Sexual Dimorphism in the Control of Amebic Liver Abscess in a Mouse Model of Disease

Hannelore Lotter,1,4† Thomas Jacobs,2† Iris Gaworski,2 and Egbert Tannich1

Department of Molecular Parasitology1 and Department of Immunology,2 Bernhard Nocht Institute for Tropical Medicine, 20359 Hamburg, Germany

INFECTION AND IMMUNITY, Jan. 2006, p. 118–124 Vol. 74, No. 1

Accepted 27 September 2005

Amebic liver abscess (ALA) is the most common extraintestinal manifestation of human infection by the enteric protozoan parasite Entamoeba histolytica. In contrast to intestinal infection, ALA greatly predominates in males but is rare in females. Since humans are the only relevant host for E. histolytica, experimental studies concerning this sexual dimorphism have been hampered by the lack of a suitable animal model. By serial larval passage of cultured E. histolytica trophozoites in gerbils and mice, we generated amebae which reproducibly induce ALA in C57BL/6 mice. Interestingly, all animals developed ALA, but the time courses of abscess formation differed significantly between the genders. Female mice were able to clear the infection within 3 days, whereas in male mice the parasite could be recovered for at least 14 days. Accordingly, male mice showed a prolonged time of recovery from ALA. Immunohistology of abscesses revealed that polymorphonuclear leukocytes and macrophages were the dominant infiltrates, but in addition, γδ-T cells, NK cells, and natural killer T (NKT) cells were also present at early times during abscess development, whereas conventional αβ-T cells appeared later, when female mice had already cleared the parasite. Interestingly, male and female mice differed in early cytokine production in response to ameba infection. Enzyme-linked immunospot assays performed with spleen cells of infected animals revealed significantly higher numbers of interleukin-4-producing cells in male mice but significantly higher numbers of gamma interferon (IFN-γ)-producing cells in female mice. Early IFN-γ production and the presence of functional NKT cells were found to be important for the control of hepatic amebiasis as application of an IFN-γ-neutralizing monoclonal antibody or the use of NKT knockout mice (Vα14NKT, Jα 18−/−) dramatically increased the size of ALA in female mice. In addition, E. histolytica trophozoites could be reisolated from liver abscesses of Jα18−/− mice on day 7 postinfection, when wild-type mice had already cleared the parasite. These data suggest that the sexual dimorphism in the control of ALA is due to gender-specific differences in early cytokine production mediated at least in part by NKT cells in response to E. histolytica infection of the liver.

Entamoeba histolytica, the causative agent of human amebiasis, is endemic in most tropical and subtropical countries and is considered to be responsible for tens of millions of cases of dysentery and liver abscesses each year (32, 33). Following fecal-oral transmission, the parasite colonizes the large bowel, where it multiplies and may persist for months or even years as an asymptomatic luminal gut infection (3). Only in about 10% of infected individuals does the parasite penetrate the intestinal mucosa and induce colitis or disseminate to other organs, and it most commonly disseminates to the liver, where it induces abscess formation (13). At present, the factors that trigger ameba invasion are largely unknown.

Another unresolved enigma in amebiasis is the fact that the occurrence of amebic liver abscesses (ALA) is age and sex dependent. In children, the dominant clinical symptoms associated with E. histolytica infections are restricted to the gut, and development of ALA is extremely rare (16). More than 95% of all ALA cases occur in adults (1, 3, 4). However, the disease greatly predominates in males, and the male-to-female ratio is between 2:1 and 7:1 depending on age. The risk for development of ALA in females is more or less equally distributed among the different age groups, with a slight increase in women who are more than 60 years old. In contrast, the risk for ALA in males increases after puberty, and the peak incidence is at approximately 40 years of age. This sexual dimorphism for risk of ALA is independent of the prevalence of the parasite, which is usually higher in children and adult females than in adult males (4). Moreover, it appears to be independent of cultural or ethnic background as it has been observed in individuals in all parts of the world where amebiasis is endemic, as well as in travelers from countries where it is not endemic (1, 23, 30).

