A Severe Combined Immunodeficient (SCID) Mouse Model for Infection with *Entamoeba histolytica*

By Paul R. Cieslak,* Herbert W. Virgin IV,*‡§ and Samuel L. Stanley, Jr.*‡

*From the Departments of *Medicine, ‡Molecular Microbiology, and §Pathology, Washington University School of Medicine, St. Louis, Missouri 63110*

Summary

We used severe combined immunodeficient (SCID) mice to study resistance to invasive infection with *Entamoeba histolytica*. Seven of seven SCID mice developed liver abscesses when challenged intrahepatically with virulent HM1:IMSS strain *E. histolytica* trophozoites. Only one of seven similarly challenged immunocompetent congenic C.B-17 mice developed an abscess. Adoptive transfer of polyclonal rabbit anti-*E. histolytica* antiserum, but not preimmune rabbit serum, completely protected 7 of 12 SCID mice from intrahepatic challenge with ameba. These results demonstrate that lymphocyte-based immunity is important in protection against amebic liver abscess, and that anti-*E. histolytica* antibody can protect against amebic infection in this system. The SCID mouse may provide a powerful model for studying the components of protective immunity to invasive amebiasis.

The protozoan *Entamoeba histolytica* causes an estimated 36,000,000 cases of disabling colitis or liver abscess and kills at least 40,000 people annually, ranking it third worldwide among parasitic causes of death (1). Despite intensive research over the past two decades, the precise mechanisms of protective immunity to amebiasis have not been defined (2). Part of the problem has been the lack of a suitable animal model.

SCID mice lack functional B and T cells (3), a defect that can be corrected adoptive transfer of normal murine splenocytes (4). SCID mice have intact macrophage and NK cell-mediated immunity (5, 6). SCID mouse models have proved useful in studies of resistance to a number of viral (7–9), bacterial (10, 11), helminth (12), and protozoan pathogens (13, 14). We report here the establishment of a SCID mouse model for amebic liver abscess, and the use of this model to demonstrate that immune serum protects against visceral *E. histolytica* infection.

Materials and Methods

Cells. *E. histolytica*, strain HM1:IMSS (15), passaged three times through hamster liver, was kindly provided by Dr. V. Tsutsumi (Center for Research and Advanced Studies, National Polytechnical Institute, Mexico City, Mexico). The strain was maintained in our laboratory by subculturing twice weekly in axenic BI-S-33 medium (16) and passaged bimonthly through hamster liver to ensure continued virulence (17).

Animals. C.B-17-SCID mice and immunocompetent congenic C.B-17 mice were bred in a barrier facility at Washington University School of Medicine. Lack of infection with adventitious pathogens was documented using sentinel mice, intermittent serologic assessment of retired breeder C.B-17 mice, and inoculation of tissues from retired breeder SCID mice into C.B-17 recipients followed by serologic testing for murine viral pathogens.

Hepatic Inoculation. Log-phase (72-h) cultures *E. histolytica* HM1:IMSS trophozoites were chilled on ice for 5 min. Trophozoites were pelleted by centrifuging at 500 *g* for 5 min, counted on a hemocytometer, and resuspended in 100 μl BI-S-33 medium to yield a final concentration of ~2.5×10^6 amebas/100 μl. Tubes containing amebas were kept on ice pending inoculation, which occurred within 5–10 min after resuspension.

SCID mice and C.B-17 controls, weighing 20–25 g, were anesthetized intraperitoneally with 58 mg/kg ketamine and 8.7 mg/kg xylazine. After povidone-iodine scrub, a vertical incision, 1–1.5 cm in length, was made in the anterior abdominal wall. The peritoneal cavity was subsequently entered, and the 100 μl amebic inoculum (2.5×10^6 trophozoites) was administered by direct intrahepatic injection from a 1-ml tuberculin syringe via 26-gauge needle so that a visible bleb was raised. The peritoneum was closed with 4–0 chromic gut sutures and the abdominal wall with 7-mm Michel clips. The animals were returned to their cages and killed 7 d later. The entire liver was removed, weighed, and any abscess detected was resected and weighed. The percentage of liver abscessed was calculated as the weight of the abscess divided by the liver weight before abscess removal. Specimens for histology obtained from each abscessed and visually normal liver were fixed in formalin, sectioned, and stained with hematoxylin and eosin.

