Medium-Dependent Activity of Immune Serum on *Brucella*-Infected Macrophages

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Medium constituents affect the inhibitory action of anti-*Brucella melitensis* serum during in vitro infection of oil-induced peritoneal macrophages of the guinea pig with *B. melitensis.*

The phagocytic capacity of rabbit peritoneal macrophages depends upon the extent of macrophage activation (3), which in turn depends in part upon the nature of the reagent used to produce the macrophage-containing exudate in the peritoneal cavity of the animal (4). Peritoneal macrophages from *Brucella melitensis* immune animals called forth by mineral oil resist the lytic action of *B. melitensis*, behaving like macrophages in an exudate stimulated by injection of killed *brucellae* into the peritoneal cavity. Intraperitoneal inoculation of glycogen into immunized animals yields cells that are lysed, responding as though hypersensitive. Fetal bovine serum (FBS) in contact with normal macrophages raises their capacity to limit bacterial growth (2). Handling, such as chilling, washing, or treatment with antibiotics, influences the uptake of bacteria, penetration of antibiotic, and subsequent bacterial survival in phagocytic cells (8).

We observed that specific antiserum itself affects the ability of macrophages to ingest and to destroy intracellular *brucellae*, whereas others also studying *brucella* infection have found antiserum to be ineffective, even detrimental (6). Upon studying factors that might influence the intracellular killing of *brucellae* by guinea pig peritoneal macrophages induced by sterile mineral oil, we found that the basal support medium influences the interaction between rabbit anti-*brucella* serum and macrophages, thereby altering the intracellular *brucella* growth curves.

Six media (Table 1) were each mixed with an equal volume of a 1:25 dilution of anti-*brucella* rabbit serum. Other portions of the six media were mixed with normal rabbit serum and a third set received no addition of serum. Guinea pig macrophages were harvested in Tyrode solution

**Table 1. Influence of medium on inhibitory effects of immune serum during infection of normal guinea pig macrophages by *B. melitensis* Rev I**

<table>
<thead>
<tr>
<th>Medium constituents</th>
<th>Bacterial plate count at day 2 after addition</th>
<th>Resistance index$^a$ (log$_{10}$ B/BNRS/BIRS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None (log$_{10}$ B)</td>
<td>1/25 NRS (log$_{10}$ B)</td>
</tr>
<tr>
<td>Tyrode-NGPS, 30%</td>
<td>2.860</td>
<td>3.574</td>
</tr>
<tr>
<td>Hanks-NGPS, 30%</td>
<td>3.068</td>
<td>2.222</td>
</tr>
<tr>
<td>Medium 199-FBS, 15%</td>
<td>4.022</td>
<td>3.759</td>
</tr>
<tr>
<td>Medium 199-FBS, 15%</td>
<td>3.068</td>
<td>2.910</td>
</tr>
<tr>
<td>TLMH-FBS (20%) with glutamine + thyroxine$^d$</td>
<td>3.515</td>
<td>3.284</td>
</tr>
<tr>
<td>Tyrode-NRS, 40%</td>
<td>1.618</td>
<td>NN$^e$</td>
</tr>
</tbody>
</table>

$^a$ Resistance index = log$_{10}$ B per ml in NRS/log$_{10}$ B per ml in IRS, where log$_{10}$ B per ml represents the log$_{10}$ increase in *brucella* colonies per ml in 2 days at 37 C.

$^b$ B, *brucella*.

$^c$ All sera were heated for 15 min at 65 C. NGPS, normal guinea pig serum; IRS, immune rabbit serum, 4 weeks after subcutaneous infection of *10* smooth Rev 1 bacilli; NRS, normal rabbit serum; FBS, fetal bovine serum.

$^d$ Tris, modified Hanks, lactalbumin hydrolysate, 20% FBS, according to Fong (5), was further modified by addition of 0.002 M glutamine and 1.38 µM of thyroxine per ml.

$^e$ NN, addition not necessary.
from the peritoneal cavity 5 days after an intra-peritoneal inoculation of sterile Klebsiella, washed in Tyrode solution four times, and exposed to a suspension of *B. melitensis* Rev 1 (1) in either normal or anti-brucella serum in the various media. Bacterial plate counts were made at intervals from replicate samples of disrupted macrophages (7). The extent of intracellular growth varied dramatically in the 48-hr observation period, depending on the medium in which the parasitized macrophages were maintained; the resistance index, representing the degree of intracellular brucella inhibition in the presence of extracellular immune serum, varied from −0.090 (no effect) in Hanks-guinea pig serum to 2.649 (highly inhibitory) in medium 199-FBS (Table 1).

The differences were not correlated with macrophage survival (after 4 days), with brucellar growth in the absence of macrophages, or with the presence of immune serum in the medium itself. In Hanks medium, intracellular growth was greatly dependent on the calcium concentration (Table 2). Increasing the Ca²⁺ tended to reduce growth in 1:25 normal rabbit serum and to increase growth in the immune, the net effect being a lessening of the differences between immune and normal systems. In the case of Hanks or medium 199, the diametrically opposed results might have led us to different conclusions regarding the inhibitory action of antisera on intracellular growth of brucellae. These factors should be taken into consideration when broad comparisons are made of conflicting studies on serum-mediated macrophage responses.

Although we have presented data from an in vitro model in which heated serum from heterologous sources was used, these results in general resemble those we have obtained with autologous sera and unheated serum.

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### LITERATURE CITED