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Immune Response to *Giardia duodenalis*

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INTRODUCTION

Giardia

Although its first description was attributed to the microscopist Antonie van Leeuwenhoek (1632 to 1723), Vilem Lambl (1824 to 1895), a Czech physician, was credited with the discovery in 1859 of the flagellate *Giardia*. The name *lamblia* was given to the species by Blanchard in 1888 (121). *Giardia*, a flagellated protozoan, inhabits the upper part of the small intestine of its host and has a direct life cycle. After the host ingests cysts, which are the infective stage, the trophozoites (Fig. 1) emerge from the cysts in the duodenum and attach to the small intestinal mucosa. They undergo mitotic division in the intracellular lumen; some will encyst to protect themselves and will be eliminated from the host in the feces. Cysts can survive for 3 months in water at 4°C (120, 121). They are transmitted to a new host through contaminated water or food or by person-to-person or animal-to-person contact. The inoculum required for infection in humans is between 10 and 100 cysts (155).

Interest in this group of protozoa began only 20 years ago, when *Giardia* organisms were isolated from mammal, bird, and amphibian hosts (105). Initially, assignment of a species name to *Giardia* was based on the animal host species from which the organism was isolated. Filice (66) rejected this concept of host specificity and proposed to use the morphology of the trophozoite microtubular organelles known as the median body (Fig. 1) to classify species into three groups: (i) the amphibian group (*G. agilis*), which has a long teardrop-shaped median body; (ii) the rodent and bird group (*G. muris*), which has two small, rounded median bodies; and (iii) the human group (*G. duodenalis* = *lamblia* = *intestinalis*), in which the single or double median bodies resemble the claw of a claw hammer (Fig. 1). Organisms of the *duodenalis* group have been described not only in humans but also in other mammals, birds, and reptiles.

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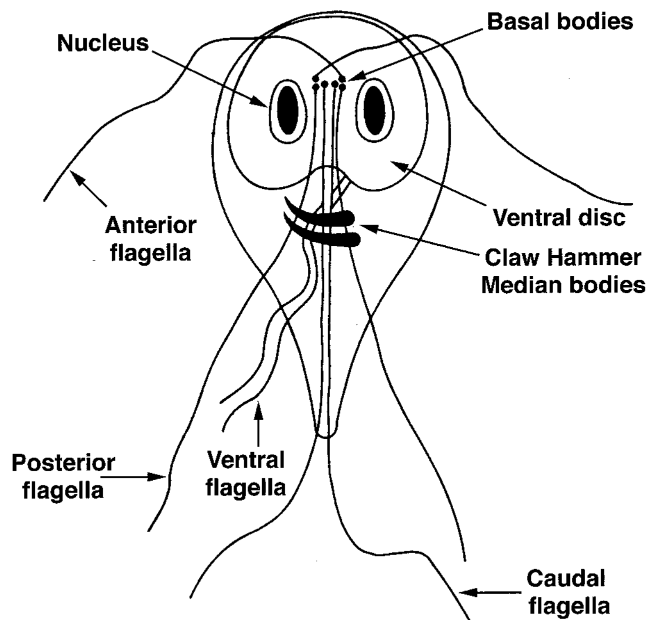


FIG. 1. Trophozoite of the *G. duodenalis* type of organism.

Giardia trophozoites recently isolated from the great blue heron (56) and budgerigar (58) were given the names of *G. ardea* and *G. psittaci*, respectively, because these species were found to be distinct from *G. duodenalis* when examined by electron microscopy. However, these new species share many of the characteristics of the *duodenalis* organism group (58). It is likely that new *Giardia* species will be described in the future. In this review, because Filice's (66) classification is followed, the name "*G. duodenalis*" is used to describe the human type of *Giardia*.

Giardiasis

In humans, the clinical effects of *Giardia* infection range from the asymptomatic carrier state to a severe malabsorption syndrome. In fact, it was only in the late 1970s that *Giardia* was recognized to cause pathology. In a clinical study in 1978, Kulda and Nohynkova concluded that this parasite can cause disease in humans based on symptoms such as malabsorption and the pathology observed in the upper part of the small intestine in patients from whom the organism was isolated (105). In 1981, the World Health Organization added *Giardia* to its list of parasitic pathogens (197).

Factors possibly contributing to the variation in clinical manifestations include the virulence of the *Giardia* strain (8, 136), the number of cysts ingested, the age of the host, and the state of the host immune system at the time of infection. The clinical diagnosis of giardiasis is difficult since symptoms are nonspecific and resemble those of a number of other gastrointestinal ailments. Clinical features may range from diarrhea to constipation, nausea, headache, and flatulence (121, 199). Moreover, the symptoms observed vary with the life cycle stage of the parasite. The incubation period may last 12 to 19 days and is marked by the first detection of cysts in the feces (97). This period is followed by the acute phase, where a variety of symptoms signal the onset of the disease. If the immune system of the host is fully developed and healthy, the acute phase usually resolves spontaneously and the symptoms will disappear. Unfortunately, in certain cases, in spite of a healthy and fully developed immune system, the acute phase develops into a chronic stage. In these situations, the symptoms of the disease will reappear for short and recurrent periods (199). There are also some asymptomatic patients who pass cysts in their feces. In one study, it was found that between 60 and 80% of infected children in day care nurseries and their household contacts have asymptomatic giardiasis (101). Asymptomatic individuals are an important reservoir for spread of the infection.

The histopathological changes occurring at the mucosal sites range from minimal to severe enough to cause enteropathy with enterocyte damage, villus atrophy, and crypt hyperplasia (65). The reasons for these variations are similar to those mentioned above as possible factors contributing to the variation of clinical manifestation. Shortly after the trophozoites leave the stomach of their new host in response to low pH, excystation will take place. Using their flagella and ventral disc, trophozoites released in the upper part of the small intestine move to the microvillus-covered surface of the duodenum and jejunum, where they attach themselves (88, 116), and play a role in the onset of the pathology (22, 34, 124). The suction force created by this mode of attachment may damage the microvilli and interfere with the process of food absorption (88, 116). Eventually, the rapid multiplication of the trophozoites by binary fission creates a physical barrier between the intestinal epithelial cells and the lumen of the intestine, interfering with the process of absorption of nutrients.

Since it is difficult to access the intestinal mucosa of humans without using invasive procedures, our knowledge of the mucosal pathology caused by *Giardia* is limited. The trophozoites do not usually penetrate the epithelium (65). However, when the conditions are favorable, trophozoites may invade tissues such as the gallbladder and the urinary tract (73, 122). Mucosal invasion by trophozoites has also been observed in the mouse model of the disease (114, 145). The migration of trophozoites from the lumen of the intestine into surrounding tissues is, however, an unusual occurrence in humans and mice.

The jejunal morphology ranges from normal to subtotal villus atrophy, and a correlation between the degree of villus damage and malabsorption has been reported (21, 35, 124, 125, 201). In humans, polymorphonuclear leukocytes and eosinophils have been detected (202). These changes revert to normal after treatment or when the parasite has been eliminated by the immune system. On the other hand, Brandborg et al. found normal jejunal histology with absence of inflammatory cells in symptomatic patients (with diarrhea) (29). A higher incidence of giardiasis has been reported in hypogammaglobulinemic patients (200); it appears that more severe damage to the villus is present in the hypogammaglobulinemic patients than in those with a normal immune system (65). Interestingly, the degree of villus pathology observed in patients with AIDS is comparable to that in immunocompetent patients (103), although AIDS patients are deficient in $CD4^+$ T cells. Furthermore, AIDS patients do not appear to be more susceptible than healthy persons to giardiasis (166). For a review of the effects of *G. duodenalis* on the structure, kinetics, and function of absorptive intestinal cells and other epithelial cells and a correlation with morphological injury and physiological alterations, the reader is referred to the review by Buret et al. (34).

ANTIGENS OF *GIARDIA*

Polypeptides

The identification of *G. duodenalis* antigens that play a role in acquired immunity has been difficult for a variety of reasons: (i) usually the trophozoites do not invade the tissues (if there

is a stimulation of the immune system, it remains localized); (ii) antigenic variation on the surface membrane of trophozoites has been reported (see the following section); (iii) investigators have used different isolates of *Giardia*, different antibody reagents, and a variety of assays in studies of the immune response to *Giardia*; and (iv) it is difficult to compare the results obtained by different laboratories. Crude antigenic extracts prepared from *G. duodenalis* trophozoites cultured in vitro have revealed different polypeptides depending on the techniques used to characterize them. For example, a minimum of 20 distinct Coomassie blue-staining bands ranging in molecular mass from 14 to 125 kDa were obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (126). However, high-performance liquid chromatography showed five distinct fractions, and when they were used as antigens in an enzyme-linked immunosorbent assay (ELISA) to detect specific antibodies in the serum of immunized rabbits, the assay was positive only with the higher-molecular-mass fractions (126). These findings indicate that many polypeptides detected by SDS-PAGE are probably not playing a role in the immune response.

On the other hand, SDS-PAGE has been useful in demonstrating similarities in the antigen profiles of *G. duodenalis* isolates from the same geographic area (196). Since *G. duodenalis* is a ubiquitous organism, it is possible that the antigenic profiles of isolates from different geographic areas will vary. Surprisingly, analysis of the molecular mass of polypeptides from crude extracts of trophozoites obtained from different geographic isolates shows that there are many similarities. For example, similarities were reported among the proteins in isolates from Afghanistan, Puerto Rico, Ecuador, and Oregon. Their molecular masses ranged from 12 to 140 kDa (167). In this case, it is not surprising that the antigenic profiles of *G. duodenalis* isolates from a same geographic area have also revealed many similarities among them (196).

Nash and Keister (132) were able to classify 19 isolates of *G. duodenalis* into three groups by comparing the reactivity of antibodies raised against excretory-secretory (ES) products released in vitro in the culture medium by each isolate. Five isolates showed major antibody cross-reactivity, and 11 showed moderate antibody cross-reactivity. Three isolates released identical ES products. Similarities were also observed in the antigens present on the surface of the trophozoites of the 19 isolates even if the patients had been infected in different geographic areas (132). None of these studies of antigenic profiles in geographic areas were able to identify a single dominant protein among the isolates.

The identification of a *G. duodenalis* trophozoite major surface antigen that is present on all isolates will be an asset for the development of immunodiagnostic tests or for the design of a vaccine. The existence of a dominant surface antigen on the trophozoite of *G. duodenalis* was first reported by Einfeld and Stibbs (55). The characterization of this 82-kDa antigen revealed that it was pronase and periodate modifiable and heat labile (55). Using surface iodinated techniques, Edson et al. (54) identified an 88-kDa major trophozoite surface antigen which they claim is similar to the 82-kDa polypeptide reported by Einfeld and Stibbs. Antibodies to the 88-kDa polypeptide were detected in the sera of infected patients, but no clear correlation was established between the appearance of specific serum antibodies to *G. duodenalis* major antigens and protective immunity. Unfortunately, only two anti-*G. duodenalis* human sera were used in their study (54). The identification of a major surface antigen of approximately 80 kDa is an interesting finding. It is not known if this major antigen is also present in isolates from different geographic areas. Antigens with different molecular masses were identified from isolates obtained from symptomatic and asymptomatic patients. By using immunoblotting, 65- and 70-kDa antigens were identified in the feces of gerbils infected with strains obtained from symptomatic and asymptomatic patients, respectively (127, 128).

