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REVIEW ARTICLE

Pathogenesis of Acute Experimental Liver Amebiasis

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Classical descriptions of the pathology of amebiasis portray the parasite as the cause of tissue damage and destruction, and in recent years a number of amebic molecules have been identified as virulence factors. In this review we describe a series of experiments that suggest a more complex host–parasite relation, at least during the early stages of acute experimental amebic liver abscess in hamsters. The problems of extrapolating experiments *in vitro* to explain observations *in vivo* are discussed. The role of amebic cysteine proteases is examined and evidence presented to suggest that they are primarily related not to tissue damage but to amebic survival, which is required for the progression of the lesion. Inflammation is shown to be not only the major cause of tissue damage but also an absolute requirement for amebic survival in the liver, whereas complement and ischemia are not involved in the disappearance of the parasite in the absence of inflammation.

Key Words: Experimental liver abscess, Hamster, Cysteine proteases, E-64, Inflammation, Complement, Ischemia.

Introduction

Since the classic publication of Councilman and Lafleur (1) in 1891 entitled *Amoebic Dysentery* and based on the clinicopathological study of 14 autopsy cases, the pathology of the human disease has been periodically reviewed and adequately illustrated several times (2–8). A survey of the literature published on the subject since the last comprehensive review available failed to produce any new or significant information. Such a result is not something to celebrate, because many questions about the pathology of human amebiasis are yet to be answered. But it seems that their more likely explanations will not come from more and more careful and sophisticated morphological studies (although surprises are not excluded), but rather from cellular, biochemical, immunological and other molecular probings of the host–parasite relation, both in human and in experimental models of the disease. Instead of restating the well-known facts of the pathology of human amebiasis, we have focused this review on some of the work performed in our laboratory exploring the mechanisms of tissue damage and destruction in early amebic lesions, using as a model the acute experimental liver abscess produced in hamsters (AEALAH) by the intraportal injection of axenically grown trophozoites of *E. histolytica* strain HM1-IMSS.

Background

For many years (actually, since 1891), *E. histolytica* has been etiologically connected with the disease known as human amebiasis (9). It is interesting that some of the very first observations of the presence of the parasite in the affected patients (feces, pulmonary secretions) (10,11) were not considered as significant, but rather as opportunistic or coincidental infections.

However, the pioneering human experiments of Walker and Sellards (12) in 1913 established the causal relation between the parasite and the disease, as reliably as was possible at that time. Further studies of the pathology of human amebiasis led to the definitive proof that *E. histolytica* was a true pathogen and strongly suggested that tissue damage in amebic disease was caused by the

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parasite (13,14). The pathogenesis of the lesions was believed to be directly secondary to the histolytic properties of amebas that, upon arrival in the intestine or other tissues, induced extensive and often progressive necrosis with little reactive inflammation (15). Early suggestions that amebic proteases were responsible for tissue destruction were inconclusive, and although gelatinase (16,17), glutaminase (18) and casease (19) activities were demonstrated in extracts of pathogenic amebas, the substrates used were denatured and no proteases capable of degrading proteins in their native state were observed. In addition, the same proteases were present in nonpathogenic amebas. A hyaluronidase was also described in amebas (20,21), but careful studies failed to reveal any correlation between such enzyme and parasite virulence (22).

In 1970, in a monograph on human amebiasis one of the authors (5) wrote:

> ''Therefore, it must be concluded that, although the more probable mechanism of amebic tissue penetration and cellular destruction is enzymatic lysis, there still is no evident proof of it, and the name ''histolytica'' is yet to be justified on basis firmer than pure morphologic inference.''

Since that time a vast literature has accumulated incriminating several amebic molecules, some with hydrolytic and other biologic activities, as directly responsible for cell and tissue damage in amebiasis. Those more frequently mentioned are amebapore (23,24), an 18-kDa protein with no enzyme activity but with the ability to create ion channels in cell membranes; a galactose and *N*-acetyl-D-galactosamine (Gal/GalNAc)-specific lectin, also non-enzymatic but that mediates adhesion of the parasite to colonic mucins (25,26); phospholipase A (27) and collagenase (28), proteolytic enzymes with better-defined substrates and at least 20 genetically different cysteine proteinases (EhCPs) (29,30) with a broad protein-substrate spectrum (31). A critical analysis of the relevant information supporting the claim that each of the amebic molecules mentioned plays a significant role in cell and tissue destruction in both experimental and human amebiasis reveals the following problems.

In Vitro Experiments

Purified amebapore has a cytotoxic effect on several cell lines, provided it is tested in acid pH (5.6-6.0 u) (32). Purified EhCPs have a cytopathic effect on monolayers of HeLa cells (33), BHK cells (31), and human fibroblasts (34). A 30-kDa EhCP is effectively cytolytic on dead rat and hamster hepatocytes and this activity is blocked by E-64, a specific inhibitor of such enzymes (35). Decrease of EhCP5 expression induced in *E. histolytica* by antisense mRNA correlates with decreased phagocytosis but cytopathic effect and hemolytic activity remain unchanged (36). Overexpression of EhCP2 in both *E. histolytica* and *E. dispar* effectively increases the cytopathic activity of those two amebic species (37).

Experiments using purified amebic molecules tested against different cell lines under *in vitro* conditions adequate for cell culture provide interesting results, but they are far removed from the *in vivo* situations in amebic disease. Major differences in pH, O_2 concentration, and the presence of a very different environment with many additional components may completely change the nature of the results obtained *in vitro*.

In Vivo Experiments

E. histolytica trophozoites grown axenically and in the presence of E-64 (38) or laminin (39) have a decreased capacity to produce liver abscesses in immunodeficient mice (SCID). Lysates of virulent *E. histolytica* lower the transepithelial electric resistance (TER) in the cecum of gerbils, and this effect is inhibited by E-64 (40). Decrease in EhCP5 expression, induced in *E. histolytica* by means of antisense mRNA, correlates with a decreased capacity of the parasite to induce liver abscesses in hamsters (41) and to cause inflammation, to secrete Il-2 and to convert proIl-1 to Il-1 in human intestine transplanted to SCID mice (42).

The experiments mentioned above and others similar in design and results certainly suggest that some amebic molecules, especially EhCPs, may play a role in tissue damage in amebiasis. But the evidence is far from conclusive because in many of those experiments the effect of the amebic manipulation on the viability and other functions of the parasite were not tested, and in those few that included such observations it was clear that both growth and viability of amebas were compromised (43). CPs are present in many other species of parasites (44), and when their enzymatic activity is blocked with inhibitors their survival and nutritional metabolism are severely damaged (45). Thus, the results of *in vivo* experiments with interference of EhCPs could be equally interpreted as indicating an important role of such enzymes in the survival of the parasite, which is necessary for the initiation and progression of tissue damage, probably related to other molecular mechanisms of the parasite and/or of the host.

That the host is involved in tissue damage and destruction in amebiasis has been suspected for a long time, since the existence of ''healthy carriers'' was experimentally established in humans by Walker and Sellards in 1913 (12). That virulent *E. histolytica* isolated from the feces of a patient suffering from clinical amebiasis fails to produce the disease in healthy subjects when they are infected with such parasite and become cyst passers, and these cysts are further fed to other healthy subjects and some of them develop the full disease while others remain asymptomatic, was considered proof that some hosts either lack something that the parasite needs to cause the disease or have something that actively prevents them from doing it. Whatever the