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Opsonization In Vitro of *Giardia lamblia* Trophozoites

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The ability of peritoneal rabbit macrophages from immunized and nonimmunized animals to phagocytose *Giardia lamblia* trophozoites in the presence of serum was studied and compared in an in vitro system. The rabbits which served as the source of immune serum and macrophages were injected repeatedly at multiple sites (intramuscularly, subcutaneously, and intradermally) with a mixture of *G. lamblia* trophozoites and Freund complete adjuvant. In the presence of normal rabbit serum, a low level of phagocytosis of *Giardia* trophozoites by normal and immune macrophages was observed. In the presence of hyperimmune rabbit serum, an increased phagocytic activity of both types of macrophages occurred. The opsonic activity was similar whether whole serum or purified immunoglobulin G was used and whether or not these were heat inactivated. *G. lamblia* trophozoites in suspension were shown to be agglutinated in the presence of hyperimmune serum. Tests employing serial dilutions of hyperimmune serum resulted in a parallel loss of opsonifying and agglutinating activities. It is suggested that opsonization in vivo may play a role in the ability of the host to limit infection by these organisms.

Our knowledge of the role of host humoral factors and cellular factors in limiting the spread of *Giardia* infection is incomplete. The idea that both may be involved is strengthened by observations of the course of giardiasis in several types of immunodeficient hosts. Thus, humans with dysgammaglobulinemia are likely to have severe *Giardia* infection (2, 3, 6, 7, 9-13). Further, although *Giardia* infection in most strains of immunocompetent mice is self-limited, with resolution in 4 to 7 weeks, followed by resistance to subsequent infection (18), congenitally athymic mice fail to resolve their infection even after 10 weeks (20). The life span of these nude mice when infected with *Giardia* and *Hexamita (Spironucleus)* spp. is short but may be lengthened if they are cured of their intestinal protozoan infections (5). Numbers of excreted *Giardia* cysts are reduced (and in some nude mice completely eliminated) if the mice are injected with lymphoid cells obtained from normal donor mice infected with *Giardia* spp. (17). Radulescu et al. (15) have observed *Giardia* trophozoites in the tissues and organs of rodents simultaneously infected with *Plasmodium berghei;* this suggests that the result of this suprainfection in these animals was a diminished ability to resist invasion. *Giardia* trophozoites have also been found in tissues of the small intestinal wall of mice which had been exposed once to irradiation (24).

Owen and colleagues (Abstr. Annu. Meet. Am. Gastroent. Assoc. 1980, 78, p. 1232), in a transmission electron microscope study of *Giardia* infection in normal mice, noted that *Giardia* trophozoites were engulfed by pseudopods extending from macrophages situated beneath the basal lamina or totally within the epithelium. They observed degraded trophozoites in the dome epithelium in phagolysosomes of macrophages surrounded by lymphoblasts. This mechanism may be one route by which macrophages process antigen for presentation to lymphocytes.

This paper is concerned with the role of the interaction of macrophages and specific antibody in limiting the spread of *Giardia* infection.

MATERIALS AND METHODS

Parasites. *G. lamblia* trophozoites, isolated in Portland, Oreg., and designated the Portland 1 strain, were cultured in vitro in HSP-3 medium (4). Trophozoites in 72-h cultures were detached from the culture tube glass by chilling the tubes at 4°C for 30 min.

Serum preparation. The immune serum used in this study was collected from four New Zealand male rabbits (2,000 g each); each rabbit received a series of injections consisting of a mixture of 10 mg of lyophilized *G. lamblia* trophozoite antigen and Freund complete adjuvant. A total of nine injections were given to each rabbit (three injections [intramuscular, subcutaneous, and intrademal] on three occasions at 21-day intervals). Each rabbit thus received a total of 30 mg of antigen. Animals were bled 4 weeks after the last injection. Both unheated and heat-inactivated (56°C, 30 min) hyperimmune serum and immunoglobulin G (IgG) purified from this serum by diethylaminoethyl column chromatography (26) were used in these studies. Nonimmune serum was obtained from 12 New Zealand rabbits which had not received *Giardia* antigen.

