Progress report

Pathogenesis of amoebiasis

In recent years the species complex of amoebae infesting man which produce four-nucleate cysts has been divided into three groups. First the amoebae, described previously as the small race E. histolytica, are separated into a distinct species E. hartmanni based on size and absence of virulence. Second, amoebae which are indistinguishable morphologically from true E. histolytica but are able to grow and multiply at temperatures below 37°C are known as E. histolytica-like amoebae. The optimum temperature for their growth is about 26°C. These isolates have, until recently, only been discovered in North America. Attempts to find such strains in Great Britain have so far failed, although a report of the isolation of low-temperature E. histolytica-like amoebae from Sicily has appeared. The third group consists of true E. histolytica. Amoebae of this group were previously described as the large race and only these amoebae are responsible for invading tissue in man. This progress report concerns the pathogenicity of true E. histolytica.

Differences in Virulence between Different Isolates

Entamoeba histolytica lives primarily as a commensal in the large intestine feeding mainly on bacteria, though it may occasionally feed on superficial cells of the intestinal mucosa. However, clinical manifestations due to the infection appear when the amoebae invade the mucosa and ulceration develops. The amoebae are able to migrate through the tissue without eliciting a cellular response and if a suitable site is reached, usually the liver, one or more abscesses are formed.

It is now well established that the virulence of isolates of amoebae established in culture can be determined by the intracaecal inoculation of laboratory animals, particularly weanling rats. The observed virulence correlates with the presence or absence of the clinical symptoms in man. The geographical distribution of amoebiasis shows that although the infection is world wide, disease is generally limited to the warmer regions of the world. Therefore virulent isolates generally originate overseas, whilst indigenous isolates in Great Britain are avirulent. However, not all isolates made in an area where disease occurs are virulent; avirulent strains also occur. Epidemiological evidence suggests that the explanation for this heterogeneity relates to the instability of the invasiveness of a population of amoebae rather than to the existence of two distinct races of amoebae distinguishable from one another only by their invasive properties.

Amoeba-bacteria Relationship

Since the amoebae are dependent on bacteria for their growth in vitro and in vivo, their interrelationships have been much studied. Exchanging bacterial
flora of isolates of different invasiveness or adding pathogenic bacteria to in vitro cultures of amoebae did not alter the virulence of an isolate of E. histolytica. The conclusion from this work is that the amoebae of each isolate are responsible for the characteristic invasiveness of each isolate. However, there is some work which indicates that the virulence can be increased by certain bacteria such as Clostridium perfringens.

The availability of germ-free animals has led to a partial elucidation of the amoeba-bacteria relationship. It was found that the amoebae would not colonize the bacteria-free guinea-pig caecum even when the redox potential was lowered with the use of chemical reducing agents. Some colonization was produced after the caecal mucosa had been damaged, though the amoebic lesions were small and the infections were not lethal. Normal E. histolytica infections were only produced if the germ-free guinea-pigs were mono-contaminated with Clostridium perfringens or Bacterium subtilis.

The relationship has been further studied employing the recently discovered techniques for culturing E. histolytica axenically. The studies with axenic amoebae confirmed the necessity of associating amoebae with living bacteria to show invasive properties. The speculation is that the virulence factor may be an episomal-like character derived from bacteria and incorporated into the amoebae possibly by phagocytosis. It may be commented that the test of virulence employed was artificial since amoebae were injected into the liver of hamsters. While the results with the more natural intracaelal inoculation may be similar, it would be useful to repeat the experiments with the intestinal infection.

Instability of Virulence

Continued cultivation in vitro invariably results sooner or later in a loss of virulence as shown first by a lower degree of ulceration and second by reduction of infectivity. Lost virulence can be recovered by a series of liver or intestinal passages. It is also possible to increase the virulence by the addition of cholesterol to the amoebae in culture or to the experimental host. The mechanism of the action of cholesterol is not understood.

The state of the host also plays a part in determining the reaction to infection with E. histolytica. Thus changes in diet and induced avitaminoses of experimental animals result in more severe infections (see Neal for references). In these instances, it is unlikely that the increase of virulence can be easily explained by changes in the bacterial flora. Some authors believe that the outcome of an amoeba-host encounter is primarily determined by the host and not by the parasite.

It is clear, therefore, that invasiveness is an unstable character in E. histolytica. On the basis of present evidence, it is my view that amoebae are normally avirulent, living in the intestinal lumen, and under some stimulus change to a virulent form, resulting in ulceration in the large intestine which produces symptoms in the patient. The nature of the stimulus is still not understood.

Mechanism of Invasion

The study of enzymes likely to be related to the pathogenic action of amoebae has not yielded any clues as to the mechanism. A hyaluronidase and a
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Proteinase closely related to trypsin have been identified, but have not been linked to virulence.\(^{20}\)

Leucocytes and other cells are killed by contact with amoebae\(^{21,28}\). This activity could be used as an *in-vitro* model for further studies on the mode of action of the amoebae to gain access to the intestinal mucosa.

Analysis of antigens has not yet revealed any clear-cut differences between virulent and avirulent strains of *E. histolytica*.

Electron microscope studies of amoebae of *E. histolytica* revealed several features which may be of interest in regard to pathogenicity. In amoebae obtained from the human colon a 'fuzzy coat' was observed which had not been seen in amoebae cultured *in vitro*\(^{28}\). Similarly a filamentous structure was observed in amoebae from experimentally infected rats but not from the same isolate of amoebae grown *in vitro*\(^{24}\). A well documented piece of work is the discovery of a lysosome active on the surface of the amoeba\(^{22}\). The necessity for contact between amoebae and cells was attributed to the presence on the surface of the amoeba of a lysosome. It was situated in a depression of the surface membrane and at the bottom of the depression was a hair-like projection which triggered the release of the lysosome contents. Contact between the amoebae and the tissue cells resulted in death of the tissue cells. Further work is necessary to determine if the differing virulence can be interpreted in the light of this new discovery.

**Comments**

The most profitable line of research on the virulence of *E. histolytica* at the present time would appear to follow the *in-vitro* models of virulence with either the leucocyte model of Jarumilinta\(^{21}\) or the tissue culture models of Eaton and his colleagues\(^{28}\). However, at the present time infection of laboratory animals is still necessary to determine the degree of invasiveness of *E. histolytica*.

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**References**

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