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Review

Current Problems of Amebiasis in Japan and Recent Advances in Amebiasis Research

Tomoyoshi Nozaki*

Department of Parasitology, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo 162-8640, Japan

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CONTENTS:

SUMMARY: Amebiasis is epidemic in two major populations in Japan: male homosexuals and institutionalized people. Currently available diagnostic, chemotherapeutic, and prophylactic measures and their problems are discussed. Recent advances in basic research on amebiasis are also described with new findings of unique metabolisms and intracellular structures and organization. Discoveries and analyses of the unique features presented by this parasitic protist help in our elucidation of the pathogenic mechanisms of the parasite and may eventually lead to the development of new drugs and vaccines against amebiasis.

1. Introduction

The eukaryotic protozoan *Entamoeba histolytica* is a major cause of morbidity and mortality worldwide (1). There are an estimated 50 million cases of invasive amebiasis and 40,000-110,000 death annually (1). The most common clinical presentation of amebiasis is amebic dysentery and colitis; extraintestinal abscesses (i.e., hepatic, pulmonary, and cerebral) are, however, also common and often lethal. Infection of the human host occurs upon ingestion of water or food contaminated by the cyst form of the parasite. These cysts then excyst to form ameboid trophozoites that subsequently colonize the large bowel. Colonization of the human gut by *E. histolytica* most commonly results in an asymptomatic intestinal infection similar to that resulting from the closely related commensal *E. dispar* spp. (2). In approximately 5-10% of those infected with *E. histolytica*, disease and symptoms including bloody diarrhea, fever, tenesmus, and colitis develop. However, the rest of the infected individuals remain asymptomatic. Immunological, biological, and genetic mechanisms that contribute to whether or not a disease develops have not been elucidated.

The aim of this review is to summarize the current situation regarding amebiasis in Japan, and to highlight the remaining problems of diagnosis, treatment, and intervention. Recent advances in basic research to understand the pathogenesis and biochemical and biological peculiarities of this organism will also be discussed.

*Corresponding author: Tel: +81-3-5285-1111 ext. 2733, Fax: +81-3-5285-1173, E-mail: nozaki@nih.go.jp

2. Epidemiology of amebiasis in Japan

Cases of Amebiasis have often been found in two groups in Japan: in male homosexuals (3-7) and in institutionalized people, including the residents of institutions for the mentally retarded (8-13). The high prevalence of amebiasis in male homosexuals is a unique characteristic of amebiasis in Japan. In the early 1980s, a high percentage of the male homosexuals in the United States (U.S.) and Europe were reported to be infected with *E. histolytica*. However, neither the presence of clinical symptoms nor positive serology correlated with the presence of the parasites in the stool (14,15). Furthermore, biochemical and molecular characterization of the isolates from the infected individuals showed that the amoebas from the male homosexuals were not *E. histolytica*, but exclusively *E. dispar*, a non-pathogenic species morphologically indistinguishable from *E. histolytica* (16-18). It is now widely accepted that the male homosexuals in western countries are infected with *E. dispar*, but not with *E. histolytica*. This is in sharp contrast to the situation in Japan, where the male homosexual population exhibited symptoms attributable to invasive amebiasis and seropositiveness to the *E. histolytica* antigens (19,20). Furthermore, zymodeme analysis revealed that the isolates from Japanese male homosexuals have zymodemes indicative of *E. histolytica* (21). A high correlation between seropositiveness to the *E. histolytica* antigens and that to *Treponema pallidum* hemagglutination test

(TPHA) was reported for Japanese male homosexuals (5,19), strongly suggesting that amebiasis in this population is a sexually transmitted disease.

The prevalence of amebiasis in institutionalized people, e.g., the residents of institutes for the mentally retarded, has also been reported several times since late 1980s in Japan (8-13). A high prevalence of amebiasis in institutions was also reported in the U.S., and believed to be associated with typical abnormal behavior of the retarded (22). Besides the residents of institutes and daycare centers, a small percentage of health care workers are also found to be infected (e.g., 2%, [12]). There are also quite a few cases of imported invasive amebiasis, which presumably constitutes up to 20% of all cases in Japan (e.g., [7]). In contrast to the adult domestic cases, which are almost exclusively male, there is no reported bias on gender in the imported cases. However, it has been shown by field studies conducted in Vietnam that males have a higher risk of developing liver abscesses than females (Tannich et al., unpublished), suggesting that gender bias regarding a susceptibility to amebic liver abscess exists.

3. Current problems of amebiasis

(1) Diagnosis

A majority of all diagnoses of amebic infection have been, and still are, based on light microscopic demonstration of hematophagous trophozoites in stool or liver aspirate. Physicians and lab technicians need to be careful not to overdiagnose *E. histolytica* by mistakenly identifying non-pathogenic amoebae, macrophages, or white blood cells. Unfortunately, microscopy does not differentiate between *E. histolytica* and *E. dispar*. This is not usually a problem in diagnosing invasive amebiasis, since a demonstration of hematophagous trophozoites in the specimen represents a definitive diagnosis. However, asymptomatic cyst passers of either the pathogenic *E. histolytica* or *E. dispar* need to be differentiated, since cyst passers of *E. dispar* do not require treatment. In contrast, asymptomatic *E. histolytica* cyst passers must be treated since they are a potential public health hazard, and long-term carriage could lead to later acute invasive disease.

A conventional method of differentiating the two ameba *E. histolytica* and *E. dispar* is cultivation and propagation of the isolated amoebae, followed by zymodeme profiling by isoenzyme electrophoresis. However, these procedures are time- and labor-consuming, and therefore are routinely performed in only a few laboratories in Japan, including the Department of Tropical Medicine and Parasitology, Keio University School of Medicine. However, antigen-capture enzyme-linked immunosorbent assays (ELISA) using monoclonal antibodies that distinguish between *E. histolytica* and *E. dispar* are commercially available (e.g., Techlab, Inc., Blacksburg, Va, USA). Alternatively, to detect parasite DNA in the specimen, a method to specifically amplify either *E. histolytica* or *E. dispar* genomic DNA by polymerase chain reaction (PCR) is available in some laboratories (e.g., Department of Infectious Diseases, Tokai University School of Medicine, and Keio University).

Infection with the pathogenic *E. histolytica* is often, but not necessarily always, associated with positive serologic tests, including gel diffusion precipitin test, hemagglutination test, immunofluorescence assay, and ELISA. These tests can be performed either at private laboratories, universities, or national institutes, where reagents are available. Some kits, including hemagglutination (*E. histolytica* HA, Japan Lyophilization Laboratory, Tokyo, discontinued) and indirect immunofluorescence assay (Ameba-spot IF, #72901, Biomerieux, Tokyo), are commercially available.

