

The Mouse Model of Amebic Colitis Reveals Mouse Strain Susceptibility to Infection and Exacerbation of Disease by CD4⁺ T Cells¹

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Amebic colitis is an important worldwide parasitic disease for which there is not a well-established animal model. In this work we show that intracecal inoculation of *Entamoeba histolytica* trophozoites led to established infection in 60% of C3H mice, while C57BL/6 or BALB/c mice were resistant, including mice genetically deficient for IL-12, IFN- γ , or inducible NO synthase. Infection was a chronic and nonhealing cecitis that pathologically mirrored human disease. Characterization of the inflammation by gene chip analysis revealed abundant mast cell activity. Parasite-specific Ab and cellular proliferative responses were robust and marked by IL-4 and IL-13 production. Depletion of CD4⁺ cells significantly diminished both parasite burden and inflammation and correlated with decreased IL-4 and IL-13 production and loss of mast cell infiltration. This model reveals important immune factors that influence susceptibility to infection and demonstrates for the first time the pathologic contribution of the host immune response in amebiasis. *The Journal of Immunology*, 2002, 169: 4496–4503.

Entamoeba histolytica is an intestinal protozoan that infects up to 8% of the world's population (1). Colitis is the most common manifestation of disease and accounts for the majority of the estimated 50,000 annual deaths attributed to the parasite (2). Both humoral and cell-mediated immune (CMI)³ responses have been observed in patients with amebiasis. A mucosal secretory IgA response against the parasite adhesin is associated with acquired immunity, whereas, paradoxically, a serum IgG response is associated with increased susceptibility to repeat infection (3, 4). Correlations of CMI responses with immunity are more limited. Humans with amebic liver abscess exhibit robust parasite-specific T cell proliferation and amebicidal IFN- γ production, yet such studies originated from hospitalized patients who required antibiotic therapy for cure (5). In vitro a protective role for IFN- γ , TNF- α , and NO in phagocyte killing of *E. histolytica* trophozoites has been demonstrated (6, 7).

The paucity of animal models of intestinal infection has detracted from the ability to define the mechanisms of innate and acquired immunity in amebic colitis. The naturally transmissible cyst form of the parasite has never been successfully cultured in

vitro; therefore, attempts at experimental intestinal infection have relied on challenge with the invasive trophozoite form. Since Lösch reproduced intestinal amebiasis in dogs with human dysenteric stool in 1875 there have been numerous attempts at an intestinal model using oral or intrainestinal inoculation of trophozoites into outbred, inbred, and immune-deficient animals. Difficulties have arisen in achieving either durable infection beyond 10 days (8, 9) or reproducibility (10). Ghosh and colleagues (11–13) reported a promising model using the C3H mouse with intracecal inoculation of laboratory strain trophozoites, characterized by systemic Ab and delayed-type hypersensitivity responses to the parasite and infiltrating IgA⁺ cecal lymphocytes. Yet fundamental questions of whether innate immunity can resist establishment of infection and whether parasite-specific humoral or CMI responses can protect from disease have remained unanswered.

In this work intracecal inoculation of mice with *E. histolytica* trophozoites has been used to explore the nature and function of the immune response in amebic colitis. BALB/c and C57BL/6 mice were innately resistant to intestinal challenge with *E. histolytica*. Infection in C3H mice was a chronic cecitis, characterized by massive inflammation and epithelial ulceration. A deleterious acquired CD4⁺ T cell response exacerbated disease in these animals, as evidenced by parasite burden and intestinal pathology.

Materials and Methods

Animals

Six-week-old female C3H/HeJ, C3H/HeOuJ, BALB/c, C57BL/6, as well as IFN- γ , IL-12 p40, and inducible NO synthase (iNOS) knockout mice were purchased from The Jackson Laboratory (Bar Harbor, ME); C3H/HeN mice were purchased from the National Cancer Institute (Frederick, MD). Golden Syrian hamsters were purchased from Harlan (Indianapolis, IN). Animals were maintained under specific-pathogen-free conditions at the University of Virginia and all protocols were approved by the Institutional Animal Care and Use Committee.

Parasites and Ags

Trophozoites were cultured in trypsin-yeast-iron (TYI-S-33) medium (14). Laboratory strain HM1:IMSS trophozoites were passaged through hamster

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³ Abbreviations used in this paper: CMI, cell-mediated immune; MLN, mesenteric lymph node; SAA, soluble amebic Ag; iNOS, inducible NO synthase.

liver (15) then grown in the bacterial flora of the xenic strain CDC:0784 supplemented with 0.01% erythromycin (Sigma-Aldrich, St. Louis, MO). Log-phase growth trophozoites were iced and spun ($900 \times g$ for 5 min) before intracecal inoculation. *E. histolytica* lectin was purified as described (16). Soluble amebic Ag (SAA) was obtained from the supernatant of axenic trophozoites washed in sterile HBSS, lysed by freeze-thaw, and spun ($10,000 \times g$ for 10 min). Both lectin and SAA were endotoxin free using the *Limulus* amoebocyte lysate assay (<0.1 EU/ml).