Clark and Holberton (44) introduced methods to study *Giardia* molecules from pure fractions of plasma membranes. After purification of the cell membrane preparation by centrifugation on a Percoll gradient, a major band was found at 75 kDa. The investigators concluded that the antigen corresponded to the iodinatable and antibody-precipitated 82-kDa antigen reported earlier by Einfeld and Stibbs (55). In addition, 22-, 54-, and 58-kDa polypeptides were identified. Interestingly, the 54- and 58-kDa proteins comigrated with α- and β-tubulins. The authors concluded that tubulin is a constituent of *Giardia* membranes and appears in a different form from the tubulin found in microtubules (44). It is possible that all these different polypeptides observed on the surface membrane of trophozoites in the early literature were in fact variant surface proteins described in the late 1980s by Nash et al. (137); this would also explain the difficulty encountered in the isolation of dominant antigens.

Genes that encode surface membrane proteins of trophozoites have been cloned. Sequence analysis of a gene encoding a 72.5-kDa protein revealed a single open reading frame specifying a hydrophilic cysteine-rich protein with an amino-terminal signal peptide and a postulated hydrophobic membrane-spanning anchor region near the carboxyl terminus (67). The cysteine residues (58 of 84 residues) were in a Cys-Xaa-Xaa-Cys motif dispersed 29 times throughout the sequence. The authors hypothesized that the abundance of cysteine residues suggests that the native proteins on the parasite surface may contain numerous disulfide bonds. These bonds would confer resistance to intestinal-fluid proteases and to the detergent activity of bile salts, thereby helping the parasite survive in the hostile environment of the intestine (67). Upcroft et al. (183) have expressed *Giardia* antigens in *Escherichia coli* by cloning *G. duodenalis* genomic DNA into pUC vectors. Expressed proteins were part of the organelles of the trophozoite. For example, a 32-kDa protein which is associated with the spiral part of the ventral disc was also found in the flagella and axonemes. Other proteins expressed by the clones covered the surface of the trophozoites or were associated with the coat (183).

Cyst antigens detected in human feces have a molecular mass varying between 21 and 49 kDa (71). Similar antigens were also detected in immunoblots of parasites cultured in vitro in encysting medium. These polypeptides are not found in the trophozoites (71). Monoclonal antibodies (MAbs) raised against cyst antigens were able to recognize polypeptides ranging from 29 to 45 kDa in immunoblot and immunofluorescence assays. The polypeptides appeared within 8 h of exposure of the trophozoites to encystation medium (37, 193). These investigators concluded that the molecules appearing early during encystation represent potential targets for strategies directed at inhibiting the process of encystation. Genes that express protein components of the cyst wall have been identified. One of the cloned genes expresses an acidic, leucine-rich 26-kDa polypeptide (CWP1) that contains 5.3 tandemly arranged copies of a degenerate 24-amino-acid repeat (129). Interestingly, the levels of the transcripts from the cyst wall protein gene increase more than 100-fold during encystation. Cyst wall protein expression also increases dramatically during encystation. Before CWP1 is incorporated into the nascent cyst wall, it is contained within encystation-specific vesicles of encysting trophozoites. CWP1 was not observed in nonencysting trophozoites (129). Another gene expressing a different cyst

wall protein has been cloned. The novel 39-kDa polypeptide (CWP2) is also expressed during encystation; unlike CWP1, CWP2 has a 121-residue COOH-terminal extension (113). These studies of polypeptides of *G. duodenalis* trophozoites and cysts demonstrate the antigenic complexity of this intestinal parasite and the challenge it provides to the immune system of its host.

Heat Shock Proteins

Heat shock proteins (HSP) are synthesized by mammal, bacterium, protozoan, helminth, and even plant cells in response to stresses such as an abrupt rise in temperature, pH, or other stressful treatment. These proteins help the cell to survive the stress. *Giardia* trophozoites live in the intestine, a habitat where stresses are likely to occur. Few studies have been done on HSP in giardiasis, and the role they may play in the immune response has yet to be defined. HSP have been detected on the surface membrane of trophozoites. The synthesis of [^{35}S]methionine-labeled proteins of 30, 70, 83, and 100 kDa was increased at 43°C (110). During in vitro encystation, several stage-specific proteins were recognized in immunoblots by antisera raised against antigens of the HSP60 family from *Mycobacterium bovis* and HSP70 from *Plasmodium falciparum* (152). The detection of HSP in encysting cells is interesting. *Giardia* trophozoites have developed a way of surviving for a certain period in the harsh environment of the host small intestine. However, the phenomenon of encystment may represent an escape mechanism for the trophozoites at the time when the immune system detects the presence of this invader attaching itself to the intestinal mucosal surface. At present, little is known about how and when the trophozoites turn on genes to build the cyst structure. Whether HSP plays a role in the phenomenon of encystation is unknown.

Lectins

Lectins are glycoproteins that bind to specific sugars and oligosaccharides and are linked to glycoproteins or glycolipids present on the cell surface of eukaryotes. Trophozoites of *G. duodenalis* have surface membrane lectins with specificity for D-glucosyl and D-mannose residues (61). Ward et al. (194) have identified and characterized taglin, a mannose-6-phosphate binding, trypsin-activated lectin from the trophozoite membrane. Activation of *G. duodenalis* lectin by proteases from the human duodenum has been reported (108). After activation, the lectin agglutinated intestinal cells to which the parasite adheres in vitro. The lectin was specific for mannose-6-phosphate and was bound to the plasma membrane of *Giardia* (108). A systematic analysis of *G. duodenalis* trophozoite surface carbohydrate residues with lectins and glucosidases of known sugar specificity has revealed that *N*-acetyl-D-glucosamine is the only detectable saccharide on the plasma membrane (192). The biological functions of lectins are unknown, but it appears that they play a role in the mechanisms of attachment of the trophozoites at the site of colonization (61). The role that lectins play in the immune response to *Giardia* is unknown. The immunobiology of the *N*-acetyl-D-galactosamine surface lectin of *Entamoeba histolytica* is well known (38). This lectin binds to mucin for colonization and prevents the trophozoites from making contact with the underlying surface of the epithelium (181). Taglin, a lectin present on the surface membrane of *Giardia*, does not bind to mucin. It is also unknown if taglin is able to transform the local lymphocytes into blast cells. In this case, it is unlikely that lectins are important in the immune response to giardiasis.

Giardins

Giardins are unique proteins of *Giardia* cells; to date, nothing in the literature indicates the presence of similar proteins in the cytoskeletons of other cell types. In contrast to surface membrane antigens of trophozoites, structural proteins of *G. duodenalis* appear to be highly conserved among isolates. For example, analysis of the amino acid sequence of a 33-kDa protein located in the ventral disk and axostyle revealed a single open reading frame of 813 bp (6). The giardins are defined as a family of ~30-kDa structural proteins found in microribbons attached to microtubules in the disk cytoskeleton of *Giardia* trophozoites (46). Using SDS-PAGE, Crossley and Holberton (47) characterized the proteins from the axonemes and disk cytoskeleton of *G. duodenalis* trophozoites. In addition to tubulin and the 30-kDa disk protein, at least 18 minor components copurify with the two major proteins in Triton-insoluble structures (47). The 30-kDa polypeptide accounts for about 20% of the organelle proteins on gels. In continuous 25 mM Tris-glycine buffer, this polypeptide migrates as a close-space doublet and was given the name of giardin. Peattie et al. (147) have studied the molecular aspects of giardins and have found giardins at the edges of disk microribbons of the trophozoite; they named these particular proteins α-giardins. In a subsequent study (141), more than one giardin was present at the edges of the disk. The giardins were renamed α1-giardin, α2-giardin, and γ-giardin. Sequence analysis comparison revealed that the genes coding for the α-giardins had 81% identity at the nucleotide level and 77% identity at the predicted amino acid level (141). The interest in giardins as primary antigens in the immune response to *Giardia* stems from the fact that they form a family of proteins unique to this parasite. They also represent a large proportion of the proteins found in the organelle of attachment (ventral disk) of the parasite to its host. They are surface antigens, and they are probably the first set of antigens detected by the local immune system after attachment of the parasite to the mucosal surfaces. No studies have been reported on the role played by giardins in immunity in giardiasis.

Tubulin

Tubulin determinants have been localized separately in the disk cytoskeleton and flagella (180). After tubules were fixed in formalin, α-tubulin was detected in the flagella, ventral disk, funis, and median body (45). However, unfixed tubules showed different antigenic structures. For instance, disk microtubules were not stained by antitubulin antibodies. Crossley and Holberton (47) have identified at least five isoelectric variants of *G. duodenalis* tubulin. These molecules may represent a primary target for the immune system since they are found in many organelles. The role they play in immunity has not been studied.

ANTIGENIC VARIATION

Antigenic variation represents a mechanism whereby selected viruses, bacteria, and parasites evade the immune response of the host. By the time the host has developed a protective immune response to the antigens originally present, the latter have been replaced in a few surviving organisms by new antigens. Antigenic variation affects the surface antigens of the infectious agents in which it occurs.

Antigenic Variation in Giardiasis

Nash et al. (137) were first to report the phenomenon of antigenic variation in giardiasis. Some characteristics of this phenomenon in giardiasis are as follows: (i) certain epitopes are reexpressed in clones, suggesting the presence of a favored set in the repertoire of epitopes; (ii) the repertoires of variant surface proteins (VSPs) may differ among isolates; and (iii) the same epitope detected on the surfaces of independent isolates is present in molecules with different molecular masses (134, 135, 138). In contrast to other parasites in which the phenomenon has been observed, antigenic variation in giardiasis was first observed as a phenomenon occurring in vitro. Most of the studies on antigenic variation were done with the WB isolate obtained from a symptomatic individual infected in Afghanistan. Clones of the WB isolate of *G. duodenalis* were exposed in vitro to a cytotoxic MAb which reacts with a 170-kDa surface antigen (137). Analysis of progeny and clones of the progeny by different assays failed to detect the high-cysteine 170-kDa antigen. In a subsequent study, it was demonstrated that the loss of this antigen was associated with the appearance of a new 64-kDa surface antigen (3). Specific variants have been detected after 12 generations of in vitro growth of the WB isolate (133). The abundant, highly variable VSPs which cover the surface of trophozoites have been confirmed (204), and these VSPs are capable of binding ^{65}Zn in vitro. The finding of a cysteine-rich protein(s) in *Giardia* trophozoites (3, 7) was not unexpected, since *Giardia* has a high nutritional requirement for cysteine (69). The gene VSPA6 coding for the 170-kDa surface antigen has been cloned (3). This gene consists of three regions: a short 5′ region containing a hydrophobic leader, a repeat region comprising 4,056 nucleotides and 20.8 repeats, and a 3′ region containing a region of homology to the other VSPA6 genes (2). Antigenic variation at the surface membrane of trophozoites occurs frequently in *Giardia* isolates. These antigens are made of cysteine-rich proteins (6, 33), which are controlled by 20 to 184 genes (133). In contrast to African trypanosomiasis, where genes controlling variant surface antigens are expressed in telomere-associated sites, the VSP genes controlling the VSPs in *Giardia* are not telomere associated (138).

Biological Significance

The importance of antigenic variation as a parameter in the immune response to *Giardia* was realized when the phenomenon was documented in vivo in humans, mice, and gerbils (10, 77). Gerbils were inoculated orally with live trophozoites of *G. duodenalis* clone WB Cl-6E7, which expresses a major 179-kDa surface membrane protein. By day 7 postinfection, this protein was no longer detected on the surface of trophozoites and had been replaced by a series of new antigens, including a major protein at 92 kDa (10). When immunocompetent BALB/c mice were infected with a cloned human isolate of *G. duodenalis*, trophozoites removed from the small intestine had lost a major surface epitope by day 22 postinfection (77). Gottstein and Nash hypothesized that B-cell-dependent mechanisms are most likely to be responsible for the surface antigen switch (77). In contrast, the trophozoites removed from the guts of infected athymic nude and *scid* mice still expressed the major surface membrane epitope at the same level on day 25 postinfection. Interestingly, the initial antigenic surface variant remained unchanged after encystment and subsequent excystments by infection in a new host (138). The facts that antigenic variation was not observed in athymic mice and the initial surface variant antigens remained unchanged after encystation indicate that the phenomenon of antigenic variation in giardiasis is driven by the immune system of the host.