(2) Treatment

As previously mentioned, *E. dispar* cyst passers require no treatment. This may pose the medical care provider with a question: whether or not asymptotic cyst passers need to be treated, because most physicians and clinical lab workers do not have immediate access to laboratories where isoenzyme characterization and PCR are routinely performed. However, consultation with these laboratories is strongly encouraged because asymptomatic *E. histolytica* cyst passers are a potential threat to public health, and there is also have a possibility of future relapse. Freezing a stool specimen normally preserves the integrity of parasite DNA for at least a few months for future identification of the infecting parasites by a variety of PCR-based methods, and therefore is strongly recommended. After a diagnosis is made, the *E. histolytica* cyst passers should be treated with one of the lumen-acting amebacides described below.

The commonly used anti-ameba drugs metronidazole (Fragyl) and tinidazole (Fasigyn) have been proven to be effective against invasive amebiasis, including both intestinal and extraintestinal amebiasis. Extensive clinical experience and epidemiologic data showed that these compounds are not carcinogenic or mutagenic in humans (23). Chloroquine, emetine, and dehydroemethine have been successfully added to metronidazole treatment for the rare patient not responding to metronidazole or tinidazole alone (24,25). However, these compounds are well absorbed from the intestinal lumen, and, therefore, are generally not effective in the treatment of asymptomatic cyst passers unless therapy is prolonged for a minimum of 10 days. With shorter courses of metronidazole therapy, patients may suffer a relapse of invasive amebiasis months later (24,25). The lumen-acting drugs diloxanide furoate (Furamide), iodoquinol (Diodoquin), and paromomycin (Humatin) are often used to treat asymptomatic cyst passers and also to prevent relapses from persistent cysts after invasive amebiasis. Successful treatment of the cyst passers with metronidazole only or with metronidazole and diloxanide furoate on 5 consecutive days followed by 5 more days of metronidazole was also reported for several Japanese cases (11,13). Whether or not treatment is effective for each cyst passer could depend upon biological differences in the virulence of the infecting strain and/or genetic differences in the human immune background (see below).

The fact that not only institutionalized people, but also a fraction of health care workers are infected, suggests that transmission to healthy individuals actually occur. As this transmission could pose a serious threat to public health, those infected need to be identified and properly treated. To prevent transmission among homosexual males, people should be made aware of transmission by oral-anal sexual practices and of the relation between infection and sexual promiscuity.

(3) Vaccine

E. histolytica exhibits a rather simple developmental transition in its life cycle, having only two known stages, trophozoite and cyst. It has been suggested that a great proportion of proteins are expressed in both developmental stages, providing logistic identification of target molecules expressed in both stages. There are no insect vectors or secondary hosts involved in the transmission of amebiasis. Furthermore, as *E. histolytica* is an extracellular parasite, an antibody is predicted to play a potential important role in vaccine-induced

immunity to amebic infection. All these suggest that we have a very good chance of developing a successful amebiasis vaccine. Several recombinant proteins have been tested for their efficacy in the gerbil model, including serine-rich *E. histolytica* protein (SREHP) (26), Gal/GalNAc-inhibitable lectin (27), and Peroxiredoxin (28). Animal studies using these recombinant proteins demonstrated the feasibility of developing a recombinant parenteral vaccine to prevent amebic liver abscess (29-38). Since *E. histolytica* first adheres to intestinal epithelial cells to cause disease, mucosal anti-amebic antibodies should play a critical role in defense. Stanley and colleagues, therefore, utilized an antigen delivery and presenting system that is capable of stimulating mucosal IgA antibody production. They first produced the dodecapeptide repeat of SREHP fused to the cholera toxin B subunit. When this antigen was orally administered, IgA anti-ameba antibodies were induced in the stool and in bile, and the numbers of antibody-secreting cells producing anti-SREHP antibodies were also significantly increased in mesenteric lymph nodes and the spleen (32). They subsequently explored the use of attenuated strains of *Salmonella* as carriers to deliver the SREHP or Gal/GalNAc lectin protein to induce mucosal and cell-mediated anti-amebic immune responses (33, 38, 39), showing that in the gerbil model, an oral vaccine combining the recombinant amebic proteins and the attenuated *Salmonella* strain can provide protection against amebic liver abscess. Successful utilization of the *Salmonella* and the *Vibrio* strains (35) for delivery of vaccine antigens provides the possibility of a combination amebiasis/typhoid fever and amebiasis/cholera vaccine.

Although all of these studies appear promising, there are still a number of important issues to be resolved. First, all of these studies were conducted to assess protection against amebic liver abscess with only one animal-model gerbil. Consequently, it is largely unknown whether or not the observed protection in the gerbil reflects the potential success of an ameba vaccine against human intestinal amebiasis. Second, the natural polymorphism of these target molecules remains unknown. Therefore, it is not known whether immunization with recombinant proteins from one reference strain can protect gerbils against heterologous *E. histolytica* strains. Third, currently used attenuated *S. typhi* strains still have, as shown in previous clinical trials in humans, side effects such as fever and diarrhea with vaccination. Thus, a truly innocuous host strain needs to be developed.

4. Recent developments in basic research on amebiasis

(1) Virulence factors

Quite a few virulence factors have been identified in the last 10-15 years. These factors include cysteine proteinases (40-44), Gal/GalNAc inhibitable lectin (45-49), amebapore (50-54), and phosholipase (55-57), and details on each factor should be consulted in the original references.

Antioxidative mechanisms of *E. histolytica* are also a part of the virulence function, as the parasite has to detoxify the host oxidative stress in order to survive in oxygen-rich tissues and organs, including the liver, lung, and brain. In contrast to other eukaryotic cells, *E. histolytica* lacks glutathione and glutathione-dependent enzymes (58). L-Cysteine is the major thiol in *E. histolytica* and is presumed to play an important role in antioxidative defenses (58,59). L-Cysteine has been shown to be essential in its attachment to the matrix (60), elongation, motility, and growth in vitro (61,62). As shown in Fig. 1, *E. histolytica* possesses a sulfur assimilatory cysteine biosynthetic pathway, where inorganic sulfur is fixed into an organic compound, L-cysteine, in the pathway that is absent in its mammalian hosts (63-66). Biochemical and reverse genetic studies of the major enzymes of this pathway showed that this pathway plays an important role in monitoring total cellular thiol concentrations and as a part of an antioxidative mechanism (65). Since this pathway is absent in mammalian hosts, it is a good target for developing a new chemotherapeutic. Peroxiredoxin (formally called as 29- or 30-kDa cysteine-rich protein [67-69]) is a ubiquitous antioxidant conserved from prokaryotes to higher eukaryotes, and has also been shown to be involved, in amoeba, in an antioxidative mechanism (70,71) together with NADPH: flavin oxidoreductase (70).