Intracecal inoculation

We anesthetized mice with ketamine/xylazine, shaved their abdomens, incised the skin and peritoneum, exteriorized the cecum, and injected 150 μ l of trophozoite pellet ($\sim 1 \times 10^7$ trophozoites) into the proximal, middle, and apical cecum. Sham-challenged mice were injected with 150 μ l of trophozoite culture supernatant. Cecae were blotted, the peritoneum was sutured, and the skin was stapled. Mice were kept on 37°C warming blankets throughout. Survival was $\geq 90\%$ in all strains.

Pathology of murine amebic colitis and immunohistochemistry

Mice were sacrificed, the cecum was fixed in 10% buffered formalin phosphate or Hollande's fixative and then cut into four to six equal cross-sections and paraffin embedded, and 4- μ m slides were stained with H&E or periodic acid-Schiff. Cecal thickness was measured at two or more sites with an ocular micrometer at $\times 40$ magnification. For *c-kit* immunohistochemistry rabbit polyclonal Ab to human *kit* (cross-reacts with mouse *c-kit*; Research Diagnostics, Flanders, NJ) was used at a 1/250 dilution followed by avidin-biotin peroxidase, diaminobenzidine, and hematoxylin. PBS was used as negative control and revealed no background staining.

Pathology was scored blindly for each section as follows and averaged for each mouse. Numbers of ameba were scored 0–5 (0, none; 1, rare and difficult to locate; 2, occasional, up to 10% of the lumen occupied by ameba; 3, moderate, up to 25% of lumen occupied; 4, heavy, up to 50% of lumen occupied; 5, virtually complete occupation of the lumen by ameba). Extent of ulceration was measured by estimating the percentage of the luminal circumference that was ulcerated (0–100%). Degree of inflammation was scored 0–5 (0, normal; 1, mucosal hyperplasia, mild to moderate increase of lymphocytes in mucosa and submucosa with no neutrophil infiltration; 2, increased chronic inflammation, with spotty infiltration of neutrophils, not involving the entire thickness of the mucosa; 3, increased chronic inflammation with marked increase in neutrophil infiltration, involving full thickness of mucosa; 4, marked neutrophil infiltration of mucosa and submucosa, with tissue architecture intact; 5, complete destruction of cecal architecture by inflammation).

Fecal Ag detection

The *E. histolytica* II stool ELISA kit (TechLab, Blacksburg, VA) was used according to the manufacturer's instructions. Three fecal pellets per mouse were assayed within 1 h of collection and background was subtracted from sample OD₄₅₀.

Serum and fecal Ab determinations

Serum was obtained from orbital plexus blood and fecal samples were prepared from four stool pellets per mouse vortexed into a suspension with protease inhibitors (Complete; Roche, Mannheim, Germany) and spun at $900 \times g$ and then $10,000 \times g$. Ab levels were assayed in duplicate by ELISA (17) using lectin-coated plates (3.5 μ g/ml) and HRP-conjugated anti-mouse IgG, IgG1, IgG2a, IgA, and IgE secondary Abs (Southern Biotechnology Associates, Birmingham, AL). Background ELISA OD from the serum of naive mice was subtracted from sample ELISA OD.

Mesenteric lymph node (MLN) proliferation assays and cytokine determinations

Single-cell suspensions of MLNs, spleens, and PBMCs were prepared as described (18). MLN cells (1×10^5) were cultured in round-bottom 96-well plates (Costar, Corning, NY) in Dulbecco's complete medium supplemented with 10% FCS, 100 U/ml penicillin, 100 μ g/ml streptomycin, 100 μ g/ml gentamicin, and 5×10^{-5} 2-ME for 72 h at 5% CO₂, with or without 0.1 μ g/ml lectin, 100 μ g/ml SAA, or 0.1 μ g plate-bound anti-murine CD3 ϵ (145-2C11), then pulsed for 12 h with 10 μ Ci [³H]thymidine, and proliferation was counted using a Trilux scintillation counter (Wallac, Turku, Finland). Ag-specific proliferation was determined after subtracting background proliferation in medium alone. Supernatants were pooled at

72 h and analyzed in duplicate for IL-4, IL-13, and IFN- γ production using ELISA sets (R&D Systems, Minneapolis, MN) per the manufacturer's instructions.

RNA protection analysis and Affymetrix gene chip analysis

Total RNA was isolated from rinsed cecal tissue and MLN with the Qiagen RNeasy kit (Qiagen, Valencia, CA). RNase protection analysis was performed using the mck-1b template (BD PharMingen, San Diego, CA) per the manufacturer's instructions. Affymetrix gene chip analysis (Affymetrix, Santa Clara, CA) was performed per the manufacturer's instructions using murine MgU74Av2, MgU74Bv2, and MgU74Cv2 arrays. The in vitro transcription reaction product was purified and analyzed by gel electrophoresis to confirm the size range. Results were analyzed using D-chip analysis software (19), which reported average difference of hybridization intensity between perfect match and mismatch and fold change between the three infected vs three sham-challenged cecal samples.