Since humans are the only relevant host for E. histolytica, attempts to study the mechanism underlying the observed ALA-associated gender differences have been hampered by the lack of suitable animal models. In recent years, several rodent species have been successfully used as models to study the development of ALA. Although amebic liver lesions could be provoked by direct inoculation of E. histolytica trophozoites into the livers of rabbits, Syrian hamsters, or Mongolian gerbils (5, 6, 14, 20), these models had substantial limitations, such as a lack of suitable tools for studying the immune response of infected animals and the fact that gender differences in response to amebic infections were not observed in these laboratory animals. In addition, several immunodeficient mouse strains have been used, including SCID mice and gamma in-
terferon (IFN-γ) and inducible nitric oxide synthase knockout mice. From these studies it was concluded that polymorphonuclear leukocytes are the major constituent of the early wave of infiltrating cells during the onset of ALA and that the containment of trophozoites is dependent on the production of IFN-γ, leading to the production of amebicidal NO (25–27, 29). Although immunocompetent mice were also found to develop ALA, gender-specific differences were not assessed.

Here we describe experimental induction of ALA in immunocompetent C57BL/6 mice and show that female mice were more resistant to development of ALA than male mice. This resistance was associated with significantly faster clearance of $E.\;histolytica$ trophozoites from the liver and an accelerated time of recovery from ALA. Interestingly, during the early phase of ALA development, spleen cells from female mice elicited higher IFN-γ levels than spleen cells from male mice elicited, whereas male mice contained higher numbers of interleukin-4 (IL-4)-producing cells. The importance of IFN-γ for the control of liver abscess formation in female mice was demonstrated by application of an IFN-γ neutralizing antibody. Moreover, we present evidence that a source of early IFN-γ production is natural killer T (NKT) cells, which were found to be required for the control of ALA in female mice.

**MATERIALS AND METHODS**

**Cultivation of parasites and infection of mice.** For all experiments $E.\;histolytica$ trophozoites of isolate HM-1:IMSS were used, and these trophozoites were cultivated axenically in TYI-S-33 medium (10). Animal infections were performed with 3-month-old C57BL/6 mice using a protocol previously described by Chadee and Meerovitch (6), with minor modifications. For some experiments J:s18−/− mice, which lack Vn14NK1 cells (7), were used. In brief, mice were anesthetized by intramuscular injection of a mixture containing 20 μl of xylazine (2%), 40 μl of ketamine hydrochloride (5%), and 20 μl of phosphate-buffered saline (PBS). Subsequently, laparotomy was performed by vertical incision (about 1.0 cm) to visualize the liver, and 1 × 105 cultured $E.\;histolytica$ trophozoites from the early log phase of growth suspended in 25 μl of PBS were injected into the left liver lobe using an insulin syringe. The peritoneum and the abdominal wall were sealed with resorbable thread, and the skin was closed with wound clips. At times indicated below, animals were sacrificed and analyzed for the presence of ALA. Abscesses occurred in two forms, single nodules and multiple closely related nodules. For comparisons, the diameters of abscessed areas within the presence of the liver were measured, and the results were expressed using the following scores: 0, no abscess; 1, pinhead; 2, <5.0 mm; and 3, >5.0 mm.

**Immunohistochemistry.** Fluorescein isothiocyanate (FITC)-labeled anti-CD4, anti-CD8, anti-CD3, anti-CD26, anti-αβ-TCR, anti-γδ-TCR, anti-DX-5, anti-GR1, anti-CD11b, and anti-CD11c were purchased from Becton Dickinson (Heidelberg, Germany). FITC-labeled anti-NK1.1 was obtained from CALTAG (Burlingame, CA). All antibodies were used at a dilution at which nonspecific staining was not observed with a control specimen. For immunohistochemistry ALA-containing liver lobes were embedded in tissue-freezing medium (Leica, Nussloch, Germany), frozen in liquid nitrogen, and stored at −70°C until use. Cryosections (6 μm) were stained with the FITC-labeled primary antibody, followed by anti-FITC-AlexaFluor488 (Molecular Probes, Eugene, Oreg.). NK cells and NKT cells were differentiated by double staining with anti-NK-1.1 or anti-GR1, anti-CD3, anti-CD11c, and anti-CD26 using the FITC-labeled primary antibody and anti-CD4, anti-CD8, anti-CD3, anti-CD26, anti-NK-1.1, or anti-GR1, anti-CD3, anti-CD11c, and anti-CD26 with the biotinylated secondary antibody, followed by avidin-conjugated peroxidase. Spots were developed with substrate buffer (100 mM Tris [pH 7.5]; 3.3'-diaminobenzidine, 800 μg/ml; NiCl2, 400 μg/ml) and were analyzed with an ELISPOT reader (Bioreader 2000). Data were expressed as the number of cytokine-producing cells per 1 × 105 cells (spot-forming units).

