

Amebic Infection and the Host Immune Response

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Abstract

Entamoeba histolytica is known as the causative agent of amebiasis. Amebic dysentery is the primary clinical syndrome and then the ameba may penetrate to other organ to produce abscesses. Such abscesses can be found at liver lung, brain and skin etc. (1-3). Amebic liver abscess is the most common extraintestinal amebiasis.

E. histolytica infection occurs in 10 percent of the world population (480 million cases per year). Fewer than 10 percent (36 million cases per year) and approximately 5 percent of those infected develop clinical symptoms and invasive amebiasis respectively. More than 40,000 patients per year die from amebiasis. Among parasitic disease the mortality rate of amebiasis is only ranks behind malaria and schistosomiasis (4-6). The mortality rate of the extraintestinal amebiasis is 7-14 percent (3). (J Infect Dis Antimicrob Agents 1995;12:137-9.)

Amebiasis is worldwide distribution, and commonly found in tropical and temperate zones especially in the area of bad sanitation (1,2,7). The high risk groups for invasive amebiasis are the poor in least developed parts of the world, the very young, the malnourished, pregnant women, those with other serious underlying illnesses (6). In the United States approximately 20-30 percent of the male homosexuals were reported as a high risk group of amebic dysentery. Other high risk groups in developed countries are recent immigrants and institutionalized patients (1,7). However all *E. histolytica* isolated from 19 patients with AIDS were found to be non-pathogenic zymodemes. In addition, 9 out of 14 patients with diarrhea also harbored other pathogens such as *Cryptosporidium species* and *Isospora belli* (7). Amebiasis is easily transmitted through tap water in which the chlorine content cannot kill cysts of *E. histolytica* (1). In Thailand, the Ministry of Public Health reported that during 1988 to 1992 the number of amebic patients is approximately 1,700-3,600 cases per year and 0-31 patients died per year (8).

The morphologic study of *E. histolytica* revealed many stages : trophozoite, precyst, cyst, metacyst and

metacystic trophozoite. Metacyst is the infective stage and can be found in formed stool. Cyst form is round or oval shape, 8-20 μm in diameter, chitinous cell wall, one to four nuclei (four nuclei when mature, rarely up to eight) glycogen in a distinct vacuole in the mature and often with chromatoid bodies in the immature cyst but disappearing in the mature. Nuclei is morphologically similar to those of trophozoites. Trophozoite is ameboid form, 15-50 μm in diameter, one distinct nucleus, no mitochondria, golgi, rough endoplasmic reticulum, typical lysosome or organized cytoskeleton and usually ingests red blood cells, white blood cells or bacteria. The organisms grow well in the condition of 5 percent oxygen (4,9).

Amebiasis is transmitted by the ingestion of food or water contaminated with cysts of *E. histolytica* or by fecal-oral transmission. The amebic cyst is resistant to acid in the stomach, excystation to become trophozoites occurs in the small intestine and trophozoites colonize in the colon. The trophozoites adhere to the colonic mucous blanket, penetrate into deep tissues and secrete numerous proteases being active against basement membrane constituents and collagen (6). When the

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protective mucous layer is depleted, the epithelial barrier is disrupted. Then the intestinal mucosa is destroyed, mucous bloody diarrhea developed. The trophozoites with ingested red blood cells can be found in the diarrhea stool (1). Trophozoites are rapidly killed by the exposure to a variety of agents including antibiotics, antidiarrhea preparations, tap water and barium sulfate. So fresh stool should be microscopically examined for ameboid movement of trophozoites within 20 minutes (7). Cysts can be detected in the stool of an asymptomatic cyst passer and a patient with amebic liver abscess who has no dysentery (4). Diamond et al, suggested that there were two species of *E. histolytica*: pathogenic and non-pathogenic species. The pathogenic ameba is a pathogen of variable virulence. It causes disease as mentioned above and may cause massive tissue destruction in several extraintestinal organs (4,10). Patients with amebic liver abscesses characteristically have an acute symptoms of less than 10 days. However a subacute to chronic course up to 6 months is also reported (7). The pathogenic *E. histolytica* can be isolated from both symptomatic and asymptomatic patients but the non-pathogenic is never found associated with invasive amebiasis (10). The detection of amebae in culture is more sensitive than by the microscopic examination but cannot be available in some laboratories. There are limitations of the direct detection of amebae in abscess fluid by microscopy. Therefore, the current diagnosis method relies mainly on serological methods (7,11). A monoclonal antibody specific to the gene encoding the 30 kDa molecule of pathogenic *E. histolytica* was identified. The polymerase chain reaction (PCR) was found to be a sensitive method by Tachibana et al in 1992 to detect the pathogenic *E. histolytica* DNA in liver abscess fluids (14).

