Cytopathogenicity of Entamoeba histolytica

C. Gitler, Edna Calef and I. Rosenberg

| **Email alerting service** | Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here** |

Cytopathogenicity of *Entamoeba histolytica*

By C. Gitler, Edna Calef and I. Rosenberg

Department of Membrane Research and Unit of Parasitology, The Weizmann Institute of Science,
Rehovot, 76100, Israel

The lesions induced in man by *Entamoeba histolytica* are characterized by massive tissue injury in the absence of major local signs of a host immune response. The amoeba damages surrounding cells preferentially by contact-mediated cytolysis. Recently, a presumptive aetiological factor underlying this process has been identified. It is a protein, amoebapore, capable of spontaneous incorporation into host cell membranes. Therein it induces high conductance ion-channels which rapidly collapse the cellular transmembrane potential and lead to a prelytic state.

Amoebapore is present within the amoeba in a highly aggregated state in a small, dense particle. It is shed into the medium in a particulate form by a stimulus-mediated process. Release is enhanced by addition of concanavalin A, lipopolysaccharide or the calcium ionophore A23187. Surface-labelling of intact amoeba, followed by fractionation of the homogenate in self-generating Percoll gradients, identified two labelled fractions, the plasma membrane and a particulate fraction sedimenting in the region of intracellular particulate amoebapore. This latter fraction appears to be material in the process of exocytosis.

A highly immunogenic surface lipid has been identified and shown to be involved in the rapid surface redistribution of immune complexes, their shedding and endocytosis. The relevance of these findings to the immunoprophylaxis of amoebiasis is discussed.

Introduction

Invasive amoebiasis is characterized by the massive destruction of host tissues by the parasite *Entamoeba histolytica*, which, if unattended, will progressively endanger the life of the affected individual. Epidemiological studies indicate that some 10 % of the world's population is infected with *E. histolytica* (World Health Organization 1969). Of these, some 15 % will have symptomatic amoebiasis (Trissl 1982). It has been estimated that 1.6–2.1 % of patients entering the general hospital in Mexico City have liver abscesses (Alvarez Alva & Loza Saldivar 1971). Autopsy serial studies indicated that amoebiasis was the seventh leading cause of death in Guatemala and the fourth in Mexico (Sepulveda 1970). That the incidence of the disease is related to the frequency of exposure can be derived from the fact that infection can reach 70 % in patients resident in mental institutions (Sexton *et al.* 1974) and 31.7 % of 126 homosexuals studied in one New York hospital (Kean *et al.* 1979). This can be compared to the average incidence of infection in the United States, which was about 4 % (Juniper 1971).

Clinical signs of the disease are accompanied in most cases with high titres of circulating antibodies directed mainly against surface determinants of the amoeba (Aust Kettis *et al.* 1983). Recent evidence indicates that surface lipids of the amoeba are strongly immunogenic in man (Calef & Gitler 1984). The evidence suggests that the immune response does not alter the course of the disease. In fact, the antibody levels may correlate with the severity of the clinical involvement. Even though the serum antibodies may persist over as long as 20 years following

infection, there is no evidence (Brandt & Tamayo 1970) that they protect against reinfection and dysentery (invasion of the intestinal mucosa). Opinions differ (Sepulveda & Martinez Palomo 1984) with regards to the reoccurence of invasive amoebiasis (extraintestinal lesions). Amoebae appear to evade immune attack by shedding or internalizing antigen–antibody complexes (Aust Kettis & Sundqvist 1978; Calderon *et al.* 1980).

A puzzling aspect of the disease resides in the large disparity between the number of infected individuals passing cysts and those showing overt clinical symptoms. This has led to the concept that the amoeba can exist commensally in some individuals (lumenal amoebiasis). The reason why some individuals acquire the disease while others are simply carriers is not completely understood. Compelling evidence suggests that strains of *Entamoeba* exist that are avirulent while others are found to be highly virulent (Sargeaunt *et al.* 1982). However, even within a single cloned isolate, virulence can vary with the time of subculture, with the presence of various components in the growth media and with the passage through an animal host. Whether the observed differences are phenotypic or genotypic is not known. Methodology is required for the genomic characterization of the different isolates to clarify these essential points. This would then allow the assessment of the role of the local host defences in preventing access of the amoeba into the host tissues.

Animal models have been described that duplicate more or less the human pathology including dysentery and invasive amoebiasis. The problem residing mainly in the variable virulence of the axenic cultures (for a review see Ravdin & Guerant 1982). It is of interest, however, that protection against invasive amoebiasis has been reported using as a model the development of liver abscesses. Exposure to live amoeba or immunization with crude amoeba fractions protected against the challenge resulting from the injection of trophozoites directly into the liver of hamsters (Trissl 1982).

The main damage caused by the amoeba resides in the destruction of host tissues. The virulent trophozoite kills cells by a rapid process that involves, first, the intimate but transient contact between the amoebae and the surrounding cells. Next, the amoeba liberates in the area of contact cytopathic factors that damage the target cell. Third, the trophozoite will ingest parts or the total afflicted cell. Fourth, the amoebae liberate in the site of contact and in the area of the ingested cell, digestive enzymes that result in the lysis of the ingested cell. If this sequence could be dissected thoroughly and the participating elements identified, they might permit the design of immune strategies to neutralize the cytolethality of *E. histolytica*. However, the ultimate preventive goal would be to limit access of the amoeba into the host's intestinal mucosa. This will require a long lasting local immune protective response.