**Neutralization of IFN-γ.** Neutralizing monoclonal antibody against IFN-γ was purified from supernatants of hybridoma cells (rat XMG 1.2) using HitTrap protein G columns (Pharmacia, Uppsala, Sweden). The neutralizing capacity was regularly checked by incubation of the purified antibody with recombinant mouse IFN-γ, followed by an anti-IFN-γ cytokine enzyme-linked immunosorbent assay (Becton Dickinson, Heidelberg, Germany). Ten female mice per group were immunized by intraperitoneal application at day −1 of either 150 μl PBS, 500 μg of neutralizing monoclonal antibody against IFN-γ, or 500 μg of isotype-matched control antibody (rat monoclonal antibody immunoglobulin G1 [IgG1]; Dianova, Hamburg, Germany).

**FIG. 1.** Comparison of amebic liver abscess progression in male and female C57BL/6 mice. (A) Eight animals of each gender were intraperitoneally challenged with 105 $E.\;histolytica$ trophozoites at each time point. Animals were sacrificed at the times indicated, and the relative sizes of the abscesses were calculated by using the following scores: 0, no abscess; 1, pinhead; 2, <5.0 mm; and 3, >5.0 mm.

(a) Comparison of amebic liver abscess progression in male and female C57BL/6 mice. (A) Eight animals of each gender were intraperitoneally challenged with 105 $E.\;histolytica$ trophozoites at each time point. Animals were sacrificed at the times indicated, and the relative sizes of the abscesses were calculated by using the following scores: 0, no abscess; 1, pinhead; 2, <5.0 mm; and 3, >5.0 mm. According to the Mann-Whitney U test, female mice had significantly smaller abscesses than male mice on day 7 ($P < 0.05$), on day 14 ($P < 0.0001$), and on day 21 ($P < 0.0001$). (B) Reisolation of $E.\;histolytica$ trophozoites from liver abscess lesions of C57BL/6 mice. Infected animals were sacrificed at the times indicated, the entire liver was removed from each animal, and abscesses were dissected and dissolved in TYI-S-33 medium. The bars indicate the percentages of animals with positive ameba cultures. According to a paired $t$ test, there were significant differences between male and female mice on day 7 ($P < 0.0001$) and on day 14 ($P < 0.001$). * $P < 0.05$; ** $P < 0.005$; *** $P < 0.0001$. 

For enzyme-linked immunosorbent assay (ELISPOT) analysis, matched pairs of either anti-IFN-γ or anti-IL-4 antibodies were used (Becton Dickinson, Heidelberg, Germany). Spleen cells from infected or noninfected control mice (2 × 105 cells/well) were incubated with medium alone or with medium supplemented with 3 μg per ml of anti-CD3 for 24 h on ELISPOT plates (MultiScreen HA; Millipore, Bedford, MA) that were previously coated with the appropriate capture antibody. Analysis was performed as described by the manufacturer. Cytokine-producing cells were detected using biotinylated anti-IFN-γ, followed by avidin-conjugated peroxidase. Spots were developed with substrate buffer (100 mM Tris [pH 7.5]; 3.3'-diaminobenzidine, 800 μg/ml; NiCl2, 400 μg/ml) and were analyzed with an ELISPOT reader (Bioreader 2000). Data were expressed as the number of cytokine-producing cells per 1 × 105 cells (spot-forming units).
Statistical analysis. The Mann-Whitney U test was used to compare the liver abscess sizes of male and female mice over the time period monitored, and the paired $t$ test was used to determine the difference between abscesses from male and female mice that were culture positive for *E. histolytica*. Student’s $t$ test was used for the statistical analysis of the ELISPOT data.