Collection of macrophages. Normal rabbits and rabbits hyperimmunized with *G. lamblia* trophozoite antigens were inoculated intraperitoneally with 3 ml of 3% thioglycolate to elicit a peritoneal exudate. Five days later, the animals were killed with ether. The peritoneal exudate was collected by making a small incision through the abdominal wall into the peritoneal cavity and introducing 20 to 30 ml of Hanks balanced salt solution containing 5 IU of heparin per ml. The resultant macrophage-Hanks balanced salt solution suspension was harvested, and the macrophages were washed with three successive centrifugations (280 × *g*, 4°C, 5 min) and finally suspended in RPMI 1640 tissue culture medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 20% calf serum–50 IU of penicillin–50 μg of streptomycin per ml.

Cultivation of macrophages for phagocytic test. Volumes (1 ml each) of the macrophage suspension (2×10^6 ml) were dispensed into Leighton tubes containing cover slips (8 by 30 mm). After 2 h at 37°C, the culture fluid was withdrawn, and the cover slips were gently rinsed in situ. The cover slips then were overlaid with 1 ml of medium and incubated at 37°C in a 5% CO_2–95% air atmosphere until used; this incubation never exceeded 4 h. Viability of the macrophages was determined by their ability to ingest latex particles and *Staphylococcus aureus*.

Passive sensitization of parasites. A suspension of *G. lamblia* trophozoites, obtained as described above, was sedimented at 800 × *g* and then washed and suspended in pH 7.0 phosphate-buffered saline to a concentration of 8×10^6 parasites per ml. Trophozoite suspension (1 ml) was mixed with 1 ml of homologous rabbit antiserum and incubated for 30 min at 4°C, and then the trophozoites were washed twice with phosphate-buffered saline by centrifuging at 800 × *g* for 5 min.

Phagocytic test. A suspension of passively sensitized parasites was added to tubes containing macrophage monolayers on cover slips at a parasite/macrophage ratio of approximately 4:1. After incubation at 37°C for intervals of 5, 30, 60, and 120 min and 24 h, cover slips were removed, washed gently in phosphate-buffered saline, fixed for 10 min in methanol, stained with Giemsa stain, and examined.

We have defined the phagocytic index (PI) for these studies as the number of macrophages counted which had adhering or engulfed particles divided by the number of counted macrophages.

Agglutination test. A suspension of unsensitized trophozoites was mixed with an equal volume of diluted hyperimmune serum and allowed to stand at 4°C for 30 min, and microscopic observations were made at 200×. We considered the agglutination titer to be the highest dilution of immune serum in which 50% or more of the parasites were agglutinated.

RESULTS

After 1 h in the presence of antitrophozoite serum, macrophages, whether from immunized or control rabbits, were observed to be attached to or to ingest 85 to 90% of *Giardia* trophozoites, whereas in the presence of normal rabbit serum, this capacity was in the range of 10 to 12% (Table 1). The phagocytic activities of macrophages from normal (PI = 0.85) and immunized animals (PI = 0.90) were similar. The macrophage activity towards the trophozoites was similar whether whole serum or purified IgG was used and whether or not these had been heat inactivated.

The effect of diluting anti-*Giardia* serum on the phagocytic activity of macrophages is summarized in Fig. 1. The PI remained above 0.60 up to a serum dilution of 1:512; further dilution resulted in a sharp decrease in PI. A similar

TABLE 1. *Phagocytic activity of peritoneal macrophages from rabbits immunized with G. lamblia trophozoites and from nonimmunized rabbits*

Source of peritoneal macrophages[a]	Serum reactant[b]	Particles offered for phagocytosis	PI[c]
Immunized rabbit	Hyperimmune serum	*Giardia* trophozoites	0.90
Nonimmunized rabbit[d]	Hyperimmune serum	*Giardia* trophozoites	0.85
Nonimmunized rabbit	IgG purified from hyperimmune serum	*Giardia* trophozoites	0.92
Immunized rabbit	Normal rabbit serum[d]	*Giardia* trophozoites	0.10
Nonimmunized rabbit	Normal rabbit serum	*Giardia* trophozoites	0.12
Nonimmunized rabbit	Fetal bovine serum	*Giardia* trophozoites	0.14
Nonimmunized rabbit	Normal rabbit serum	*S. aureus*	0.93
Nonimmunized rabbit	None	*S. aureus*	0.80
Nonimmunized rabbit	None	Latex particles	0.70
Nonimmunized rabbit	None	*Giardia* trophozoites	0.13
Immunized rabbit	None	*Giardia* trophozoites	0.12

[a] Peritoneal macrophages, collected and cultivated as described in the text, were obtained from separate rabbits to conduct each of the 11 experiments described.