Contact-dependent cytolysis

The manner whereby the amoeba damages the host tissues is not known in detail. It is not, however, the simple phagocytosis of the surrounding cells. Detailed analysis of amoeba destroying monolayer cell cultures shows that the process is initiated by a contact-dependent cytolethal stage followed by phagocytosis. Cells not in contact with the amoeba remain intact (Knight *et al.* 1975; McCaul 1977; McCaul & Bird 1977; McCaul *et al.* 1977; Bos 1979; Ravdin *et al.* 1980). These studies suggest the following sequence of events.

Transient contact

The amoeba transiently contacts several cells in its vicinity and only when these are seen to bleb and round up will the amoeba proceed to ingest them. Electron microscope analysis at this stage indicates that close apposition is established between the plasma membrane of the amoeba and of the target cells. In some cases it has been reported that erosion of the intervening membranes may occur. It is difficult to decide whether what is observed represents the transient contact or the initial stages of phagocytosis.

Cytolethal effect

After contact, the target cells are damaged as shown by extensive blebbing, loss of the normal shape, release of markers such as radioactive chromium and indium oxine and uptake of trypan blue (McCaul 1977; McCaul *et al.* 1977; Ravdin *et al.* 1980). An important observation has been made (Ravdin *et al.* 1980) that upon exposure to virulent amoeba, a significantly greater number of target cells, relative to the control, were stained by trypan blue. This is strong evidence that the amoeba induces contact-dependent cytolethal changes in more cells than those that it ingests.

McCaul (1977) described the changes occurring in the target cells following contact with virulent trophozoites as similar to those seen after the administration of substances that depolymerize microtubuli. When amoeba are added to epithelial cells, one of the early effects observed is the widening of the intercellular spaces owing to the opening of the occluding junctions along the upper margin of the lateral borders of the cells (Martinez Palomo 1982).

Phagocytosis and cell digestion

It is not clear whether the target cells have to be damaged to be ingested by the amoeba. In-depth observations of *in vitro* interaction indicate that the amoeba seldom ingests cells at first contact.

Phagocytosis of the whole cell or pinching-off of cellular segments is a rapid process that does not diminish the activity of the trophozoites. Cinematography reveals that the trophozoites gain momentum following interaction with the target cells. Fusion of digestive vesicles with the internalized cell leads to its ultimate digestion.

The contact-mediated cytolethal effect occurs over a time interval of minutes. This places stringent requirements on the factor or factors that could mediate the process leading to the cellular damage. An agent acting on the plasma membrane capable of drastically increasing the permeability to ions would be a good candidate for this role.

The role of amoebapore in contact-dependent cytolysis

The addition to artificial planar lipid bilayers of minute amounts of axenic medium that has supported growth of *E. histolytica* (conditioned medium) or of the total amoeba homogenate results in an almost immediate and progressive rise in the conductance across the bilayer that can reach a value as high as five orders of magnitude greater than that of the original bilayer (Lynch *et al.* 1980, 1982; Young *et al.* 1982). The increased conductance resulted from the *spontaneous* incorporation of amoeba-derived ion channels into the lipid bilayer. The ion channel

forming fraction is a small molecular mass protein named *amoebapore*. Presumptive evidence suggests that amoebapore may be the aetiological factor leading to contact-dependent cytolysis induced by the amoeba.

Characteristics of amoebapore

Amoebapore in a fully dissociated form has an apparent molecular mass of 13 000–14 000 Da (Lynch *et al.* 1982). It appears not to have disulphide bonds or free sulphydryl groups. It exists within the trophozoite in a highly aggregated state in a small-diameter, highly dense particle, that has been separated from the many digestive vesicles present within the cell (Rosenberg & Gitler 1984). However, complete characterization of this particle has, as yet, not been possible because it cannot be totally resolved from the amoeba ribosomes. Amoebapore can be completely dissociated from this particle by treatment with high salt (Rosenberg & Gitler 1984) and partly by repeated freezing and thawing (Young *et al.* 1982). Gel filtration shows that under these conditions amoebapore exists almost exclusively as a dimer, although it tends to reassociate into higher molecular mass aggregates (Rosenberg & Gitler 1984; Young *et al.* 1982). The protein bears a cationic charge at physiological pH because it is not retained by an anion exchange resin. It is highly hydrophobic molecule binding readily to resins and Sepharose containing attached phenyl moieties and is only eluted with high organic solvent concentrations.

The incorporation of amoebapore into lipid bilayers results in the formation of high-conductance ion channels (1.6 ± 0.2 nS in 1 M NaCl and -10 mV) that are moderately cation selective and voltage dependent (Lynch *et al.* 1982). Available preparations have not been sufficiently pure to establish the nature of the subunit interaction in the membrane to form the ion-channels. However, the dimers are conductive and association beyond the dimeric state only increases the total conductance (Young *et al.* 1982). Addition of *Streptomyces griseus* protease to the same side from which the amoebapore incorporated into the lipid bilayer, results in the return of the conductance nearly to the values existing before amoebapore addition. The presence of the protease on the side opposite that from which amoebapore was added had no effect in our studies (Lynch *et al.* 1980, 1982) and a slight effect in those of Young *et al.* (1982). These findings are of interest because they indicate that (i) amoebapore penetrates into the bilayer but protrudes only slightly, if at all on the inner side; (ii) the *in situ* sensitivity of amoebapore to the protease implies either that a significant portion of the molecule remains exposed at the bilayer surface or alternatively, that it exists in a reversible equilibrium between the bound and the unbound form. The latter would be the one labile to proteolysis; (iii) amoebapore does not damage the lipid, since after proteolysis the original impedance is regained.