RESULTS

Time course of amebic liver abscess formation in male and female C57BL/6 mice. To examine whether there are gender differences in immunocompetent mice in response to amebic infection of the liver, groups of eight C57BL/6 male and female mice were challenged by intrahepatic inoculation of axenically cultured *E. histolytica* trophozoites. Prior to injection into mice, amebae had been regularly passaged through gerbil livers in order to maintain virulence. On day 7 postinfection, livers were removed from the various animals and inspected for the presence of abscesses. The results indicated that there were clear differences between the two groups. All female mice had only residual lesions that were pinhead size at the site of ameba injection, and these lesions did not contain any viable parasites, as revealed by microscopy and culture. In contrast, 75% of male mice developed abscesses comparable to those usually seen in the gerbil model. In addition, abscesses of male mice contained viable *E. histolytica* trophozoites, which could be reisolated and grown in culture. Repetition of the experiment using a mouse-adapted *E. histolytica* strain, and liver abscess formation was assessed in eight animals per group and time on days 3, 7, 14, 21, and 35 postinfection (Fig. 1A). The results indicated that all animals sacrificed on day 3 or 7 had developed abscesses, which on average were larger in male mice than in female mice. During the following weeks both groups of mice were apparently able to control the infection and resolved their abscesses. However, resolution of abscesses was considerably slower in male mice than in female mice. On day 14 postinfection all male mice examined had abscesses which were larger than those found on day 3 or 7. In contrast, only four of eight female mice had abscesses on day 14, and the abscesses were significantly smaller than those of male mice ($P < 0.001$) (Fig. 1A). On day 21 none of the female mice examined had liver lesions, whereas all male mice had clear abscesses, although they were smaller than those seen at earlier times. On day 35, no signs of liver abscess were detected in female mice, whereas some residual lesions were still present in male animals.

Ameba culture revealed that in female mice viable parasites were present only on day 3 postinfection and not at any later time (Fig. 1B). In contrast, amebae could be reisolated from the majority of male mice until day 14, but they were absent in liver tissues from day 21 or 35 (Fig. 1B). This result was confirmed by periodic acid-Schiff staining of liver sections, which...
were predominantly GR1 infection (data not shown). From day 7 on, infiltrating cells appeared to be virtually absent during this very early phase of infection of abscess resolution were different for the two genders. Immunohistological staining of the lymphocyte population on days 3 and 7 postinfection indicated that there was accumulation of CD3+ T cells is expressed in spot-forming units (SFU).

FIG. 3. Time courses of IL-4 (A) and IFN-γ (B) production in male and female mice with ALA. Cytokine-producing spleen cells were determined by ELISPOT assays. Spleen cells from male or female mice with ALA were cultivated on antibody-coated plates for 24 h either in the presence of medium alone or in the presence of anti-CD3. Plates were developed with the appropriate secondary antibodies, followed by the detection reagent. The number of cytokine-producing cells is expressed in spot-forming units (SFU).

indicated that intact trophozoites were absent in female mice at any time later than day 3 postinfection (data not shown).

Characterization of immune cells infiltrating amebic liver abscesses in C57BL/6 mice. Histological examination of liver abscesses during the period between day 3 and day 35 postinfection using classical staining methods (Giemsma stain, hematoxylin and eosin, periodic acid-Schiff stain) revealed no differences in the composition of cellular infiltrates between male and female mice. The regeneration of the liver tissue proceeded similarly in the two groups, although the time courses of abscess resolution were different for the two genders. Immunohistological staining of the lymphocyte population on days 3 and 7 postinfection indicated that there was accumulation of γ,δ-T cells, NK cells, and NKT cells in the central abscess area (Fig. 2A to C). Cells that expressed anti-CD3 or a conventional α,β-T cell receptor in the absence of NK-1.1 appeared to be virtually absent during this very early phase of infection (data not shown). From day 7 on, infiltrating cells were predominantly GR1+ neutrophils (Fig. 2D) and CD11b+ macrophages (Fig. 2E); in addition, a large number of conventional α,β-T cells were present in the abscess center (Fig. 2F). These cells were found to coexpress CD3 but not NK-1.1 (data not shown).