Ravdin et al, 1981 (15) found that the low temperature (4°C) could prevent killing of target cells by trophozoites and amebic effector was divisible into discrete adherence and cytolytic events. They also found that two carbohydrate monomers, galactose (Gal) or N-acetyl-D-galactosamine (Gal NAc) could completely inhibit amebic adherence at mM concentrations.

Patients recovered from either amebic colitis or liver abscess is believed to have substantial immunity against a recurrence of invasive amebiasis (6). The high titre of *E. histolytica* antibodies could be detected on the fifth day after the onset of the illness (16). There is no effect of the antibodies on the progression of the disease but the result is to contribute to acquired

immunity following therapy. The pathogenic *E. histolytica* trophozoites are resistant to lytic effects by the complement system, the non-pathogenic *E. histolytica* disappears within 5-12 months in asymptomatic patients. This may due to either the development of specific immunity or the competition with other intestinal microflora (6). The antibodies to pathogenic *E. histolytica* was detected from the serum of every patient (16). Nearly all of these positive cases developed high titre of antibodies which was not detected in the asymptomatic patients infected with non-pathogenic *E. histolytica*. Only 20 percent of asymptomatic patients developed positive antibodies against non-pathogenic *E. histolytica* and only 2-4 percent of these patients developed a high titre. So non-pathogenic *E. histolytica* induces very poor humeral immune response (17). Ravdin et al, 1990, determined the 170 kDa antigen as the heavy subunit of the adherence lectin. They also found that over 95 percent of immune sera studied recognized the heavy subunit of immunoaffinity purified Gal/Gal NAc adherence lectin, none of the sera studied contained antibodies to the 35 kDa light subunit of the adherence lectin. This Gal/Gal NAc adherence lectin plays a central role in amebic adherence, cytolysis with highly conserved antigenicity. As a result it is an excellent candidate for use as a subunit vaccine (6). The specific cell mediated immune responses in patients with amebic liver abscess are effective *in vitro* against *E. histolytica*. The humoral immune response or polymorphonuclear leukocytes do not appear to prevent the invasion by *E. histolytica* trophozoites. *In vitro*, axenic trophozoites kill human neutrophils, monocytes, lymphocytes and monocyte-derived macrophages. The protection in animals by vaccination or prior infection appear to be due to a cell mediated immune response. In addition, the depression in cell mediated immunity caused by steroid therapy, neonatal thymectomy, splenectomy, radiation and anti-lymphocyte or anti-macrophage globulin or silica treatment, resulted in an increased incidence and extent of experimental invasive amebiasis (18).

Salata et al, 1986, demonstrated that the absolute lymphocyte count and the percentage of mature circulating T lymphocytes (OK T₃- staining) in patients treated for amebic liver abscess were similar to controls, but the ratio of the T₄ to T₈ decreased (18). The study indicated that amebic proteins induced patient lymphocytes to secrete lymphokines effective in activating normal macrophages to kill virulent amebic trophozoites *in vitro*

and the antigen-specific activation of patient T lymphocyte's cytotoxic activity occurred. The lymphocytes from individuals with acute infection and from non-infected controls were not cytotoxic. Lymphokines produced by lymphocytes obtained from patients cured of amebic liver abscess contained high levels of gamma interferon, and in the presence of *E. histolytica* antigen could activate monocyte-derived macrophages to kill axenic trophozoites (18). The amebicidal activity of these lymphocytes was dependent upon the direct contact and mediated by the OK T₈ positive subset of cells. The purified Gal/Gal NAc adherence lectin could induce blastogenesis, lymphokine production and amebicidal activity in lymphocytes obtained from individuals whose sera are the anti-lectin antibody positive, and who have a prior history of invasive amebiasis. As other parasitic infections, invasive *E. histolytica* infection induces an antigen-specific immunosuppressive state. Therefore, the sera from patients with acute infection have a suppressor factor which can selectively inhibit lymphocytic blastogenesis and lymphokine production in response to total soluble *E. histolytica* antigen (6).

All of this recent information and the under investigation of the host immunity in amebiasis will heighten the researchers to carry on their works to the global aim of immunoprophylaxis. The advance of the discoveries on the host factors and the immune mechanisms are helpful for the vaccine development and treatment. The success of the works will fulfil their hope to have the efficient amebiasis vaccine in developing countries and other countries through out the world.

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