The incorporation of amoebapore does not depend on the nature of the lipid. Equivalent results were obtained with the synthetic neutral diphytanoylphosphatidylcholine and with azolectin or egg lecithin. Amoebapore readily incorporates into multi- or single lamellar liposomes where it can be shown to lead to a rapid collapse of the transmembrane potential by enabling the rapid equilibration of cations down their concentration gradients. This property has been used as the basis for a simple assay to identify its presence and to quantify its distribution (Loew *et al.* 1980, 1983).

The properties of amoebapore suggest that it may function as one of the causal agents in the amoeba-induced contact-mediated cytolysis. We have developed a working hypothesis that

depicts the manner whereby the initial contact could lead to the participation of amoebapore in the damage of the host cell.

Cell–cell recognition

It is now generally agreed that the first step in the cytolethal sequence involves specific recognition of the host cell by the amoeba. As mentioned above, electron microscopy indicates extensive cell–cell contact between amoeba and target cells. Virulent *E. histolytica* have been reported to contain two surface lectin-like molecules. Adherence of the amoeba to a human intestinal cell line, to baby hamster kidney cells and to erythrocytes was specifically inhibited by oligosaccharides of *N*-acetylglucosamine, by purified bacterial peptidoglycan or by the precoating of the cells with wheat germ agglutinin (Kobiler & Mirelman 1980; Orosco *et al.* 1982). Adherence of trophozoites to Chinese hamster ovary cells was inhibited by *N*-acetylgalactosamine (Ravdin & Guerant 1981). These latter authors reported further that the presence of millimolar GalNAc resulted in the complete inhibition of the destruction of a monolayer of Chinese hamster ovary cells by the amoeba (see also Mirelman *et al.* 1983).

Transmembrane stimulation and secretion

It is proposed that the cell–cell contact induces the transmembrane stimulation of the amoeba leading to the focused secretion or exteriorization of active antitarget agents including amoebapore at the site of contact.

The original observations of Lynch *et al.* (1980, 1982) showed that a significant amount of amoebapore was detected in conditioned media. Furthermore, the addition to intact trophozoites of the calcium ionophore A-23187, resulted in the liberation of amoebapore into the extracellular fluid to the extent of some 10% of that present in the cell. A similar stimulation of the secretion of amoebapore was induced by the exposure of the trophozoites to lipopolysaccharide from *E. coli* or concanavalin A (Young *et al.* 1982).

These findings suggest that cell–cell recognition might trigger the flow of calcium into the amoeba and that the rise in intracellular calcium in turn induces secretion. In this regard, it is of interest to note that contact-mediated cytolysis was abrogated by the presence in the medium of EDTA or EGTA and was diminished by the action on the amoeba of the calcium channel inhibitor bepridil (Ravdin *et al.* 1982). The availability of highly active calcium channel inhibitors (Glossmann *et al.* 1982) should clarify whether indeed calcium channels exist in the amoeba. A channel would be capable, in the short contact period, of allowing sufficient calcium to flow into the amoeba for the stimulus-secretion to occur.

The term 'secretion' was used above, but some characteristics of the release of amoebapore and other amoeba derived components into the extracellular space requires comment. Amoebapore is present within the trophozoite in a highly aggregated state in a small (less than 100 nm), dense (1.19 relative density) particle. It is discharged in a form that is largely sedimentable (Rosenberg & Gitler 1984). It has not been established whether the intracellular amoebapore-containing particle is surrounded by a membrane. Recently, the observation was made that concanavalin A added to trophozoites also stimulated the release of neutral, thiol-activated proteases which sedimented in a large part at 10000 *g*. Trophozoites remained intact because under the same conditions, the cytoplasmic alcohol dehydrogenase of the amoeba was not liberated into the medium (Yanovsky & Gitler 1984). Bos *et al.* (1980) found that a toxin activated by cysteine and inhibited by iodoacetamide was secreted to the medium of

trophozoites incubated in physiological saline. We have also observed that the amount of amoebapore and neutral proteases externalized by the amoeba was significantly greater when the trophozoites were incubated in physiological saline than when incubated in the growth medium.

Additional evidence supporting the release of structured material was derived from the following experiments. Lactoperoxidase-labelling of intact amoeba followed by homogenization and separation of the subcellular components in self-generating Percoll gradients showed that the majority of the label was associated with a fraction found in the lower third of the gradient, the plasma membrane. This fraction also contained the majority of the concanavalin A-binding glycoproteins and of the intrinsic membrane proteins labelled with iodonaphthylazide (Rosenberg & Gitler 1984). In addition, a second lactoperoxidase labelled fraction, almost completely devoid of concanavalin A binding glycoproteins, was consistently found in the top third of the gradient. This component, consisting of some 10–12 labelled polypeptides, represents material on the cell surface distinct from the membrane. It is reasonable to suggest that it may constitute material exteriorized during exposure of the cells to physiological saline solutions during the labelling procedure.

