Effect of Anti-Kupffer Cell Serum on Phagocytosis and Humoral Antibody Formation

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To ascertain the effects of selective impairment of the fixed macrophage compartment on host defense, rabbit anti-rat Kupffer cell serum was raised and tested for its ability to depress phagocytosis and to serve as an immunosuppressant. The ability of the anti-Kupffer cell serum (AKS) to depress intravascular clearance rates of gelatinized RE test lipid emulsion indicated that the antiserum can functionally impair overt phagocytic activity. The phagocytic impairment was manifested by a significantly decreased liver phagocytosis of the lipid emulsion, whereas only a slight decrease in phagocytosis by the spleen was noted. When titered in vitro, AKS was cytotoxic to hepatic and splenic macrophages. Although AKS induced some degree of immunosuppressive activity, it was not as effective as antilymphocytic serum in suppressing the humoral immune response of rats to sheep red blood cells. Selectivity in the cytotoxic activity of AKS was manifested by toxicity for macrophages but no toxicity for thymocytes or splenic lymphocytes. It is suggested that AKS as a specific antimacrophage serum may be useful for assessing the contribution of the reticuloendothelial system to host defense physiology and metabolism.

Although peritoneal (7, 8) and splenic (6) macrophages have been demonstrated to play a major role in immunogenesis, relatively little is known of Kupffer cell involvement in immune phenomena. The recent development of a new method for the isolation of Kupffer cells (18, 19) which exhibit active phagocytosis and metabolism (19) prompted the use of these isolated Kupffer cells to develop an antimacrophage serum to assess the contribution of the hepatic macrophage to immunogenesis.

Antiperitoneal macrophage serum has been employed to ascertain the effects of macrophage removal on humoral or cellular immune responses, or both (1, 3, 11, 13, 14). Antimacrophage serum impairs the phagocytic event as determined by decreased intravascular clearance rates of colloidal materials (11, 14), intraperitoneal phagocytosis of foreign red blood cells (3), and in vitro phagocytosis of a variety of particulate materials (3, 11, 13). The effects of antimacrophage serum on the immune responses, however, remain a matter of controversy.

Since peritoneal macrophages have been employed as the antigen for the induction of antimacrophage serum (1, 3, 11, 13, 14, 17), variations in the immunosuppressive activity of the antiperitoneal macrophage serum (1, 3, 14) may be partially explained by the high variability of the harvested peritoneal cellular population used as antigen. The cellular population of peritoneal exudates varies significantly with the time of procurement after intraperitoneal injection of a stimulating irritant (15) and, indeed, whether an irritant is used. Although Hirsch et al. (11) did not employ an irritant before harvesting peritoneal cells, Argyris and Plotkin (1) and Panijel and Cayeux (17) harvested cells 7 days and 10 min, respectively, after stimulation. The immunosuppressive activity of antiperitoneal macrophage serum, reported by some of these investigators (1, 17), may therefore be related to the number of lymphocytes present in the cellular harvest, i.e., the antilymphocytic activity of antimacrophage serum (14). Similarly, lack of immunosuppressive activity (3, 14) may be related to the type of phagocytic cell present within the cell harvest fluid, i.e., macrophages which process antigens (7–9, 16) or polymorphonuclear leukocytes which are not effective antigenic processors. Macrophages and leukocytes can be found in various proportions in peritoneal exudate washouts (15).

Since the Kupffer cells comprise the bulk of the reticuloendothelial system (RES; 2), the ability to immunologically remove these cells from the host defense system should provide a mechanism to define the role that Kupffer cells
play in host defense physiology and hepatic function. In addition, it would appear to be advantageous to employ a relatively homogenous macrophage cellular population for the induction of antimacrophage serum.