Comparison of IFN-γ production and IL-4 production during ALA development. Previous studies have shown that IFN-γ is a key mediator for induction of amebicidal activity of immune cells. Therefore, we compared the cytokine profiles for spleen cells of male and female ALA mice by performing ELISPOT assays at the early phase (days 3 and 7) and the late phase (days 21 and 35) of infection (Fig. 3). At the early times, cytokine production was most probably due to innate immune mechanisms, whereas at the late times a contribution by the adaptive immune response had to be considered. This was corroborated by analysis of the activation marker CD25 on splenic T cells by flow cytometry. On day 21 postinfection an increased number of T cells were found to express this activation marker, whereas almost no induction was found on day 7 postinfection (data not shown). Interestingly, on day 3 or 7 postinfection, significantly higher numbers of IFN-γ-producing cells were found in the spleens of female ALA mice, whereas male mice contained elevated numbers of IL-4-producing cells. In contrast, on day 21 postinfection, this dichotomy was not observed, and male and female mice contained similar numbers of IL-4- and IFN-γ-producing cells.

Analysis of IFN-γ and functional NKT cells for early control of ALA in female mice. To test whether the increased early production of IFN-γ by female ALA mice might be important

| Mouse strain | No. of mice with abscesses | Abcess score (mean ± SE) | No. of mice positive for E. histolytica/100% of mice with abscesses | % of cultures positive for abscesses |
|--------------|---------------------------|-------------------------|-------------------------------------------------------------------|-----------------------------------|
| Jα 18+/+    | 10/11                     | 2.1 ± 0.3*              | 7/10                                                               | 70.0b                             |
| C57BL/6      | 7/11                      | 1.1 ± 0.3               | 1/7                                                                | 14.3                              |
| wild type    |                           |                         |                                                                   |                                   |

* Different from wild-type C57BL/6 mice at P < 0.05 (Mann-Whitney U test).

a Different from wild-type C57BL/6 mice at P < 0.01 (Mann-Whitney U test).
for the observed resistance to *E. histolytica* infection, we administered a neutralizing anti-IFN-γ antibody (XMG1.2) 1 day prior to intrahepatic challenge. The results indicated that in contrast to sham-immunized female mice or mice treated with a rat isotype control antibody (monoclonal antibody IgG1), anti-IFN-γ-treated animals developed significantly larger abscesses (Fig. 4).

NKT cells, which are present in the liver at a high level, are known to be an important source of early cytokine production. Therefore, development of ALA in mice lacking functional NKT cells (Vα14/NKT cells) was investigated using Jo18<sup>-/-</sup> mice. Inoculation of *E. histolytica* trophozoites into the livers of Jo18<sup>-/-</sup> female mice resulted in abscesses that were significantly larger than those in female wild-type mice (*P* < 0.05). Interestingly, viable trophozoites could be reisolated from liver abscesses of 70% of Jo18<sup>-/-</sup> female mice on day 7 postinfection, when the majority of wild-type mice (86%) had already cleared the parasite (*P* < 0.01) (Table 1). ELISPOT analysis of spleen cells derived from female Vα14/NKT cell-deficient Jo18<sup>-/-</sup> mice at day 7 postinfection revealed that there were significantly fewer IFN-γ-producing cells compared to spleen cells of infected wild-type mice (*P* < 0.002) (Fig. 5).

**FIG. 5.** IFN-γ production in female Vα14/NKT cell-deficient Jo18<sup>-/-</sup> mice after induction of ALA. Cytokine production from spleen cells was determined by ELISpot assays. Spleen cells either from uninfected C57BL/6 or Vα14/NKT cell-deficient Jo18<sup>-/-</sup> mice or from the same mouse strains 7 days after intrahepatic challenge with *E. histolytica* trophozoites (right panel) were cultivated on antibody-coated plates for 24 h in the presence of medium alone (−) or in the presence of anti-CD3 (+). Plates were developed with the appropriate secondary antibody, followed by the detection reagent. The number of cytokine-producing cells is expressed in spot-forming units (SFU). Each group consisted of five animals. p.i., postinfection.

**DISCUSSION**

This study resulted in three major findings. First, immuno-competent C57BL/6 mice reproducibly develop ALA when they are challenged with a mouse-adapted *E. histolytica* strain. Second, this animal model shows that there is clear sexual dimorphism with regard to the control of liver abscess formation. Third, IFN-γ and functional NKT cells are required to mediate primary resistance in female mice. In previous studies of mice the workers used cultured *E. histolytica* trophozoites that had been passaged through livers of hamsters or SCID mice (17, 18, 26, 28). However, a strain adapted to immunocompetent mice was not established, and gender differences were not addressed.