These results taken together indicate that true secretion does not occur. Rather, stimulated amoeba expose a complex of active factors to the exterior which, operating together or in an ordered manner, could have an enhanced cytopathic effect on the target cell.

Effect of amoebapore on target cells

Experiments with *Fundulus* blastomeres impaled with voltage recording and current passing electrodes showed that they exhibited a striking decrease in the input membrane resistance when exposed to *E. histolytica*-derived particulate fractions in concentrations equivalent to those used to obtain the *in vitro* bilayer effects (Lynch *et al.* 1980). The addition of partly purified amoebapore to macrophages or lymphocytes resulted in the rapid fall of the cell surface potential as measured by the equilibrium distribution of the lipid soluble cation tetraphenyl phosphonium (Young *et al.* 1982).

These results demonstrate that amoebapore rapidly incorporates into the plasma membrane of cells and induces marked changes in its permeability to cations. Amoebapore-induced channels are highly permeable to potassium, sodium, protons and to calcium. Its unit conductance is high and the period that single channels remain open is in the range of seconds. It is clear therefore, that incorporation of such a channel into a host cell, will allow rapid equilibration of the ions down their concentration gradients. The high activity of ATPases, in a cell short-circuited by amoebapore, would result in a rapid fall in the ATP level of the affected cell. In addition, the rise in intracellular calcium would rapidly depolymerize microtubules and lead to the destruction of cell–cell junctions. Both the loss of ATP and the depolymerization of microtubuli are known to result in the rounding of cells and in the extensive blebbing of their plasma membrane.

Amoebapore meets the requirements for being the mediator of the amoeba-induced contact-mediated cytolysis. However at present, all of the evidence is indirect. That is, no experiment has as yet been performed that shows that the trophozoites indeed kill target cells by the focal exteriorization of amoebapore. What is required is a specific inhibitor of amoebapore that could be shown to block killing by the intact amoeba. The fact that the amoeba transiently contacts target cells could allow for an antiamoebapore antibody to block its

channel-forming properties and perhaps prevent cell killing. Enough pure amoebapore is only now becoming available to attempt to develop immunization schemes to obtain adequate blocking antibodies. A second approach would be to find a small molecule that could block the ion channel. Polar guanidines and amidines are being tested as candidates. The artificial lipid bilayer procedure allows for the rapid testing of molecules of different structure in their capacity to block the channel conductance.

Another problem in the postulated role of amoebapore lies in the understanding of how the amoeba itself avoids the deleterious effects of the channel-forming molecule. Incorporation of channels from an intracellular store into the plasma membrane of a large cell such as the amoeba, may induce a local depolarization leading to membrane internalization. Once the membrane containing the channel is internalized, the deleterious effect would be neutralized. An ion-permeable intracellular vesicle poses no problem. It is possible that cells like macrophages, which have a high rate of pinocytosis, recover from the effect of amoebapore by an equivalent mechanism (Young *et al.* 1982).

The above hypothesis has many parallels with observations made by Adams and colleagues on the mechanism of tumour cytolysis by activated macrophages. They found that macrophages from C57BL/6J mice, when activated by bacillus Calmette-Guérin, lysed syngeneic MCA-I sarcoma targets but not syngeneic embryo fibroblasts. Cytolysis appeared to be mediated by a neutral protease secreted at the site of contact with the tumour cell. Macrophages incubated in the absence of the tumour secreted the lytic factor into the medium, which could be enriched by gel filtration. The purified fraction also contained the proteolytic activity. Secretion was found to be enhanced by the contact of the macrophage with the tumour cell. It is interesting that thioglycolate-activated macrophages could not induce cytolysis except when previously exposed to bacterial lipopolysaccharide (Adams 1980; Adams *et al.* 1980; Johnson *et al.* 1981). The cytolytic effect observed in these experiments differed from the amoeba process in that prolonged exposure to the protease was required for the cytolysis to take place. In this regard it is noteworthy to add that slow-acting cytolytic neutral proteases have also been purified from the amoeba (Lushbaugh *et al.* 1978, 1979; Bos *et al.* 1980; McGowan *et al.* 1982). These have been shown to be secreted into the extracellular space. In addition, virulent strains of *E. histolytica* have been found to contain an active type I collagenase (Munoz *et al.* 1982). The level of enzyme activity correlates with the virulence of the amoeba substrain. It is secreted into the surrounding media and is found bound to the membrane in a form that can be dissociated by high salt (Gadasi & Kessler 1983). It is unlikely that these proteases participate in the contact cytolysis. Rather, they seem to be involved mainly with the capacity of the amoeba to penetrate into the host tissues and to digest the cells during phagocytosis. Their effect would appear to be too slow to account for the rapid onset of amoeba contact cytolethality.