The Variant Protein VSPH7

Neonatal ZU.ICR mice infected with trophozoites of *G. duodenalis* clone GS/M-83-H7 expressing the variant protein VSPH7 transiently produced milk immunoglobulin A (IgA) antibodies against a variant-specific 314-amino-acid N-terminal region of VSPH7. These IgA antibodies exhibit a strong parasiticidal effect on VSPH7-type trophozoites both *in vitro* and *in vivo*. Not only are they promoting antigenic variation in clone GS/M-83-H7, but also they influence the early course of the infection in mice (174). VSPH7 consists of two antigenically distinct fragments: a unique, variant-specific 314-amino-acid N-terminal region which elicits a low antibody response that is preferentially detectable during the early phase of infection, and a 171-amino-acid C-terminal region which elicits a high antibody response during the later phase or after resolution of infection (130). Again, these results provide a good example of the complexity of the immune response to *Giardia* antigens. A low antibody response was detected against a specific epitope during the early phase of the infection, while a higher antibody response was obtained against a different epitope in the late phase of the infection. The immunogenicity of VSPH7 in adult female ZU.ICR mice was studied after peroral immunization with a recombinant vaccine (173). For this purpose, the biocarrier *Salmonella enterica* serovar Typhimurium strain LT2M1C was used to deliver the VSPH7 antigens to the mucosal site. The vaccination induced VSPH7-specific IgG1, IgG2a, and IgG2b antibodies in the serum whereas IgA antibodies were detected from supernatants of in vitro-maintained intestinal-cell conglomerates. The authors concluded that the live attenuated serovar Typhimurium strain LT2M1C is an ideal antigen delivery system, since the specific systematic and local antibody responses were similar to those induced by experimental or natural infections of mice with *G. lamblia* clone GS/M-83-H7. Unfortunately, the authors did not determine if the mice immunized with the biocarrier serovar Typhimurium were protected against a challenge infection with *G. lamblia*.

Immune Response in Animal Models

The variety of humoral and cellular immune responses stimulated during the occurrence of antigenic variation has been studied by using the mouse and gerbil animal models of the disease. The predominant anti-*Giardia*-specific antibodies are of the IgM and IgG isotypes, whereas the $CD4^+$ T lymphocytes isolated from mouse Peyer's patches (PP) show a predominant proliferative response to the antigens (75). On the other hand, spleen and mesenteric lymph node (MLN) cells did not show any lymphoproliferative response and no specific anti-*Giardia* IgA antibodies were detected. These results show that in a natural infection the lymphoid cells responding to the antigenic stimulation are located along the intestinal mucosal surfaces. The variant surface antigens of *G. duodenalis* have been localized on the surface membrane of the trophozoites, and they are usually associated with the presence of a thick cell coat (149). The entire surface of the organism is usually covered by the thick surface coat containing the variant surface protein, but on some trophozoites the thick surface coat is absent (149). It is not known if the absence of a thick surface membrane is associated with an absence of antigenic variation.

EFFECTOR MECHANISMS OF THE IMMUNE RESPONSE

Our understanding of the mechanisms of the immune response in giardiasis comes from four sources: (i) in vitro studies involving the growth of axenically grown *G. duodenalis* trophozoites together with immune cells from a variety of hosts; (ii) studies of mice infected with their natural parasite, *G. muris*; (iii) animal models involving *G. duodenalis*-infected adult gerbils or weanling mice; and (iv) studies of humans naturally infected with *Giardia* or those who have volunteered to be infected with *Giardia* (62).

Human Innate Immunity

In some patients, giardiasis resolves within a few days, while in others the symptoms last for years, even in the presence of circulating antibodies in serum or secretory antibodies at mucosal sites and the cell-mediated immunity. Because of its biological characteristics, it is likely that nonimmune factors play a role in susceptibility to infection or in the duration and severity of the disease. For example, normal human milk kills *G. duodenalis* trophozoites independently of specific secretory IgA antibodies (68). A number of laboratories have demonstrated one giardiacidal factor present in milk, such as conjugated bile salts (70), unsaturated fatty acids (160), or free fatty acids (154). When grown in vitro in the presence of human milk, trophozoites can be protected from its giardiacidal effect by addition of intestinal mucus to the culture medium (203). *G. duodenalis* trophozoites are killed by products of lipolysis present in human duodenal and upper jejunal fluid (50). Aley et al. (11) have also reported that human neutrophil defensins and indolicidin have antitrophozoite activities when they are added to the culture medium. These results demonstrate the importance of nonimmune mechanisms in the control of the parasite population in the intestine. On the other hand, mechanisms of innate immunity may protect the parasite from destruction. For example, mucus has been reported to protect the trophozoites from being killed by lipolytic products present in the intestinal fluid (205).

Mechanisms of Acquired Immunity in Humans

Both humoral and cell-mediated immune responses have been reported to occur in human giardiasis (4). However, little is known about the mechanisms involved in this immune response because most of our knowledge is based on the mouse model of disease involving a rodent source of *Giardia* (*G. muris*). Also, studies of the immunological aspects of the host-parasite relationship with *G. duodenalis* types of organisms were done in vitro with culture media developed for growing lymphoid cells, not *Giardia* trophozoites (4, 62, 78). The culture of trophozoites under inappropriate conditions has also made the parasite more vulnerable to immunological attack. Because of this, the interpretation of many in vitro studies of the effector mechanisms implicated in the immune response to *G. duodenalis* trophozoites is problematic.

The lethal effect of human serum for *G. duodenalis* trophozoites appears to be dependent on the presence of an intact classical pathway of complement. Human sera containing anti-*G. duodenalis* antibodies killed more than 98% of the parasites in vitro (84). The killing effect of human sera was abrogated when the sera were chelated with EDTA or heat inactivated at 56°C for 30 min, conditions known to inactivate complement. These results were confirmed in another study, where sera, obtained from infected humans, containing anti-*G. duodenalis* trophozoite antibodies of the IgM class and complement lysed the trophozoites (51); these authors concluded that the activation of the classical pathway of complement produced the lysis. Since *Giardia* trophozoites reside in the lumen of the intestine, it is unlikely that the above mechanisms play a role in controlling parasite numbers within the intestine. However, lysis of trophozoites by specific antibodies in the presence of complement may play a role in limiting the invasion of tissues by trophozoites. The humoral arm of the immune system has been reported to play a role in infected patients. For example, the jejunal-plasma immune response to *Giardia* involves a decrease in the number of IgA cells and an increase in the number of IgM cells (104).

The functional importance of mucosal-associated lymphoid tissue is indicated by its large population of antibody-producing plasma cells that are secreting primarily IgA antibodies. However, cell-mediated immunity also plays an important role at the mucosal sites. Lymphocytes are found in large numbers in the lamina propria, in PP, and within the epithelial layer. Many of these cells are T cells of different phenotypes. Since *Giardia* antigens are T-cell-dependent antigens, the role played by cell-mediated immunity at mucosal sites has been studied. Due to the invasive techniques required for harvesting cells at the mucosal sites, studies of cell-mediated immunity studies in human giardiasis have been done with lymphocytes circulating in the blood. Specific cellular immune responses to *G. duodenalis* antigens have been reported. A lymphocyte proliferative response was obtained by stimulating human peripheral blood leukocytes with antigens obtained from homologous or heterologous isolates (76). As predicted, the higher stimulation indices were obtained with the homologous parasite antigens. Experiments designed to study the role played by human mononuclear cells as effector mechanisms against *Giardia* have produced contradictory results. Aggarwal and Nash (9) determined the cytotoxicity of mononuclear cells to *Giardia* by using a thymidine assay and found that *G. duodenalis* trophozoites died spontaneously without the presence of mononuclear cells and, surprisingly, that the presence of mononuclear cells increased the ability of the parasite to survive. On the other hand, Hill and Pearson (87) reported the opposite results. They found that incubation of *Giardia* cells with mononuclear cells and the addition of 20% immune serum increased the ingestion of parasites eightfold, indicating that opsonization exists in giardiasis. Killing of trophozoites was attributed to the oxidative microbicidal activity of phagocytes. Human neutrophils and monocytes are able to interfere with the in vitro attachment of *Giardia* trophozoites to the sides of culture tubes, demonstrating that the adherence mechanism of the parasites may be a feasible target for immunological attack (48). When trophozoites encyst, they lose their property to attach to substrate (64). Since encystation coincides with the immune system expulsion, one can speculate about whether neutrophils and/or other effector mechanisms of the local immune response play a role in the phenomenon of encystation.

MOUSE MODEL

The *G. muris*-mouse model of giardiasis, described by Roberts-Thomson et al. (157) in the mid-1970s, has provided a powerful tool to study the immune effector mechanisms that occur during *Giardia* infection. The selection of the mouse over other animal models for the study of immune mechanisms in giardiasis has considerable advantages: (i) adult mice are being infected with their natural parasites; (ii) a considerable variety of reagents and technologies exists for the study of the immune response in mice; and (iii) immunologically well-defined inbred strains of mice are available. The mouse model of

giardiasis has been useful for the understanding of not only the immune mechanisms of giardiasis but also the immunological phenomena at mucosal intestinal sites. The natural habitat of *G. muris* trophozoites is the mouse small intestine, where it resides in the lumen or attached to the epithelium. This protozoan lives extracellularly and, like *G. duodenalis*, does not invade host cells or tissues.

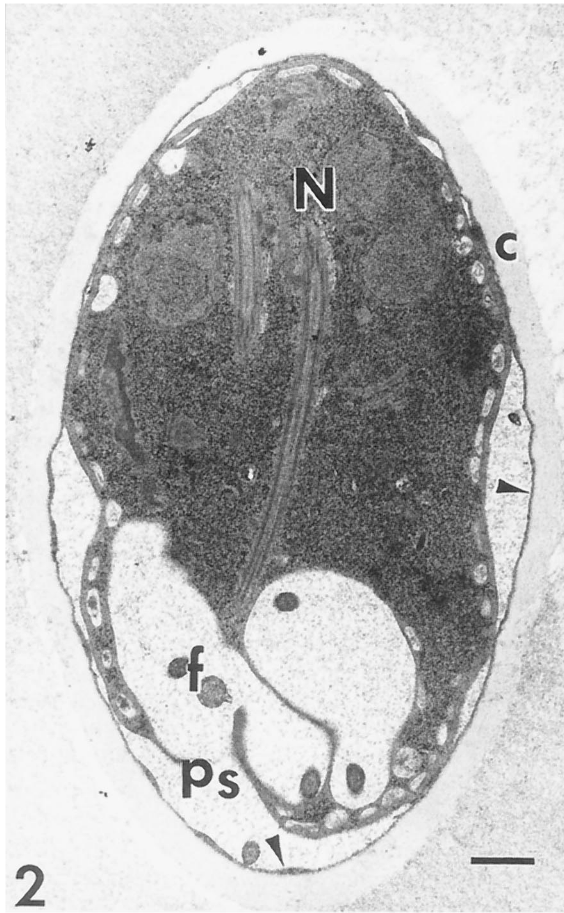
Immune Response in Susceptible and Resistant Mice

