Does the Eosinophil Have a Protective Role in Amebiasis?

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While normal human eosinophils are destroyed in vitro by virulent Entamoeba histolytica, notwithstanding the presence of antibodies and complement, activated eosinophils promptly destroy the parasite although dying also at the end of the process. To study the possible in vivo participation of eosinophils in invasive amebiasis, we compared the induction of experimental amebic abscess of the liver (AAL) in gerbils (Meriones unguiculatus) previously made eosinophilic through Toxocara canis antigen injection and in normal control gerbils. After intraportal inoculation of $10^5$ ameba trophozoites (6 and 24 hr), the ratio of gerbils with AAL, as well as the number and size of the microabscesses was comparable in eosinophilic and control gerbils. However, at 96 hr the number and size of the microabscesses were significantly smaller ($p<0.05$) in eosinophilic gerbils. On the other hand the actuarial AAL survival curve up to 45 days post-amebic inoculation was significantly ($p<0.05$) shifted to the right in controls. These results suggest that antigen-induced eosinophilia may exert a protective effect against AAL in gerbils.

Key words: eosinophilia - Entamoeba histolytica - amebic abscess of the liver - gerbils

Amebiasis is a major health problem in developing countries. Entamoeba histolytica infects 5 x $10^8$ people worldwide, more than 90% of them living in West and South East Africa, China, SE Asia, México, the western portion of South America and India (Smyth 1994). Only 10% of all infected individuals eventually develop invasive amebiasis ranging from mild (i.e. self-limited) or serious intestinal amebiasis, to life-threatening hepatic invasion (Walsh 1986).

It has been suggested that there are in fact two different species of Entamoeba. One, the classic pathogenic E. histolytica discovered by Schaudinn in 1903. The other, the non-pathogenic E. dispar described by Brumpt in 1925, based mainly on epidemiological considerations. The concept of two Entamoeba species began to be objectively established by functional in vitro studies (Martínez-Palomo 1982, Mirelman & Chayen 1990) andzymodeme patterns (Sargeaunt 1988), culminating now with molecular genetic data (Tannich & Burchard 1991, Tannich 1996).

Thus, the majority of ameba infected individuals may actually harbour E. dispar, leaving the E. histolytica as the agent of the relatively rare invasive intestinal and extraintestinal disease. Amebic abscess of the liver (AAL) on the other hand, occurs in less than 1% of individuals with invasive intestinal amebiasis (Martínez-Palomo et al. 1993).

Why the vast majority of individuals ($\geq99\%)$ appear to be resistant to extraintestinal invasion remains unknown but some inroads have now been made: a significant increase in the SCO1 complement and in HLA-DR3 was found in 160 Mexican mestizos (adults and children) with AAL when compared to healthy controls, to adult patients with amebic rectocolitis or to asymptomatic amebic carriers (Arellano et al. 1996). DNA subtyping is now in process to confirm these findings.

Studies of human amebiasis and of experimental animal models of amebic disease have suggested that cell-mediated rather than humoral immune mechanisms, are responsible for acquired immunity to AAL (De León 1970, Salata & Radvin 1986, Kretschmer & López-Osuna 1990). Moreover, axenically grown trophozoites of E. histolytica kill in vitro normal human neutrophils and eosinophils (not-withstanding the aid by antibody and complement), normal monocytes and lymphocytes, without suffering changes in their own viability (Jarumilinta & Kradolfer 1964, Artigas et al. 1966, Guerrant et al. 1981, Salata et al. 1985, 1987, López-Osuna et al. 1986, López-Osuna & Kretschmer 1989). However, if macrophages are activated beforehand, they become capable of ef-
fectively destroying virulent amebas, even though finally succumbing in the process as well (Salata et al. 1985). In our laboratory we found that the same occurs with activated human peripheral blood eosinophils (López-Osuna et al. 1992).

There is no circulating eosinophilia in most protozoan infections, yet this is not necessarily an argument against their possible participation in AAL, specially because eosinophils are regularly found in the early inflammatory stages of experimental amebic liver disease (Tsutsumi et al. 1984), and since the bulk of eosinophils in the body normally dwells, not intravascularly but in tissues (Weller 1991). We therefore decided to study in vivo the role of eosinophils, by inducing eosinophilia in gerbils (M. unguiculatus) through four injections of an innocuous *Toxocara canis* antigen, prior to the induction of experimental AAL with $1 \times 10^5$ intraportally injected ameba trophozoites (Velázquez et al. 1995, López-Osuna 1995, Velázquez 1996). This relatively small inoculum of amebas (compared to the usual $1 \times 10^6$) was chosen in order to maximize the eosinophil’s chances to reveal its eventual protective role against *E. histolytica*. Gerbils reached their eosinophilia peak at 21 days (after the first injection of the antigen) (Fig.1 and Table) at which time they and their controls (i.e.normal eosinophil number) received the intraportal injection of *E. histolytica* HM1-IMSS in saline. Animals were then sacrificed at 6, 24, 96 hr and - those surviving - at 45 days post inoculation.

The livers revealed comparable histopathologic amebic lesions at 6 and 24 hr in both groups. At 96 hr, however, microabscesses were significantly fewer and smaller in the eosinophilic group (p<0.05)(Fig. 2). In the 45 days actuarial AAL curves, eosinophilic gerbils revealed a better survival rate and had fewer abscesses than their normal controls (p<0.05) (Fig.3).

Serum IL-5 levels (ELISA Endogen test) were measured during these experiments but no significant changes were observed at the chosen times (Table).

In order to confirm these findings, we are now studying AAL in eosinopenic gerbils. A search is also presently under way for specific eosinophil granule proteins (MBP, ECP, EPO) in the livers of eosinophilic gerbils in order to ascertain the true presence or absence of this leucocyte (Gleich et al. 1994). Much remains to be learned in order to establish if eosinophils actually play a role in invasive amebiasis. Screening carefully those gerbils that escape (or recover) from the formation of amebic abscesses (rather than studying only those with AAL) appears now to be mandatory. The traditionally eschewed subject of active participation of eosinophils in protozoan diseases (trypanosomiasis, malaria and leishmaniasis) has become an important landmark in today’s immunoparasitology. Invasive amebiasis could apparently join this group.
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