Amoebal surface determinants and immunology

Amoebal immune avoidance is intimately related to the properties of its plasma membrane. Evidence indicates that the plasma membrane exists in a dynamic state. This is characterized by high lateral diffusion of surface elements allowing (i) rapid antibody- and lectin-induced redistribution of surface complexes; (ii) their shedding; and (iii) their internalization. The amoeba has the remarkable property of not being denuded of surface antigens by this process. Pinocytosis rates are such that the total membrane is internalized roughly every 20 min. This

requires continuous recirculation of the membrane determinants which could replenish those lost as immune complexes. Until recently, the specific surface antigenic determinants were undefined. An amoeba lipid and a lipopeptidophosphoglycan have been found to contribute significantly to the antigenicity of the amoeba surface. Immune sera of patients with liver abscess contain significant amounts of antisurface antibodies directed against the amoeba lipid and lipopeptidophosphoglycan. In addition, they contain antibodies directed against surface proteins (Parkhouse *et al.* 1978; Aust Kettis *et al.* 1983).

The surface lipid and lipopeptidophosphoglycan

The persistence of surface antigens after repeated antibody-induced surface capping could readily be explained if the surface antigenic determinant was a lipid. The plasma membrane of *E. histolytica* contains some 10^8–10^9 lipid molecules. The redistribution brought about by antibody binding to some 10^5 or 10^6 determinants would clearly not denude the surface of antigenic determinants. It therefore was of interest to determine whether the lipids of *Entamoeba histolytica* were immunogenic and whether immune sera of patients with invasive amoebiasis or rabbit immune sera against the total trophozoite contained anti (surface lipid) antibodies. The availability of a simple and reproducible radioimmunoassay for lipids (Smolarsky 1980) facilitated the identification of a unique, highly immunogenic amoeba lipid (Calef & Gitler 1984). The amoeba lipids were extracted by classical procedures and plated in microtitre plates. The presence of antigenic lipids was determined by the radioimmunoassay with immune sera from patients with invasive amoebiasis, or with rabbit immune sera against total trophozoites. In addition, potent antilipid immune rabbit sera were obtained by the intraperitoneal injection of liposomes made from the amoeba lipids. Results identified high titres of antilipid antibodies in all sera tested. After separation by thin layer chromatography, each distinct lipid species was plated and the radioimmunoassay with the immune sera was repeated. In this manner a unique antigenic lipid was identified. Its preliminary characterization indicates that it is an aminoglycophospholipid representing some 3.8 % of the total amoeba lipid.

Pure antiaminoglycophospholipid antibodies were affinity purified by a novel procedure which involved their selective adsorption by antigen-containing liposomes followed by elution with n-butanol (Calef & Gitler 1984). The antilipid antibodies were found neither to cross-react with the total lipids extracted from the growth media nor with those of *Tetrahymena pyriformis* which, like *Entamoeba* contain a significant portion of phosphono-derivatives. Surprisingly, however, they showed very strong cross reactivity with a lipopeptidophosphoglycan purified from the amoeba by the phenol method of Westphal & Jann (1965). The lipopeptidophosphoglycan has been reported to contain 85 % sugars including glucose, galactose, xylose, fucose and aminosugars. The major amino acids were aspartic and glutamic acids (8 %); some 2.5 % of fatty acids and 1 % phosphate (Isibasi *et al.* 1982). If the antigenic lipid is a part of the lipopeptidophosphoglycan, it must be covalently attached. Thin-layer chromatography of the isolated lipopeptidophosphoglycan did not result in the release of material with a chromatographic behaviour equivalent to that of the aminoglycophospholipid. Kumate and coworkers (Isibasi *et al.* 1982) had previously shown that immune sera from patients with amoebiasis reacted strongly with the lipopeptidophosphoglycan.

Self-generating Percoll gradient fractions containing the subcellular organelles and the plasma membrane of *E. histolytica*, were extracted either with chloroform-methanol or with phenol. The extracts were assayed for the presence of the lipid and lipopeptidophosphoglycan,

respectively, by the radioimmunoassay with the immune sera. Results indicate a parallel distribution of both entities in all the trophozoite subcellular membranes, including the plasma membrane (Calef & Gitler 1984). For the time being there is no way of distinguishing whether the interactions with immune sera as described are with the antigenic aminoglycophospholipid, with the lipopeptidophosphoglycan or with both.

The surface proteins

Table 1 shows the characteristics of the plasma membrane proteins of *E. histolytica* HM-1:IMSS (Rosenberg & Gitler 1984). The exofacial surface proteins were identified by their labelling with lactoperoxidase and by the binding of concanavalin A to the glycoproteins that were separated by gel electrophoresis (see also Aley *et al.* 1980; Aust Kettis *et al.* 1983). The proteins containing lipid embedded domains (l.e.d.) were labelled from within the lipid bilayer with [^{125}I]-5-iodonapthalene-1-azide (Gitler & Bercovici 1980). An impressive number of

