Antibody-dependent macrophage-mediated cytotoxicity against *Entamoeba histolytica*

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Summary. Interactions between trophozoites of *Entamoeba histolytica* and peritoneal exudate macrophages from unsensitised and antigen-sensitised animals were studied *in vitro*. Normal macrophages killed trophozoites to some extent. This killing capacity was enhanced by prior sensitisation of the animals with specific antigen. Incorporation of anti-amoebic antiserum in the amoeba-macrophage mixture greatly enhanced the killing capacity of macrophages. Fraction one (F-I) of a crude amoebic extract was most effective in enhancing the cytotoxicity of macrophages by prior sensitisation and anti-F-I serum was the most effective antiserum. The cytotoxicity-inducing capacity of the immune serum resided in the IgG but not in the IgM fraction.

Introduction

Cellular immunity plays an important role in amoebic infections (Savanat *et al.*, 1973; Ortiz-Ortiz *et al.*, 1975; Harris and Bray, 1976; Jain *et al.*, 1980; Vinayak *et al.*, 1980), whereas humoral immune responses have little influence on the outcome of the disease (Vinayak *et al.*, 1981). Although macrophages are important in host defence against various bacterial infections (Mackaness, 1962; Ruskin *et al.*, 1969; Behin *et al.*, 1979), their role in amoebic infection has not been well documented. Recently, Ghadirian and Meerovitch (1982a, 1983) suggested that macrophages may be important in host defence against hepatic amoebiasis. We have already reported on the cytotoxic effect of lymphocytes against *Entamoeba histolytica* in association with anti-amoebic serum (Vinayak *et al.*, 1984). Because macrophages also participate in antibody-dependent cellular cytotoxicity against various parasites (Capron *et al.*, 1982), we studied the ability of macrophages to destroy *E. histolytica* in the absence or presence of anti-amoebic serum.

Materials and methods

Trophozoites

*E. histolytica* (NIH:200) was grown in TPS-I medium (Diamond, 1968) under axenic conditions.

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with MEM and the chambers re-incubated for 3 h. The macrophages formed a monolayer on the lower coverslips.

Cytotoxicity assays

The cytotoxic potential of sensitised and unsensitised macrophages was studied by incubating trophozoites of *E. histolytica* with macrophages in a fixed ratio (1:50), with or without anti-amoebic serum (Anti-CAE or Anti-F-I), for 4 h at 37°C in air plus CO₂ 5%. Trophozoites alone (with and without serum) were used as controls. After incubation the percentage of viable trophozoites was determined by a trypan blue dye exclusion method and phase-contrast microscopy (Guerrero et al., 1976); 20 microscope fields (× 20) were counted at random.

Effect of 2-mercaptoethanol

To determine the nature of the immunoglobulin involved in the cytotoxicity reaction, 50 µl of 0.2 M 2-mercaptoethanol was mixed with 50 µl of immune serum and added to the amoeba-macrophage mixture to give a final concentration of 0.03 M.

Isolation of IgG and IgM

IgM was isolated from anti-CAE and anti-F-I by chromatography on Sephadex G-200 (Lamon et al., 1975). The first half of the first fraction was pooled and the protein precipitated with 45% ammonium sulphate. The ammonium sulphate was removed with Sephadex G-25. IgG was isolated by DEAE-cellulose ion-exchange chromatography (Hudson and Hay, 1980), followed by ammonium sulphate precipitation. Both IgM and IgG were checked for purity and activity and their cytotoxicity-inducing capacity determined.

Results

Cytotoxicity of macrophages

The table shows that 23.9 ± 2.5% of trophozoites were killed by unstimulated macrophages. Stimulation of macrophages *in vivo* with specific antigens resulted in enhanced killing capacity. CAE-stimulated macrophages killed 40.37 ± 2.0% amoebae and F-I-stimulated macrophages killed 61.0 ± 3.5% amoebae (table). The cytotoxic potential of macrophages was significantly enhanced when anti-amoebic antiserum was added to the amoeba-macrophage mixture. Almost all trophozoites (99.08 ± 1.82%) were killed by the combination of unstimulated macrophages and anti-F-I serum. Similarly, F-I stimulated macrophages killed 100 ± 1.5% of amoebae in the presence of anti-F-I serum (table). Inactivation of antiserum did not alter the ability of anti-amoebic serum to induce cytotoxicity of macrophages.