In vivo CD4 cell depletion and flow cytometry

One milligram of rat IgG2a anti-murine CD4 (GK1.5) or purified rat IgG (catalog no. I8015; Sigma-Aldrich) was injected i.p. on days -1, +2, +5, and +8 relative to intracecal challenge. Flow cytometry was performed using FITC-labeled Abs to murine CD4 (GK1.5), CD8 α (53-6.7) (both from BD PharMingen), and Ig H and L chains (Rockland, Gilbertsville, PA), and to rat Ig κ (RG7) as previously described (18). Staining with anti-rat Ig κ indicated no masking of CD4 by persistent GK1.5 Ab.

Statistics

Group means were compared by the Student *t* test or the alternate Welch test and infection rates were compared by Fisher's exact test. All *p* values are two-tailed.

Results

Intracecal inoculation of *E. histolytica* trophozoites leads to amebic colitis in C3H/HeJ mice

Intracecal injection of trophozoites into C3H/HeJ mice led to chronic cecal infection in 60% (112 of 186) of mice as determined by histopathology (Fig. 1A). Infection did not spontaneously clear and was documented to persist beyond 18 mo postchallenge. Infected ceca were thickened and contracted on gross inspection. Histopathologic changes were evident as early as 4 days after challenge, including crypt hyperplasia, epithelial ulceration, and submucosal infiltration. At such early time points viable ameba were usually seen only at areas of epithelial ulceration, yet by 3 wk postinoculation ameba had extended into the lumen. Inflammation was severe and contained plasma cells, neutrophils, and mast cells. By 10 wk inflammation obscured the entire mucosa and morphologically resembled human colitis (Fig. 1B). Disease was limited to the cecum (the most common segment affected in humans; Ref. 20), which the gastrointestinal tract was able to bypass, perhaps explaining why infected mice did not become ill or lose weight. The model mirrored human infection in important ways: the morphology of the inflammatory infiltrate, the burden of trophozoites within the mucosa without submucosal invasion or liver abscess formation (these are the exception in human amebic colitis; Ref. 21), and the absence of parasite cyst development with invasive infection.

Approximately 40% (74 of 186) of *E. histolytica*-challenged C3H/HeJ mice did not develop chronic colitis; these mice were classified as "challenged/uninfected" mice. The resistance in these mice occurred rapidly. Fecal parasite Ag was negative in 82% of challenged/uninfected mice by 4 days (100% by 16 days, Fig. 1C). Fecal Ag excretion was thus measured after the first week for the purposes of detecting and quantifying successful infection, and the sensitivity of the test was 58% (152 of 263) for a single measurement and 90% (43 of 38) for three measurements. Cecal pathology was entirely normal at all time points in challenged/uninfected mice as demonstrated by cecal thickness (Fig. 1D).