TABLE 1. *ENTAMOEBA HISTOLYTICA* PLASMA MEMBRANE PROTEINS

band	molecular mass/kDa	lacto-labelled†	Con A-labelled‡	l.e.d.-proteins§
0.1	205 (9)	−	+	−
0.2	195 (8)	−	+	−
1.0	154	+ (1)	+ (2)	+
2.0	147	+ (2)	+ (1)	+
2.1	129	−	+	−
2.2	120	−	−	−
2.3	115	−	+ (8)	−
2.4	110	−	−	+ (8)
3.0	100	+ (7)	+	−
3.1	92	+	+ (7)	−
3.2	85	−	+ (3)	−
3.3	83	+	+ (4)	−
3.4	76	+	+ (5)	−
3.5	73 (7)	−	−	−
4.0	70 (6)	−	+	−
4.1	69	−	+	−
4.2	67	−	+	−
4.3	61	−	+ (6)	+
5.0	57 (3)	+ (6)	+	−
5.1	54	−	−	+ (2)
6.0	50 (4)	−	+	−
6.1	48	+	−	−
6.2	45	+	−	−
6.3	42	−	+ (10)	+ (6)
6.4	40	−	+	−
7.0	37 (5)	+ (4)	+ (11)	+ (3)
8.0	34	+ (5)	+ (9)	+ (9)
9.0	31	+	−	+ (4)
9.1	30	+	−	−
10.0	25 (1)	−	−	−
11.0	24 (2)	+ (3)	−	+ (1)
11.1	19	−	−	+ (5)
11.2	15	+	−	+ (7)
11.3	13	+	−	−

Proteins labelled by: † [^{125}I]lactoperoxidase; ‡ Concanavalin A and § [^{125}I]iodonaphthaleneazide (Rosenberg & Gitler 1984). The numbers in parentheses indicate the order according to the intensity of the bands.

concanavalin A binding glycoproteins were detected, only about half of which were also labelled with lactoperoxidase. A number of polypeptides are likely to be transmembrane proteins since they are exofacial surface proteins with polypeptide domains in contact with the bilayer lipids (bands 1, 2, 4.3, 6.3, 7, 8, 9, 11 and 11.2). This pattern suggests that the amoeba contains an extensive glycocalix made up of extrinsic glycoproteins probably anchored to the surface by their interaction with some of the transmembrane proteins or with the lipopeptidophosphoglycan (Martinez Palomo 1982).

Parkhouse *et al.* (1978) used immune sera to precipitate membrane glycoproteins. A major band of 81 and minor bands of 150, 30 and 20 kDa were identified. Aust Kettis *et al.* (1983) observed variable interaction of plasma membrane proteins with immune sera from patients with invasive amoebiasis. However, by immunoblotting the amoeba proteins with human antiamoeba serum, they could identify bands of 150, 120, 88, 74, 68, 42, 37 and 18 kDa. The most prominent bands were those of molecular mass 88000 and 42000 (bands 3.1 and 6.3 of table 1). Comparative studies with rabbit immune sera showed reaction with all the bands detected by the human sera except those of 150 and 120 kDa. Additional immunoreactive bands were found of 74, 56, 30 and 23 kDa.

Antibody-induced surface antigen redistribution

Addition of heat-inactivated immune sera or of concanavalin A to intact trophozoites induced rapid redistribution of the complexes on the cell surface forming aggregates found mainly in the uroid region (Martinez Palomo 1982). The capped material was either shed or internalized. This process in the amoeba is distinct from that of surface immunoglobulin redistribution (Stall & Knopf 1978) in that the immediate readdition of the antibodies or of the lectin shows that the surface has not been denuded of antigenic determinants (Trissl *et al.* 1977; Aust Kettis & Sundqvist 1978; Calderon *et al.* 1980).

By using the pure antilipid antibodies, repeated capping could be performed (Calef & Gitler 1984). Adsorption of immune sera from patients with invasive amoebiasis or of rabbit anti-(total amoeba homogenate) with liposomes containing the antigenic lipid, greatly decreased the titre required to obtain indirect immunofluorescent staining of intact amoebae. This demonstrated that the antilipid (or anti lipopeptidophosphoglycan) antibodies represent a significant fraction of the total antibodies directed against the surface components of the virulent *Entamoeba histolytica*.

As mentioned above, Parkhouse *et al.* (1978) and Aust Kettis *et al.* (1983) found that immune sera contain antibodies directed against amoeba surface proteins. The addition of antilipid antibodies does not inhibit concanavalin A binding. Since the lectin binds to the majority of the trophozoite surface glycoproteins and can induce repeated capping, it is clear that the antigenic proteins also participate in the capping, shedding and internalization.

Serrano & Reeves (1975) determined that the rate of pinocytosis of *E. histolytica* was 5–9 μl min^{-1} ml^{-1} of cells. Yanovsky (unpublished results) determined the pinocytotic rate by means of sucrose internalization to be 8.3 μl. Calculation of the time required to internalize the total surface (Steinman *et al.* 1976) gives values on the order of 20 min. It is likely that the high rate of internalization and externalization of membrane that is evident from the pinocytotic rates can lead to the rapid replenishment of the antigenic moieties depleted by exposure to immune sera.

A major part of the surface redistribution involves the antigenic lipid and the lipopeptido-

phosphoglycan. Immune complexes containing these elements must be continuously formed and shed. The difficulties in identifying the shed materials and the components of the soluble immune complexes, could derive from this fact. It will be important to establish their role in the curtailment the host's immune attack on the amoeba.

It would be expected that the formation of surface antigen–antibody complexes should result in the rapid activation of the direct complement system. The majority of axenically cultivated trophozoites exposed to immune sera were rapidly lysed. However, some amoeba were resistant and retained this property on subsequent incubations (Calderon & Tovar Gallegos 1980). It is of interest that trophozoites isolated from the intestine of a patient with invasive amoebiasis were found to be resistant to lysis by the patient's own serum (Trissl 1982).