Nature of antibodies

2-Mercaptoethanol had no effect on the capacity of anti-amoebic serum to induce cytotoxicity (figs 1

![Table. Killing of trophozoites by unstimulated and antigen-stimulated macrophages in the absence or presence of anti-amoebic serum](image)

<table>
<thead>
<tr>
<th>Effector cells</th>
<th>Percentage (±s.d.) of trophozoites killed in the presence of</th>
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<tbody>
<tr>
<td></td>
<td>NGPS</td>
</tr>
<tr>
<td>Unstimulated macrophages</td>
<td>23.9 ± 2.5</td>
</tr>
<tr>
<td>CAE-stimulated macrophages</td>
<td>40.37 ± 2.0</td>
</tr>
<tr>
<td>F-I-stimulated macrophages</td>
<td>61.0 ± 3.5</td>
</tr>
</tbody>
</table>

N.D. = Not Done.
NGPS = Normal Guinea Pig Serum.
KILLING OF *E. HISTOLYTICA* BY MACROPHAGES

Fig. 2. Capacity of isolated immunoglobulins from anti-F-I to induce cytotoxicity by peritoneal exudate macrophages: NGPS (口), anti-F-I (■), 2-mercaptoethanol-treated anti-F-I (■). IgG (■) and IgM (●).

Fig. 3. Immunoelectrophoresis of isolated IgG and IgM from anti-CAE against R/A/GPS. (A) IgG, (B) IgM, (C) NGPS.

Fig. 4. Interaction between macrophages and *E. histolytica* in the presence of anti-F-I. Many macrophages can be seen attached to the trophozoite (×1500).

that IgM did not induce cytotoxicity of macrophages.

Physical contact between macrophages and trophozoites was required to induce killing by the former (fig. 4) and more than one macrophage attached to a trophozoite in the presence of anti-F-I serum.

Discussion

High levels of anti-amoebic antibodies have been reported in cases of hepatic amoebiasis (Kessel *et al.*, 1965; Prakash *et al.*, 1970; Vinayak, 1975). However, anti-amoebic antibody titres showed little or no correlation either with virulence indices or with severity of the disease (Vinayak *et al.*, 1981) indicating that humoral immunity alone had little or no role to play. On the other hand, cellular immunity is depressed during amoebic infection (Ortiz-Ortiz *et al.*, 1975; Jain *et al.*, 1980) and experimental immunosuppression with steroids and anti-lymphocytic serum resulted in more severe lesions in animals (Vinayak *et al.*, 1982). Because a high degree of protection is achieved by immunisation with partially purified antigens (Krupp, 1974; Vinayak *et al.*, 1980), and vaccination not only augments antibody production but also primes the effectors of cellular immunity, there may be an association between the two arms of the host defence system. We found that unstimulated macrophages killed amoebic trophozoites in vitro. The killing capacity of the resident macrophages was greatly enhanced by antigen sensitisation or by addition of anti-amoebic serum. The F-I fraction of CAE was the most effective inducer of macrophage cytotoxicity by prior sensitisation and by anti-F-I serum. This in-vitro cytotoxic potential correlated well with protection studies with F-I (Krupp, 1974; Vinayak *et al.*, 1980).
Peritoneal exudate cells of vaccinated animals are not phagocytosed by amoebae but kill them (Ghadirian and Meeroxvich, 1982b). Similar result were obtained in our experimental system. However, we have shown that macrophage cytotoxicity could also be induced by anti-amoebic serum. Furthermore, this capacity of the immune serum resided in the IgG fraction—2-mercaptoethanol treatment did not alter it and purified IgG was equally effective. Involvement of IgG in antibody-dependent cellular cytotoxicity against parasites has been shown previously (Capron et al., 1982).

The existence of Fc receptors of IgG on macrophages has been well documented (Unanue, 1981). It is conceivable that the IgG of an immune serum binds to the surface of the trophozoite by its Fab portion and to the macrophage by its Fc portion, thus signalling the macrophage to kill the trophozoite. It seems that attachment of macrophages to the trophozoite is necessary to induce cytotoxicity. It was observed that in the presence of anti-F-I serum a large number of macrophages attached to trophozoites, often completely surrounding them, and that the absolute number of trophozoites (dead and alive) was significantly decreased (p<0.001) after incubation for 4 h with macrophages in the presence of anti-F-I serum. Trophozoites were disintegrated, sometimes beyond recognition, by macrophages and it is thought that macrophages break down trophozoites before engulfing them.

We believe that specific anti-F-I antibodies may be important, in association with effector cells of the immune system, in combating invading trophozoites of E. histolytica. Specific cell-mediated immune responses are depressed after amoebic infection (Jain et al., 1980) and it is probable that a deficiency in one arm of the immune system is responsible for the effectiveness of E. histolytica as a parasite.

It has been shown that patients with hepatic amoebiasis have circulating immune complexes in their serum (Pillai and Mohimen, 1982). These may bind to the Fc receptors on the macrophages, rendering them unavailable for binding to IgG of immune serum. This is being studied in our laboratory.

REFERENCES


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