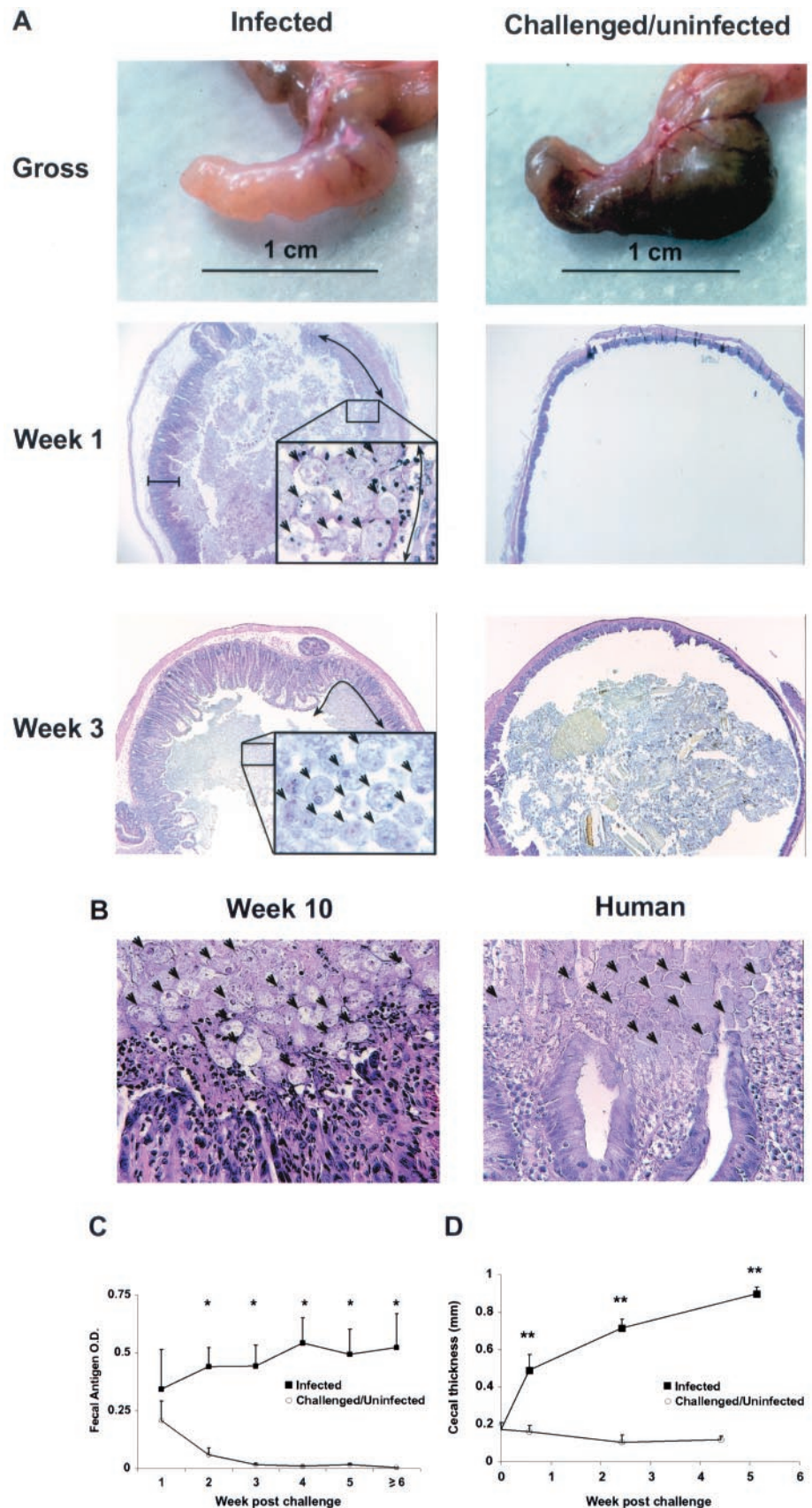


FIGURE 1. Chronic amebic colitis in C3H/HeJ mice after intracecal challenge with *E. histolytica*. **A**, On gross inspection infected mouse ceca were thickened and contracted compared with challenged/uninfected ceca, here shown at 1 mo postinoculation. During wk 1 of successful infection histopathology revealed rapid mucosal hyperplasia (bracket) and submucosal infiltration not present in challenged/uninfected mice (histopathology are at $\times 40$, H&E). Trophozoites (arrowheads in all figures) were seen only at areas of ulceration (arrows in all figures) early after infection; however, by wk 3 ameba were both ulcerating the epithelium and occupying the lumen (*insets*, $\times 1000$). **B**, By wk 10 of infection complete destruction of the mucosal architecture could be seen, a pattern typical of severe human disease (H&E, both $\times 400$). **C** and **D**, Fecal Ag excretion and cecal thickness was measured in infected vs challenged/uninfected mice as verified by histopathology during the weeks indicated. Data are shown as mean weekly Ag OD or mean cecal width \pm SE. *, $p < 0.002$; **, $p < 0.0007$.

Susceptibility to intestinal infection with E. histolytica is parasite and mouse strain dependent

The infection rate in C3H/HeJ mice was higher for trophozoites cultured in bacterial flora before intracecal challenge (15 of 39 vs

8 of 48, $p = 0.03$) and ultimately 60% (112 of 186) with a trophozoite strain that was in vivo passaged through hamster liver abscess. Using these same trophozoites we found that C3H/HeOuJ and C3H/HeN strains had similar infection rates and disease

severity (Table I). We quantified disease severity in infected mice by fecal Ag excretion and scoring of histopathology. Cross-sections of infected ceca were examined histopathologically for numbers of ameba, extent of ulceration, and severity of inflammation as detailed in *Materials and Methods*. The comparable infection rates and disease severities by histopathology of the C3H/HeOuJ strain ruled out the defective LPS signaling of the C3H/HeJ (22) as the explanation for its susceptibility. C57BL/6 mice and BALB/c mice were relatively resistant to initial infection; however, when it occurred (in 4 of 27 C57BL/6 mice) fecal Ag and disease severity was again similar to that of C3H/HeJ (Table I). To understand whether innate resistance in the C57BL/6 or BALB/c strains was due to more robust innate production of IL-12, IFN- γ , or NO we tested susceptibility in genetically deficient mice but found that resistance persisted. Thus, there were mouse strain-dependent differences in the initial susceptibility to infection but not in the development of amebic colitis once successful infection occurred.

During chronic infection, parasite-specific Ab and cellular proliferative responses were robust

Given the significant inflammation in murine amebic colitis, yet the failure of acquired immunity to clear the infection once established, we sought to characterize this nonhealing immune response in the C3H/HeJ mouse. We measured Ab against the *E. histolytica* adherence lectin, a major virulence factor, by ELISA. Mice at 5–7 wk duration of infection produced significantly higher-titer serum antiadherence lectin IgG, IgG1, IgG2a, IgE, and fecal IgA than challenged/uninfected mice (Fig. 2A). Gross inspection of infected mice revealed enlarged MLNs, although flow cytometry of MLN revealed similar percentages of CD4⁺, CD8⁺, and B lymphocytes among infected, challenged/uninfected, and wild-type mice (mean 41 ± 1.4 , 17 ± 0.4 , 18 ± 1.1 ; $n = 11$). However, the MLN cells from infected mice demonstrated significantly higher proliferation in response to SAA than those from challenged/uninfected or wild-type mice (Fig. 2B).