[b] Although these serum reactants were unheated, heating (56°C, 30 min) of these reactants did not result in a significant change in PI.

[c] Each PI represents the result of an average of five determinations, each in turn arrived at by examining 100 macrophages on a separate cover slip.

[d] The rabbits which served as the sources of these reactants were free of *Giardia* infection at the time that these experiments were conducted; the possibility cannot be excluded that earlier they had been infected with *Giardia* and then recovered.

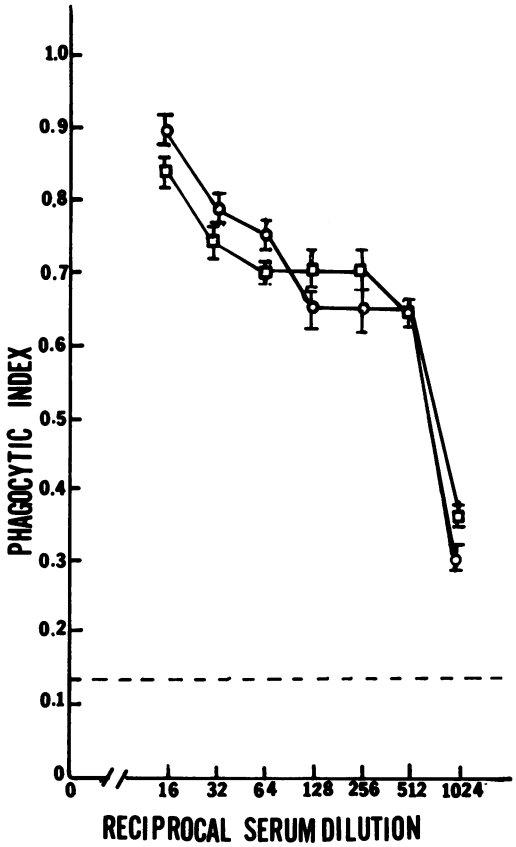

FIG. 1. *Titration curves of the phagocytic activity against G. lamblia trophozoites, after exposure to various dilutions of antiserum, of macrophages from hyperimmune* (○) *and nonimmune* (□) *animals. Each point represents the average of five determinations, each in turn arrived at by examining 100 macrophages on a separate cover slip; the five macrophage preparations used to determine each point were obtained from a single animal. The dotted line represents base-line phagocytosis which occurred in the absence of serum; horizontal bars represent standard errors of the mean.*

effect of antiserum dilution was noted for the ability of the antiserum to agglutinate trophozoites; the agglutination titer of the hyperimmune serum was 1:512.

The effect of the time of contact of macrophages to sensitized trophozoites on the PI was also studied. After 30 min, most macrophages could be seen attached to at least one trophozoite (Fig. 2); the PI was 0.70. Thereafter, parasites could be seen inside macrophages; the PIs at 1, 2, and 3 h were 0.85, 0.91, and 0.85, respectively. After 3 h of contact, ingested trophozoites became unidentifiable in the cytoplasm of the macrophages, the last recognizable organelles being the nucleus and adhesive disk. The effect of time of contact of macrophages to unsensitized trophozoites was quite different. At all contact intervals from 30 min (Fig. 3) to 3 h, the PI remained at about 0.10.

DISCUSSION

The extent of phagocytosis of *Giardia* trophozoites by macrophages in these experiments was high in the presence of immune serum and low in its absence. The high opsonizing activity observed in the presence of either immune serum or specific IgG, whether heat inactivated or not, suggests that the attachment of macrophages to *Giardia* trophozoites is mediated by an immune complex, constituents of which are antibodies from the serum and antigens of the parasite membrane, and that trophozoites are opsonized as a result of coupling with antibody.

In studies of *Cryptococcus neoformans,* Kozel and McGaw (8) showed that purified IgG in the absence of complement acts as an opsonin primarily by facilitating attachment of parasite to phagocyte; a similar mechanism apparently operates here.