The first evidence of the involvement of the immune system in the elimination of *Giardia* in primary infection was reported by Roberts-Thomson et al. (157), who showed that athymic nude mice develop a prolonged *Giardia* infection. Reconstitution of these mice with lymphocytes restored a normal pattern of elimination of the parasite at 7 weeks. Among many mouse strains, some mice have been identified as being particularly susceptible to *Giardia* infection, developing a prolonged elimination phase or even persistent infection (27). For instance, in contrast to the resistant B10.A and DBA/2 mice, the infection in susceptible A/J and C3H/He mice is characterized by a short latent period, a high cyst output during the acute phase of infection, and a relatively long period of resolution of infection. The immunological basis for prolonged or chronic infection in susceptible mouse strains has not yet been elucidated. It has been reported that susceptible C3H/He mice recognize different antigen recognition patterns from resistant BALB/c mice (60). For example, a crude trophozoite antigenic extract bound to wheat germ agglutinin used to vaccinate BALB/c mice failed to induce protection (60). On the other hand, no differences were observed in the giardiacidal activity of spleen, MLN, and peritoneal lymphoid cells from susceptible or resistant mice (17) and no apparent relationships were found between this capacity to mount cell-mediated or humoral effector immune responses and their ability to control the infection (25). These observations highlight the complexity of the immunological aspect of the host-parasite relationship. Noninfected and infected resistant mice have a greater capacity to recruit cells into the peritoneal cavity after thioglycolate injection than do compared to susceptible mice (18, 165). The quantitative differences observed in the inflammatory responses in resistant infected mice were related to functional differences in phagocytosis and a greater capacity to respond to chemotaxis in vitro (18). The involvement of immune system mechanisms to explain prolonged infection became puzzling when it was found that susceptible adult female C3H/He mice could protect their suckling young and develop higher antibody responses than resistant adult female BALB/c mice (182). Moreover, following treatment with metronidazole to eliminate the trophozoites from the intestine, susceptible C3H/He mice became resistant to challenge infection (182). Recently, Venkatesan et al. (184) reported no differences in the timing, titer, or specificity of secretory or serum antibodies to *G. muris* between susceptible and resistant strains of mice. However, when serum IgG subclass responses were compared, the resistant strain produced IgG2a while the susceptible strain produced IgG1. According to these authors, these results suggest differential involvement of T-helper (Th) 1 and Th 2 subsets of lymphocytes (184). When cells harvested from MLN were stimulated with concanavalin A, gamma interferon (IFN-γ) and interleukin-5 (IL-5) were secreted by cells from the resistant strain but only IL-5 was secreted by cells from the susceptible strain (184). The lack of secretion of IFN-γ by MLN cells from the susceptible strain is interesting because it may explain why this intestinal parasite is particularly susceptible in these mice. IFN-γ is recognized as playing a role not only in the proliferation of B cells but also in the switch from one Ig to another. Furthermore, if hypersensitivity reactions are playing a role in the control of the infection at the gut level, the nonsecretion of IFN-γ by MLN cells would affect this mechanism of defense.

Humoral Effector Mechanisms in Animals

The expulsion of *G. muris* from the small intestines of infected mice is closely associated with the appearance of anti-*G. muris* IgA antibody in intestinal secretions (169). Parasite-specific IgA and IgG antibodies bind to *G. muris* trophozoites colonizing the small intestine (83). The percentage of trophozoites with adherent neutrophils increases in the presence of anti-*Giardia*-specific IgG serum antibodies or immune mouse milk or secretory IgA antibodies (99). Phagocytosis of trophozoites by macrophages increases after incubation with immune serum (17, 98, 99, 150) or immune mouse milk (99). On the other hand, bone marrow-derived macrophages from C3H/HeN mice pretreated with recombinant IFN-γ ingest significantly larger numbers of *G. duodenalis* trophozoites than do untreated macrophages (23). The classical pathway of complement can be activated by immune complexes containing IgM or IgG antibodies, and it appears that anti-*Giardia*-specific antibodies of the IgM or IgG isotypes support the lytic effect of complement on *Giardia* cells. Deguchi et al. (51) have reported that *G. duodenalis* trophozoites sensitized with anti-*Giardia* antibodies of the IgM class are lysed. Butscher and Faubert (36) obtained similar results with *G. muris* trophozoites sensitized to similar antibody isotypes. Moreover, an IgG1 MAb was found to bind *in vitro* to the surface of trophozoites, flagella, and flagellar insertions (36). This MAb was able to lyse *G. muris* trophozoites in the presence of exogenous complement, and when administered directly into the duodenum of mice, it significantly reduced the number of trophozoites during the acute phase of the infection (24). The main target for this MAb was a 35-kDa Triton-soluble glycoprotein located on the surface membrane of the trophozoite (24, 36). Finally, the role of complement in lysing *Giardia* cells was also demonstrated with a MAb which recognized proteinaceous cyst antigens and was able to abolish the formation of the cyst when added to the culture medium together with a source of complement (37). All these studies show that anti-*Giardia* antibodies in the presence of an exogenous source of complement can effectively lyse trophozoites and encysting cells in vitro. Unfortunately, the complement proteins are absent in the lumen of the intestine. The only source of complement near the intestinal lumen would come from the few macrophages present in the deep invagination of the M cells which are located in the mucous membrane.

Although the role played by T and B lymphocytes in the control of the infection is well documented, there is only one study reported in the literature on the cytokines produced by $CD4^+$ T cells in response to *Giardia* antigenic stimulation. When *Giardia* trophozoite proteins were used to challenge PP and spleen cells removed from infected mice, IL-4, IL-5, and IFN-γ were not detected in the culture supernatant (52). However, when the cells were challenged with concanavalin A, all three cytokines were detected. The release of IL-4 and IL-5 by the spleen and PP cells in the culture supernatant confirms the role played by antibodies of the IgA isotype in the control of giardiasis. Two conclusions can be drawn from these experiments. First, it appears that *Giardia* proteins are poor immunogens since they were not able to stimulate lymphoid cells adequately for the production of lymphokines. A weak lymphocyte proliferation was observed when a *G. muris* crude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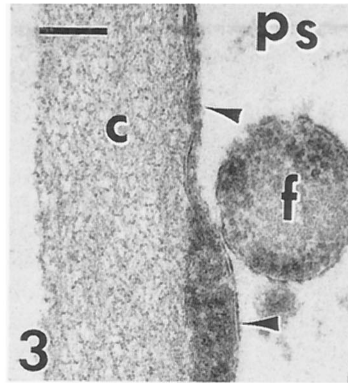
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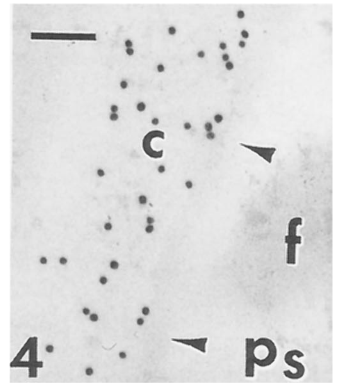
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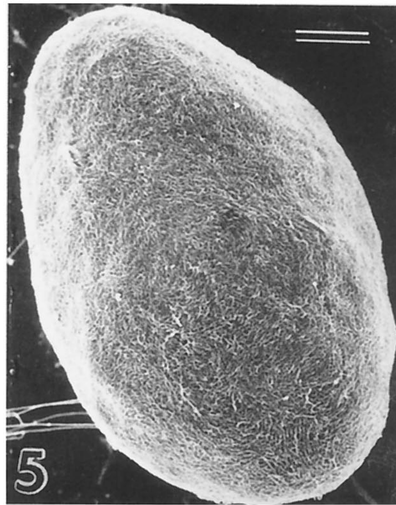
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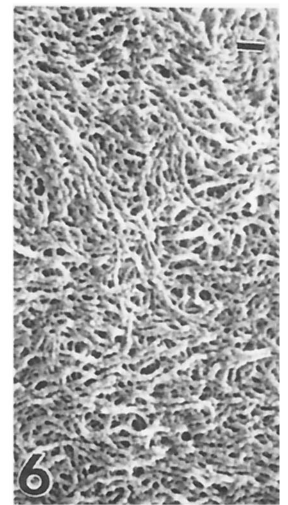
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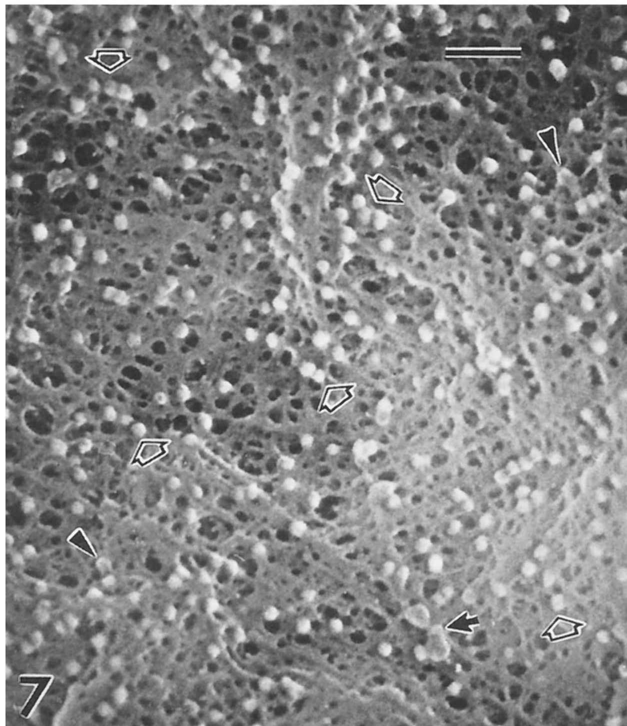
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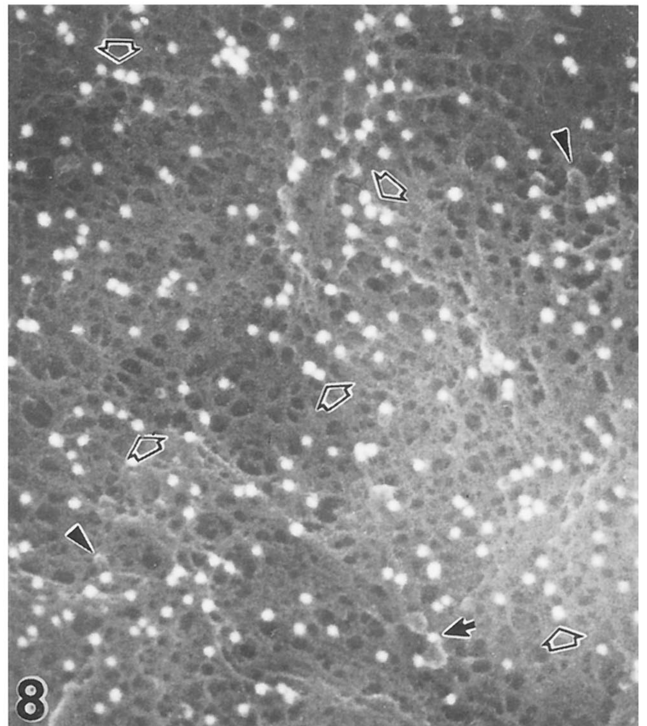
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extract from trophozoites was used to stimulate PP cells from noninfected mice in vitro (86). Second, the relative success of *G. muris* in completing its life cycle in a primary infection might be due, in part, to poor stimulation of Th1 and Th2 immune responses. The Th1-type immune response is virtually absent in the primary infection. In vitro studies have shown the central role played by macrophages and IFN-γ in the killing of trophozoites (23).