Virulent *Entamoeba histolytica* glycoproteins are devoid of sialic acid (Feria Velasco *et al.* 1973). It would be expected therefore, that the amoebae should activate the alternative pathway of complement. Indeed, Capin *et al.* (1978) reported that sera from patients with invasive amoebiasis were depleted of C3 while containing normal levels of C1q. It seems likely that the amoebae have developed a means of inactivating the C3 cascade.

Immune response in humans and experimental animals

The general concensus about acquired immunity in humans has been summarized by Trissl (1982). The high titres of humoral antibodies present in patients with dysentery and invasive amoebiasis do not alter the course of the disease nor do they prevent reinfection. This is further supported by the finding that the incidence of the disease increases with age.

It is not clear whether commensal carriers, many of which are asymptomatic, remain apparently healthy because of acquired resistance. No comparison has been made to determine whether sera of individuals with lumenal amoebiasis contain antibodies against different surface elements than those with clinical disease. The local antibody levels in these individuals are not known. A transient rise in IgA coproantibodies has been reported (Trissl 1982). In addition, specific antiamoeba secretory IgA has been reported to be present in mother's milk (Grundy *et al.* 1983).

In contrast, several reports have appeared indicating that animals may acquire resistance to the induction of liver abscesses following exposure to live amoeba or immunization with fractions derived from the trophozoite (Ghadirian *et al.* 1980). Aust Kettis *et al.* (1983) reported the interesting finding that immune rabbit sera interact with different amoeba surface proteins than those detected by human immune sera. In no case where protection was reported have the authors described the nature of the immune reactions involved.

Concluding remarks

In amoebiasis there is no evidence to date that the immune system of the human host is involved in preventing infection or modifying the course of the disease. Although the exact mechanisms remain unknown, immune avoidance must play an important role. Surface redistribution of the antigenic determinants is likely to be central to the process. The finding that lipids are major participants in binding and removing antibodies from the surface, suggests that closer scrutiny of their involvement might yield interesting results.

The pathology of amoebiasis results from the amoebal contact-mediated cytolysis of host tissues. Identification of the participating elements including the surface lectins, amoebapore and proteolytic enzymes may provide targets for immune and chemotherapeutic strategies.

REFERENCES

Adams, D. O. 1980 *J. Immunol.* **124**, 286–292.

Adams, D. O., Kao, K.-J., Farb, R. & Pizzo, S. V. 1980 *J. Immunol.* **124**, 293–300.

Aley, S. B., Scott, W. A. & Cohn, Z. A. 1980 *J. exp. Med.* **152**, 391–404.

Alvarez Alva, R. & de la Loza Saldivar, A. 1971 *Arch. Inv. Med. (Mexico)* **2** (Suppl. 1), 327–332.

Aust Kettis, A. & Sundqvist, K. G. 1978 *Scand. J. Immunol.* **7**, 35–44.

Aust Kettis, A., Thorstensson, R. & Utter, G. 1983 *Am. J. trop. Med. Hyg.* **32**, 512–522.

Bos, H. J., 1979 *Trans. R. Soc. trop. Med. Hyg.* **73**, 120.

Bos, H. J., Leijendekker, W. J. & van den Eijk, A. A. 1980 *Expl Parasitol.* **50**, 342–348.

Brandt, H. & Perez Tamayo, R. 1970 *Hum. Pathol.* **1**, 351–385.

Calderon, J., Munoz, M. de L. & Acosta, H. M. 1980 *J. exp. Med.* **151**, 184–193.

Calderon, J. & Tovar Gallegos, R. 1980 *Arch. Inv. Med. (Mexico)*. **11**, (suppl. 1), 241–243.

Calef, E. & Gitler, C. 1984 *J. Immunol.* (Submitted.)

Capin, N. R., Jimenez, M. Zamaona, G. & Ortiz Ortiz, L. 1978 *Arch. Inv. Med. (Mexico)*. **9** (suppl. 1), 297–303.

Feria Velasco, A., Martinez Zedillo, G., Trevino Garcia Manzo, N. & Gutierrez Pastrana N. D. 1973 *Arch. Inv. Med. (Mexico)*. **4** (suppl. 1). 33–38.

Gadasi, H. & Kessler, E. 1983 *Infect. Immun.* **39**, 528–531.

Ghadirian, E., Meerovitch, E. & Hartman, D.-P. 1980 *Am. J. trop. Med. Hyg.* **29**, 779–784.

Gitler, C. & Bercovivi, T. 1980 *Ann. N.Y. Acad. Sci.* **346**, 199–211.

Glossmann, H. Ferry, D. R., Lubbecke, F., Mewes, R. & Hofmann F. 1982 *Trends Pharmacol. Sci.* **3**, 431–436.

Grundy, M. S., Cartwright-Taylor, L., Lundin, L., Thors, C. & Huldt, G. 1983 *J. clin. Microbiol.* **17**, 753–758.

Isibasi, I., Santa Cruz, M., Ramirez, A. & Kumate, J. 1982 *Arch. Inv. Med. (Mexico)*. **13** (suppl. 3), 51–56.

Johnson, W. J., Whisnant, C. C. & Adams, D. O. 1981 *J. Immunol.* **127**, 1787–1792.

Juniper, K. Jr. 1971 *Bull. N.Y. Acad. Med.* **47**, 448–461.

Kean, B. H., Williams, D. C. & Luminais, S. K. 1979 *Br. J. vener. Dis.* **55**, 375–378.