Passive Immunization. Immune serum was obtained from a rabbit vaccinated with HM1:IMSS trophozoites (18), and stored at −20°C until use. SCID mice were injected intraperitoneally with 300 μl immune rabbit serum or an equivalent amount of preimmune serum

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/92/12/1605/05 $2.00
Volume 176 December 1992 1605–1609

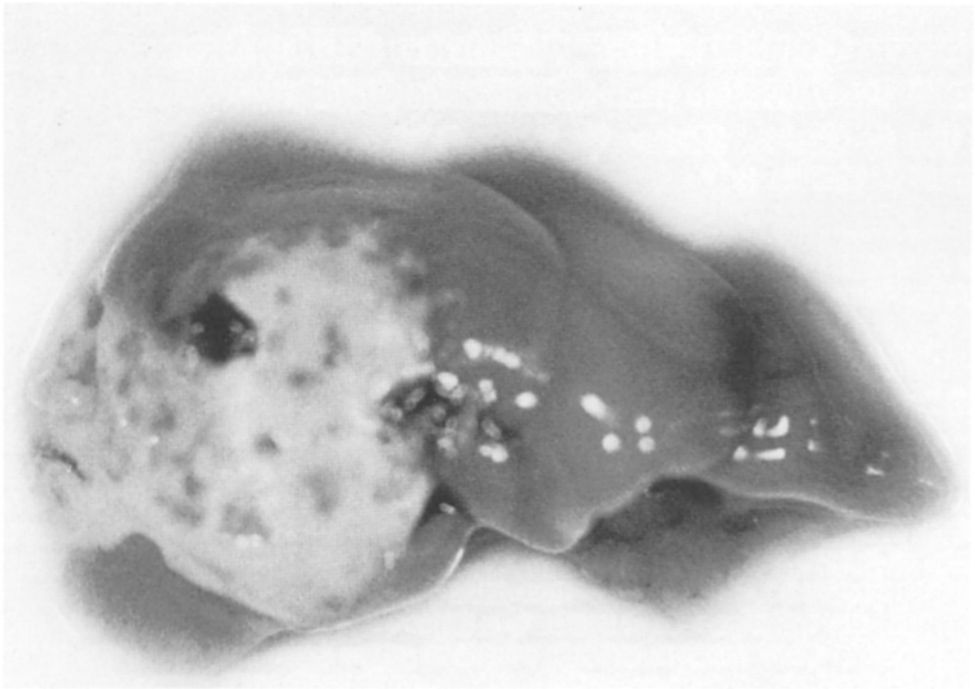

Figure 1. Gross appearance of amebic liver abscess in a SCID mouse.