Usefulness of Specific Antibodies in Studies on Encystation

The process of encystation is a key step in the *Giardia* life cycle that allows this intestinal protozoan to survive between hosts during person-to-person, waterborne, or food-borne transmission. To my knowledge, the existence of serum or local antibodies at the gut level against cyst antigens in infected patients has never been reported. The absence of antibodies against novel molecules appearing on the surface membrane of the encysting trophozoite is not surprising. Encystation is a complex phenomenon occurring over a short period and is probably not detected by the local immune system. In spite of the apparent absence of antibodies against encysting molecules in a natural infection, I believe that studying the immunogenicity of the latter is important since they offer immunological strategies for stopping, or at least decreasing, the spread of the infection in the environment.

Our knowledge of the formation of the cyst structure was limited until polyclonal antibodies and MAbs specific to cyst molecules were developed and used in studies of cyst wall formation. Using immunofluorescence and immunogold staining, Erlandsen et al. (57) studied the chronological events taking place during encystment. The phenomenon begins with the formation of an intracellular and extracellular phase, which requires a minimum of 14 h. The extracellular phase is initiated with the appearance of cyst wall antigens on small protusions of the trophozoite membrane, which enlarge to form "caplike structures" with progression to formation of the cyst wall. Caplike structures are detected over the entire surface of the trophozoites, including the adherence disk and flagella (57, 59). Late stages in encystment include a "tailed" cyst, in which some of the flagella are not fully retracted into the cyst. After encystation is completed, the cyst wall is composed of filamentous and membranous portions and is separated from the cytoplasm of the trophozoite by the peritrophic space (Fig. 2 through 8). These observations confirm the findings of earlier investigators (37, 64, 71, 118, 151, 192). Using monospecific antibodies to a VSP antigen (TSA 417), which is a type 1 integral membrane protein that covers the entire surface of the trophozoite, and a MAb against a cyst wall protein (8C5), McCaffery et al. (118) observed the transport of the epitopes that bind to these two specific antibodies during encystation. In preencysting cells, both proteins are localized on the nuclear-envelope endoplasmic reticulum cisternae, and cytoplasmic membrane cisternae, thereby reflecting their site of synthesis. However, only epitope 8C5 is localized on the encystation-specific vesicles (ESV). The ESV are the equivalent of caplike structures described by Erlandsen et al. (57). These large secretory vesicles form only during encystation, and they transport cyst antigens (Fig. 9) to the nascent wall (118). In contrast, only TSA 417 was found on the outer surface of the plasmalemma of trophozoites, encysting cells, and underlying the walls of many cysts (Fig. 9 and 10). As encystation progresses (Fig. 10), TSA 417 disappears from the plasmalemma and its level in the lysosome-like peripheral vesicles and other large cytoplasmic vesicles is increased (118).

Preexposure of cysts to polyclonal rabbit antiserum against purified cyst wall proteins or to wheat germ agglutinin inhibits excystation by more than 90% (119). The investigators concluded that the ligand binding cyst wall epitopes inhibit encystation, most probably by interfering with the proteolysis of cyst wall glycoproteins.

Cell-Mediated Effector Mechanisms in Animals

Many of the cellular events of the intestinal mucosal site in response to parasite antigens are under the complex regulation of T cells. Heyworth et al. (79) found that most of the cells harvested from the intestinal lumen of mice infected with *G. muris* were lymphocytes mixed with a small number of macrophages. When the cells were identified by immunofluorescent staining, approximately 50% of the intraluminal leukocytes were shown to be T lymphocytes. The kinetics of intraepithelial lymphocyte (IEL) and lamina propria lymphocyte (LPL) response during *G. duodenalis* infection in weanling mice have been studied. An increase in the numbers of suppressor and $CD8^+$ T cells in the IEL and LPL tissues was observed during the latent period; the numbers peaked during the acute phase and decreased during the elimination phase. In contrast, the number of $CD4^+$-T-cell subsets remains small during the first two phases of the infection and increases significantly during the elimination phase (189). Meanwhile, the number of IgA-plasma cells in the lamina propria declined during the latent and acute phases of infection and increased during the elimination phase (189). Investigators concluded that induction of

FIG. 2. Transmission electron micrograph of a *G. muris* cyst fixed in the presence of 1% bovine serum albumin. The cyst wall is composed of filamentous (c) and membranous (arrowheads) portions and is separated from the cytoplasm of the trophozoite by the peritrophic space (ps). N, nucleus; f, flagellum. Original magnification, ×16,000. Bar, 0.5 μm. Reprinted from reference 59 with permission of the publisher.

FIG. 3. Transmission electron micrograph of the filamentous (c) *Giardia* cyst wall showing the course of individual filaments. The membranous portion of the cyst wall (arrowheads) separates the filamentous portion from the underlying peritrophic space (ps). f, flagellum. Original magnification, ×80,000. Bar, 0.1 μm. Reprinted from reference 59 with permission of the publisher.

FIG. 4. Thin section of *G. muris* cyst wall, comparable morphologically to that in Fig. 3 but immunostained with rabbit polyclonal antiserum (R-AGLMB) and goat anti-rabbit IgG labeled with 15-nm colloidal gold. Specific staining with immunogold is detected over the filamentous (c) portion of the cyst wall, and no labeling is seen on the membranous portion of the cyst wall (arrowheads) or in the peritrophic space (ps). f, flagellum. Original magnification, ×80,000. Bar, 0.1 μm. Reprinted from reference 59 with permission of the publisher.

FIG. 5. Low-voltage field emission SEM of a *G. muris* cyst taken at 1.5 kV, illustrating the filamentous nature of the cyst wall. Original magnification, ×9,500. Bar, 1 μm. Reprinted from reference 59 with permission of the publisher.

FIG. 6. Higher magnification of the filamentous cyst wall of the *G. muris* cyst seen in Fig. 5. Individual filament populations, ranging from 7 to 20 nm, are easily discerned and appear to form a tightly interwoven mesh. Original magnification, ×39,500. Bar, 0.1 μm. Reprinted from reference 59 with permission of the publisher.

FIG. 7 and 8. SEI (Fig. 7) and BEI (Fig. 8) of the filamentous cyst wall of *G. muris* immunocytochemically labeled with rabbit antiserum to the *Giardia* cyst wall (R-AGLMB) and goat anti-rabbit IgG coupled to 15-nm colloidal gold. A comparison of the SEI and BEI taken at 10 kV by FESEM reveals the one-to-one correspondence (open arrows) between the 15-nm immunogold complexes associated with the filamentous cyst wall as seen by surface topography (Fig. 7) or by atomic-number contrast (Fig. 8). In some instances, 15-nm immunogold particles were not obvious with SEI (small solid arrow) but were easily detected by BEI, as (Fig. 8). Other particles detected by SEI (arrowheads) were shown not to be immunogold by BEI. Original magnification, ×80,000. Bar, 0.05 μm. Reprinted from reference 59 with permission of the publisher.

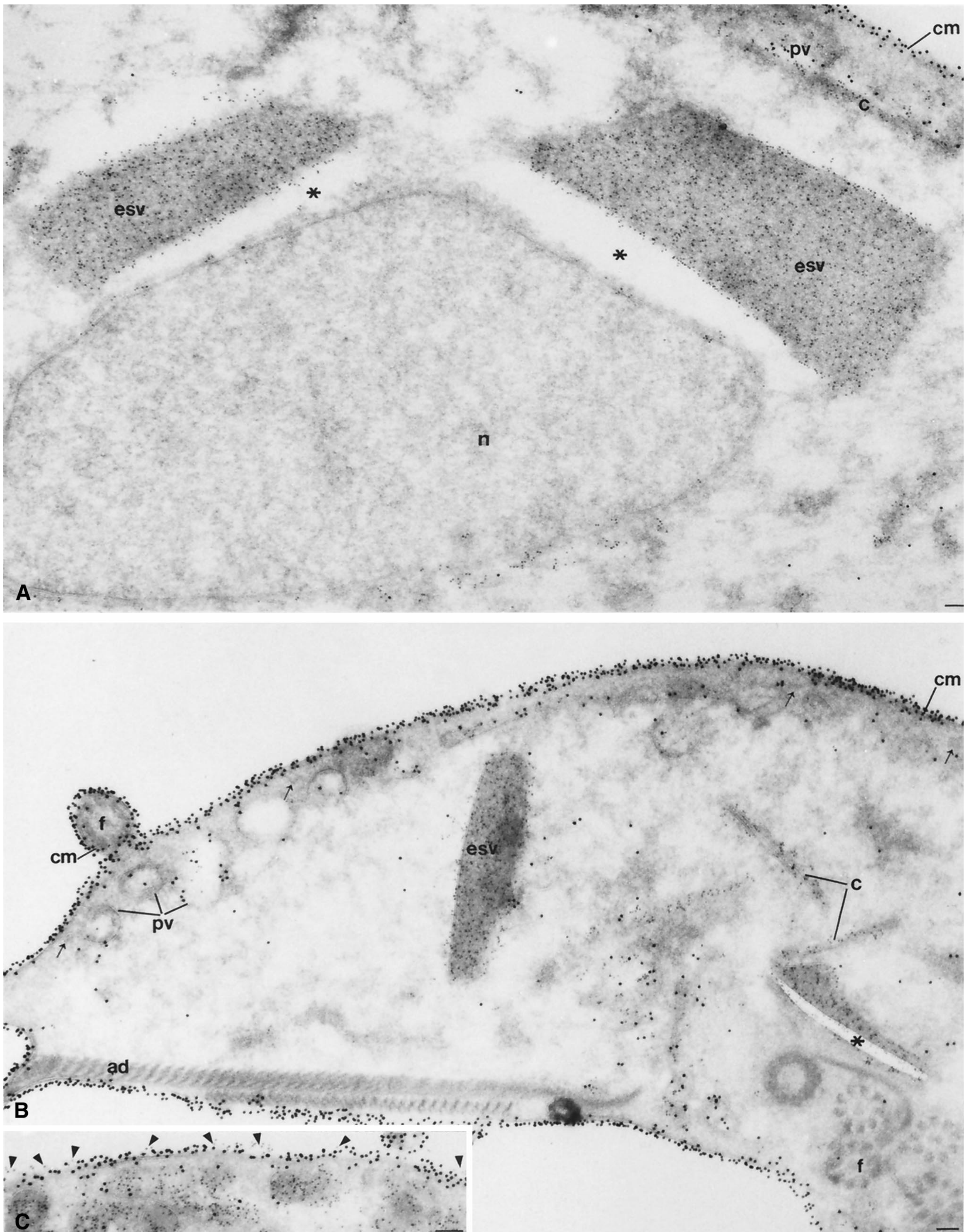


FIG. 9. (A to C) Ultrathin cryosections of 15-h encysting cells, doubly immunolabeled with 8C5 (5-nm Au) and TSA 417 (10-nm Au). (A) 8C5 is localized to large encystation specific vesicles (esv), membrane cisternae (c), nuclear envelope, and peripheral vacuoles (pv) and is absent from the cell surface. TSA 417 is found in cisternae, peripheral vacuoles, and cell membrane (cm). The displacement of the ESV from the perinuclear cisternae is probably due to a sectioning artifact. (B and C) At 15 h, TSA 417 still dominates the cell surface and is also present in the peripheral vacuoles and cisternae. 8C5 is concentrated in the ESV, peripheral vacuoles, and cisternae and is just beginning to appear on the cell surface (arrowheads). Note that the external flagellum (f) cell is covered with membrane and TSA 417. Bars, 0.1 μm. Reprinted from reference 118 with permission of the publisher.

