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Amoebic disease

Entamoeba histolytica and *E. dispar*: comparison of molecules considered important for host tissue destruction*

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Abstract

Entamoeba histolytica and *E. dispar* are genetically distinct but closely related protozoan species. Both colonize the human gut but only *E. histolytica* is able to invade tissues and cause disease. Comparison of the 2 species may help to elucidate the specific mechanisms involved in the pathogenicity of *E. histolytica*. During the last few years, various amoeba molecules considered to be important for pathogenic tissue invasion have been identified and characterized, such as a galactose-inhibitable surface lectin, pore-forming peptides and cysteine proteinases. This review summarizes present knowledge about the structure and function of these molecules, with emphasis on the differences between *E. histolytica* and *E. dispar*.

Keywords: amoebiasis, *Entamoeba histolytica*, *Entamoeba dispar*, pathogenicity

Introduction

The introduction of deoxyribonucleic acid (DNA)-based methodologies into *Entamoeba* research during recent years has confirmed the 2 species concept, separating *Entamoeba dispar* from *E. hisolytica* (DIAMOND & CLARK, 1993). This has brought to an end a long-lasting debate between *Entamoeba* cognoscenti and paved the way for more sophisticated studies on the epidemiology, diagnosis and treatment of human amoebiasis. In addition, since only *E. histolytica* is pathogenic for humans but both amoeba species are highly similar in genetic background, cell biology and host range (for both, humans are the only relevant host), the comparison between *E. histolytica* and *E. dispar* provides an interesting area of research for identifying and analysing pathogenicity factors of an intestinal protozoan parasite.

E. histolytica is characterized by its extraordinary capacity to destroy human tissues leading to massive and sometimes lethal pathological alterations such as ulcerative colitis or abscesses of various organs, most commonly the liver (RAVDIN, 1995). During recent years, due to the developments of suitable protocols to culture *E. histolytica* trophozoites axenically (DIAMOND *et al.*, 1978), the mechanisms involved in tissue destruction have been studied *in vitro*. Following these investigations, *E. histolytica* has been found to be 'the most remarkable and potent of all killer cells' (WARREN, 1988). For example, incubation of *E. histolytica* trophozoites with activated macrophages in a ratio of 1:500 (amoeba: target cells) kills virtually all macrophages within minutes. Similarly, within the same short period of time the amoebae completely disrupt monolayers of cultured fibroblasts. These and other studies in conjunction with biochemical investigations have indicated that at least 3 functions of the amoebae are important for pathogenic tissue invasion (for a review, see HORSTMANN *et al.*, 1992). Pathogenicity is viewed as the result mainly of (i) adherence of the amoeba to host cells predominantly mediated by a galactose- and N-acetylgalactosamine-inhibitable surface lectin, (ii) killing of host cells by pore-forming peptides known as amoebapores, and (iii) proteolysis of the host's extracellular matrix mediated by cysteine proteinases. During the last few years, the various molecules have been purified from *E. histolytica* lysates and characterized at the molecular level.

The galactose-inhibitable surface lectin

Adherence of the amoeba to host cells is a prerequisite for tissue destruction, since killing of cells requires an intimate contact between *E. histolytica* trophozoites and the target cells. In addition, adherence is also important for the colonization of the large intestine in that amoeba bind to colonic mucins (RAVDIN,. 1989). Interestingly, both forms of adherence appear to be mediated by the same amoeba surface receptor, which is a lectin inhibitable by galactose and N-acetyl galactosamine (RAVDIN & GUERRANT, 1981; CHADEE *et al.*, 1987). The structure, function and immunogenicity of the receptor have been studied in considerable detail (for review see RAVDIN, 1989; MCCOY *et al.*, 1994). It is a membrane associated glycoprotein consisting of heavy (170 kDa) and light (35 kDa) subunits (PETRI *et al.*, 1989). Molecular cloning of the 2 subunits revealed that each of them is encoded by multiple genes (MANN *et al.*, 1991; TANNICH *et al.*, 1991a, 1992; MCCOY *et al.*, 1993a; RAMAKRISHNAN *et al.*, 1996). Primary structure analysis of the large subunit indicated an interesting domain structure and an extraordinary high content of cysteine residues within the C terminal two-thirds of the molecule. Besides structural requirements, the high cysteine content is believed to be important to prevent rapid cleavage and degradation of the receptor by intestinal proteases. The most unusual feature of the lectin is its membrane association. Whereas the large subunit is linked by a classical membrane anchor, the light subunit is inserted via a glycophospholipid-inositol moiety (MCCOY *et al.*, 1993b). So far, the sugar-binding site of the lectin has not been identified. However, antibodies to certain epitopes have been shown to inhibit amoebic adherence to target cells and to colonic mucin (PETRI *et al.*, 1990). Therefore, the lectin was considered as one of the leading candidates for a vaccine to prevent amoebiasis. Consequently, several laboratories have investigated the vaccine efficiency of recombinant lectin fragments using the gerbil model for amoebic liver abscess (ZHANG *et al.*, 1994; SOONG *et al.*, 1995; LOTTER *et al.*, 1997). The results indicated that immunization with certain parts of the cysteine-rich region are indeed protective and prevent liver abscess formation. However, it remains to be determined whether a lectin-based vaccine is able to prevent amoe-

