Effect of Immunoglobulin M from Normal Human Serum on
Leishmania donovani Promastigote Agglutination, Complement-
Mediated Killing, and Phagocytosis by Human Monocytes

THOMAS R. NAVIN,† EDWARD C. KRUG,‡ AND RICHARD D. PEARSON*

Division of Geographic Medicine, Department of Medicine, University of Virginia School of Medicine, Charlottesville, Virginia 22908

Received 19 November 1988/Accepted 20 December 1988

Serum from healthy, nonimmune humans contained immunoglobulin M (IgM) antibodies that agglutinated Leishmania donovani promastigotes, activated complement, and enhanced promastigote ingestion by human monocytes. The findings indicate that IgM antibodies have the capacity to affect the initial interaction of L. donovani promastigotes with human host defenses.

Early studies of the effects of nonimmune human serum on Leishmania donovani indicated that promastigotes are susceptible to complement-mediated lysis following activation of the classical pathway (3, 6). The observations suggested that cross-reacting or natural antibodies are present in the sera of people who have not had prior exposure to L. donovani or other pathogenic members of the family Trypanosomatidae. We therefore explored the interaction between L. donovani promastigotes and components of normal human serum (NHS), especially immunoglobulin M (IgM).

A Sudanese strain of L. donovani (MHOM/SU/00/S3) was cloned by limiting dilution and maintained by weekly passage in modified minimal essential medium to which 10% heat-inactivated (HI; 56°C for 30 min) fetal bovine serum (FBS), penicillin, and gentamicin were added (1). Promastigotes were harvested in the mid-logarithmic phase, washed twice in phosphate-buffered saline (PBS), and resuspended in modified minimal essential medium at 10⁶/ml. The concentration of motile, unagglutinated promastigotes was determined by counting in a hemacytometer the number of parasites that occurred singly or in pairs. Parasite viability was assessed by measuring the uptake of [3H]leucine or [3H]uracil as described previously (5).

NHS and monocytes were obtained from healthy North American adults with no history of exposure to L. donovani. Serum samples from 12 donors were pooled and were used fresh or after heat inactivation at 56°C for 30 min. In some studies sera were adsorbed against 10⁶ promastigotes per ml at 4°C for 30 min. IgG and IgM were prepared from NHS by immunoaffinity chromatography by using goat IgG raised against human IgM (μ-chain specific) or human IgG (γ-chain specific) (Sigma Chemical Co., St. Louis, Mo.) linked to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden). Antibodies were eluted from the columns with 1.5 M potassium thiocyanate, washed in PBS, and adjusted to a final concentration equivalent to that in 10% NHS. IgM- and IgG-deficient serum samples were prepared by applying serum samples to the columns and washing them with PBS. The eluate was dialyzed extensively and reconstituted to the original serum concentration. Antibody concentrations were measured by radial immunodiffusion. Serum was considered antibody deficient if no precipitant ring was observed. This indicated that less than 11 μg of antibody was present per ml. IgA was obtained from Sigma.

Incubation of L. donovani promastigotes with HI-NHS resulted in parasite agglutination (Table 1). Within 1 h after the addition of 10% HI-NHS, 43% of the promastigotes were agglutinated in groups of three or more, and after 3 h, 82% of the promastigotes were agglutinated. Free promastigotes were rarely observed after 6 h. The uptake of [3H]leucine or [3H]uracil by agglutinated parasites was comparable to that of control parasites in medium alone, indicating that HI-
NHS agglutinated but did not kill the promastigotes. The addition of FBS to control medium resulted in the multiplication of promastigotes and an increase in amino acid uptake. When HI-NHS was added to promastigotes in FBS, [3H]leucine and [3H]uracil uptakes were similar to those in medium alone.

HI-NHS alone or with FBS resulted in a persistent decrease in the number of unagglutinated promastigotes in cultures that were followed for 5 days (Fig. 1). Similar results were obtained with serum from 27 donors that was studied individually (data not shown). Human umbilical cord serum, which is naturally deficient in IgM, did not cause promastigote agglutination and supported promastigote growth, although not as well as FBS did. The addition of 10% FBS or hypoxanthine (100 μM) to umbilical cord serum resulted in maximal growth, suggesting that umbilical cord serum may have lower concentrations of purines or higher concentrations of xanthine oxidase than FBS does. In addition, NHS heated at 65°C for 45 min to destroy antibodies did not agglutinate promastigotes and supported growth to a degree similar to that in umbilical cord serum. Finally, agglutination was not observed after HI-NHS was absorbed three times against promastigotes (Fig. 2).

The addition of purified IgM to promastigote cultures produced agglutination at 24 h equivalent to that of HI-NHS; thereafter, the number of unagglutinated promastigotes increased until by day 5 the number of promastigotes was equal to that in cultures with FBS (Fig. 3). IgM-deficient serum produced no detectable parasite agglutination. Ne-
TABLE 1. Uptake of \([^{1}H]\)leucine and \([^{1}H]\)uracil by *L. donovani* promastigotes in the presence of various serum samples*

<table>
<thead>
<tr>
<th>Serum sample</th>
<th>cpm (mean ± SE)</th>
<th>Concns (promastigotes/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>([^{1}H])leucine</td>
<td>([^{1}H])uracil</td>
</tr>
<tr>
<td>No serum</td>
<td>834 ± 112</td>
<td>1,077 ± 144</td>
</tr>
<tr>
<td>10% HI-NHS</td>
<td>936 ± 221</td>
<td>1,125 ± 266</td>
</tr>
<tr>
<td>10% FBS</td>
<td>1,589 ± 305</td>
<td>1,761 ± 301</td>
</tr>
<tr>
<td>10% FBS plus 10% HI-NHS</td>
<td>1,013 ± 85</td>
<td>934 ± 390</td>
</tr>
</tbody>
</table>