CD4^{+} T cells during the elimination phase concomitant with an increase in the number of lamina propria IgA-plasma cells results in the elimination of the parasite from the gut. Villus atrophy and crypt hyperplasia were observed in the duodenum of gerbils infected with *G. duodenalis* trophozoites (22) and mice infected with *G. muris* (30). Crypt mitotic rates have been reported to double during the acute phase of *Giardia* infections in mice (115). It has been hypothesized that T lymphocytes directly or indirectly control the cycling time of crypt stem cells as well as the factors that orchestrate their differentiation along different lines (14). Again, these observations reinforce the role of cell-mediated immunity in the immune response in gerbils.

PP T- and B-cell subset populations have been studied in susceptible BALB/c mice infected with *G. muris*. In this peripheral lymphoid organ, the number of leukocytes doubled during the course of the infection but returned to control levels as the infection was eliminated from the intestine (40). The CD4^{+} and T-suppressor subsets represent 34.1 and 6.2%, respectively, of the total population in PP in noninfected mice; these percentages did not change after the infection with *G. muris*. On the other hand, the number of PP secretory IgM (sIgM) B cells increases rapidly in infected BALB/c mice to reach a maximum at the end of the latent period, whereas the number of sIgA B cells increases later to reach a maximum during the acute phase (41). The switching from the IgM to the IgA isotype confirms the importance of the Th2 subset and mast cells in the self-cure phenomenon. Both types of cells are recognized to secrete IL-5, which promotes the switching to the IgA isotype.

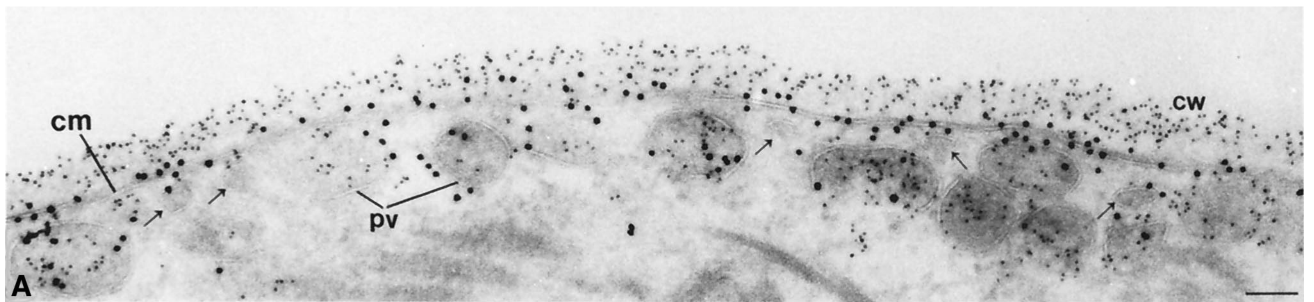
The role played by macrophages in the immune response to *Giardia* is well documented. In the mouse model of the disease, invading *G. muris* trophozoites were found in the epithelium near dying or desquamating columnar cells (146). Macrophages beneath the basal lamina extended pseudopods into the epithelium, trapping invading *G. muris* trophozoites and enclosing them in phagolysosomes. Macrophages containing digested trophozoites were surrounded by rosettes of lymphoblasts in the epithelium (146). On the other hand, in nude mice there was apparent hyperplasia of macrophages, which filled the follicle domes and resulted in more frequent entrapment of *G. muris*, but no contact occurred between the macrophages and lymphoblasts in the epithelium (146). Murine mononuclear cells isolated from collagenase-treated PP by adherence to glass ingested a significantly larger number of *G. duodenalis* trophozoites when incubated with immune mouse serum than nonstimulated cells did (85). Similar results were obtained by Belosevic and Faubert (17), who reported that macrophages isolated from the peritoneal cavities of susceptible A/J or resistant B10.A mice ingested a significantly larger number of *G. muris* trophozoites when incubated with immune mouse serum. Interestingly, no differences were found in the capacity of A/J and B10.A mice to mount a cell-mediated immune response, but their efficacy in eliminating the infection was different (17). It appears that the association of *Giardia* with macrophages elicits mainly an oxidative response (85). The capacity of mice infected with *Giardia* to mount an inflammatory response was studied in vitro and in vivo. The B10.A mice exhibited a greater capacity to recruit cells into the peritoneal cavity than did the A/J mice (18). The recruitment of inflammatory cells by both strains of mice was higher during the acute and elimination phases of infection. In vitro, the macrophages from the B10.A mice were more phagocytically active and were more chemotactically responsive than those of A/J mice during the acute and elimination phases of the infection (18). The role that macrophages play in acquired immunity has not been determined with unanimity. The trophozoites inhabit the lumen of the intestine, and the macrophages located in the pocket of the M cells are not recognized to migrate into the lumen of the intestine. Nevertheless, studies done in vitro have shown the killing capacity of these cells. In vivo they could play a dual role: first, a role as a guardian in case the trophozoites invade the mucosa, and second, an indirect role by secreting IL-5.

Acquired Resistance in Animals

In the mouse model, acquired resistance was observed when CF-1 Swiss mice were partially protected against challenge with 1,000 *G. muris* cysts 6, 12, and 18 weeks after the primary infection (158). Similar results were reported by Brett and Cox (30) with CBA mice. Underdown et al. (182) showed that BALB/c and C3H/He mice, drug cured at 5 and 10 weeks after primary infection, were completely protected against a challenge of 1,000 cysts. Belosevic and Faubert (26) did a temporal study of acquired resistance in CD-1 and inbred mice infected with *G. muris*. In the first set of experiments, these investigators terminated the first infection by treating the infected mice with metronidazole on day 3, 6, 12, 24, or 48. In the second set of experiments, the first infection was allowed to last 30, 60, 90, 120, or 150 days. In each case, the mice were challenged 10 days later with 1,000 cysts. In all cases, a significant reduction in both cyst and trophozoite numbers in the small intestine was obtained. The acquired resistance in inbred strains was similar to that in the outbred Swiss mice. These results show that mice can acquire significant resistance to *G. muris* even after a 3-day period of contact with the parasite and that the resistance may last up to 150 days.

Like many humans, most gerbils infected with *G. duodenalis* cysts or trophozoites undergo the self-cure phenomenon. Usually, no cysts can be detected in the feces after 40 days postinfection. The absence of cysts in stool after this period does not necessarily means that the trophozoites have been eliminated from the small intestine. It is possible that the trophozoites are present in small numbers; therefore, the number of cells encysting will also be small, not allowing their detection even after concentration procedures have been used to increase the sensitivity of detection by routine diagnostic methods. If this is the case, the self-cure phenomenon in giardiasis may not represent a state of sterile immunity in the infected host. The hypothesis of nonsterile immunity in giardiasis has been tested in the laboratory. Gerbils were treated with hydrocortisone acetate on day 50 or 70 or at 7 months postinfection. A recrudescence of the infection as evidenced by passage of cysts in stool was observed in the treated gerbils (109). These results confirm the hypothesis. The injection of hydrocortisone provoked an immunosuppression in the gerbils, as evidenced by a significantly reduced number of plaque-forming cells in response to sheep erythrocytes (SRBC) (109). The opportunistic *Giardia* took advantage of the weakness of the immune system of its host and began to multiply again.

Immunity acquired by animals experimentally infected in the laboratory and challenged with the same isolate appears to be of long duration. Mongolian gerbils infected with 1,000 *G. duodenalis* trophozoites of the WB strain were protected against reinfection for up to 8 months after primary infection (20, 26, 109). To date, there is no report in the literature on the level of resistance of humans to a secondary infection with *Giardia*. Nevertheless, protective immunity is suggested by the self-limiting nature of most infections and by the lower prevalence of giardiasis in adults in areas where the disease is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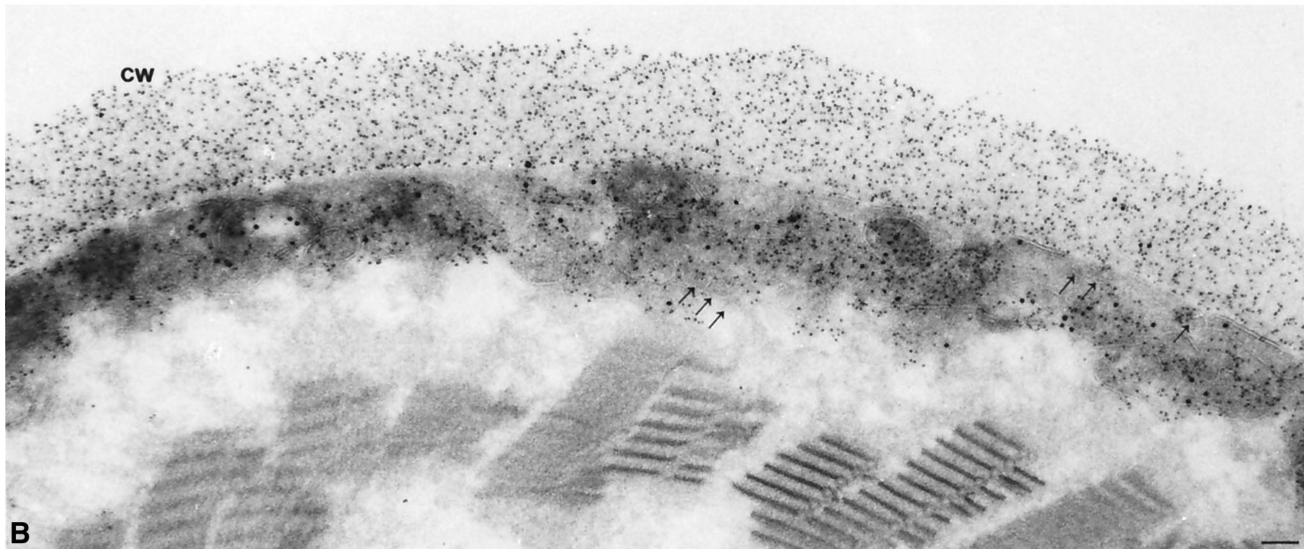
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endemic compared with symptomatic infections in travelers to the same areas, who are newly exposed (13).

Passive Transfer of Immunity

Transfer of immune serum containing IgG and IgA antibodies against *G. muris* from BALB/c mice to syngeneic recipients prior to inoculation with cysts of *G. muris* does not confer protection against infection in the recipient mice. Underdown et al. (182) and Erlich et al. (60) reported failure to transfer resistance to *G. muris* following repeated injections of a relatively large volume of immune serum (1.5 ml/mouse/week). On the other hand, antibodies directed against *G. muris* trophozoites have been used as therapeutic agents during ongoing infections in mice. When the MAb was administered directly into the duodenum of the infected mice, the number of trophozoites in the small intestine was reduced during the late-latent and acute phases of the infection (24, 36). In vitro the activity of the IgG1 MAb was directed against the flagella and the surface membrane of the trophozoite. The transfer of spleen cells from inbred NMRI mice infected with *G. duodenalis* to syngeneic recipients prior to infection resulted in a significant decrease in both the numbers of cysts released and the numbers of trophozoites in the small intestine (190).