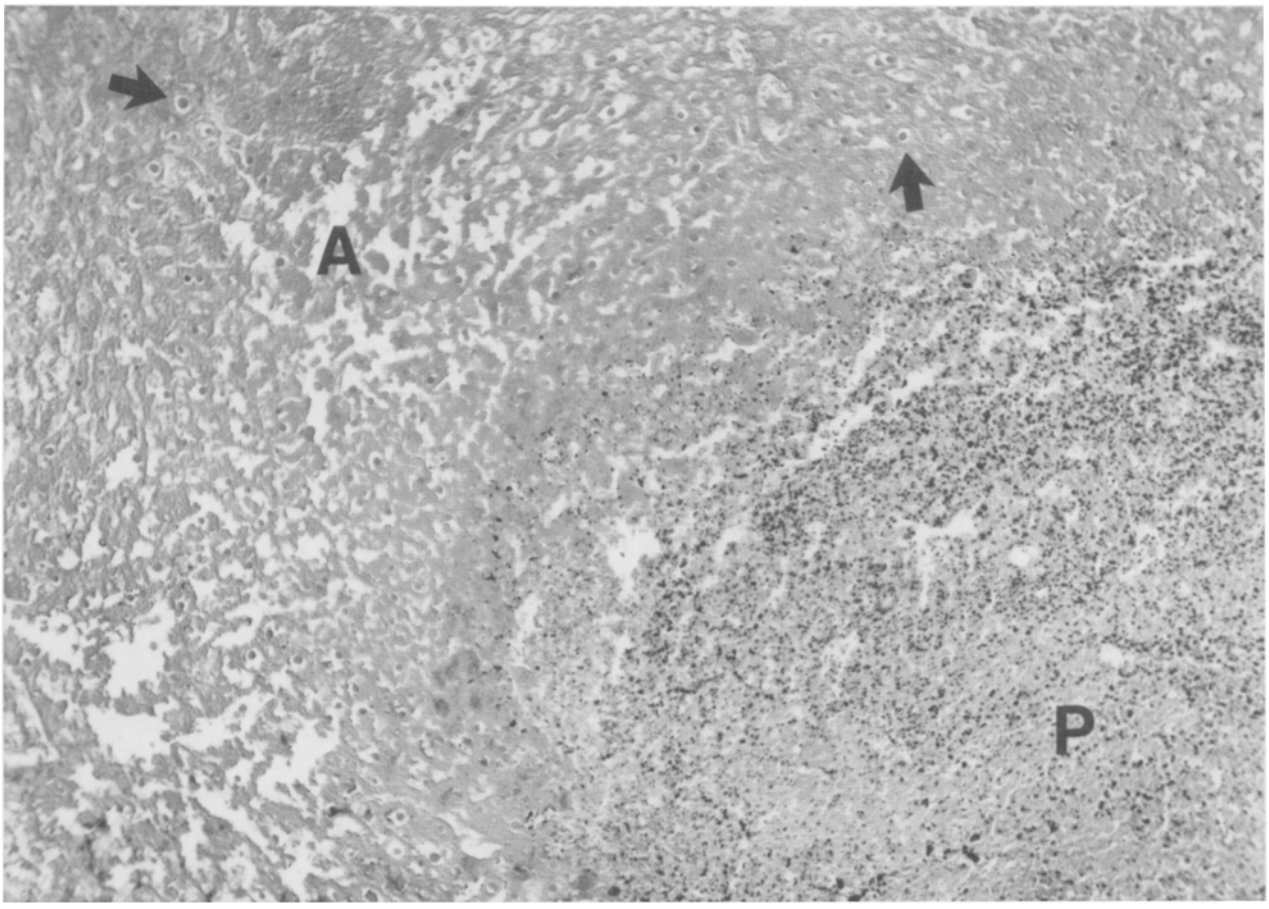

Figure 2. Photomicrograph of hematoxylin and eosin-stained section of *E. histolytica* liver abscess from SCID mouse. Amebic trophozoites (*arrows*) can be seen in areas of necrosis within the abscess (*A*). An intense polymorphonuclear infiltrate is seen in the liver parenchyma (*P*) adjacent to necrotic areas. ×150.

24 h before intrahepatic challenge with 2.5 × 10^6 HM1:IMSS *E. histolytica* as described above.

Results and Discussion

Inbred mouse strains are generally resistant to amebic infection (19, 20). In contrast we found that seven of seven SCID mice developed liver abscesses 1 wk after inoculation of virulent HM1:IMSS *E. histolytica*. Only one of the seven congenic immunocompetent C.B-17 mice developed a liver abscess ($\chi^2 = 10.5$, $p < .001$). The use of virulent hamster liver-passaged ameba appears to be necessary for the establishment of amebic liver abscess in SCID mice, since equivalent quantities of clonally derived HM1:IMSS trophozoites which were avirulent in hamster and gerbil liver abscess models were incapable of causing abscesses in SCID mice (data not shown).