* Each condition started with 10^6 promastigotes per ml. After 6 h of incubation labeled amino acids were added. The number of unagglutinated *L. donovani* promastigotes was counted at the midpoint of the experiment (12 h after the addition of the amino acids), and the reactions were stopped 24 h after the addition of the amino acids. Values represent the mean of three experiments, each of which was performed in triplicate.

ther isolated IgG nor IgA affected promastigote agglutination or growth. Indirect immunofluorescence microscopy demonstrated IgM deposits on unagglutinated promastigotes that were exposed to HI-NHS (Fig. 4). The effects of IgM and IgG on the interaction of promastigotes with human monocytes were prepared as described previously (4) and incubated with promastigotes and HI-NHS, IgM, IgG, or medium alone at 37°C for 1 h at a promastigote-to-phagocyte ratio of 10:1. Monocytes phagocytized promastigotes even in the absence of serum, but the number of ingested parasites more than doubled in the presence of HI-NHS or IgM (Fig. 5). Conversely, the addition of IgG did not affect ingestion. The effects of IgM on complement-mediated promastigote lysis were then assessed. Fresh NHS rapidly killed promas-

FIG. 1. Growth of *L. donovani* promastigotes over 5 days in the presence of various serum samples: 10% FBS (●), 10% HI-NHS (●), no serum (○), 10% HI human umbilical cord serum (▲), 10% HI-NHS plus 10% FBS (□), and 10% HI-human umbilical cord serum plus FBS (○). For Fig. 1, 2, and 3, each datum point represents the mean (± standard error of the mean) concentration of unagglutinated promastigotes determined in three experiments that were done in triplicate. The limit of detection was 10^6 promastigotes per ml, as indicated by the dashed lines.

FIG. 2. Promastigote growth in the presence of NHS absorbed one, two, or three times. The following serum samples were used: 10% FBS (●), 10% HI-NHS (■), 10% HI-NHS absorbed once with 10^6 promastigotes per ml (▲), 10% HI-NHS absorbed twice with 10^6 promastigotes per ml (○), 10% HI-NHS absorbed three times with 10^6 promastigotes per ml (▲), and 10% HI-NHS absorbed 3 times plus 10% FBS (□).

FIG. 3. Promastigote growth in the presence of IgM purified from NHS. The following serum samples were used: 10% FBS (●), 10% HI-NHS (■), IgM (○), 10% HI-human IgM-free serum (▲), 10% HI-human IgM-free serum plus 10% FBS (▲), and IgM plus 10% HI-NHS (□).
FIG. 4. Photomicrographs of *L. donovani* promastigotes after incubation with 10% HI-NHS for 1 h and staining with fluorescein isothiocyanate-labeled anti-human IgM. (A) Phase-contrast optics of promastigotes showing body-body, body-flagellar, and flagellar-flagellar attachments. (B) Same field as in panel A, but with fluorescent illumination showing deposits of IgM. (C) Transposition of panels A and B showing that attachment of promastigotes corresponds with deposits of IgM. Bar, 10 μm.
tigotes (Fig. 6) (3, 6). Absorption of fresh NHS abrogated killing. IgM alone did not kill promastigotes, but when IgM was added to absorbed serum the lethal effect was fully restored. Neither IgG alone nor IgG plus absorbed serum killed promastigotes.

The data indicate that IgM, but not IgG, antibodies in the serum of people with no history of exposure to Leishmania species or related kinetoplastids can agglutinate L. donovani promastigotes, enhance their phagocytosis by human monocytes, and initiate killing of promastigotes by complement. It is unclear why nonimmune people have such antibodies. The antibodies may have arisen in response to exposure to microbes or other environmental antigens with cross-reacting, possibly carbohydrate, epitopes, or they may be natural antibodies. Likewise, the mechanism by which IgM enhances promastigote ingestion by monocytes remains speculative. Based on studies of the serum-independent attachment of L. donovani promastigotes to mononuclear phagocytes, we hypothesize that IgM may facilitate the deposition of monocyte-produced complement components and that promastigote attachment and ingestion are mediated by monocyte receptors for membrane-bound complement component C3b (2, 3, 7, 8).

The data indicate that factors other than IgM in human serum can affect promastigotes. First, human umbilical cord serum, which is deficient in IgM, supported maximal promastigote growth in vitro only after the addition of FBS or hypoxanthine. Furthermore, HI-NHS appeared to contain a nonspecific factor(s) that suppressed promastigote growth in culture.

It is difficult to extrapolate from the in vitro findings to the role that IgM plays in host-parasite interactions in vivo. On the one hand, if serum complement is present and functional at the dermal site where promastigotes are inoculated by sandflies, IgM antibodies may activate complement and result in promastigote killing. If the complement membrane attack complex (C5b-C9) is not functional, however, IgM may enhance phagocytosis of promastigotes by cutaneous mononuclear phagocytes. It is within the macrophage that L. donovani converts from its promastigote to its amastigote form and multiplies. It may be that promastigotes actually use IgM antibodies to facilitate entry into their preferred sanctuary, the macrophage.

LITERATURE CITED