MATERIALS AND METHODS

Kupffer cells were isolated from the livers of Sprague-Dawley rats by the methods of Pisano et al. (18, 19). Briefly, minced liver fragments were enzymatically digested with collagenase at 20 °C; the resulting isolated Kupffer cells were then trypsinized at 37 °C and washed with Hanks balanced salt solution (Difco). The Kupffer cells were suspended in distilled water for 5 sec to remove any red cell contaminants and then resuspended in Hanks balanced salt solution to a final concentration of 10⁶ Kupffer cells/ml.

Anti-Kupffer cell serum (AKS) was raised in rabbits by weekly intraperitoneal injections of 10⁶ Kupffer cells. Seven days after the fourth Kupffer cell injection and every injection thereafter, the rabbits were bled via the marginal ear vein and the serum was titered for a 50% cytotoxicity end point against isolated Kupffer cells by previously described methods (20). When a cytotoxic titer exceeding 1:20 was obtained, the rabbits were exsanguinated by cardiac puncture and the AKS was heat-inactivated (56 °C, 45 min). Rabbit anti-rat red blood cell activity was removed from the antiserum by three isovolumetric adsorptions with packed rat erythrocytes. The antiserum was then frozen at −20 °C.

The procedure for preparing rabbit anti-rat lymphocytic serum (ALS; 20) was essentially identical to that described for the preparation of AKS; however, rat thymocytes in Tyrode's solution were employed as antigen. Normal rabbit serum (NRS) was obtained from rabbits which had received weekly intraperitoneal injections of Hanks balanced salt solution.

The effect of NRS, AKS, or ALS on in vivo phagocytosis was assessed by the method of intravascular clearance of gelatinized RE test lipid emulsion (21). A 10% gelatinized ¹³¹I triolein-labeled RE test lipid emulsion was prepared as previously described by Di Luzio and Riggi (4) and Saba, Filkins, and Di Luzio (21). Rats received 50 mg of emulsion per 100 g of body weight, and blood samples were obtained every 2 min for 10 min for determination of intravascular clearance. Liver, lung, and spleen were assayed for ¹³¹I radioactivity in a Nuclear-Chicago Auto-Gamma System. The half-time for intravascular clearance of the lipid emulsion was determined on the basis of the rate of decrease in blood radioactivity; tissue distribution of the emulsion was calculated as the per cent injected dose of emulsion per gram of tissue and per total organ.

Hemolytic antibody titers were determined 7 days after the intraperitoneal injection of 1.0 ml of a 1% suspension of sheep red blood cells into rats which had received five daily intraperitoneal injections of 1.0 ml of either NRS, ALS, or AKS. Twofold serial dilutions of the appropriate heat-inactivated test serum were made, and the hemolytic titer toward sheep red cells is expressed as the last dilution of serum to manifest lytic activity after the addition of guinea pig serum as a source of complement.

RESULTS

The intraperitoneal injection of AKS or ALS into rats induced a significant depression of overt phagocytosis as determined by the intravascular clearance of gelatinized RE test lipid emulsion (Table 1). Phagocytic depression was manifested by an approximate 2.4-fold increase in half-time for intravascular clearance in ALS or AKS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No.</th>
<th>Intravascular removal rate (half-time)</th>
<th>Liver</th>
<th>Lung</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Per cent ID/g</td>
<td>Per cent ID/TO</td>
<td>Per cent ID/g</td>
<td>Per cent ID/TO</td>
</tr>
<tr>
<td>NRS</td>
<td>1.5</td>
<td>±1.1</td>
<td>4.0±0.3</td>
<td>41.3±1.4</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>ALS</td>
<td>6</td>
<td>31.2±1.4</td>
<td>26.7±3.0</td>
<td>0.7±0.02</td>
<td>1.0±0.3</td>
</tr>
<tr>
<td>AKS</td>
<td>6</td>
<td>32.2±3.7</td>
<td>20.4±0.2</td>
<td>1.3±0.1</td>
<td>1.8±0.1</td>
</tr>
</tbody>
</table>

Table 1. Effect of intraperitoneally administered antilymphocytic serum or anti-Kupffer cell serum on the intravascular clearance rate and tissue distribution of gelatinized RE test lipid emulsion in rats

* Rats received 1 ml of appropriate serum 4 hr before determination of intravascular clearance rate. Values are expressed as means ± standard error.