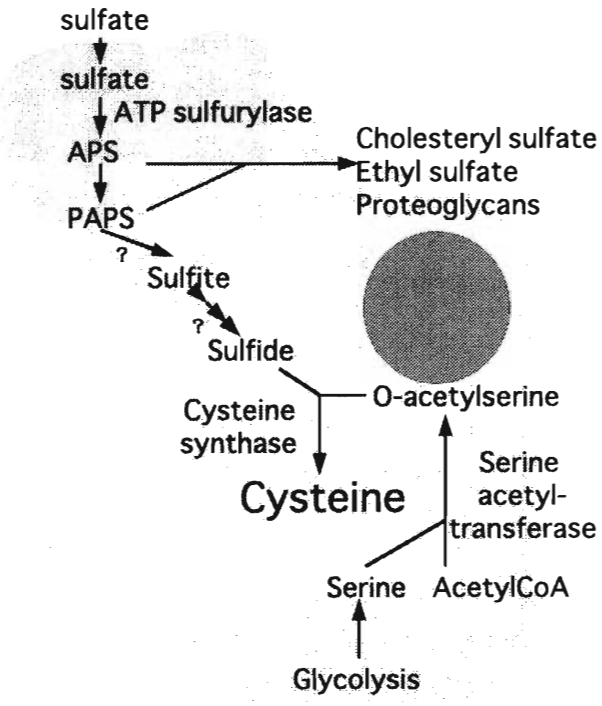

Fig. 1. A schematic representation of the sulfur assimilatory cysteine biosynthetic pathway in *E. histolytica*.

(2) Peculiar organellar structure

Entamoeba trophozoites appear to have a very primitive cytoplasmic structure (Fig. 2A). The major cytoplasmic components are the ingestive and digestive vacuoles, the residual bodies, the nucleus, and a number of smaller membranous vesicles. α-Glycogen is distributed throughout the cytoplasm. The rough endoplasmic reticulum (ER) is not present in the usual form, but is functionally replaced by shorter lengths of helically arranged ribosomes. These helices coalesce into bundles as larger crystalline arrays, forming the chromatoid bodies. The smooth ER is also poorly developed. Both typical Golgi apparatus and mitochondria are absent. The trophozoites also lack peroxisome and hydrogenosome. *E. histolytica* has long been thought to be an ancient eukaryote due to a lack of mitochondrion, a major source of ATP in most eukaryotic cells. However, a recent demonstration of two *E. histolytica* genes encoding proteins that in other eukaryotes are localized in the mitochondria, i.e., pyridine nucleotide transhydrogenase and the chaperonin cpn60, unequivocally proved that *E.*

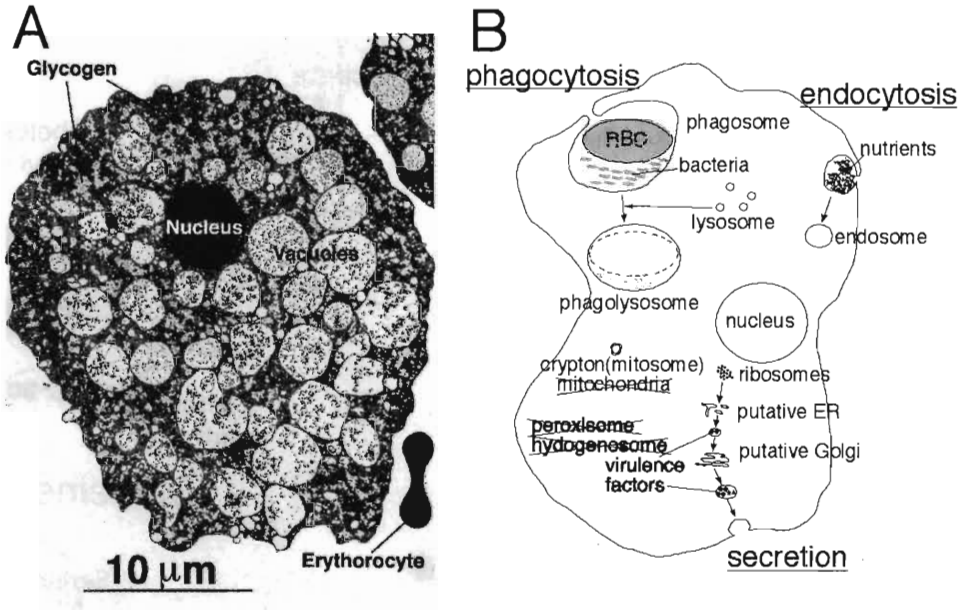

Fig. 2. (A) Transmission electron micrograph of the *E. histolytica* trophozoite. A bar represents 10 μm. (B) A schematic representation of a variety of cellular trafficking events in the *E. histolytica* trophozoite.

histolytica secondarily lost mitochondrial function during evolution (72). Although the ameba does not apparently have a structure that resembles mitochondria, immunolocalization revealed that a residual organelle called a crypton or mitosome is present in the cytoplasm (73, 74). Ghosh et al. have recently shown that this residual organelle contains double-stranded DNA and is bound by a double membrane (75), suggesting that this organelle shares some structural similarities with mitochondria. The real function of this mitochondria-like organelle will likely be revealed by further investigation. In addition to the lack of mammalian-like mitochondria, an anaerobic organelle called hydrogenosome, present in an anaerobic protozoan *Trichomonas*, is also absent in *E. histolytica*. Furthermore, amoebas are not able to use hydrogen as an electron acceptor, suggesting that hydrogenosome is not functional even if it is present. However, pyruvate:ferredoxin oxidoreductase (POR) from *E. histolytica* showed a close phylogenetic association with the POR of the enterobacteria and *T. vaginalis*, which targets its POR to hydrogenosome (76). Moreover, a gene encoding iron hydrogenase, demonstrated in hydrogenosome of *Trichomonas* (77), is present in *Entamoeba*, and its recombinant protein is enzymatically active in vitro.

Vesicular transport in *E. histolytica* is believed to share a common basic machinery with that in other eukaryotes (Fig. 2B). Common proteins involved in vesicular transport and shared by both *E. histolytica* and other eukaryotes include small GTP-binding proteins (Rab [78-80] and Arf [81]), ER chaperons BiP (81,82) and putative disulfide isomerase, signal recognition particle (Srp54) (83) and ER membrane protein Sec61, which is involved in transport from the cytosol to the ER lumen, Golgi protein Erd2 (84), and lysosomal vacuolar ATPase (85). More than ten Rab genes have been identified and characterized from *E. histolytica* (78-80). All of these Rab genes obtained by sequence homology or from EST databases show high identities to Rabs from other eukaryotes. However, domains known to interact with regulatory proteins, including GTPase activating protein and guanine nucleotide exchange factor, vary significantly from Rabs from other species. Furthermore, Rab5 from *E. histolytica*, for example, does not reverse the a growth defect of a yeast rab5-mutant, and is involved in phagocytosis, whereas, in yeast and mammalian cells, Rab5 is located in endosomes and is involved in vesicular transport from the plasma membrane to early endosome and in the homotypic fusion of early endosomes during endocytosis (78). Aley et al. showed that *E. histolytica* trophozoites possess a unique non-acidified rapidly exchanging endocytic compartment (86). These data suggest that *Entamoeba* has developed unique machinery in protein trafficking, especially in the endocytic and phagocytic pathways, that significantly differs from that of other eukaryotes, although *E. histolytica* mostly shares identical proteins for vesicular transport from ER to Golgi.

