Immunosuppressive Effect of *Entamoeba histolytica* Extract on Hamsters

Roger Carvajal, Blanca Ruiz*, and Emelio Barjau
Departamento de Biología Experimental, Facultad de Medicina, Universidad Nacional Autónoma de México, México 20, D.F., México

**Abstract.** The immune response to sheep red blood cells (SRBC) in mice and hamsters injected with an extract of *Entamoeba histolytica* was studied. Both the primary and secondary immune response, measured by anti-SRBC antibody titers, were unaltered in the mouse, while a significant depression of the primary, but not the secondary, response was observed in the hamster. The effect was greatest when the amebic extract (AE) and SRBC were injected on the same day. The number of anti-SRBC rosettes formed in the spleen cells of hamsters treated with both AE and SRBC on day 0 was measured from days 1–16. The response peaked on day 13, while cells from animals injected with SRBC alone gave a maximal response on day 5. The formation of anti-SRBC rosettes in T-lymphocyte-enriched spleen cells treated with anti-gamma globulin serum and complement was almost abolished for the duration of the experiment. It is suggested that the mechanism responsible for this immunosuppressive phenomenon could involve early interference in the afferent limb of the immune response.

**Introduction**

Among the factors which play a role in the pathogenesis of amebiasis, the immune response has been implicated in the resistance of the host to the invasion of the tissues by trophozoites. This has been supported by the findings that the number and size of amebic lesions are greater in immunosuppressed hosts (Teodorovic et al. 1963) and that the factors which affect the immune system also modify the development of the infection (Teodorovic et al. 1963; Padma 1974). Invasive amebiasis in immunocompetent subjects is assumed to occur due to an evasion of the immune response.

* Scholarhip recipient from the Centro de Investigaciones Biológicas de Baja California Sur

Offprint requests to: R.E. Carvajal, Instituto Nacional de Enfermedades Respiratorias, Apt. 091, México 14080 D.F.
by the parasite. It has been suggested that a period of immunosuppression must exist if invasion by *Entamoeba histolytica* is to take place (Harris and Bray 1976). This suggestion is strengthened by data showing that: (a) there is no definite inflammatory process surrounding the amebic focus (Brandt and Pérez Tamayo 1970); (b) the cellular immune response decreases during amebic infection (Ortiz-Ortiz et al. 1975); (c) acquired immunity to intestinal amebiasis does not exist (Brandt and Pérez Tamayo 1970); and (d) there is high frequency of candidiasis associated with experimentally-induced hepatic amebic abscesses (Gonzalez-Mendoza et al. 1976).

The possibility that immunosuppression is associated with *E. histolytica* infection, similar to the effects of other protozoa (Ramos et al. 1978; Tanabe et al. 1978), was explored in this study. The modifications of the immune response to sheep erythrocytes in the spleen cells of mice and hamsters previously injected with an extract of *E. histolytica* were determined and compared to the response of untreated animals.

**Materials and Methods**

*Animals*

Young adult male and female golden hamsters (*Mesocricetus auratus*), weighing 80–120 g, were supplied by Dr. Tena Betancourt from the National Medical Center, Instituto Mexicano del Seguro Social, Mexico City. Outbred eight-week-old CD1 mice of both sexes (25–30 g) were obtained from our animal colony.

*Amebic Extract (AE)*

A total extract of axenically grown *Entamoeba histolytica* strain HK 9 was purchased from Millipore (Lot mex-113). The animals were injected intraperitoneally with varying amounts of the extract, as determined by protein measurement, in a volume of 0.3 ml, following dilution in Hank’s balanced salt solution (HBSS). The control animals were injected with HBSS alone.

*Sheep Erythrocyte Antigen*

Sheep red blood cells (SRBC) were kept in sterile Alsever solution until needed for experiments. Mice and hamsters were injected intraperitoneally with $2 \times 10^8$ SRBC suspended in 0.2 ml HBSS on the side opposite to that of the AE inoculation. When both substances were administered on the same day, SRBC were injected 2 h after the AE.

*Determination of Immune Response*

The animals were killed by bleeding, their spleens were teased apart, and the released cells were washed by centrifugation with large amounts of HBSS. In some experiments, the cells were treated with anti-gamma globulin serum and complement to eliminate immunoglobulin bearing cells (mainly B-lymphocytes). The anti-gamma globulin serum was obtained from rabbits after six weekly intraperitoneal injections of 5 mg of hamster gamma globulin, purified by ammonium sulfate and suspended in Freund’s complete adjuvant. The serum was adsorbed with an equal volume of a mixture of dissociated and washed hamster liver and kidney cells. The serum (diluted 1:6 in HBSS) was found to lyse nonadherent bone marrow cells in the