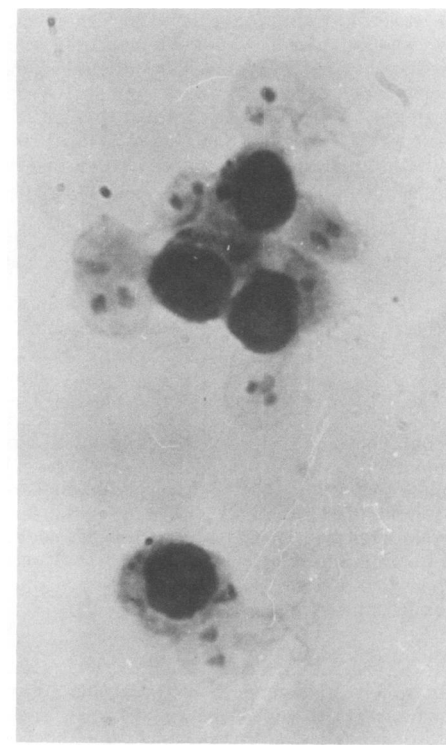

FIG. 2. *G. lamblia trophozoites and immune macrophages after 30 min of contact in the presence of hyperimmune serum. Most protozoa are in apposition to macrophages (×1,500).*

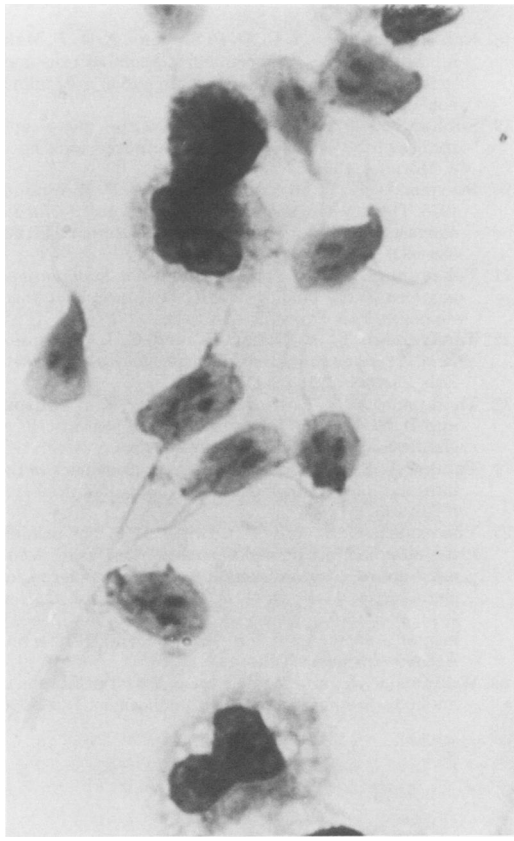

FIG. 3. *G. lamblia trophozoites and immune macrophages after 30 min of contact in the presence of normal serum. Trophozoites stained sharply; paired nuclei and flagella are apparent. Little protozoa-macrophage interaction is evident (×1,500).*

As a result of studies involving pepsin-digested IgG, Takayanagi and Nakatake (21) suggested that the Fc portion of the immune globulin molecule, which forms an antigen-antibody complex with the parasite, plays an important role in the attachment, probably by reacting with specific macrophage membrane receptor sites.

Results of the present study indicate that the observed opsonization does not involve the participation of complement. Our finding in the *Giardia*-macrophage system of a correlation between the ability of antibody to enhance in vitro phagocytosis and to agglutinate antigen has been observed in other parasite-macrophage systems. Takayanagi et al. (22), in studying a number of antisera obtained against various antigenic fractions of *Trypanosoma gambiense,* noted that only antiserum capable of agglutinating trypanosomes could enhance phagocytosis of these protozoa.

Two types of phagocytic activity against *Giardia* trophozoites have been demonstrated here (see Table 1 and Fig. 1): a nonspecific type (base line) which proceeded at a low level in the absence of serum or complement and a specific type, involving the majority of phagocytes, which required specific IgG but not complement.

Phagocytosis by host macrophages may well play a role in limiting *Giardia* infection in vivo. Peyer's patches are known to contain numerous macrophages capable of phagocytosing parasites (1, 19, 20), and Owen and colleagues (Abstr. Annu. Meet. Am. Gastroent. Assoc. 1980, 78, p. 1232) have demonstrated that such macrophages trap and degrade *Giardia* trophozoites. Several other observations strengthen the possibility that opsonization of trophozoites may play a role in limiting *Giardia* infection. We have shown that the process proceeds in the presence of specific IgG antibodies; it has been reported that, in at least some *Giardia* infections, anti-*Giardia* antibody is produced (14, 16, 25), and IgG is one of the classes of antibody produced in the jejunal lamina (23). It should be possible, in passive transfer experiments using as the host animal the mouse model described by Roberts-Thomson et al. (18), to determine in vivo the role of this antibody in preventing *Giardia* infection.

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