Abscesses in SCID mice were grossly visible and usually bulging from the liver parenchyma (Fig. 1). Histologic specimens from SCID liver abscesses revealed eosinophilic areas of necrosis with intense, predominantly polymorphonuclear inflammatory infiltrates in adjacent liver parenchyma (Fig. 2). Eosinophilic *E. histolytica* trophozoites could be seen amidst the necrotic debris (Fig. 3), and were present throughout the abscess cavity, rather than solely at the periphery of the abscess, as has been described in human liver abscesses (21). The intense neutrophilic infiltration seen in hepatic tissue bordering the SCID mouse liver abscesses is not a regular component of hepatic amebiasis in humans, but it has been reported as an early stage of abscess development in animal models (22). The appearance of neutrophils may represent a critical role for these cells early in amebic hepatic infection, which may be enhanced and longer-lasting in SCID mice because of an absence of lymphocyte function. Since our study focused on abscesses at a single time point, the natural history of abscess formation in SCID mice will require further analysis.

Whereas SCID mice developed amebic abscesses, equivalent challenge failed to produce abscesses in all but one of the congenic immunocompetent C.B-17 mice. This suggests that lymphocyte-based immunity plays a role in the resistance of immunocompetent mice to amebic liver abscess. Additionally, our data suggests that macrophage, granulocyte, and NK cell-mediated resistance is not sufficient to control invasive amebic disease in this model, since all of these components of inflammatory responses are present in SCID mice. Our current model does not speak to the role of these host defense components in controlling amebic invasion in the intestine, or spread from the intestine to the liver. In this re-