Immunosuppression in Infected Mice

Protozoan and metazoan parasites have the ability to depress the immune response of their host to heterologous antigens (49, 63, 179). *Giardia* trophozoites have been associated with immunodepression in response to heterologous antigens. Brett (31) was the first investigator to report that *G. muris* infection in mice is accompanied by a depression in the ability of the mice to mount an immune response to the thymus-dependent antigen of SRBC but not to the thymus-independent antigen trinitrophenyl lipopolysaccharide. The number of IgM and IgG plaque-forming cells and the hemagglutination titer of both IgM and IgG decreased during the acute phase of the infection. Interestingly, peritoneal exudate macrophages from infected mice were slightly less cytostatic against tumor cells at the time of the elimination phase (19, 31). Belosevic et al. (16) reported that spleen and MLN cells isolated from mice during the acute phase of the infection were less responsive to SRBC. The immunodepression was detected earlier and was more pronounced in MLN cell cultures than in spleen cell cultures. The suppressor activity was localized in the population of cells adhering to plastic. When the kinetics of anti-SRBC response in *G. muris*-infected A/J and B10.A mice were studied, differences in the response were observed. The A/J mice were significantly less responsive to SRBC antigens than were the B10.A mice, and the differences were not due to suppressor T-cell activity, since both strains had a similar ability to generate this T-cell subset (16). Administration of a soluble extract of *G. muris* trophozoites to uninfected mice also resulted in a depressed response to SRBC in both strains of mice. The authors hypothesized that since *G. muris* causes a gastrointestinal infection, the lower capacity of the MLN cells to respond to SRBC may serve as an explanation for the survival of the trophozoites in the primary infection (16). Moreover, the fact that the suppressor activity was found among the macrophage population may be indicative of the role played by macrophages in the control of the primary infection.

IMMUNOCOMPROMISED HOSTS

Humans

There are few reports in the literature regarding giardiasis in immunocompromised hosts. Studies have shown that the prevalence of *Giardia* cysts in the stools of hypogammaglobulinemic patients is significantly higher than that in immunocompetent hosts (12, 32, 107, 164, 195). Ament and Rubin (12) found that approximately 90% of the hypogammaglobulinemic patients passing *Giardia* cysts were symptomatic (with chronic diarrhea). Perlmutter et al. (148) have reported that when giardiasis is present in hypogammaglobulinemic children, it is always symptomatic. Symptomatic giardiasis has been observed in X-linked infantile congenital hypogammaglobulinemia (Bruton's syndrome) and also in the common variable (late-onset) acquired hypogammaglobulinemia (28). In the former congenital defect, the syndrome represents a pure B-cell deficiency characterized by low levels of all Igs and normal T-cell function, whereas in acquired hypogammaglobulinemia, only the IgG and IgA levels are decreased but a T-cell dysfunction may also occur. It is also important to underline that some of these hypogammaglobulinemic patients also have severe IgM deficiency (195). No significant differences were reported between the two types of hypogammaglobulinemia. These observations in immunocompromised patients confirm that the development of symptomatic giardiasis cannot be associated with a particular arm of the immune system. In fact, there are contradictory observations about the possible association of depressed secretory IgA and *Giardia* infection. Zinneman and Kaplan (205) reported that hypogammaglobulinemic patients with giardiasis had a decreased number of secretory IgA anti-*Giardia*-specific antibodies and that their infection was mild. In malnourished patients, an enhancement of giardiasis was reported (42). Serum antibody response in malnutrition is often normal, but the level of secretory IgA antibody on mucosal surfaces is reduced (42). Since it has been demonstrated that secretory IgA plays a role in immunity to the infection, this may affect the elimination of the parasite from the gut. On the other hand, Jones and Brown (95) failed to find any differences in secretory or serum-specific IgA antibody levels between hypogammaglobulinemic patients with giardiasis and a control group. Children with a severe T-cell deficiency due to thymic aplasia (Di George syndrome) or purine nucleoside phosphorylase deficiency are not more susceptible to giardiasis, and their morbidity is comparable to that in immunocompetent children (195). AIDS patients with a low $CD4^+$-T-cell count do not have persistent or severe diarrheal episodes (93). These results are surprising, since in the mouse model of the disease, the $CD4^+$ T cells and other T-cell subsets play a role in the elimination of the parasite from the small intestine (159, 175, 189). Using an enzyme-linked immunosorbent assay to detect IgM, IgG, and IgA specific to *G. duodenalis* trophozoites, Janoff et al. (92) tested sera obtained from 29 patients with

FIG. 10. Ultrathin cryosections of 24-, 48-, and 66-h cysts, doubly labeled for 8C5 (5-nm Au) and TSA 417 (10-nm Au). (A) In many 24-h encysting cells, 8C5 is localized to the cyst wall (cw), which has been deposited over the cell membrane (cm), which is decorated with TSA 417. 8C5 and TSA are both found in peripheral vacuoles (pv). The amount of TSA 417 on the cell membrane seems somewhat reduced. (B) In 48-h cysts, the cyst wall (cw) containing 8C5 has markedly increased in thickness and TSA 417 is completely absent from the cell membrane (cm). Note the small transport vesicles (small arrows). (C and D) In 66-h water-resistant cysts, TSA 417 is localized exclusively to the peripheral vacuoles and large internal vesicles and vacuoles, resembling the endosomal and prelysosomal compartments of higher eukaryotes. 8C5 is also present in many of these vesicles. Bars, 0.1 μm. Reprinted from reference 118 with permission of the publisher.

TABLE 1. Sensitivity of immunoassays used for the detection of specific antibodies to *Giardia* proteins in serum and human milk and for the detection of antigens of *Giardia* in feces

Antibody or antigen[a] and assay	Antigen or antiserum[a]	No. of positive tests[b]/total no. of samples (% positive)					Reference(s)
		IgM antibody	IgG antibody	IgA antibody	Ig[c] antibody	Feces antigen	
Serum antibodies							
ELISA	Trophozoite extract	75/128 (59)	92/128 (71)	86/110 (78)	15/43 (35)		15, 89, 90, 91, 117, 136
	Trophozoite cells		48/59 (81)				168
IFA[d]	Trophozoite cells		240/352 (68)	240/352 (68)	147/186 (79)		1, 112, 176, 191, 198
	Cyst cells	32/36 (89)	32/36 (89)	32/36 (89)	150/150 (100)		96, 156
Western blot	Trophozoite extract	47/60 (78)	57/60 (95)	39/60 (65)			15, 153
	31-kDa protein				13/13 (100)		178
	57-kDa protein	10/10 (100)	10/10 (100)	9/10 (90)			43
Immunodiffusion	Cyst extract				11/11 (100)		186
Milk antibodies							
ELISA	Trophozoite extract			38/61 (62)			140
Western-blot	Trophozoite extract	4/4 (100)	4/4 (100)	4/4 (100)			153
Feces antigen							
ELISA	GSA-65					759/779 (97)	5, 15, 94, 117, 163
ELISA	66-kDa protein					77/94 (82)	53, 185, 187
ELISA	Trophozoite extract					239/251 (95)	72, 139
CIE[e]	GSA-65					36/40 (90)	162

[a] The first is for the antibody detection, and the second is for the antigen detection.
[b] The number of tests positive is given with respect to the total number of specimens tested which were obtained from studies of persons with proven cases of giardiasis.
[c] Whole serum Ig.
[d] Immunofluorescence assay.
[e] Counterimmunoelectrophoresis.

AIDS. The patients (15 of 29) who had acute symptomatic giardiasis had significantly lower levels of specific anti-*Giardia* antibodies of all isotypes in serum than did subjects who also had giardiasis but did not suffer from AIDS. These results show that despite a suppressed immune system, the immune response to *Giardia* in AIDS patients does not seem to be very different from that in healthy individuals. Because the therapy available for giardiasis is independent of the patient's immune status, patients with AIDS do not have to suffer from prolonged symptomatic *G. duodenalis* infection (92). It is probably for this reason that giardiasis is not listed among the opportunistic parasitic infections affecting AIDS patients (100).

Usually, clinical studies are required to establish if recrudescence of preexisting opportunistic infections is an important cause of morbidity when immunosuppressive therapy is given to patients in areas where the infection is endemic. To date, there are no reports in the literature on the effects of drugs such as corticosteroids, cyclosporin A, and other immunosuppressive agents of cell-mediated immunity on the outcome of *Giardia* infections in humans.

Animals

Stevens et al. (175) have shown for the first time the importance of thymus-dependent lymphocytes in the clearance of primary infections and in subsequent reinfection with *G. muris*. Hypothymic (nude) mice failed to eliminate the infection from the intestine, and a chronic state of the disease appeared. Unlike most strains of mice, which acquire resistance to reinfection (26, 40, 106, 107, 157), nude mice are not resistant to challenge infection with *G. muris* (175). The reconstitution of nude mice with thymus, MLN, or spleen cells from heterozygous thymus-intact controls results in rapid resolution of the infection (159). The total number of leukocytes, $CD4^+$ and $CD8^+$ T cells, and macrophages present in the intestinal lumen of *Giardia*-infected immunocompetent mice and nude mice was compared. Although the total number of leukocytes harvested was similar in the two strains of mice, the number of $CD4^+$ T cells was smaller in nude mice (80). According to Carlson et al. (39), the impaired capacity of nude mice to clear the infection results from a deficiency of $CD4^+$ T cells. In contrast, no differences were observed between the numbers of luminal $CD8^+$ T cells and macrophages (80). The authors also found a much smaller number of $CD4^+$ T cells in PP of nude mice than of immunocompetent mice. BALB/c mice depleted of $CD4^+$ T cells do not eliminate trophozoites from the gut, whereas those depleted of $CD8^+$ T cells are able to clear the infection normally (82). Therefore, the role played by the $CD4^+$-T-cell population in the elimination of the infection in the mouse model is different from the role played by the $CD4^+$-T-cell population in humans.

Natural killer (NK) cells are present in the mouse intestinal mucosa, but the role they play in the clearance of the infection is unknown (177). Beige mice, which are deficient in NK cells, are able to clear *G. muris* infection at similar rates to those found for immunocompetent C57BL/6J mice (81). Mice with a *G. muris* infection and treated with corticosteroids (131) or cyclosporin A (21) have increased numbers of cysts released in feces compared with nontreated mice. Similar results were obtained with gerbils treated with corticosteroids and infected with *G. duodenalis* (109). In contrast to what occurs in human infections, the importance of cell-mediated immunity in the

control of giardiasis in the animal models is well established (4).

The role of antibodies in immunity to *G. muris* has been investigated with immunocompromised mice. CBA/N mice expressing the *xid* gene have a deficient B-lymphocyte function (142). Infection of CBA/N mice with *G. muris* cysts leads to a prolongation of the infection compared to the duration in normal BALB/c mice (170). Interestingly, the CBA/N mice produced high levels of IgA anti-*G. muris* antibodies in serum and gut secretions, while the anti-*Giardia* IgG antibodies in the serum were at a low level. The authors assumed that mice bearing the *xid* gene fail to produce IgA antibodies of appropriate specificity to *Giardia* antigens, whose recognition by specific antibodies is critical for successful elimination of the trophozoites (170). The treatment of mice from birth with rabbit anti-IgM sera results in IgM, IgA, and IgG deficiencies in the serum and gut secretions (74, 171). The effects of this treatment on the primary infection with *G. muris* were studied in BALB/c and (C57BL/6 × C3H/He) F_1 mice. The treated mice showed no specific anti-*G. muris* antibodies in the serum or gut washings, and the infections became chronic, with a high load of trophozoites present in the intestine and a prolonged cyst excretion (171). These results show the importance of B cells in the elimination of the parasite from the intestine; they also indicate that the nonspecific elimination of IgM antibodies at birth has a profound effect on the outcome of giardiasis in mice.

The treatment of weanling mice with cortisone prior to infection with *G. duodenalis* results in a reduction in the numbers of $CD4^+$ T cells and IgA-producing cells in the intestine. In spite of this immunosuppressive therapy, which should have increased the trophozoite load in the intestine, a significant reduction in the number of trophozoites was obtained (102). The authors concluded that control of the infection in the absence of $CD4^+$ T cells and IgA antibodies was due to an unaltered IgM antibody response (102). The decrease in the villus-to-crypt ratio, together with the decrease in disaccharidase activity usually observed during the acute phase of giardiasis, is more severe in cortisone-treated mice (22, 102). It appears that in the animal model of the disease, immunodepression leads to a more severe infection.