(3) Prokaryote-like energy metabolisms

E. histolytica is a unique eukaryote with respect to energy metabolism (87,88). As a mitochondia-lacking, and anaerobic or microaerophilic eukaryote, *E. histolytica* largely depends on glycolysis and fermentation for its energy production. *E. histolytica* lacks several typical eukaryotic glycolytic enzymes. Pentose phosphate shunt enzymes are completely missing. Another unusual feature of glycolysis is the utilization of pyrophosphate instead of ATP in several enzyme reactions: phosphofructokinase (89), phosphoenolpyruvate carboxytransphosphorylase (90), pyruvate phosphate dikinase (91), and acetate kinase. *E. histolytica* also possesses fermentation enzymes that resemble those of anaerobic bacteria. These enzymes include POR, ferredoxin, and alcohol dehydrogenase. Phylogenetic analyses strongly suggest that these amebic genes encoding fermentation enzymes are laterally transferred from bacteria that amebae have phagocytosed (76). This so-called "net hypothesis" is supported better than "tree hypothesis", which proposes that these amebic genes were present in a common eukaryotic ancestor that lived under anaerobic conditions, but were lost from other eukaryotes because fermentation enzymes are not necessary for growth in the presence of oxygen (76).

5. Remaining problems in amebiasis research

Why does disease occur only in 5-10% of those infected? This has been a long-lasting question since the first description of amebiasis in 1875 by Lesh (Losch) ([92], for history of amebiasis also see [93,94]), and we still do not have a proper answer to it. There are three possible explanations. 1. Commensal theory. *E. histolytica* is usually viewed as an innocuous gut commensal. However, with a yet-unconfirmed stimulus (e.g., nature of gut flora, dietary implications), it turns to a tissue-invading form that lost its capacity for cyst production. Thus far there is no direct evidence supporting this hypothesis of a possible conversion from a non-virulent to a virulent type, such as an identification of differentially expressed pathogenic factors. 2. Host immune theory. It depends on the genetic background of host immune systems with regard to whether amebiasis develops in the infected individual. It was reported that individuals with a certain type of human leucocyte antigen (HLA) in Mexican mestizo (DR3) are more susceptible to amebic liver abscess (95). Although no such association was found between HLA types and amebic colitis (96), the contribution of the host genetic background on the susceptibility to infectious diseases is widely known (e.g., [97,98]). In *E. histolytica* infections, macrophages play a crucial role in host defense involving both oxidative (e.g., O_2^- and H_2O_2) and non-oxidative (e.g., tumor necrosis factor [TNFα] and nitric oxide [NO]) mechanisms (99-103). Therefore, it is reasonable to investigate whether polymorphisms of the genes involved in TNFα and NO production are associated with susceptibility to amebiasis. 3. Spectrum of virulence among the *E. histolytica* isolates. Not all ameba strains are equally virulent: i.e., there is a spectrum for the strength of virulence in the *E. histolytica* population. It is known, based on zymodeme characterization, that there are at least three *E. histolytica* subtypes (104). Recent studies on the diversity of the *E. histolytica* field isolates revealed that genetic polymorphisms of several genes, including SREHP and chitinase, exist in *E. histolytica* (105). Clark and his colleagues have also initiated an investigation of clonal diversity within *E. histolytica* spp. using novel polymorphic alleles (Clark, unpublished).

There are more than a dozen unanswered questions that are not discussed in this review. These questions were also summarized by Graham Clark in the opening lecture at the XIVth Seminar on Amebiasis, Mexico City, 2000. Important basic questions regarding amoeba biology, molecular biology, and biochemistry include: Are there any other stages, including a sexual stage, in its life cycle? How are encystation and excystation controlled? What is the ploidy of amoeba? How are chromosomes organized in amoeba? Where did amoebic genes originate? How does signal transduction control a variety of processes in amoeba? How does amoeba recognize itself to avoid killing itself? Clinical and epidemiological questions to be answered include: How common is a mixed infection of *E. histolytica* and other parasites? Is there protective immunity against amebiasis? What is the dose necessary for infection? What triggers extra-intestinal propagation of amoebae?

As *E. histolytica* genome database is rapidly growing, enormous information regarding unique and, therefore, applicable aspects of metabolisms, biological pathways, and gene expression should be readily available. Such information from genomics, together with conventional research results, should help in further exploring possible targets in the development of new chemotherapeutic and prophylactic measures. Considering the rapid occurrence of multidrug resistant strains of protozoan and prokaryotic pathogens, e.g., *Plasmodium* and *Mycobacteria*, drug-resistant strains of *E. histolytica* will probably emerge in the future. Therefore, we need to continue to look for parasite-specific cellular events not only to elucidate pathogenic mechanisms of the parasite, but also to eventually develop measures to intervene in transmission of the disease.