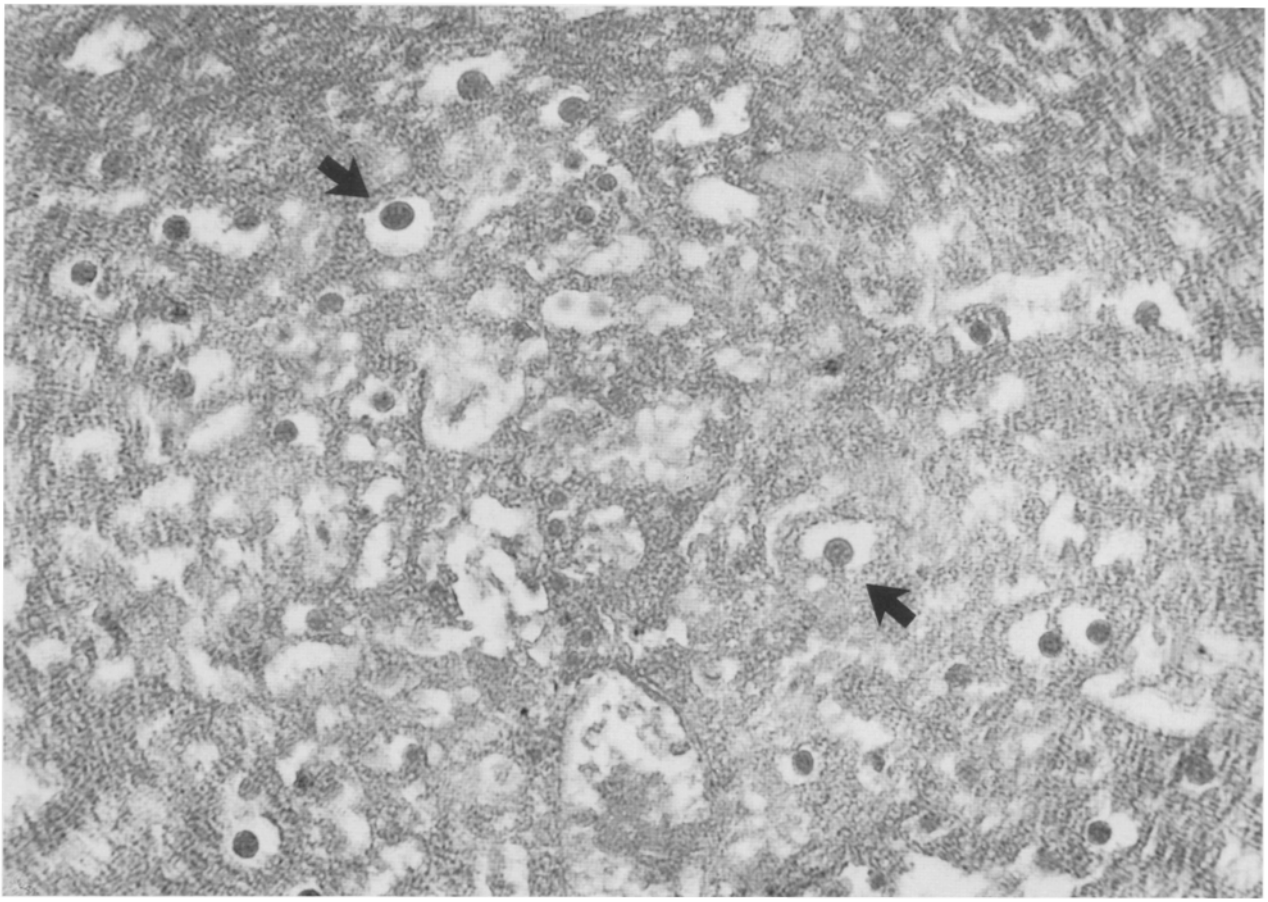

Figure 3. High power detail of hematoxylin-eosin–stained section of amebic liver abscess in SCID mouse demonstrating multiple *E. histolytica* trophozoites (*arrows*) within necrotic debris. ×600.

gard, it should be noted that in preliminary studies direct intracecal inoculation of more than 10^6 virulent HM1:IMSS trophozoites failed to establish intestinal infection or disease in SCID mice (data not shown). This suggests that the mechanisms that render other inbred mouse strains resistant to intestinal infection with *E. histolytica* are intact in SCID mice.

We subsequently used the SCID mouse model to investigate whether passive transfer of *E. histolytica*-immune serum would be sufficient to protect against amebic liver abscess. We found that a single dose of an *E. histolytica*-immune rabbit serum administered 24 h before intrahepatic challenge with amebic trophozoites provided complete protection from liver abscess in 7 of 12 (58%) of SCID mice (Table 1). Preimmune antiserum was not protective, as nine of nine control SCID mice developed amebic liver abscesses ($\chi^2 = 7.875$, $p < .01$). Antibody has not generally been considered to play an important role in resistance to amebiasis (2). Our results, however, are consistent with those of Swartzwelder and Avant (23), who found in an intestinal model of amebiasis that the infection rate of dogs inoculated per anum decreased from 85 to 30% after passive transfer of immune dog serum. In addition, Sepúlveda et al. (24) have reported that hamsters passively immunized with *E. histolytica*-immune human serum that were then challenged intrahepatically with virulent *E. histolytica* developed smaller liver abscesses than unimmunized controls.

The mechanisms by which antibody conferred protection remain unclear. Antibody-dependent cell-mediated cytotoxicity (ADCC)[1] is one possibility. Neutrophils and eosinophils, both of which are intact in the SCID mouse, have been implicated in the antibody-dependent killing of schistosome parasites (25, 26). Provision of *E. histolytica*-immune serum to SCID mice may allow ADCC directed against ameba to occur.

[1] *Abbreviation used in this paper:* ADCC, antibody-dependent cell-mediated cytotoxicity.