The results presented here indicate that female mice cleared the infection considerably faster than male mice cleared the infection, but there were no obvious gender differences in the morphology and histopathology of abscesses. Abscesses in C57BL/6 mice showed no tendency to spread to other lobes of the liver. They occurred either as single or multifocal abscesses similar to those found in human amebic infections (21). In human ALA, ameba trophozoites can be found within the abscess area and also in hepatic tissue distal from the abscess zone (29). In the C57BL/6 model, trophozoites were found only within or in the vicinity of the abscess area, and they were never detected in nonabscessed liver parenchyma. This might have simply been due to differences in the routes of infection as in the animal model parasites are directly inoculated into the liver, whereas in human hepatic infection it is believed that amebae continuously spread into the liver via the portal vein after evasion from the intestinal tract.

Liver sections of patients with subclinical ALA or at the beginning of ALA development are usually not examined (11). Thus, there is little information about the composition of cellular infiltrates at very early stages of human amebic infections of the liver. However, similar to the mouse model on day 7 or 14, biopsies of fully developed human ALA have been reported to contain massive infiltrates of neutrophils and monocytes within and around the parasite-infected tissue. In addition, CD4<sup>+</sup> and CD8<sup>+</sup> T cells have been described in human ALA, but they are usually in areas more distant from amebic trophozoites (29).

Another interesting result of this study is the finding that male and female ALA mice differ significantly in the production of cytokines, such as IFN-γ and IL-4, during the early phase of abscess development. Spleens of male mice were found to contain elevated numbers of IL-4-producing cells, whereas female mice had higher numbers of IFN-γ-positive lymphocytes. Gender-dependent differences in innate resistance that correlate with cytokine production have also been described in the SCID mouse model for *Toxoplasma gondii*. In this model, male mice were resistant and produced higher levels of IFN-γ and IL-12 than female mice (24, 31).

The initial higher IFN-γ level of females in the mouse model for ALA presented here might explain the capacity of females to rapidly clear amebic trophozoites from the infected tissue, which is further suggested by our finding that application of an IFN-γ-neutralizing antibody reverses the relative resistance of ALA development in female mice. A number of previous studies have shown the importance of IFN-γ in the defense against *E. histolytica* infection. IFN-γ or tumor necrosis factor alpha-stimulated neutrophils were found to be able to kill *E. histolytica* trophozoites in vitro (9). Likewise, after stimulation with recombinant IFN-γ or lipopolysaccharide macrophages were reported to exhibit strong amebicidal activity (8, 19). In vivo, targeted disruption of the IFN-γ receptor gene in SCID mice
led to an increase in the size of experimentally induced liver abscesses, as was found in mice with disruption of the gene encoding the inducible NO synthase (26). The increased ratio of proinflammatory cytokines is likely to allow female mice to trigger production of more NO and thus increase their resistance to ALA, whereas the high ratio of anti-inflammatory cytokines in male mice might dampen amebicidal mechanisms. However, the cellular source for IFN-γ and IL-4 remains to be determined. In the present study we found that the number and distribution of macrophages and neutrophils that infiltrate the liver of ALA mice were apparently similar in male and female mice. Thus, not only the mere quantity but also the degree of activation and cytokine production of these cells, particularly during the very early phase of abscess development, might influence susceptibility or resistance. The finding that activation markers on splenic T cells were not induced and the finding that CD11c-positive dendritic cells appeared in the abscess region when female mice had already cleared the infection indicate that an early, organ-specific response is critical for the observed gender differences. In contrast to conventional T cells, γδ-T cells and NKT cells, as well as NK cells, were present in the center of the abscess at early stages of abscess development. These cells are known for their capacity to produce anti-inflammatory cytokines and thus exert regulatory functions, as well as their capacity to produce large amounts of IFN-γ upon activation (2, 12). In this context, NKT cells appear to be particularly important as IFN-γ production by this lymphocyte population is strongly promoted by the female sex hormone estradiol (15). NKT cells recognize lipid antigens that are presented by CD1d molecules on antigen-presenting cells. Recently, several microbial lipids that bind to CD1d and activate NKT cells were isolated. Especially, invariant NKT cells that are defined by expression of the Vα14-Jα18 TCR-α chain participate in the recognition of microbial lipid antigens, and it was recently shown that mice lacking CD1d-restricted NKT cells were more susceptible to certain pathogens (22). Likewise, the results presented here indicate that in contrast to wild-type mice, mice lacking Vα14/NKT cells are highly susceptible to the development of ALA, and in addition, spleen cells from these mice produced less IFN-γ during the early phase of infection. Production of IFN-γ may directly trigger amebicidal mechanisms or promote the activation of other cells that may act synergistically to control amebic infection of the liver.