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bic disease not only in artificially infected rodents but also in naturally infected humans.

Comparison between *E. histolytica* and *E. dispar* revealed that both species possess nearly identical lectin receptors which bind with the same efficiency to target cells and to colonic mucin as well (BURCHARD & BILKE, 1992; DODSON *et al.*, 1997; PILLAI *et al.*, 1997). Thus it has been speculated that the main physiological role of the receptor is to mediate amoebic colonization of the intestine. Nevertheless, inhibition of amoeba-induced cell killing by specific sugars or anti-lectin antibodies gives good reasons to assume that the galactose-inhibitable lectin constitutes an essential component for *E. histolytica* pathogenicity. However, additional factors are required to permit amoeba-induced tissue destruction.

The pore-forming peptides—amoebapores

Within seconds after close contact between amoebae and target cells has been established, swelling and massive blebbing of target cells occur, a process which is irreversable and finally leads to target cell lysis (RAVDIN, 1995). Since this kind of cell death resembles that mediated by cytotoxic T lymphocytes (TSCHOPP & NABHOLZ, 1990), it was concluded that a channel-forming protein of the amoeba is involved in target cell killing, which was termed amoebapore after a corresponding activity could be identified in *E. histolytica* lysates (YOUNG *et al.*, 1982; LYNCH *et al.*, 1982). However, it took almost 10 years until the protein was purified and further characterized (LEIPPE *et al.*, 1991, 1992; ANDRÄ & LEIPPE, 1994). It is a peptide of 77 amino acid residues forming a compact structure of 4 amphipathic α-helices which are stabilized by 3 disulphide bonds. The molecule is able to bind to and insert into membranes, where the amoebapore monomeres tend to oligomerize and form water-filled channels through which ions and other small molecules can pass (LEIPPE *et al.*, 1991). As a consequence, the internal milieu of the cell is changed totally, which ultimately results in its lysis. Three isoforms, amoebapore A, B and C, have been identified which all are stored within granules found in large numbers within the amoeba's cytoplasm (LEIPPE *et al.*, 1994b). The 3 isoforms are rather similar in structure but are present in different quantities within the cell. Amoebapore A is the most abundant, followed by B and C with a ratio of approximately 35:10:1. *In vitro*, all 3 amoebapores were found to kill various human cell lines in a dose-dependent manner, but amoebapore C exhibited substantially higher cytolytic efficacy than the other 2. Besides killing of nucleated cells another function of the pore-forming amoeba molecules is their potent activity against various bacteria species by perturbing the integrity of bacterial cytoplasmic membranes (LEIPPE *et al.*, 1994a, 1994b; ANDRÄ *et al.*, 1996). *E. histolytica* trophozoites have an enormous capacity to phagocytose bacteria (up to 1000 bacteria per hour), which implies that they possess a mechanism by which bacteria can be continuously engulfed and then efficiently killed and degraded. Since amoebae do not possess antimicrobial mediators such as oxygen metabolites, they appear to rely on an oxygen-independent mechanism as a first line of defence. The amoebapores are considered to be part of this mechanism and might exert their antibacterial activity in conjunction with lysozymes, which have been recently characterized and localized within the amoeba granules (JACOBS & LEIPPE, 1995). Thus killing of bacteria appears to be the primary physiological function of amoebapores. Therefore, it was not surprising that this class of molecules was detected in *E. dispar* by LEIPPE *et al.* (1993). Genes for all 3 isoforms were found in this amoeba species with high similarities to those of *E. histolytica*. However, on the protein level only amoebapore A and B could be detected in *E. dispar* lysates, and in reduced concentrations compared to *E. histolytica*; amoebapore C is virtually absent from *E. dispar*, which presumably explains the reduced capacity of *E. dispar* to destroy nucleated cells.