Chronic amebic colitis was associated with mucosal production of IL-4, IL-13, and mast cell proteases

We analyzed the production of cytokines from the in vitro stimulated MLN cultures for IL-4, IL-13, and IFN- γ by ELISA (Fig. 3A). This revealed significantly higher IL-4 and IL-13 production in response to SAA or anti-CD3 in infected compared with challenged/uninfected mice. Significantly higher quantities of IFN- γ were produced in response to anti-CD3 in the MLN cultures from

infected mice, but parasite-specific IFN- γ production was negligible (<0.1 ng/ml). RNase protection analysis of MLN RNA from infected vs sham-challenged mice at 10 wk postinoculation confirmed increases in IL-4 with minimal and perhaps diminished IFN- γ expression (Fig. 3B).

To examine the cytokine profile at the site of infection, RNase protection analysis was performed on cecal RNA from the same infected and sham-challenged mice (Fig. 3B). Increased levels of IL-4 and IL-13 and minimal IFN- γ mRNA expression were again observed in infected mice. Thus, the consistent finding in MLN and cecum was the production of IL-4 and IL-13 in response to *E. histolytica* infection; while infected MLN were capable of producing IFN- γ , production was not observed in response to parasite Ag or in situ in the infected cecum. The elevated serum anti-lectin IgG2a (dependent upon IFN- γ for its production) might therefore represent Ag-specific skewing of cytokine production (lectin vs SAA) or dissociation between splenic and MLN parasite-specific cytokine production, as has been described in gastrointestinal helminth infections (23).

To more broadly characterize gene expression during the inflammation of murine amebic colitis, we performed Affymetrix gene chip hybridization on cecal RNA from the same three infected and three sham-challenged mice. Differences in expression by gene chip correlated with visual differences in expression by RNase protection for 89% (8 of 9) of genes (the exception was IL-15, where gene chip indicated decreased expression in infected mice; average difference was 69.3 ± 3.3 vs 164 ± 5.0 for controls ($p < 0.0001$), while RNase protection showed increase in MLN and decrease in cecum). Of the total of 9645 genes or expressed sequence tags probed by gene chip, mast cell proteases accounted for 4 of the 20 most overexpressed genes in infected mice vs controls (Fig. 3C). With 95% confidence, expression of mast cell protease 2, mast cell protease-like protein, mast cell chymase 2, and mast cell protease 1 were ≥ 25 -, 23-, 8.4-, and 7.3-fold increased, respectively ($p < 0.0007$). There was no evidence for increased expression of the mast cell-inducing cytokines IL-3 or IL-9 by gene chip (both cytokines scored as absent in five of six mice) or the mast cell effector cytokine TNF- α (scored as absent for six of six mice by gene chip). Furthermore, there was no evidence for an increase in the Th3/Tr1 T cell population by TGF- β 1 or IL-10 expression levels (both cytokines scored as absent for five of six mice). Differences in production of these cytokines due to post-transcriptional regulation or using other detection techniques remain possible.

Table I. Murine susceptibility to intestinal *E. histolytica* infection and disease^a

Mouse Strain	Genotype	Infection Rate (%) ^b	Disease Severity			
			Fecal Ag OD	Cecal ameba score	Ulceration (%)	Inflammation score
C3H/HeJ	WT	60 (186)	0.76 \pm 0.22	3.4 \pm 0.5	54 \pm 10	3.8 \pm 0.3
C3H/HeOuJ	WT	57 (7)	0.78 \pm 0.13	3.5 \pm 0.3	78 \pm 8.8	4.0 \pm 0.4
C3H/HeN	WT	25 (8)	0.57 \pm 0.33	ND	ND	ND
C57BL/6	WT	15 (27)*	0.72 \pm 0.08	4.6 \pm 0.3	48 \pm 2.5	3.6 \pm 0.3
	IL 12 ^{-/-}	0 (10)*	N/A	N/A	N/A	N/A
	iNOS ^{-/-}	0 (6)*	N/A	N/A	N/A	N/A
BALB/c	WT	0 (9)*	N/A	N/A	N/A	N/A
	IFN- γ ^{-/-}	0 (7)*	N/A	N/A	N/A	N/A

^a All mice were challenged with an identical strain of trophozoite in xenic culture. Infection rate was determined by histologically verified amebic colitis. Disease severity in infected mice was quantified by fecal Ag measurements during wk 2, 3, and 4 and scoring of histopathology at 1 mo as detailed in *Materials and Methods*. Disease severity data are shown as mean \pm SE ($n = 5, 4, 2$, and 3 for C3H/HeJ, C3H/HeOuJ, C3H/HeN, and C57BL/6, respectively). WT, Wild-type. ND, Not done, as pathology was not available at 1 mo. N/A, Not applicable, as no mice were successfully infected.