In contrast to the early phase of abscess development, the analysis of the late phase, which is characterized by activation of the adaptive immune system, revealed similar levels of IFN-γ and IL-4 in the two genders. At this time we detected CD4+ and CD8+ T cells in the vicinity of the abscesses, which were likely responsible for cytokine production in this phase of infection.

Taken together, the data presented here suggest that an early IFN-γ burst by innate immune mechanisms specific to female mice induce accelerated clearance of E. histolytica infection, and thus abscess formation is limited. Male mice that lack this early IFN-γ burst but produce elevated levels of antagonizing IL-4 in response to ameba infection require development of adaptive immune mechanisms to control the parasite, which prolongs persistence of amebae within the tissues considerably. Consequently, E. histolytica trophozoites can multiply within the liver, and thus larger and more clinically relevant lesions are induced. Since the C57BL/6 mouse model mimics at least in part the course of ALA development in humans, this model may provide a valuable tool to further elucidate the mechanism(s) responsible for gender differences in amebiasis.

ACKNOWLEDGMENTS
We thank Klara Tenner-Racz and Paul Racz for helpful discussions and support in histology and Claudia Marggraff for skillful technical assistance. M. Taniguchi is gratefully acknowledged for providing Jα14 mice.

E.T. is supported by the Deutsche Forschungsgemeinschaft (TA1107/3).

