Neutrophils Play a Critical Role in Early Resistance to Amebic Liver Abscesses in Severe Combined Immunodeficient Mice

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Animal models of liver abscess formation with *Entamoeba histolytica* suggest that the neutrophil is the first cell of the host immune system to interact with the invading ameba. In vitro studies have suggested that lysis of neutrophils by virulent amebae may exacerbate the damage seen in amebic liver abscesses. To investigate the role of neutrophils in vivo, we used the severe combined immunodeficient (SCID) mouse model of amebic liver abscess formation and compared liver damage in neutrophil-depleted and control mice. We found that neutrophil-depleted animals have significantly larger amebic liver abscesses at early stages of infection and that abscesses in neutrophil-depleted SCID mice lack the prominent inflammatory cell ring seen in amebic liver abscesses in control SCID mice. These data suggest that neutrophils play a protective role in the early host response to amebic infection of the liver.

The protozoan parasite *Entamoeba histolytica* is the cause of amebic dysentery and amebic liver abscesses, diseases associated with significant morbidity and mortality worldwide. Amebic liver abscesses develop when *E. histolytica* trophozoites invade through the intestinal mucosal and submucosal layers and enter the portal circulation, allowing them to establish infection in the liver. Studies with animal models of amebic liver abscess formation utilizing either portal-vein inoculation of amebae or direct hepatic inoculation of amebae have shown that neutrophils are the earliest host defense cells to contact amebae within the liver, appearing as early as 1 h after inoculation (12, 13). The role played by these neutrophils in amebic disease has been unclear. *E. histolytica* trophozoites can lyse neutrophils in vitro, with a resultant release of neutrophil contents (7, 10). It has been hypothesized that the damage seen in amebic liver abscesses may arise in large part from mediators released from neutrophils which were lysed by amebic trophozoites (9, 11, 12). This concept has been supported by in vitro studies, where monolayers of either liver- or intestine-derived cell lines were incubated with either *E. histolytica* trophozoites alone or *E. histolytica* trophozoites and neutrophils (1, 10). Damage to the monolayer was significantly greater when neutrophils were present. Here, we describe the use of a severe combined immunodeficient (SCID) mouse model of amebic liver abscess formation (2) and the neutrophil-depleting monoclonal antibody RB6-8C5 (8) to study the role of neutrophils in amebic liver abscess formation in vivo. We found that neutrophils play a protective role against SCID liver damage, as neutrophil depletion is associated with significantly larger areas of liver damage, especially at early stages of infection.

Historically, the hamster and gerbil models have been the animal models most frequently used for the study of amebic liver abscess formation. Unfortunately, there are relatively few immunologic reagents available for either of these species. This lack of immunologic reagents was part of the rationale for developing a mouse model of amebic liver abscess formation. We have previously used this model to define the pathological

findings of amebic liver abscess formation in SCID mice 7 days after hepatic challenge with amebae (2). Abscesses were characterized by an extensive neutrophilic infiltrate surrounding regions of necrosis containing amebic trophozoites, with a ring of inflammatory cells and fibrosis surrounding the necrotic areas and providing a clear demarcation between the diseased area and normal hepatic tissue. Since neutrophils appeared to be the predominant cell type around abscesses at the 2- to 7-day time point, and because of the controversy surrounding the role of neutrophils in the tissue damage seen with amebic liver abscesses, we looked at the effect of depleting neutrophils with the monoclonal antibody RB6-8C5.

For these studies, we used a standard protocol of inoculation of 100 μ l of TYI-S-33 medium without serum containing 10⁶ HM1:IMSS amebae directly into the liver parenchyma of SCID mice. Animals were either treated intraperitoneally with daily injections of 150 μ g of protein-A-purified monoclonal antibody RB6-8C5 or an equal volume of phosphate-buffered saline. At the time of sacrifice the peripheral blood of all animals was analyzed by fluorescence-activated cell sorter staining to ensure that neutrophil depletion by more than 95% was achieved. At sacrifice, the livers of the animals were analyzed for the presence of abscesses, and the percentage of liver abscessed was assessed by dividing the weight of the abscessed portion by the total liver weight. Samples were also taken at this time for routine histologic analysis. As shown in Table 1, amebic liver abscesses were significantly larger in neutrophil-depleted SCID mice at the 48-h time point than in control

TABLE 1. Percentage of liver abscessed after challenge with 10⁶ amebic trophozoites^a

Day postinfection	% of liver abscessed after treatment with:		P value
	RB6-8C5	Saline	
2	46.3 $\pm$ 6.1 (n = 6)	12.6 $\pm$ 7.7 (n = 7)	0.007
4	29.4 $\pm$ 7.3 (n = 5)	9.4 $\pm$ 2.2 (n = 5)	0.03
7	15.9 $\pm$ 5.1 (n = 10)	7.4 $\pm$ 3.4 (n = 12)	0.17

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^a Mice received 150 μ g of monoclonal antibody RB6-8C5 against murine neutrophils or an equivalent volume of saline. Mice were sacrificed on the indicated days, and abscessed liver and normal liver were weighed to determine the percentage of the liver occupied by abscesses.