^b Total numbers appear in parentheses.

*, $p < 0.05$ compared to C3H/HeJ.

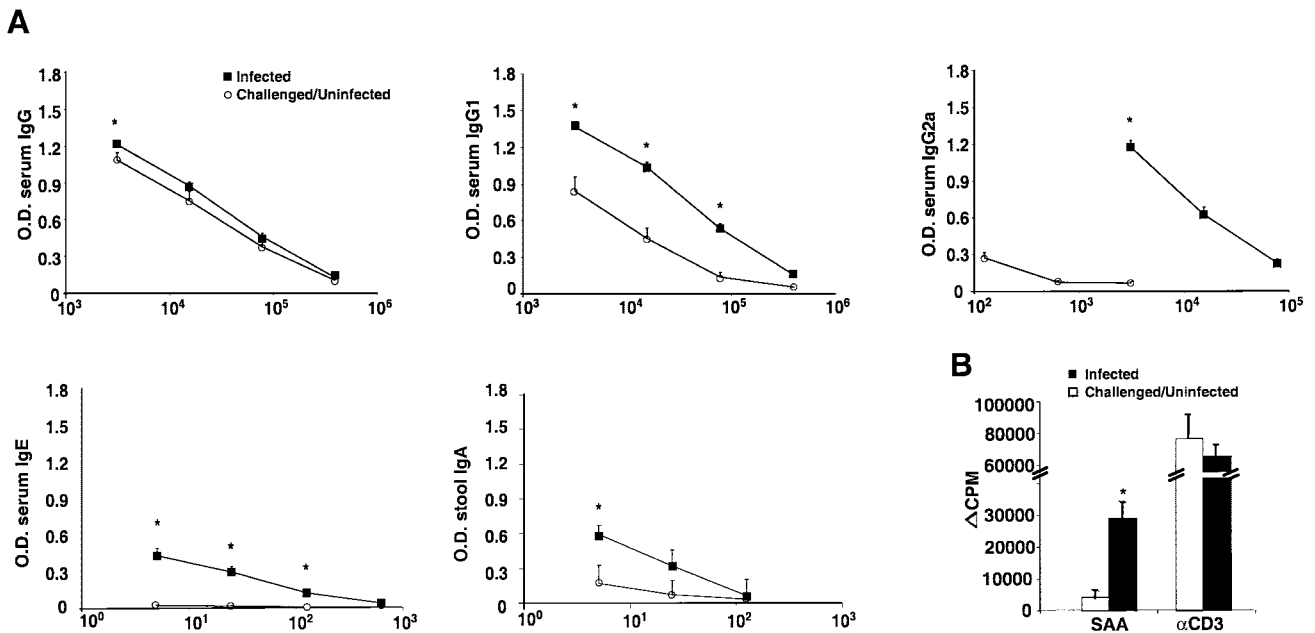


FIGURE 2. Elevated humoral and cellular immune responses to *E. histolytica* in infected mice. **A**, Sera and stool preparations were obtained from infected and challenged/uninfected mice 5–7 wk after intrarectal challenge and analyzed for serum IgG, IgG1, IgG2a, IgE, and stool IgA to *E. histolytica* lectin by ELISA at the dilutions indicated on the x-axis. Data are analyzed as antilectin OD minus background OD from naive mice. Data are shown as mean \pm SE ($n \geq 8$ mice per group). *, $p < 0.05$. **B**, MLN proliferative responses to SAA or anti-CD3 were measured by overnight [³H]thymidine incorporation minus background in infected vs challenged/uninfected mice 7 wk after challenge. Data are shown as mean \pm SE ($n = 24$ infected and 4 challenged/uninfected mice). *, $p < 0.01$. Naive mice had undetectable proliferation in response to SAA ($n = 4$).

CD4⁺ T cell depletion paradoxically decreased parasite burden and intestinal inflammation