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REFERENCES

1. World Health Organization (1995): The World Health Report 1995: Bridging the Gaps.World Health Organization, Geneva.
2. Diamond, L. S. and Clark, C. G. (1993): A redescription of *Entamoeba histolytica* Schaudinn, 1903 (Emended Walker, 1911) separating it from *Entamoeba dispar* Brumpt, 1925. J. Eukaryot. Microbiol., 40, 340-344.
3. Takeuchi, T., Okuzawa, E., Nozaki, T., Kobayashi, S., Mizokami, M., Minoshima, N., Yamamoto, M. and Isomura, S. (1989): High seropositivity of Japanese homosexual men for amebic infection letter. J. Infect. Dis., 159, 808.
4. Takeuchi, T., Miyahira, Y., Kobayashi, S., Nozaki, T., Motta, S. R. and Matsuda, J. (1990): High seropositivity for *Entamoeba histolytica* infection in Japanese homosexual men: further evidence for the occurrence of pathogenic strains. Trans. R. Soc. Trop. Med. Hyg., 84, 250-251.
5. Maeda, K., Yoshikawa, K., Terao, S., Yamamoto, S. and Umeyama, K. (1990): Clinical investigation of 10 patient with amebic dysentery. Nippon Shokakibyo Gakkai Zasshi, 87, 1525-1531 (in Japanese).
6. Fujihara, T., Nagai, Y., Kubo, T., Seki, S. and Satake, K. (1996): Amebic liver abscess. J. Gastroenterol., 31, 659-663.
7. Ohnishi, K. and Murata, M. (1997): Present characteristics of symptomatic amebiasis due to *Entamoeba histolytica* in the east-southeast area of Tokyo. Epidemiol. Infect., 119, 363-367.
8. Kaneda, Y., Nagakura, K., Tachibana, H., Tanaka, T. and Sasao, M. (1988): *Entamoeba histolytica* infection in a rehabilitation center for mentally retarded persons in Japan. Scand. J. Infect. Dis., 20, 687.
9. Nagakura, K., Tachibana, H., Tanaka, T., Kaneda, Y., Tokunaga, M., Sasao, M. and Takeuchi, T. (1989): An outbreak of amebiasis in an institution for the mentally retarded in Japan. Jpn. J. Med. Sci. Biol., 42, 63-76.
10. Nagakura, K., Tachibana, H., Kaneda, Y., Suzuki, H., Sasaoka, K., Kobayashi, S. and Takeuchi, T. (1990): Amebiasis in institutions for the mentally retarded in Kanagawa Prefecture, Japan. Jpn. J. Med. Sci. Biol.,

43, 123-131.
11. Sahara, K., Hori, W., Masuda, T., Ohata, K., Yamazaki, F., Akahane, S., Hattori, H., Yuasa, H., Hashizume, I., Tanaka, Y., Nagakura, K. and Tachibana, H. (1996): Invasive amebiasis at an institution for the mentally retarded in Shizuoka Prefecture. J. Jpn. Assoc. Infect. Dis., 70, 1-6 (in Japanese).
12. Abe, N., Nishikawa, Y., Yasukawa, A. and Haruki, K. (1999): *Entamoeba histolytica* outbreaks in institutions for the mentally retarded. Jpn. J. Infect. Dis., 52, 135-136.
13. Terai, K., Masuda, T. and Miyamoto, H. (1999): Survey of amebiasis at an institution for the mentally retarded in Shizuoka Prefecture. J. Jpn. Assoc. Infect. Dis., 73, 626-627 (in Japanese).
14. Allason-Jones, E., Mindel, A., Sargeaunt, P. and Williams, P. (1986): *Entamoeba histolytica* as a commensal intestinal parasite in homosexual men. N. Engl. J. Med., 315, 353-356.
15. Goldmeier, D., Sargeaunt, P. G., Price, A. B., Munday, P. E., Billington, O., Dixon, I., Borriello, P., Carder, J. M., Shaw, A., Hilton, J. and Jefferies, D. J. (1986): Is *Entamoeba histolytica* in homosexual men a pathogen? Lancet, 1, 641-644.
16. Sargeaunt, P. G., Oates, J. K., MacLennan, I., Oriel, J. D. and Goldmeier, D. (1983): *Entamoeba histolytica* in male homosexuals. Br. J. Vener. Dis., 59, 193-195.
17. Sargeaunt, P. G. (1984): Amoebiasis in homosexual men letter. Gut, 25, 1434.
18. Allason Jones, E., Mindel, A., Sargeaunt, P. and Williams, P. (1986): *Entamoeba histolytica* as a commensal intestinal parasite in homosexual men. N. Engl. J. Med., 315, 353-356.
19. Takeuchi, T., Kobayashi, S., Asami, K. and Yamaguchi, N. (1987): Correlation of positive syphilis serology with invasive amebiasis in Japan. Am. J. Trop. Med. Hyg., 36, 321-324.
20. Takeuchi, T., Matsuda, H., Okuzawa, E., Nozaki, T., Kobayashi, S. and Tanaka, H. (1988): Application of a micro enzyme-linked immunosorbent assay (ELISA) to detection of anti-amebic antibody in various forms of amebic infection. Jpn. J. Exp. Med., 58, 229-232.
21. Nozaki, T., Motta, S. R., Takeuchi, T., Kobayashi, S. and Sargeaunt, P. G. (1989): Pathogenic zymodemes of *Entamoeba histolytica* in Japanese male homosexual population. Trans. R. Soc. Trop. Med. Hyg., 83, 525.
22. Brooke, M. M. (1963): Epidemiology and control of amebiasis in institutions for the mentally retarded. Am. J. Ment. Defic., 68, 187-192.
23. Koch-Weser, J. and Goldman, P. (1980): Drug therapy: metronidazole. N. Engl. J. Med., 303, 1212-1218.
24. Bassily, S., Farid, Z., el Masry, N. A. and Mikhail, E. M. (1987): Treatment of intestinal *E. histolytica* and G. lamblia with metronidazole, tinidazole and ornidazole: a comparative study. J. Trop. Med. Hyg., 90, 9-12.
25. Simjee, A. E., Gathiram, V., Jackson, T. F. and Khan, B. F. (1985): A comparative trial of metronidazole v. tinidazole in the treatment of amoebic liver abscess. S. Afr. Med. J., 68, 923-924.
26. Stanley, S.L., Jr., Becker, A., Kunz Jenkins, C., Foster, L. and Li, E. (1990): Cloning and expression of a membrane antigen of *Entamoeba histolytica* possessing multiple tandem repeats. Proc. Natl. Acad. Sci. USA, 87, 4976-4980.
27. Petri, W. A., Jr., Smith, R. D., Schlesinger, P. H., Murphy, C. F. and Ravdin, J. I. (1987): Isolation of the galactose-binding lectin that mediates the in vitro adherence of *Entamoeba histolytica*. J. Clin. Invest., 80, 1238-1244.
28. Torian, B. E., Flores, B. M., Stroeher, V. L., Hagen, F. S. and Stamm, W. E. (1990): cDNA sequence analysis of a 29-kDa cysteine-rich surface antigen of pathogenic *Entamoeba histolytica*. Proc. Natl. Acad. Sci. USA, 87, 6358-6362.
29. Zhang, T. and Stanley, S. L., Jr. (1994): Protection of gerbils from amebic liver abscess by immunization with a recombinant protein derived from the 170-kilodalton surface adhesin of *Entamoeba histolytica*. Infect. Immun., 62, 2605-2608.
30. Zhang, T., Cieslak, P. R. and Stanley, S. L., Jr. (1994): Protection of gerbils from amebic liver abscess by immunization with a recombinant *Entamoeba histolytica* antigen. Infect. Immun., 62, 1166-1170.
31. Stanley, S. L., Jr., Blanchard, J. L., Johnson, N., Foster, L., Kunz Jenkins, C., Zhang, T., Tian, K. and Cogswell, F. B. (1995): Immunogenicity of the recombinant serine rich *Entamoeba histolytica* protein (SREHP) amebiasis vaccine in the African green monkey. Vaccine, 13, 947-951.
32. Zhang, T., Li, E. and Stanley, S. L., Jr. (1995): Oral immunization with the dodecapeptide repeat of the serine-rich *Entamoeba histolytica* protein (SREHP) fused to the cholera toxin B subunit induces a mucosal and systemic anti-SREHP antibody response. Infect. Immun., 63, 1349-1355.
33. Zhang, T. and Stanley, S. L., Jr. (1996): Oral immunization with an attenuated vaccine strain of *Salmonella typhimurium* expressing the serine-rich *Entamoeba histolytica* protein induces an antiamebic immune response and protects gerbils from amebic liver abscess. Infect. Immun., 64, 1526-1531.
34. Seydel, K. B., Braun, K. L., Zhang, T., Jackson, T. F. and Stanley, S. L., Jr. (1996): Protection against amebic liver abscess formation in the severe combined immunodeficient mouse by human anti-amebic antibodies. Am. J. Trop. Med. Hyg., 55, 330-332.
35. Ryan, E. T., Butterton, J. R., Zhang, T., Baker, M. A., Stanley, S. L., Jr. and Calderwood, S. B. (1997): Oral immunization with attenuated vaccine strains of *Vibrio cholerae* expressing a dodecapeptide repeat of the serine-rich *Entamoeba histolytica* protein fused to the cholera toxin B subunit induces systemic and mucosal antiamebic and anti-*V. cholerae* antibody responses in mice. Infect. Immun., 65, 3118-3125.
36. Sultan, F., Jin, L. L., Jobling, M. G., Holmes, R. K. and Stanley, S. L., Jr. (1998): Mucosal immunogenicity of a holotoxin-like molecule containing the serine-rich *Entamoeba histolytica* protein (SREHP) fused to the A2 domain of cholera toxin. Infect. Immun., 66, 462-468.
37. Zhang, T. and Stanley, S. L. (1999): DNA vaccination with the serine rich *Entamoeba histolytica* protein (SREHP) prevents amebic liver abscess in rodent models of disease. Vaccine, 18, 868-874.
38. Lotter, H., Khajawa, F., Stanley, S. L., Jr. and Tannich, E. (2000): Protection of gerbils from amebic liver abscess by vaccination with a 25-mer peptide derived from the cysteine-rich region of *Entamoeba histolytica* galactose-specific adherence lectin. Infect. Immun., 68,