Table 1. *Amebic Liver Abscess Sizes, Given as Percent Liver Abscessed in SCID Mice Receiving E. histolytica-immune Rabbit Serum vs. Preimmune Rabbit Serum*

Immune serum		Preimmune serum	
Mouse	Abscess size	Mouse	Abscess size
	%		%
1	No abscess	1	10.8
2	No abscess	2	3.9
3	13.2	3	1.8
4	3.8	4	16.6
5	No abscess	5	7.9
6	No abscess	6	1.9
7	No abscess	7	32.6
8	5.2	8	10.4
9	5.2	9	8.8
10	8.7		
11	No abscess		
12	No abscess		

Complement-dependent mechanisms are another possibility. Virulent *E. histolytica* are known to be resistant to lysis by human complement in the absence of detectable antibody (27). These virulent strains may be lysed by mouse complement in the presence of anti-*E. histolytica* rabbit serum (which can fix mouse complement).

We have described a new and potentially valuable model for the study of the immunology of amebiasis. Furthermore, a protective role for humoral immunity was found. The establishment of a SCID mouse model for amebic liver abscess provides a means for further analysis of the contributions of humoral and cell-mediated immunity to protection against infection with *E. histolytica*.

We are indebted to Dr. Victor Tsutsumi and colleagues for providing the virulent HM1:IMSS *E. histolytica*, and for assistance with surgical technique. We thank Dr. Paul Swanson for reviewing histological specimens, and Dr. Tonghai Zhang and Lynne Foster for technical assistance.

P. Cieslak is supported by training grant 5T32AI-07172 from the National Institutes of Health. S. Stanley, Jr., is supported in part by Public Health Service grant AI-30084 and World Health Organization MIM/15/181/174.

Address correspondence to Dr. Samuel L. Stanley, Jr., Department of Medicine, Campus Box 8051, 660 South Euclid Avenue, St. Louis, MO 63110.

Received for publication 17 July 1992 and in revised form 24 August 1992.

References

1. Walsh, J.A. 1986. Problems in recognition and diagnosis of amebiasis: estimation of the global magnitude of morbidity and mortality. *Rev. Infect. Dis.* 8:228.
2. Salata, R.A., and J.I. Ravdin. 1986. Review of the human im-