Because CD4⁺ T cells are a primary source of IL-4 and IL-13, we depleted CD4⁺ T lymphocytes to test their impact on the course of infection. Mice were administered 1 mg of anti-CD4 mAb (vs control rat IgG) i.p. on days -1, +2, +5, and +8 relative to inoculation with *E. histolytica*, which resulted in >90% CD4⁺ T cell depletion from PBMCs, splenocytes, and MLN by FACS analysis during wk 1 and 4 postchallenge (data not shown). Mice were sacrificed at wk 4 and, as expected, the depletion of CD4⁺ T cells had no effect on the infection rate (5 of 17 in CD4-depleted mice vs 8 of 18 control mice, $p = 0.5$), because this appeared dependent on innate immune responses. However, fecal Ag was paradoxically decreased in the CD4-depleted/infected vs control/infected mice (Fig. 4A). Furthermore, on histopathology, while CD4-depleted/infected mice still demonstrated crypt hyperplasia and mild submucosal infiltration, the numbers of ameba, extent of ulceration, and degree of inflammation were each significantly decreased compared with control/infected mice (Fig. 4B; representative photomicrographs are shown in Fig. 4, C vs D). Immunohistochemistry on cecal tissue for the mast cell marker *c-kit* additionally revealed a significant decrease in the number of *c-kit*⁺ cells per section in CD4-depleted/infected mice (171 ± 29 vs 29 ± 16 , $p = 0.005$) (Fig. 4C). CD4-depleted/infected mice had comparable numbers of *c-kit*⁺ cells as uninfected and non-CD4-depleted mice (data not shown). Although surface *c-kit* expression can be found on other cells such as precursor T cells and intraepithelial lymphocytes (24), the *c-kit*⁺ cells had mast cell morphology on adjacent H&E-stained sections. Correlation with cytokine production was obtained by stimulating MLN with SAA or anti-CD3, and CD4-depleted/infected mice demonstrated significantly lower IL-4 and IL-13 production but a similar IFN- γ pattern compared with control/infected mice (Fig. 4E). Thus, severity of amebic colitis correlated with Ag-specific IL-4 and IL-13 production and submucosal mastocytosis.

Discussion

The most important conclusion from this study is that the acquired CD4⁺ T cell response in murine amebic colitis increases parasite burden and exacerbates disease. Although unprecedented in amebiasis, the role for CD4⁺ T cell responses in contributing to parasitic disease is well established in murine leishmaniasis and schistosomiasis (25, 26). There are also a growing number of examples in which normal parasite development requires the presence of host CD4⁺ lymphocytes (27) or benefits from CD4⁺ T cell cytokines (28).

We suspect that the mitigation in disease results from depletion of CD4⁺ T cells, because production of characteristic T cell cytokines IL-4 and IL-13 was diminished with the depletion (although a role for CD4⁺ macrophages cannot be ruled out). We thereby envision at least two mechanisms to explain how the CD4 depletion diminishes amebic colitis: 1) parasite-primed CD4⁺ T cells cause intestinal inflammation through effector cytokine production including IL-4 and/or inducing mastocytosis or 2) the CD4⁺ depletion acts primarily through decreasing parasite numbers.

This first concept of host CD4⁺ T cells directly mediating intestinal inflammation is well established from mouse models of inflammatory bowel disease (a condition that can resemble amebic colitis pathologically). These are heterogeneous models of either spontaneous or induced inflammation, most of which correlate with Th1 cytokine production (29). Yet Th2 models of intestinal inflammation exist, with a direct role for unbalanced IL-4 production documented in the pathogenesis of oxazolone-induced, TCR- α knockout, and trinitrobenzene sulfonic acid colitis in the BALB/c mouse (30–32). Furthermore, even in Th1 models of colitis, late-stage disease has been characterized by elevated IL-4 and IL-13 production (33). It is of interest that, similar to this model of amebic colitis, the Th2 models of inflammatory bowel disease are generally characterized by their colonic location (as opposed to small bowel) and by a heavily disrupted epithelium. It is postulated