4416-4421.
39. Cieslak, P. R., Zhang, T. and Stanley, S. L., Jr. (1993): Expression of a recombinant *Entamoeba histolytica* antigen in a *Salmonella typhimurium* vaccine strain. Vaccine, 11, 773-776.
40. Stanley, S. L., Jr., Zhang, T., Rubin, D. and Li, E. (1995): Role of the *Entamoeba histolytica* cysteine proteinase in amebic liver abscess formation in severe combined immunodeficient mice. Infect. Immun., 63, 1587-1590.
41. Keene, W. E., Petitt, M. G., Allen, S. and McKerrow, J. H. (1986): The major neutral proteinase of *Entamoeba histolytica*. J. Exp. Med., 163, 536-549.
42. Bruchhaus, I., Jacobs, T., Leippe, M. and Tannich, E. (1996): *Entamoeba histolytica* and *Entamoeba dispar*: differences in numbers and expression of cysteine proteinase genes. Mol. Microbiol., 22, 255-263.
43. Jacobs, T., Bruchhaus, I., Dandekar, T., Tannich, E. and Leippe, M. (1998): Isolation and molecular characterization of a surface-bound proteinase of *Entamoeba histolytica*. Mol. Microbiol., 27, 269-276.
44. Ankri, S., Stolarsky, T. and Mirelman, D. (1998): Antisense inhibition of expression of cysteine proteinases does not affect *Entamoeba histolytica* cytopathic or haemolytic activity but inhibits phagocytosis. Mol. Microbiol., 28, 777-785.
45. Petri, W. A., Jr. (1996): Recent advances in amebiasis. Crit. Rev. Clin. Lab. Sci., 33, 1-37.
46. Dodson, J. M., Clark, C. G., Lockhart, L. A., Leo, B. M., Schroeder, J. W. and Mann, B. J. (1997): Comparison of adherence, cytotoxicity, and Gal/GalNAc lectin gene structure in *Entamoeba histolytica* and *Entamoeba dispar*. Parasitol. Int., 46, 225-235.
47. Saffer, L. D. and Petri, W. J. (1991): Role of the galactose lectin of *Entamoeba histolytica* in adherence-dependent killing of mammalian cells. Infect. Immun., 59, 4681-4683.
48. Braga, L. L., Ninomiya, H., McCoy, J. J., Eacker, S., Wiedmer, T., Pham, C., Wood, S., Sims, P. J. and Petri, W. A., Jr. (1992): Inhibition of the complement membrane attack complex by the galactose-specific adhesion of *Entamoeba histolytica*. J. Clin. Invest., 90, 1131-1137.
49. Vines, R. R., Ramakrishnan, G., Rogers, J. B., Lockhart, L. A., Mann, B. J. and Petri, W. A., Jr. (1998): Regulation of adherence and virulence by the *Entamoeba histolytica* lectin cytoplasmic domain, which contains a beta2 integrin motif. Mol. Biol. Cell, 9, 2069-2079.
50. Leippe, M., Ebel, S., Schoenberger, O. L., Horstmann, R. D. and Muller-Eberhard, H. J. (1991): Pore-forming peptide of pathogenic *Entamoeba histolytica*. Proc. Natl. Acad. Sci. USA, 88, 7659-7663.
51. Leippe, M. (1995): Ancient weapons: NK-lysin, is a mammalian homolog to pore-forming peptides of a protozoan parasite letter. Cell, 83, 17-18.
52. Leippe, M., Andra, J. and Muller Eberhard, H. J. (1994): Cytolytic and antibacterial activity of synthetic peptides derived from amoebapore, the pore-forming peptide of *Entamoeba histolytica*. Proc. Natl. Acad. Sci. USA, 91, 2602-2606.
53. Leippe, M., Bahr, E., Tannich, E. and Horstmann, R. D. (1993): Comparison of pore-forming peptides from pathogenic and nonpathogenic *Entamoeba histolytica*. Mol. Biochem. Parasitol., 59, 101-109.
54. Bracha, R., Nuchamowitz, Y., Leippe, M. and Mirelman, D. (1999): Antisense inhibition of amoebapore expression in *Entamoeba histolytica* causes a decrease in amoebic virulence. Mol. Microbiol., 34, 463-472.
55. Vargas-Villarreal, J., Olvera-Rodriguez, A., Mata-Cardenas, B. D., Martinez-Rogriguez, H. G., Said-Fernandez, S. and Alagon-Cano, A. (1998): Isolation of an *Entamoeba histolytica* intracellular alkaline phospholipase A2. Parasitol. Res., 84, 310-314.
56. Ravdin, J. I., Murphy, C. F., Guerrant, R. L. and Long-Krug, S. A. (1985): Effect of antagonists of calcium and phospholipase A on the cytopathogenicity of *Entamoeba histolytica*. J. Infect. Dis., 152, 542-549.
57. Long-Krug, S. A., Fischer, K. J., Hysmith, R. M. and Ravdin, J. I. (1985): Phospholipase A enzymes of *Entamoeba histolytica*: description and subcellular localization. J. Infect. Dis., 152, 536-541.
58. Fahey, R. C., Newton, G. L., Arrick, B., Overdank Bogart, T. and Aley, S. B. (1984): *Entamoeba histolytica*: a eukaryote without glutathione metabolism. Science, 224, 70-72.
59. Mehlotra, R. K. and Tekwani, B. L. (1999): Molecular basis of defence against oxidative stress in *Entamoeba histolytica* and *Giardia lamblia*. Microbes Infect., 1, 385-394.
60. Gillin, F. D. and Diamond, L. S. (1980): Attachment of *Entamoeba histolytica* to glass in a defined maintenance medium: specific requirement for cysteine and ascorbic acid. J. Protozool., 27, 474-478.
61. Gillin, F. D. and Diamond, L. S. (1980): Attachment and short-term maintenance of motility and viability of *Entamoeba histolytica* in a defined medium. J. Protozool., 27, 220-225.
62. Gillin, F. D. and Diamond, L. S. (1981): *Entamoeba histolytica* and *Giardia lamblia*: effects of cysteine and oxygen tension on trophozoite attachment to glass and survival in culture media. Exp. Parasitol., 52, 9-17.
63. Nozaki, T., Asai, T., Kobayashi, S., Ikegami, F., Noji, M., Saito, K. and Takeuchi, T. (1998): Molecular cloning and characterization of the genes encoding two isoforms of cysteine synthase in the enteric protozoan parasite *Entamoeba histolytica*. Mol. Biochem. Parasitol., 97, 33-44.
64. Nozaki, T., Arase, T., Shigeta, Y., Asai, T., Leustek, T. and Takeuchi, T. (1998): Cloning and bacterial expression of adenosine-5'-triphosphate sulfurylase from the enteric protozoan parasite *Entamoeba histolytica*. Biochim. Biophys. Acta, 1429, 284-291.
65. Nozaki, T., Asai, T., Sanchez, L. B., Kobayashi, S., Nakazawa, M. and Takeuchi, T. (1999): Characterization of the gene encoding serine acetyltransferase, a regulated enzyme of cysteine biosynthesis from the protist parasites *Entamoeba histolytica* and *Entamoeba dispar*. Regulation and possible function of the cysteine biosynthetic pathway in *Entamoeba*. J. Biol. Chem., 274, 32445-32452.
66. Nozaki, T., Tokoro, M., Imada, M., Saito, Y., Abe, Y., Shigeta, Y. and Takeuchi, T. (2000): Cloning and biochemical characterization of genes encoding two isozymes of cysteine synthase from *Entamoeba dispar*. Mol. Biochem. Parasitol., 107, 129-133.
67. Reed, S. L., Flores, B. M., Batzer, M. A., Stein, M. A., Stroeher, V. L., Carlton, J. E., Diedrich, D. L., Torian, B. E. and Reeves, R. E. (1992): Molecular and cellular characterization of the 29-kilodalton peripheral membrane protein of *Entamoeba histolytica*: differentiation