mune mechanisms directed against *Entamoeba histolytica*. *Rev. Infect. Dis.* 8:261.
3. Bosma, G.C., R.P. Custer, and M.J. Bosma. 1983. A severe combined immunodeficiency mutation in the mouse. *Nature (Lond.).* 301:527.
4. Custer, R.P., G.C. Bosma, and M.J. Bosma. 1985. Severe combined immunodeficiency (SCID) in the mouse: Pathology, reconstitution, neoplasms. *Am. J. Pathol.* 120:464.
5. Czitrom, A.A., S. Edwards, R.A. Phillips, M.J. Bosma, P. Marrack, and J.W. Kappler. 1985. The function of antigen-presenting cells in mice with severe combined immunodeficiency. *J. Immunol.* 134:2276.
6. Lauzon, R.J., K.A. Siminovitch, G.M. Fulop, R.A. Phillips, and J.C. Roder. 1986. An expanded population of natural killer cells in mice with severe combined immunodeficiency (SCID) lack rearrangement and expression of T cell receptor genes. *J. Exp. Med.* 164:1797.
7. Minagawa, H., S. Sakuma, S. Mohri, R. Mori, and T. Watanabe. 1988. Herpes simplex virus 1 infection in mice with severe combined immunodeficiency (SCID). *Virology.* 103:73.
8. Dharakul, T., L. Rott, and H.B. Greenberg. 1990. Recovery from chronic rotavirus infection in mice with severe combined immunodeficiency: Virus clearance mediated by adoptive transfer of immune $CD8^+$ T lymphocytes. *J. Virol.* 64:4375.
9. George, A., S.I. Kost, C.L. Witzleben, J.J. Cebra, and D.H. Rubin. 1990. Reovirus-induced liver disease in severe combined immunodeficient (SCID) mice. A model for the study of viral infection, pathogenesis, and clearance. *J. Exp. Med.* 171:929.
10. Bancroft, G.J., K.C. Sheehan, R.D. Schreiber, and E.R. Unanue. 1989. Tumor necrosis factor is involved in the T-cell independent pathway of macrophage activation in SCID mice. *J. Immunol.* 143:127.
11. Schaible, U.E., R. Wallich, M.D. Kramer, C. Museteanu, and M.M. Simon. 1992. A mouse model for *Borrelia burgdorferi* infection: pathogenesis, immune response and protection. *Behring. Inst. Mitt.* 88:59.
12. Nelson, F.K., D.L. Greiner, L.D. Shultz, and T.V. Rajan. 1991. The immunodeficient *scid* mouse as a model for human lymphatic filariasis. *J. Exp. Med.* 173:659.
13. Holaday, B.J., M.D. Sadick, Z.-E. Wang, S.L. Reiner, F.P. Heinzel, T.G. Parslow, and R.M. Locksley. 1991. Reconstitution of *Leishmania* immunity in severe combined immunodeficient mice using Th1- and Th2-like cell lines. *J. Immunol.* 147:1653.
14. Mead, J.R., M.J. Arrowood, R.W. Sidwell, and M.C. Healey. 1991. Chronic *Cryptospordium parvum* infection in congenitally deficient immunodeficient SCID and nude mice. *J. Infect. Dis.* 163:1297.
15. De la Torre, M., R. de la Hoz-Couturier, L. Landa, and B. Sepúlveda. 1971. Cultivos axénicos die *Entamoeba histolytica*. *Arch. Invest. Med.* 2(Suppl. 1):165.
16. Diamond, L.S., D.R. Harlow, and C.C. Cunnick. 1978. A new medium for the axenic cultivation of *Entamoeba histolytica* and other *Entamoeba*. *Trans. R. Soc. Trop. Med. Hyg.* 72:431.
17. Bos, H.J., and R.J. van de Griend. 1977. Virulence and toxicity of axenic *Entamoeba histolytica*. *Nature (Lond.).* 265:341.
18. Stanley, S.L., Jr., A. Becker, C. Kunz-Jenkins, L. Foster, and E. Li. 1990. Cloning and expression of a membrane antigen of *Entamoeba histolytica* possessing multiple tandem repeats. *Proc. Natl. Acad. Sci. USA.* 87:4976.
19. Neal, R.A., and W.G. Harris. 1975. Attempts to infect inbred strains of rats and mice with *Entamoeba histolytica*. *Trans. R. Soc. Trop. Med. Hyg.* 69:429. (Abstr.)
20. Gold, D., and I.G. Kagan. 1978. Susceptibility of various strains of mice to *Entamoeba histolytica*. *J. Parasitol.* 64:937.
21. Brandt, H., and R. Pérez Tamayo. 1970. Pathology of human amebiasis. *Hum. Pathol.* 1:351.
22. Tsutsumi, V., R. Mena-Lopez, F. Anaya-Velazquez, and A. Martinez-Palomo. 1984. Cellular bases of experimental amebic liver abscess formation. *Am. J. Pathol.* 117:81.
23. Swartzwelder, J.C., and W.H. Avant. 1952. Immunity to amebic infection in dogs. *Am. J. Trop. Med. Hyg.* 1:567.
24. Sepúlveda, B., M. Tanimoto-Weki, and P. Calderón. 1974. Induction de immunidad pasiva antiamibiana en el hamster por la injeccion de suero immune. *Arch. Invest. Med.* 5(Suppl. 2):451.
25. Butterworth, A.E., R.F. Sturrock, V. Houba, and P.H. Rees. 1974. Antibody-dependent cell-mediated damage to schistosomula *in vitro*. *Nature (Lond.).* 252:503.
26. Dean, D.A., R. Wistar, and K.D. Murrell. 1974. Combined *in vitro* effects of rat antibody and neutrophilic leukocytes on schistosomules of *Schistosoma mansoni*. *Am. J. Trop. Med. Hyg.* 23:420.
27. Reed, S.L., P.G. Sargeaunt, and A.I. Braude. 1983. Resistance to lysis by human serum of pathogenic *Entamoeba histolytica*. *Trans. R. Soc. Trop. Med. Hyg.* 77:248.