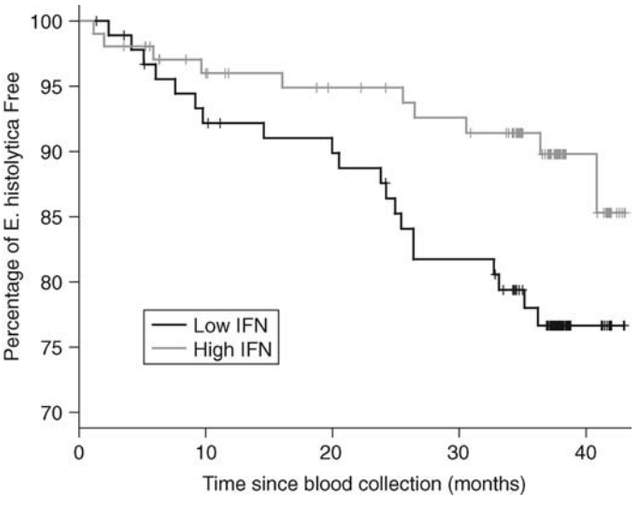

Figure 3 High levels of IFN-γ predict increased survival free of *Entamoeba histolytica* diarrhea. Peripheral blood mononuclear cells were stimulated with soluble amoebic extract and children grouped by IFN-γ production in response to soluble amebic extract (SAE) stimulation. Children were then followed for 44 months and incidence of *E. histolytica* diarrhea measured. Upper line and lower line indicate children with and without IFN-γ response above the median for all children (580 pg/mL), respectively. The two lines are significantly different: Logrank test $P = 0.03$; $n = 92$ for the low IFN-γ, and $n = 103$ for high IFN-γ groups (78). *Abbreviation*: IFN, interferon.

PARENTERAL VACCINES

The Gal/GalNac lectin plays an essential role in adherence and cytotoxicity as well as in resistance to serum complement. In addition, the lectin's cysteine-rich extracellular domain is highly conserved (81,82). The 170-kDa heavy subunit is the predominant amoebic protein recognized by immune sera of individuals cured of invasive amebiasis from geographically diverse areas including the United States, Mexico, Africa, India, and Jordan (81,82). Greater than 90% of sera from individuals with amoebic liver abscess or asymptomatic colonization with *E. histolytica* contain anti-lectin antibodies (81–83). And as mentioned above, acquired immunity in humans is associated with the production of mucosal IgA against the lectin.

In one study, 100% of gerbils immunized with purified native Gal/GalNac lectin in complete Freund's adjuvant developed high titer serum antibodies to the heavy subunit. Immune sera completely blocked amoebic adherence to CHO cells at 1/10 dilutions, and 67% of gerbils were completely protected from liver abscess following intrahepatic injection of trophozoites. Surprisingly, the remaining animals developed larger abscesses (84). Antibodies to different epitopes on the lectin's 170-kDa heavy subunit variably enhance or inhibit amoebic adherence to CHO cells and to human colonic mucin, but no differences in the development of anti-lectin antibodies or their adherence-inhibitory properties were observed in the immunized gerbils (84).

Parenteral immunization with two different recombinant peptides based on the cysteine-rich extracellular portion of the lectin's heavy subunit has been protective in the gerbil model of amoebic liver abscess. In one study, immunization of gerbils with the recombinant LC3 region and Titermax adjuvant elicited a high titer serum IgG response capable of inhibiting amoebic adherence to CHO cells. There was a 71% reduction in the number of animals with liver abscesses following intrahepatic challenge and, in contrast to abscesses following immunization with the native lectin, abscesses in the immunized gerbils that developed them were no larger than in controls (85). Similarly, Lotter et al. immunized gerbils with several recombinant peptides based on the carboxyl-terminal portion of the lectin's cysteine-rich extracellular domain. Immunization with a 115 amino acid peptide (termed 170CR2) completely prevented abscess development in 62.5% of animals and the remaining animals in this study developed significantly smaller abscesses than unimmunized controls. Antibody production to a 25 amino acid sequence within 170CR2 correlated strongly with development of protective immunity. Successful passive immunization of SCID mice with rabbit serum raised against the peptide reconfirmed the importance of humoral immunity in prevention of amoebic liver abscess (70).

Stanley et al. identified the SREHP by screening cDNA libraries (79). This protein contains multiple tandem dodecapeptide repeats reminiscent of the repetitive circumsporozoite antigens of malarial species. Indirect immunofluorescent staining localizes the native SREHP to the cell surface and to focal areas within the cytoplasm (86). Different *E. histolytica* isolates have different numbers of dodecapeptide repeats encoded within their SREHP genes (87). Western blots for the presence of anti-SREHP antibodies in patients from diverse geographical regions with acute invasive amebiasis were positive in 82%. Seropositivity ranged from 65% in Durban, South Africa, to

Table 1 Known Characteristics of Current Antiamoebic Vaccine Candidates

Amoebic protein	Putative function	Surface expression?	Conserved?	Immunogenic?	Protective in animal models?
Amoebapore	Cytolytic activity	Yes, secreted	Yes	Unknown	Unknown
Cysteine proteinase	Tissue penetration/degrades IgA, IgG, C3a, and C5a	Yes, secreted	Yes	Yes	Unknown
Gal/GalNac lectin	Adherence/complement resistance	Yes	Yes	Yes	Yes
Serine-rich *Entamoeba histolytica* protein	Possible role in adherence	Yes	No	Yes	Yes
29-kDa cysteine–rich antigen	Thiol-dependent peroxidase	Controversial, probably yes	Yes	In liver abscess only	Yes

Abbreviations: Gal/GalNac, galactose and *N*-acetyl-D-galactosamine; Ig, immunoglobulin.
Source: Adapted from Ref. 67.