between pathogenic and nonpathogenic isolates. Infect. Immun., 60, 542-549.
68. Tachibana, H., Kobayashi, S., Kato, Y., Nagakura, K., Kaneda, Y. and Takeuchi, T. (1990): Identification of a pathogenic isolate-specific 30,000-Mr antigen of *Entamoeba histolytica* by using a monoclonal antibody. Infect. Immun., 58, 955-960.
69. Tannich, E. and Burchard, G. D. (1991): Differentiation of pathogenic from nonpathogenic *Entamoeba histolytica* by restriction fragment analysis of a single gene amplified in vitro. J. Clin. Microbiol., 29, 250-255.
70. Bruchhaus, I., Richter, S. and Tannich, E. (1997): Removal of hydrogen peroxide by the 29 kDa protein of *Entamoeba histolytica*. Biochem. J., 326, 785-789.
71. Wassmann, C., Hellberg, A., Tannich, E. and Bruchhaus, I. (1999): Metronidazole resistance in the protozoan parasite *Entamoeba histolytica* is associated with increased expression of iron-containing superoxide dismutase and peroxiredoxin and decreased expression of ferredoxin 1 and flavin reductase. J. Biol. Chem., 274, 26051-26056.
72. Clark, C. G. and Roger, A. J. (1995): Direct evidence for secondary loss of mitochondria in *Entamoeba histolytica*. Proc. Natl. Acad. Sci. USA, 92, 6518-6521.
73. Mai, Z., Ghosh, S., Frisardi, M., Rosenthal, B., Rogers, R. and Samuelson, J. (1999): Hsp60 is targeted to a cryptic mitochondrion-derived organelle ("crypton") in the microaerophilic protozoan parasite *Entamoeba histolytica*. Mol. Cell. Biol., 19, 2198-2205.
74. Tovar, J., Fischer, A. and Clark, C. G. (1999): The mitosome, a novel organelle related to mitochondria in the amitochondrial parasite *Entamoeba histolytica*. Mol. Microbiol., 32, 1013-1021.
75. Ghosh, S., Field, J., Rogers, R., Hickman, M. and Samuelson, J. (2000): The *Entamoeba histolytica* mitochondrion-derived organelle (crypton) contains double-stranded DNA and appears to be bound by a double membrane. Infect. Immun., 68, 4319-4322.
76. Rosenthal, B., Mai, Z., Caplivski, D., Ghosh, S., de la Vega, H., Graf, T. and Samuelson, J. (1997): Evidence for the bacterial origin of genes encoding fermentation enzymes of the amitochondriate protozoan parasite *Entamoeba histolytica*. J. Bacteriol., 179, 3736-3745.
77. Bui, E. T. and Johnson, P. J. (1996): Identification and characterization of [Fe]-hydrogenases in the hydrogenosome of Trichomonas vaginalis. Mol. Biochem. Parasitol., 76, 305-310.
78. Saito-Nakano, Y., Yasuda, T., Shigeta, Y., Nakazawa, M., Takeuchi, T. and Nozaki, T. (2000): Identification and Characterizaton of a Rab5 Homologue in *Entamoeba histolytica*. Arch. Med. Res., 31, S155-S156.
79. Rodriguez, M. A., Garcia-Perez, R. M., Garcia-Rivera, G., Lopez-Reyes, I., Mendoza, L., Ortiz-Navarrete, V. and Orozco, E. (2000): An *Entamoeba histolytica* rab-like encoding gene and protein: function and cellular location. Mol. Biochem. Parasitol., 108, 199-206.
80. Temesvari, L. A., Harris, E. N., Stanley, S. L., Jr. and Cardelli, J. A. (1999): Early and late endosomal compartments of *Entamoeba histolytica* are enriched in cysteine proteases, acid phosphatase and several Ras- related Rab GTPases. Mol. Biochem. Parasitol., 103, 225-241.
81. Ghosh, S. K., Field, J., Frisardi, M., Rosenthal, B., Mai, Z., Rogers, R. and Samuelson, J. (1999): Chitinase secretion by encysting *Entamoeba* invadens and transfected *Entamoeba histolytica* trophozoites: localization of secretory vesicles, endoplasmic reticulum, and Golgi apparatus. Infect. Immun., 67, 3073-3081.
82. Field, J., Van Dellen, K., Ghosh, S. K. and Samuelson, J. (2000): Responses of *Entamoeba* invadens to heat shock and encystation are related. J. Eukaryot. Microbiol., 47, 511-514.
83. Ramos, M. A., Mercado, G. C., Salgado, L. M., Sanchez-Lopez, R., Stock, R. P., Lizardi, P. M. and Alagon, A. (1997): *Entamoeba histolytica* contains a gene encoding a homologue to the 54 kDa subunit of the signal recognition particle. Mol. Biochem. Parasitol., 88, 225-235.
84. Sanchez-Lopez, R., Castro, S. G., Lizardi, P. M. and Alagon, A. (1997): The secretory pathway of *Entamoeba histolytica*: characterization and expression of the ERD2 gene. Arch. Med. Res., 28, 59-61.
85. Descoteaux, S., Yu, Y. and Samuelson, J. (1994): Cloning of *Entamoeba* genes encoding proteolipids of putative vacuolar proton-translocating ATPases. Infect. Immun., 62, 3572-3575.
86. Aley, S. B., Cohn, Z. A. and Scott, W. A. (1984): Endocytosis in *Entamoeba histolytica*. Evidence for a unique non-acidified compartment. J. Exp. Med., 160, 724-737.
87. McLaughlin, J. and Aley, S. (1985): The biochemistry and functional morphology of the *Entamoeba*. J. Protozool., 32, 221-240.
88. Avron, B. and Chayen, A. (1988): Biochemistry of *Entamoeba*: A review. p. 71-86. Cell Biochemistry and Function. vol. 6. John Wiley and Sons, Ltd., New York.
89. Reeves, R. E., South, D. J ., Blytt, H. J. and Warren, L. G. (1974): Pyrophosphate:D-fructose 6-phosphate 1-phosphotransferase. A new enzyme with the glycolytic function of 6-phosphofructokinase. J. Biol. Chem., 249, 7737-7741.
90. Reeves, R.E. (1970): Phosphopyruvate carboxylase from *Entamoeba histolytica*. Biochim. Biophys. Acta, 220, 346-349.
91. Reeves, R. E. (1968): A new enzyme with the glycolytic function of pyruvate kinase. J. Biol. Chem., 243, 3202-3204.
92. Lesh, F. A. (1975): Massive development of amebas in the large intestine. Am. J. Trop. Med. Hyg., 24, 383-392.
93. Stilwell, G. G. (1955): Amebiasis: its early history. Gastroenterology, 28, 606-622.
94. Kean, B. H. (1988): A history of amebiasis. p. 1-10. *In* Ravdin, J. I. (ed.), Amebiasis: Human Infection by *Entamoeba histolytica*. John Wiley and Sons, New York.
95. Arellano, J., Isibasi, A., Miranda, R., Higuera, F., Granados, J. and Kretschmer, R. R. (1987): HLA antigens associated to amoebic abscess of the liver in Mexican mestizos. Parasite Immunol., 9, 757-760.
96. Valdez, E., del Carmen Martinez, M., Gomez, A., Cedillo, R., Arellano, J., Perez, M. E., Ramos, F., Moran, P., Gonzalez, E., Valenzuela, O., Melendro, E. I., Ramiro, M., Kretschmer, R., Munoz, O. and Ximenez, C. (1999): HLA characterization in adult asymptomatic cyst passers of *Entamoeba histolytica/E. dispar*. Parasitol. Res., 85, 833-836.
97. Hill, A. V. (1998): The immunogenetics of human infectious diseases. Annu. Rev. Immunol., 16, 593-617.