Knight, R., Bird, R. G. & McCaul, T. F. 1975 *Ann. trop. Med. Parasitol.* **69**, 197–202.

Kobiler, D. & Mirelman, D. 1980 *Infect. Immun.* **29**, 221–225.

Loew, L. M., Gitler, C. & Rosenberg, I. 1982 *Biophys. J.* **37**, 143a.

Loew, L. M., Rosenberg, I., Bridge, M. & Gitler, C. *Biochemistry, Wash.* **22**, 837–842.

Lushbaugh, W. B., Kairalla, A. B., Loadholt, C. B. & Pittman, F. E. 1978 *Am. J. trop. Med. Hyg.* **27**, 248–254.

Lushbaugh, W. B., Kairalla, A. B., Cantey, J. B., Hofbauer, A. F. & Pittman, F. E. 1979 *J. infect. Dis.* **139**, 9–17.

Lynch, E. C., Harris, A., Rosenberg, I. & Gitler, C. 1980 *Biol. Bull. mar. biol. Lab.*, Woods Hole **159**, 496–497.

Lynch, E. C., Rosenberg, I. & Gitler, C. 1982 *EMBO J.* **1**, 801–804.

Martinez Palomo, A. 1982 The biology of *Entamoeba histolytica*. In *Tropical medicine research studies series* (ed. K. N. Brown), vol. 2. Chichester: Research Studies.

McCaul, T. F. 1977 *Z. Parasitenk.* **52**, 203–211.

McCaul, T. F. & Bird, R. G. 1977 *J. Parasitol.* **7**, 383–388.

McCaul, T. F., Poston, R. N. & Bird, R. G. 1977 *Expl Parasitol.* **43**, 342–352.

McGowan, K., Deneke, C. F., Thorne, G. M. & Gorbach, S. L. 1982 *J. infect. Dis.* **146**, 616–625.

Mirelman, D., Feingold, C., Wexler, A. & Rivka, B. 1983 Interactions between *Entamoeba histolytica*, bacteria and intestinal cells. In *Cytopathology of parasitic disease. Ciba Fdn Symp.* **99**, 2–30.

Munoz, M. de L., Calderon, J. & Rojkind, M. 1982 *J. exp. Med.* **155**, 42–51.

Orosco, M. E., Martine Palomo, A., Guarneros, G. Kobiler, D. & Mirelman, D. 1982 *Arch. Inv. Med. (Mexico)*. **13** (suppl. 3), 177–184.

Parkhouse, M., Cid. M. E. & Calderon, J. 1978 *Arch. Inv. Med. (Mexico)*. **9** (suppl. 1), 211–218.

Ravdin, J. I., Croft, B. Y. & Guerrant, R. L. 1980 *J. exp. Med.* **152**, 377–390.

Ravdin, J. I. & Guerrant, R. L. 1981 *J. clin. Invest.* **68**, 1305–1313.

Ravdin, J. I. & Guerrant, R. L. 1982 *Rev. infect. Dis.* **4**, 1185–1207.

Ravdin, J. I., Sperelakis, N. & Guerrant, R. L. 1982 *J. infect. Dis.* **146**, 335–340.

Rosenberg, I. & Gitler, C. 1984 *Mol. Biochem. Parasitol.* (Submitted.)

Sargeaunt, P. G., Jackson, T. F. H. G. & Simjee, A. 1982 *Lancet* i, 1386–1388.

Sepulveda, B. 1970 *Gac. Med. Mex.* **100**, 201–254.

Sepulveda, B. & Martinez Palomo, A. 1984 Amebiasis. In *Tropical and geographical medicine* (ed. K. S. Warren & A. F. Mahmoud). New York: McGraw-Hill.

Serrano, R. & Reeves, R. E. 1975 *Expl Parasitol.* **37**, 411–416.

Sexton, D. J., Krogstad, D. J., Spencer, H. C., Jr., Healy, G. R., Sinclair, S., Sledge, C. E. & Schultz, M. G. 1974 *Am. J. Epidemiol.* **100**, 414–423.

Smolarsky, M. 1980 *J. immunol. Methods* **38**, 85–93.

Stall, A. M. & Knopf, P. M. 1978 *Cell* **14**, 33–42.

Steinman, R. M., Brodie, S. E. & Cohn, Z. A. 1976 *J. exp. Med.* **68,** 665–687.

Trissl, D. Martinez Palomo, A. Arguello, C., de la Torre, M. & de la Hoz, R. 1977 *J. exp. Med.* **145**, 652–655.

Trissl, D. 1982 *Rev. infect. Dis.* **4**, 1154–1184.

Westphal, O. & Jann, K. 1965 Bacterial lipopolysaccharides. Extraction with phenol-water and further applications of the procedure. In *Methods of carbohydrate chemistry* (ed. R. A. Whistler, J. N. BeMiller & M. L. Walfrom), vol. 5, pp. 93–95. New York: Academic Press.

World Health Organization 1969 *W.H.O. Tech. Rep. Ser.* **421**, 5–52.

Yanovsky, A. & Gitler, C. 1984 (In preparation.)

Young, J. D.-E., Young, T. M., Lu, L. P., Unkeless, J. C. & Cohn, Z. A. 1982 *J. exp. Med.* **156**, 1677–1690.