The cysteine proteinases

Numerous attempts to identify and characterize molecules involved in *Entamoeba* virulence have focused on the role of cysteine proteinases, which are the main proteolytic enzymes found in large quantities in *E. histolytica* lysates. Following these investigations, amoeba cysteine proteinases appear indeed to be responsible for a number of effects considered important for *E. histolytica* pathogenicity, as outlined by the following results: (i) cultured fibroblast monolayers are disrupted by purified *E. histolytica* cysteine proteinases (KEENE *et al.*, 1990), probably due to their ability to degrade extracellular matrix components such as fibronectin, laminin, or collagen (BRACHA & MIRELMAN, 1984; LUACES & BARRETT, 1988; SCHULTE & SCHOLZE, 1989); (ii) amoeba virulence is directly correlated with the cysteine proteinase activity of trophozoite extracts (GADASI & KESSLER, 1983; LUSHBAUGH *et al.*, 1985; REED *et al.*, 1989; KEENE *et al.*, 1990); and (iii) amoebic liver abscess formation is inhibited by specific cysteine proteinase inhibitors (LI *et al.*, 1995; STANLEY *et al.*, 1995). In addition, the importance of cysteine proteinases for amoeba pathogenicity was further supported by comparing *E. histolytica* with *E. dispar*. The latter was found to contain much less cysteine proteinase activity, apparently as a result of a lower number of cysteine proteinase expressing genes (TANNICH *et al.*, 1991b; BRUCHHAUS *et al.*, 1996). So far 6 genes (*ehcp1–ehcp6*) encoding cysteine proteinases in *E. histolytica* have been identified, 4 of which (*ehcp1*, *ehcp2*, *ehcp3*, *ehcp5*) are expressed in cultured trophozoites. N-terminal sequencing of the purified enzymes revealed that EhCP1, EhCP2 and EhCP5 are responsible for at least 90% of the total cysteine proteinase activity in *E. histolytica* (see BRUCHHAUS *et al.*, 1996). Interestingly, functional genes homologous to 2 of the *E. histolytica* genes are missing from *E. dispar*. Whereas genes homologous to *ehcp2*, *ehcp3*, *ehcp4* and *ehcp6*, with a high sequence similarity of about 95%, were found in *E. dispar*, the respective genes encoding CP1 or CP5 could not be detected in this amoeba species (BRUCHHAUS *et al.*, 1996). With regard to *Entamoeba* pathogenicity, the absence of an EhCP5-homologue in *E. dispar* seems to be of particular interest. In contrast to the other cysteine proteinases, which all are found within the amoeba's granules, EhCP5 is exceptional in that it is the only one that is localized on the amoeba surface (JACOBS *et al.*, 1998). As EhCP5 is currently the only structurally characterized member of the amoebic cysteine proteinase family that is exclusively present in *E. histolytica* and appears to be functionally unique, it is tempting to hypothesize that EhCP5 is an important factor for amoeba pathogenicity. However, further investigations are required, especially those making use of recently developed techniques of amoeba transfection (for a review, see TANNICH, 1996), to prove or disprove this hypothesis.

Conclusions

As outlined above, the amoeba molecules considered most important for host tissue destruction are present in both pathogenic *E. histolytica* and non-pathogenic *E. dispar*, calling into question their importance in *E. histolytica* pathogenicity. It appears that the various molecules are primarily used by the amoebae to colonize the human intestine and to degrade nutrients, respectively. However, higher activities of the pore-forming peptides of *E. histolytica* against nucleated cells, and the occurrence of a specific membrane-bound surface proteinase, may enable only this amoeba species to invade and destroy human tissues. On the other hand, from a biological point of view, it is not expected that *E. histolytica* possesses specific molecules exclusively for pathogenic

tissue invasion. Pathogenic behaviour itself is believed to be a 'dead-end street' for the life cycle of the parasite, since tissue forms are considered not to transform into cysts. This is most evident in cases of amoebic liver abscesses when the amoebae lose connection to the intestinal cavity. Therefore, in contrast to most other pathogenic protozoans, such as *Leishmania, Trypanosoma* or *Plasmodium, E. histolytica* has not experienced any selection pressure to develop specific molecules for survival within human tissues. The disadvantage of tissue invasion, which appears to occur accidentally, might be the reason for the lower world-wide prevalence of *E. histolytica* compared to *E. dispar*. Of the 500 million people considered to be infected with either of the 2 amoeba species, only 10% harbour *E. histolytica*.

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