antibody uptake by macrophages and that serum taken 24 hr after injection caused enhanced uptake.

It addition, it was found that a relationship existed between the degree of alteration in antibody absorption by cells from treated animals and the change induced in normal cells by serum from these treated animals [correlation coefficient \( r = 0.689 \) (Fig. 3)]. Twenty individual mice were used in determining this relationship.

Identification of enhancing and inhibiting material in mouse serum. Although it has been demonstrated that "nonspecific" cytophilic IgG may both inhibit (6, 9, 11) and enhance (11) specific cytophilic antibody uptake and although cytophilic antibodies may be released into serum by the action of bacterial lipopolysaccharide (5, 8, 10), experimental evidence suggests that IgG is not the major inhibitor or enhancing factor in lipopolysaccharide-induced changes in cytophilic antibody uptake.

When IgG levels as measured by radial im-
antibody uptake from pressed animals. Serum cytophilic antibodies to the uptake lipopolysaccharide was tested either 30 min or 24 hr after administration of lipopolysaccharide, at a time when there were marked alterations in the adsorptive capacity of their peritoneal macrophages. In addition, 10 gnotobiotic mice were found to exhibit a triphasic response to lipopolysaccharide similar in magnitude to that shown by conventional animals despite undetectable levels of immunoglobulin in their serum. The serum of these lipopolysaccharide-treated mice was also capable of enhancing cytophilic antibody uptake by normal macrophages when taken 24 hr after lipopolysaccharide administration.

Enhancing activity could not be generated in either serum or whole blood from conventional or gnotobiotic mice after exposure to lipopolysaccharide in vitro for 24 hr (0.02 mg of lipopolysaccharide was added to 0.5 ml of fresh serum or 1 ml of whole blood).

**DISCUSSION**

From these investigations, it appears that the effect of bacterial lipopolysaccharide upon the uptake of cytophilic antibody by macrophages is unrelated to several of the other effects produced by these compounds upon macrophages.

From previous studies (12), it was known that lipopolysaccharide did not exert a direct effect upon macrophage receptors. Lipopolysaccharide added to macrophage monolayers in vitro has no apparent effect upon their capacity to adsorb cytophilic antibody.

Woods et al. (14) were able to alter the rate of macrophage metabolism in vitro by exposure to lipopolysaccharide. This, taken in conjunction with the lack of in vitro response of antibody receptors and the total lack of correlation between CO₂ production under anaerobic conditions and cytophilic antibody uptake, indicates that the rate of cellular metabolism, as measured in this way, has no apparent influence upon the availability or avidity of macrophage antibody receptors.

The adsorption of macrophage cytophilic antibodies, as measured in vitro by the rosette test, is influenced by the availability of macrophage receptors. These in turn, are affected by the presence, either already on the cells or in the serum under test, of "nonspecific" cytophilic material. Such "material" may be cytophilic antibodies directed against antigens other than the ones under test. Any changes in the level of such "nonspecific" cytophilic material will be reflected in the apparent uptake of specific cytophilic antibody. It has been demonstrated that lipopolysaccharide may induce the release of preformed immunoglobulins from bone marrow and spleen (5, 8, 10). It may reasonably be assumed that such released material could contain cytophilic immunoglobulins and thus influence specific cytophilic antibody adsorption. However, this is apparently not the primary mechanism of the triphasic response to lipopolysaccharide, since such a response may be detected in gnotobiotic mice. This is not, of course, absolute evidence that immunoglobulins do not mediate the triphasic response. If, however, they do participate in this reaction, it must be considered, firstly, that they can do so in extremely small quantities, and, secondly, since the triphasic response of gnotobiotic animals was of similar magnitude to that in conventional animals, this indicates that the considerable difference in immunoglobulin levels between these two groups of animals had no significant influence upon the response.

It is, however, apparent that a factor (or factors) is present in the serum of treated mice which influences cytophilic antibody uptake. It is probably not immunoglobulin, it is not generated by addition of lipopolysaccharide to serum or whole blood, and it is probably not therefore related to any clotting factors or leukocyte pyrogens.

A factor which is known to influence macrophage activity in a similar way to cytophilic antibodies is macrophage migration inhibition factor. This is a member of a class of soluble factors released as a result of the interaction of antigen upon sensitized lymphocytes known as
“lymphokines” (4). It is possible that the enhancing factor reported here also belongs to this group and that interaction of antigen (in this case bacterial lipopolysaccharide) and sensitized lymphocytes results in its release. However, there is no evidence for the occurrence of such a reaction, and classifying this enhancing factor as a “lymphokine” at the present time is entirely speculative.

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LITERATURE CITED