IMMUNODIAGNOSIS

The immunodiagnosis of giardiasis has received much attention in the recent past. Knowledge about *Giardia* antigens and the need for improved diagnostic tests are two factors that have contributed to the increased number of publications in this area (62). A variety of assays have been used for the serodiagnosis of giardiasis. In Table 1, the results obtained by different laboratories with a variety of serological assays for the detection of *Giardia* antibodies in serum of proven cases are summarized. Proven cases of giardiasis were defined as follows: "patients passing cysts in their feces and/or presenting with one or more of the clinical symptoms of giardiasis" (15).

Sensitivity of Serological Assays

When crude extracts of trophozoites are processed for antigen usage in an ELISA, the sensitivity varies with the Ig isotype used as the second antibody. For example, when the IgM isotype was used as the second antibody, 59% of the sera from persons with proven cases tested positive, compared to only 35% when the whole Ig was used (Table 1). The ELISA has a comparable sensitivity when IgA or IgG is used as the second antibody. The use of intact trophozoites as the antigen increases the sensitivity of the IgG ELISA slightly. The sensitivity of the immunofluorescence assay (IFA) in the detection of anti-*Giardia* antibodies in the sera of persons with proven infection is comparable to that of the ELISA. However, the sensitivities of the two essays are different depending of the type of antigen used, since the sensitivity of the IFA increases when cysts are used as the antigen (Table 1). Of note, the IFA and ELISA were able to detect antibodies of the IgA and IgG isotypes at a similar level in the serum. Since *Giardia* trophozoites stimulate the production of antibodies of the IgA isotype mainly at the gut level and do not invade the tissues, one would not expect to detect anti-*Giardia*-specific antibodies of the IgA isotype in the serum at the same level as the IgG isotype.

The sensitivity of the Western blot assay is difficult to evaluate since it has been used by only a few laboratories and has been performed only on a limited number of sera from persons with proven cases. However, the sensitivity of the assay increases when purified *Giardia* proteins are used as antigens. Considering the variety of antigens stimulating the immune system of an infected patient, it is surprising that the assay is unable to detect antibodies in all the samples from the patients with proven cases of giardiasis. Identification of a common and immunodominant antigen for serodiagnostic purposes has not met with success. Studies have identified several strongly reactive antigens whose molecular masses vary immensely. For example, a major 31-kDa protein was detected in the sera of only 11 of 16 patients passing cysts in their feces, but other major bands, with molecular masses ranging from 28 to 56 kDa, were also detected in the 16 sera (178). Saliva samples taken from giardiasis patients showed 24 antigen bands with molecular masses varying between 14 and 170 kDa (161). Only one study reported 100% sensitivity of the ELISA, IFA, or Western blot technique in detecting specific antibodies in persons with proven cases of giardiasis (172). The investigators reported a significantly higher titer of circulating antibodies in symptomatic patients than in asymptomatic patients; these results confirmed the results of an earlier study (176).

The level of circulating anti-*G. duodenalis*-specific IgG, IgM, and IgA antibodies has been compared among infected persons living in Denver, Colo., and Soongnern, Thailand (91). Antibody levels detected by ELISA increased significantly during childhood in both geographic areas. The *Giardia*-specific IgA antibody levels remained elevated throughout life among adults from Thailand but decreased among adults in Denver. On the other hand, after adolescence, *Giardia*-specific IgM antibodies fell steadily with increasing age in both populations. Based on these findings, the authors concluded that the levels of *G. duodenalis*-specific IgM in adults may be useful to differentiate between recent and past infection (91).

By determining the levels of systemic and local antibodies to *G. duodenalis* in different populations, widely different immune responses in infected patients were recognized (123). Several blood and milk samples were collected simultaneously from lactating women in Texas and Mexico. Specific IgG antibodies to *G. duodenalis* were present in 77% of 153 serum samples from 27 Mexican mothers but in only 24% of 214 serum samples from Texan mothers. Secretory IgA antibodies were detected in 79% of milk samples from the Mexican population but in only 15% of milk samples from the Texan population (123). These results highlight the difference in the immune response to *Giardia* between infected patients in areas of endemic infection and other areas.

The outcome of a *Giardia* infection and humoral antibody response in humans may also vary depending on the isolate. To illustrate, enteral inoculation of healthy volunteers with 50,000 trophozoites of two distinct *Giardia* isolates having distinct

DNA restriction endonuclease patterns, surface antigens, and ES products resulted in a variety of outcomes (136). One isolate (GS/M) was obtained from a scientist from the National Institutes of Health who had typical symptoms of giardiasis. The second isolate (Isr) was obtained from a child from Bethesda, Md., who also had typical symptoms of giardiasis. The Isr isolate failed to produce an infection in healthy volunteers, while those inoculated with the GS/M isolate developed a variety of symptoms. The IgM, IgG, and IgA levels in serum and IgA levels in intestinal fluid were found in 100, 70, 60, and 50%, respectively, of the individuals infected with the GS/M isolate (136). No antibodies were detected in healthy individuals infected with the Isr isolate. This study not only shows variations in pathogenicity of *Giardia* strains in humans but also illustrates the variations in the immune response to *Giardia* protein stimulation.

The variation in the results obtained in the serological survey done in the field (123) with respect to the experimental infection of healthy individuals (136) and other studies (Table 1) demonstrates the poor sensitivity of serological assays presently available for the diagnosis of giardiasis. Therefore, the usefulness of serological assays for the diagnosis of human giardiasis is debatable. There are several reasons to explain the poor sensitivity of serological assays. (i) Geographical isolates have been identified, and they may have their own antigenic identity (89). (ii) Infection may develop into a chronic state in which the parasite may interfere with the immune system, leading to immunodepression, and this may affect the level of antibodies produced. (iii) Antigenic variation may also interfere with the production of antibodies. (iv) Many human cases of giardiasis never reach the acute stage of the infection (i.e., the period of severe diarrhea), and the type of immune response stimulated in these patients is unknown. Except for the different levels of antibodies detected, serodiagnostic assays failed to show differences in serum antibody responses between symptomatic and asymptomatic patients. Since *Giardia* trophozoites rarely invade the tissues, the systemic immune response is practically never stimulated, and searching for antibodies to *Giardia* in the serum remains an unreliable exercise. Although many commercial kits are available for detecting anti-*Giardia* antibodies in infected patients, it is unfortunate that no investigators have reported their efficacy in the literature.

Detection of Antigens in Feces

Giardiasis is usually diagnosed by the microscopic examination of stool samples for the identification of cysts ("gold standard" method). The sensitivity of this method is rather low because cysts are excreted intermittently or, in some cases, released in numbers too small to be detected (62). Therefore, a minimum of three specimens taken on three consecutive days are usually examined to obtain an acceptable sensitivity. The availability of an immunodiagnostic assay which can detect small amounts of antigens in feces would have the potential to improve the diagnosis in many ways. For example, it would be more indicative of an active giardial infection and would therefore represent a more meaningful clinical finding than the detection of antibodies in the serum. In contrast to the commercial kits available for the detection of antibodies in the serum, the sensitivity of ELISA for the detection of antigens in the stools has been evaluated by several laboratories (Table 1). The ELISA-GSA 65 detects a *G. duodenalis*-specific antigen (GSA) that is excreted in the stool. GSA has been identified in trophozoites and cysts and has an approximate molecular mass of 65 kDa (162). The ELISA-GSA 65 is available commercially as a kit, and its sensitivity and specificity are comparable to those of microscopic examination for cysts in the stool (62, 163). In fact, all studies with the ELISA-GSA 65 have reported a greater sensitivity of the immunodiagnosis assay over the microscopic examination of a single specimen (Table 1). The sensitivity of the assay varies between 95 and 100%, and 100% specificity has been reported when it was used with stools from patients infected with other intestinal parasites (15, 162). It has been reported that the ELISA-GSA 65 can detect *Giardia* infection in at least 30% more cases than the microscopic examination (163). In a recent epidemiological study of the prevalence of *G. duodenalis* infection in 328 patients admitted to the University Hospital of the West Indies for various illnesses, the commercial rapid enzyme assay for detecting antigens in a single stool specimen was compared to the formalin-ether concentration method for the detection of cysts in stool (111). The formalin-ether concentration method detected 6 cases of giardiasis, whereas the assay for detecting antigens in stool detected these 6 cases plus an additional 11 cases. These results clearly demonstrate the superior sensitivity of the rapid enzyme assay in detecting cases of giardiasis in epidemiological studies when a single specimen is analyzed. In contrast to all the serological assays used for the detection of antibodies against *Giardia* proteins, the ELISA-GSA65 for the detection of antigens in feces has demonstrated a remarkable sensitivity and specificity of 98 and 100%, respectively (15).

VACCINE

There are few studies on the induction of active immunity against *G. duodenalis*. Subcutaneous immunization of 3-week-old mice with a 56-kDa protein followed by oral immunization resulted in a lower load of trophozoites in the small intestine when the animals were challenged with 10^7 trophozoites 7 days after the last immunization (188). The immunization provoked an increase in the number of circulating $CD4^+$ T cells for a short period, but they were back to normal levels by day 30 postimmunization. Furthermore, a significant elevation in the numbers of IgA- and IgG-containing plasma cells was observed in the lamina propria and jejunum of the immunized mice (188). The subcutaneous vaccination of 6-week-old kittens with a crude extract of trophozoites of *G. duodenalis* resulted in a smaller number of cysts excreted in the feces when the animals were challenged intraduodenally with 10^6 trophozoites 14 days after the last immunization (144). The vaccination provoked an increase in the number of serum anti-*Giardia* IgG and IgA antibodies. The mucosal anti-*Giardia* IgA antibody titer in the vaccinated kittens was also increased. The experiment was repeated by vaccinating 6-week-old puppies, and the results were similar to those obtained with the kittens (143). The efficacy of these vaccination attempts was rather poor, since all of these attempts were unsuccessful in fully protecting the animals against infection in spite of the short period between the last dose of vaccine and the challenge with the live parasite.

CONCLUSIONS

The following conclusions can be drawn. The immune response plays a role in the pathology at the intestinal mucosal site. A minimum of 20 polypeptides ranging from 14 to 125 kDa have been identified from crude extracts of trophozoites. Laboratories have reported that the 82-kDa polypeptide is a major trophozoite surface antigen. Isolates from different geographic areas have antigenic similarities. Cyst antigens detected in human feces have molecular masses varying between 21 and 49 kDa. HSP, lectins, giardins, tubulin, and chitin are

other molecules of *Giardia* cells. Antigenic variation occurs in giardiasis and has been observed in vivo and in vitro. The variant surface antigens of *G. duodenalis* have been localized on the surface membrane of trophozoites; the majority of VSPs identified have an abundance of cysteine residues. Innate immunity plays a role in control of the infection. In acquired immunity, both arms of the immune system play a role in control of the infection. The IgM, IgA, and IgG-specific antibodies play a major role, as do the T-cell subsets, the macrophages, and the neutrophils. Accessory components of the immune system, like complement, play a role. Very few studies have been done on the role of cytokines. Acquired resistance to giardiasis has been well documented in animal models only. *Giardia* can depress the immune system of its host. In humans, the infection is more severe in hypogammaglobulinemic patients. However, patients with other infectious agents that can depress the immune system (e.g., AIDS) do not have a more severe infection. For several reasons, the sensitivity of serological assays in detecting *Giardia* antibodies is low, even when the assays are used to detect antibodies in the sera of persons with proven cases. Several laboratories have reported excellent sensitivity and specificity of the ELISA-GSA 65 for the detection of *G. duodenalis* antigens in the stools of infected patients. Attempts to vaccinate against giardiasis have not met with success.

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