REFERENCES
1. Acuna-Soto, R., J. H. Maguire, and D. F. Wirth. 2000. Gender distribution in amoebic and inflammatory liver abscesses. J. Infect. Dis. 183:2012–2018.
2. Bendelac, A., O. Lantz, M. E. Quinby, J. W. Yewdell, J. R. Bennink, and R. R. Bruttelwich. 1995. CD1 recognition by mouse NK1.1+ T lymphocytes. Science 268:863–865.
3. Blessum, J., I. K. Ali, P. A. Nu, B. T. Dinh, T. Q. Viet, A. L. Van, C. G. Clark, and E. Tannich. 2003. Longitudinal study of intestinal Entamoeba histolytica infections in asymptomatic adult carriers. J. Clin. Microbiol. 41: 4745–4750.
4. Blot, S., P. Van Linh, P. A. Nu, H. D. Thi, B. Muller-Myhok, H. Buss, and E. Tannich. 2002. Epidemiology of amebiasis in a region of high incidence of amebic liver abscess in central Vietnam. Am. J. Trop. Med. Hyg. 66:578–583.
5. Booy, H. J. and R. J. van de Griend. 1977. Virulence and toxicity of axenic Entamoeba histolytica. Nature 265:341–343.
6. Chadee, K., and E. Meerovitch. 1984. The pathogenesis of experimentally induced amebic liver abscess in the gerbil (Meriones unguiculatus). J. Pathol. 147:71–80.
7. Cui, J., T. Shin, T. Kawano, H. Sato, E. Kondo, Y. Kaneko, H. Koseki, M. Kanno, and M. Taniguchi. 1997. Requirement for Valpha14 NK T cells in IL-12-mediated rejection of tumors. Science 278:1623–1626.
8. Denis, M., and K. Chadee. 1989. Cytokine activation of murine macrophages for in vitro killing of Entamoeba histolytica trophozoites. Infect. Immun. 57:1750–1756.
9. Denis, M., and K. Chadee. 1989. Human neutrophils activated by interferon-gamma and tumour necrosis factor-alpha kill Entamoeba histolytica trophozoites in vitro. J. Leukoc. Biol. 45:374–378.
10. Diamond, L. S., D. R. Harlow, and C. C. Cunnick. 1978. A new medium for the axenic cultivation of Entamoeba histolytica. Trans. R. Soc. Trop. Med. Hyg. 11:431–432.
11. Erti, L. G. Laifer, P. Sendi, H. P. Ledermann, U. Fluckiger, and S. Bassetti. 2004. Low sensitivity of ultrasoundography for the early diagnosis of amebic liver abscess. Am. J. Med. 117:519–522.
12. Exley, M., J. Garcia, S. P. Balk, and S. Porcelli. 1997. Requirements for CD4+ recognition by human invariant Valpha24+ CD4-CD8 T cells. J. Exp. Med. 186:109–120.
13. Gathiram, V., and T. F. Jackson. 1989. A longitudinal study of asymptomatic carriers of pathogenic zymodemes of Entamoeba histolytica. S Afr. Med. J. 72:669–672.
14. Gil-Barbosa, M., A. Fastag de Sotr, M. de La Torre, and J. Villegas-Gonzales. 1972. Sequence of amebic hepatic lesions in the rabbit. Arch. Invest. Med. 3:349–354.
15. Gourdy, P., L. M. Aranjo, R. Zhu, B. Garmy-Susini, S. Diem, H. Laurell, M. Leite-de-Moraes, M. Dy, J. F. Arnal, F. Bayard, and A. Herbelin. 2005. Relevance of sexual dimorphism to regulatory T cells: estradiol promotes IFN-γ production by invariant natural killer T cells. Blood 105:2415–2420.
16. Hage, R., A. S. Fariqume, P. Hahn, D. M. Lyerly, and W. A. Petri, Jr. 1997. Entamoeba histolytica and Entamoeba dispar infection in children in Bangladesh. J. Infect. Dis. 175:734–736.
17. Jarillo-Luna, R. A., R. Campos-Rodriguez, and V. Tsutsui. 2002. Entamoeba histolytica: immunohistochromatographic study of hepatic amoebiasis in mouse. Neutrophils and nitric oxide as possible factors of resistance. Exp. Parasitol. 101:40–56.
18. Jarillo-Luna, R. A., R. Campos-Rodriguez, and V. Tsutsui. 2000. Morphological changes of mouse liver infected with trophozoites of Entamoeba histolytica. Arch. Med. Res. 31:521–525.
19. Lin, J. Y., and K. Chadee. 1992. Macrophage cytotoxicity against Entamoeba histolytica trophozoites is mediated by nitric oxide from i-arginine. J. Immuno 146:3999–4005.
20. Lushbaugh, W. R., A. B. Kairalla, C. B. Loadholt, and F. E. Pittman. 1978. Effect of hamster liver passage on the virulence of axenically cultivated Entamoeba histolytica. Am. J. Trop. Med. Hyg. 27:248–254.
21. Martinez-Palomo, A. 1987. The pathogenesis of amoebiasis. Parasite Immunol. 3:111–118.
22. Mattner, J., K. L. Debord, N. Ismail, R. D. Goff, C. Cantu, 3rd, D. Zhou, P. Saint-Mezard, V. Wang, Y. Gao, N. Yin, K. Hoebe, O. Schneewind, D. Walker, B. Beutler, L. Teyton, P. B. Savage, and A. Bendelac. 2005. Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. Nature 434:525–529.
23. Pham Van, L., H. Duong Manh, and H. Pham Nhu. 1996. Amebic abscess of the liver: ultrasound guided puncture. Ann. Chir. (Paris) 50:340–343.
24. Roberts, C. W., S. M. Cruickshank, and J. Alexander. 1995. Sex-determined resistance to Toxoplasma gondii is associated with temporal differences in cytokine production. Infect. Immun. 63:2549–2555.
25. Seydel, K. B., E. Li, P. E. Swanson, and S. L. Stanley, Jr. 1997. Human intestinal epithelial cells produce proinflammatory cytokines in response to infection in a SCID mouse-human intestinal xenograft model of amebiasis. Infect. Immun. 65:1631–1639.
26. Seydel, K. B., S. J. Smith, and S. L. Stanley, Jr. 2000. Innate immunity to amebic liver abscess is dependent on gamma interferon and nitric oxide in a murine model of disease. Infect. Immun. 68:400–402.

Editor: W. A. Petri, Jr.