* NRS, normal rabbit serum; ALS, rabbit anti-rat lymphocytic serum; AKS, rabbit anti-rat Kupffer cell serum.

* Expressed as per cent injected dose (ID) of emulsion per gram of tissue and per total organ (TO).

* Significant (P < 0.05) as compared to NRS-treated animals.
recipient rats as compared to NRS-treated controls. Both AKS- and ALS-induced phagocytic impairment was associated with a significant reduction in hepatic phagocytosis; ALS-treated animals localized 35% less lipid emulsion in the liver as compared to NRS controls. Rats treated with AKS manifested a greater than 50% impairment in hepatic phagocytosis. In contrast to the antisera-induced depression in hepatic phagocytosis, neither AKS nor ALS induced any significant alterations in pulmonary or splenic phagocytosis as compared to NRS-treated rats.

Since ALS induced a depression of hepatic phagocytosis, its cytotoxicity against isolated liver Kupffer cells was also determined (Fig. 1). The cytotoxicity titer of ALS towards liver Kupffer cells varied between 1:8 and 1:16, which was significantly lower than the 1:32 titer against thymocytes. When compared to the inability of NRS to exert any appreciable cytotoxic effect (Fig. 1), it is obvious that ALS manifests significant anti-Kupffer cell activity.

In contrast to the cytotoxicity of ALS towards both macrophages and thymocytes, AKS manifested significant cytotoxicity only towards macrophages (Table 2). When titered against isolated Kupffer cells, the AKS demonstrated significant cytotoxicity (Table 2). In contrast to the titer of AKS against Kupffer cells, AKS never attained 50% killing when titered against rat spleen cells or thymocytes (Table 2); however, a definite tendency towards cytotoxic activity was observed when spleen cells were employed as antigen. Slight agglutination was seen when AKS was titered against spleen cells (last dilution to show agglutination, 1:256) and thymocytes (last dilution to show agglutination, 1:512). In contrast, heavy agglutination was still seen at a 1:1,024 dilution when AKS was titered against isolated Kupffer cells. No significant cytotoxicity (titer always <10) or agglutination (last dilution always <1:32) was observed when either Kupffer cells, whole spleen cells, or thymocytes were incubated with NRS.

Since AKS was similar to ALS in reference to its depressive effects upon the RES and cytotoxicity towards isolated Kupffer cells, the ability of AKS to suppress the immune response was compared to ALS-induced immunosuppression (Table 3). Both the saline-injected and the NRS-injected animals had average titers of 1:319, whereas ALS-recipient animals had an average titer of 1:10. In contrast to control and ALS-recipient groups, rats treated with AKS re-

**Fig. 1.** Cytotoxic effect of antilymphocytic serum (ALS) on isolated Kupffer cells. Kupffer cells were incubated in vitro with either ALS or normal rabbit serum and complement for 2 hr. Viability was determined with the trypan blue dye exclusion test.

**Table 2. Cytotoxicity of anti-Kupffer cell serum upon rat Kupffer cells, spleen cells, and thymocytes**

<table>
<thead>
<tr>
<th>Dilution (−1/log2)</th>
<th>Kupffer cells</th>
<th>Spleen cells</th>
<th>Thymocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per cent dead</td>
<td>Per cent alive</td>
<td>Per cent dead</td>
</tr>
<tr>
<td>1</td>
<td>98</td>
<td>2</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>20</td>
<td>33</td>
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</tr>
<tr>
<td>4</td>
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<tr>
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<td>75</td>
<td>15</td>
</tr>
<tr>
<td>10</td>
<td>22</td>
<td>78</td>
<td>10</td>
</tr>
</tbody>
</table>