FIG. 1. Stained (hematoxylin and eosin) sections of amebic liver lesions 48 h after amebic challenge. (A) Control animal. Note inflammatory cells separating normal liver (L) from necrotic hepatocytes (N) and also surrounding amebic trophozoites (arrowheads). Magnification, $\times 83$. (B) Neutrophil-depleted animal. Note the absence of inflammatory cells and the presence of multiple amebic trophozoites (arrowheads). Magnification, $\times 83$. (C) Control animal. Note the ring of inflammatory cells (arrowhead) separating the normal liver parenchyma (L) from an area containing multiple necrotic hepatocytes (N). Magnification, $\times 415$. (D) Neutrophil-depleted animal. Note the complete absence of the inflammatory ring as regions of necrosis directly abut normal liver parenchyma. Magnification, $\times 415$.

SCID mice. Analysis of livers at 4 days postinoculation revealed a similar effect, with neutrophil depletion resulting in increased amebic liver abscess size. Analysis at day 7 showed a similar trend, although the numbers did not reach statistical significance. When similar experiments were performed on the congenic immunocompetent CB-17 mice, which are relatively resistant to amebic liver abscess formation (2), there was a trend towards increased amebic liver abscess size in neutrophil-depleted mice, but the failure to develop abscesses in many of the mice made interpretation of these results difficult (data not shown).

Histologic assessment of these *E. histolytica*-infected livers revealed that the untreated animals showed an area of necrosis surrounded by a ring of inflammatory cells that served to wall off the diseased area from normal-appearing liver parenchyma

(Fig. 1A and C). When livers from the neutrophil-depleted animals were analyzed, we saw a similar central area of pale and necrotic liver cells with scattered amebae. However, there was a complete absence of the ring of inflammatory cells surrounding the necrotic region. Instead, the necrotic region abutted directly against normal areas of the liver (Fig. 1B and D). These histologic findings suggest that neutrophils may play a critical role in the inflammatory process that walls off the amebic liver abscess. It should be noted that in both neutrophil-depleted and control SCID mice, some of the liver abscesses showed relatively few amebic trophozoites and some central coagulative necrosis, rather than the lytic necrosis which predominates in experimentally induced amebic liver abscesses in gerbils and hamsters (13). While we have also seen amebic liver abscesses with central lytic necrosis in SCID mice

(11), the present findings suggest that there could be differences in the mechanisms of *E. histolytica*-induced liver cell death seen in amebic liver abscesses in mice and those in other animals.

These data indicate that neutrophils play a significant role in controlling amebic liver abscess size in SCID mice. A possible explanation for the differences seen in vivo with our experiments and those previously seen in the in vitro system, where neutrophils appear to contribute to tissue damage, could be the activation state of the neutrophil. It has been shown in vitro that neutrophils previously activated with gamma interferon or tumor necrosis factor alpha have greatly increased amebicidal activity (4). In the SCID mouse the presence of cytokines could lead to the activation and subsequent increased efficiency of neutrophils to kill amebae.

The fact that the treated animals, as well as the untreated animals, eventually resolve these lesions suggests that other cell types must be playing a critical role in the clearance of infection. The fact that the difference in amebic liver abscess size between neutrophil-depleted and control SCID mice begins to decrease at the 7-day time point suggests that these other cell types may play their protective role later in the infection process, while neutrophils exert their effect early in the infection. Because these are SCID mice, lacking functional B and T cells, the likely candidates for protection would be macrophages or natural killer cells. We are currently attempting to distinguish between these two possibilities.

Neutrophils have been shown to play an important role in the protection against a variety of both intracellular and extracellular infectious agents, including *Listeria* and *Naegleria* (3, 5). In the instance of *Naegleria*, another genus of extracellular organisms, it has been shown that cytokine-activated neutrophils are capable of lysing amebae via a myeloperoxidase-dependent pathway (6). Furthermore, the depletion of neutrophils in an in vivo model of the disease results in significantly increased mortality upon challenge with *Naegleria*.

In summary, we have shown that in contrast to findings from some in vitro studies, neutrophils do not play an exacerbative role in liver abscess formation in vivo in the SCID mouse model of infection. Instead, neutrophils appear to play a protective role, especially at early time points, as their removal results in the formation of significantly larger abscesses. Whether this protective effect is mediated directly by killing of amebae by neutrophils or by a role of neutrophils in walling off

and thus physically containing amebic liver abscesses is currently under investigation.

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