98. Abel, L. and Dessein, A. J. (1997): The impact of host genetics on susceptibility to human infectious diseases. Curr. Opin. Immunol., 9, 509-516.
99. Denis, M. and Chadee, K. (1989): Cytokine activation of murine macrophages for in vitro killing of *Entamoeba histolytica* trophozoites. Infect. Immun., 57, 1750-1756.
100. Salata, R. A., Murray, H. W., Rubin, B. Y. and Ravdin, J. I. (1987): The role of gamma interferon in the generation of human macrophages cytotoxic for *Entamoeba histolytica* trophozoites. Am. J. Trop. Med. Hyg., 37, 72-78.
101. Lin, J. Y. and Chadee, K. (1992): Macrophage cytotoxicity against *Entamoeba histolytica* trophozoites is mediated by nitric oxide from L-arginine. J. Immunol., 148, 3999-4005.
102. Salata, R. A., Pearson, R. D. and Ravdin, J. I. (1985): Interaction of human leukocytes and *Entamoeba histolytica*. Killing of virulent amebae by the activated macrophage. J. Clin. Invest., 76, 491-499.
103. Murray, H. W., Aley, S. B. and Scott, W. A. (1981): Susceptibility of *Entamoeba histolytica* to oxygen intermediates. Mol. Biochem. Parasitol., 3, 381-391.
104. Sargeaunt, P. G., Williams, J. E., Bhojnani, R., Kumate, J. and Jimenez, E. (1982): A review of isoenzyme characterization of *Entamoeba histolytica* with particular reference to pathogenic and non-pathogenic stocks isolated in Mexico. Arch. Invest. Med., 13, 89-94.
105. Ghosh, S., Frisardi, M., Ramirez-Avila, L., Descoteaux, S., Sturm-Ramirez, K., Newton-Sanchez, O. A., Santos-Preciado, J. I., Ganguly, C., Lohia, A., Reed, S. and Samuelson, J. (2000): Molecular epidemiology of *Entamoeba* spp.: evidence of a bottleneck (Demographic sweep) and transcontinental spread of diploid parasites. J. Clin. Microbiol., 38, 3815-3821.