* Duplicate studies with normal rabbit serum indicated that rabbit serum per se had no significant cytotoxic effect upon rat Kupffer cells, spleen cells, or thymocytes.
EFFECT OF ANTI-KUPFFER CELL SERUM

TABLE 3. Effect of chronic administration of normal rabbit serum, antilymphocytic serum, and anti-Kupffer cell serum upon the production of anti-sheep erythrocyte serum by rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Animals</th>
<th>Hemolytic titer on day 7 (1/log2)</th>
<th>Avg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>10</td>
<td>2.505</td>
<td></td>
</tr>
<tr>
<td>Normal rabbit serum</td>
<td>10</td>
<td>2.505</td>
<td></td>
</tr>
<tr>
<td>Anti-Kupffer cell serum</td>
<td>5</td>
<td>2.023</td>
<td></td>
</tr>
<tr>
<td>Antilymphocytic serum</td>
<td>5</td>
<td>0.400</td>
<td></td>
</tr>
</tbody>
</table>

responded with an average hemolysin titer of 1:105.

DISCUSSION

The impairment in intravascular phagocytosis after AKS administration was equivalent to the impairment induced by the ALS administration. As with ALS, AKS-induced impairment in intravascular clearance was associated with a significant decrease in liver phagocytosis, whereas pulmonary or splenic localization of the lipid emulsion was not altered. The ability of ALS to depress RES activity has been noted by several investigators (10, 14, 20). Recent studies in our laboratory (20) have confirmed and extended the concept of ALS-induced RES depression and have suggested that the phagocytic impairment induced by ALS is attributable to the direct cytotoxic activity of ALS upon the hepatic phagocytes.

In contrast to the observation that antimacrophage serum was cytotoxic toward lymphocytes (14) when prepared by employing peritoneal cells as a source of antigen, AKS did not exert killing ability toward lymphocytes. The specificity of AKS, however, did not appear to be limited to the Kupffer cell. When AKS was titered against spleen cells, 35% of the cells were killed with a twofold dilution of the antisera. The cytotoxicity of AKS toward the spleen cells may be attributed to a selective killing of the splenic macrophage population which constitutes about 30% of the cellular elements of the spleen (5). This concept was supported by the lack of a cytotoxic effect when AKS was titered against lymphocytes derived from the thymus. Thus, AKS cytotoxicity may be directed only against macrophages.

Although both ALS and AKS manifest similar abilities to impair phagocytosis, the two antisera differed remarkably in their ability to induce an immunosuppression. The administration of ALS essentially obliterated the ability of the recipient to produce hemolysin. In contrast to the results with ALS, AKS induced only a slight reduction in the hemolysin titer.

Although other investigators have reported immunosuppression after the administration of antiperitoneal macrophage sera (1, 14, 17), it is debatable as to whether antimalphage sera inhibit humoral antibody formation (1, 14, 17), cellular immunity, (6) or both. The results reported here appear to indicate that AKS is not a very effective immunosuppressant in regard to humoral antibody formation. However, since it has been shown that humoral antibody immunosuppression by antiperitoneal macrophage serum is dependent on both the dosage of antigen employed and the time of antigen exposure in relation to the period of antiserum administration (1), it is not possible to conclusively state that AKS lacks significant immunosuppressive activity.

In view of the recently developed concept that macrophage processing of antigen is essential to the development of an immune response (7–9, 16), phagocytic impairment should be capable of suppressing the immunocompetency of the organism. Thus, it appears most likely that the AKS lacked immunosuppressive activity in this study because of its selective in vivo action against a population of macrophages, i.e., Kupffer cells which are weak antigen processors (12), and its possible inability to modify processing macrophages. Anti-Kupffer cell serum provides an immunological means to alter Kupffer cell function, permitting an evaluation of their contribution to metabolic and immune events.

ACKNOWLEDGMENTS

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LITERATURE CITED