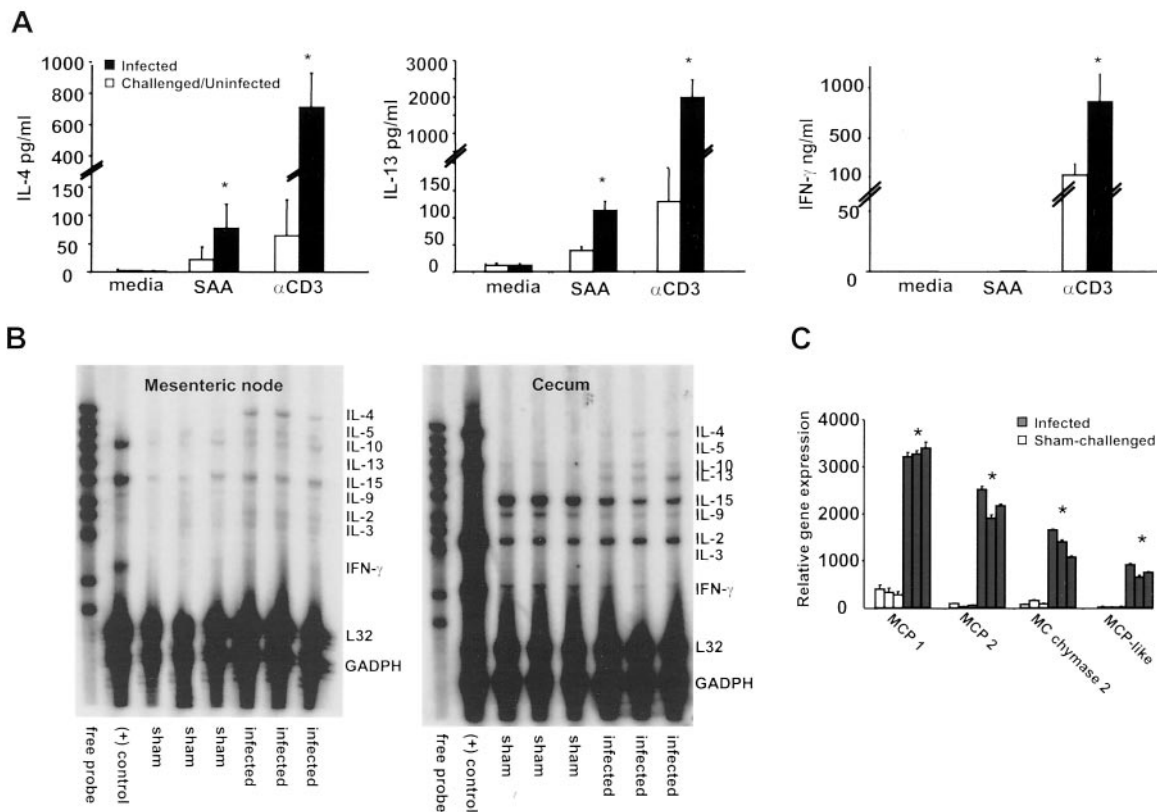


FIGURE 3. Inflammation in amebic colitis was characterized by IL-4 and IL-13 production and mast cell protease activity. *A*, MLN cells from infected and challenged/uninfected mice obtained 7 wk after challenge were cultured in the presence of medium, SAA, or anti-CD3 and supernatants were measured for production of IL-4, IL-13, and IFN- γ by ELISA. Data are shown as mean \pm SE ($n = 16$ infected and 7 challenged/uninfected mice). *, $p < 0.05$. *B*, RNase protection analysis for the cytokines indicated was performed on RNA from MLN and cecal tissue from infected vs sham-challenged controls 10 wk postchallenge. The cecal RNA was further analyzed by Affymetrix gene chip hybridization. *C*, Mast cell proteases accounted for 4 of the 20 most overexpressed genes in the three infected ceca vs three sham-challenged controls. Gene expression data are shown as average difference \pm SE ($p < 0.0007$).

that the development of Th2 intestinal inflammation depends on a combination of the gastrointestinal tract location, the specific Ag, and the mouse strain. As such, the development of amebic colitis may be a net result of the combination of the cecum and chronic Ag stimulation with *E. histolytica*. Some Th2 colitis models are marked by an anti-inflammatory suppressor TGF- β response (31); thus, given the robust ongoing cell proliferation to parasite Ag and lack of detectable TGF- β and IL-10 mRNA expression in this model, the role of an insufficient Th3 suppressor response (34) in the perpetuation of this inflammation seems reasonable.

The other plausible explanation we can envision, that CD4⁺ T cells mount a counterproductive response against the parasite, is inherently supported by the decrease in ameba numbers upon CD4⁺ depletion. Available data indicate that macrophages and neutrophils kill *E. histolytica* trophozoites more efficiently when activated by IFN- γ and/or TNF- α (35). Thus, a reasonable explanation for the increased parasite numbers in CD4⁺ T cell-depleted mice is through the down-regulatory activity of IL-4 on protective macrophage function (36).

Assuming that the primary effect of CD4⁺ T cell depletion on this model is through increasing parasite numbers, one easily finds support that this parasite can directly cause inflammation. The propensity of *E. histolytica* to destroy human tissues has been apparent since 1903, when Schaudinn named the parasite "histolytica." The ability of *E. histolytica* to damage intestinal epithelium, marked by IL-8 and IL-1 release, has been shown in vitro (37) and in vivo using the SCID-HU-INT model (38). Yet the trophozoite's action on host epithelium is complex, as we have demonstrated

previously in this model that in the infected colon intestinal cells undergo apoptosis as well (purportedly an "anti-inflammatory" process) (39).

The association of mast cell activity with deleterious immunity in this model is intriguing. In murine nematode infection, mast cells and their proteases, specifically mucosal mast cell protease-1, enhance parasite clearance (40), possibly through increased vascular and epithelial permeability. Taken with the fact that trophozoites appear to favor areas of epithelial breakdown, one wonders whether to some degree *E. histolytica* benefits from epithelial cell permeability. Mast cells could also be directly causing inflammation through production of proteases or proinflammatory cytokines, or through IgE-dependent neutrophil and monocyte recruitment (41).

This model also indicates that innate immunity can prevent establishment of infection in mice, a process that occurs both mouse-to-mouse within the C3H/HeJ strain and among the C3H, BALB/c, and C57BL/6 strains. Therefore, the mechanism of this innate protection may include combinations of genetics, environmental factors such as different bacterial flora in the intestine, and parasite factors (given that parasites grown in bacteria led to a higher infection rate). Mast cells are a critical innate immune component for survival in a cecal puncture sepsis model through Toll-like receptor-4-dependent processes (42) and TNF- α production (43), but at present this model does not support these mechanisms in protection against *E. histolytica* given the similar susceptibilities across C3H strains (Toll-like receptor-4 dominant negative or wild type) and >90% survival in all mice. Indeed, we can only conclude that

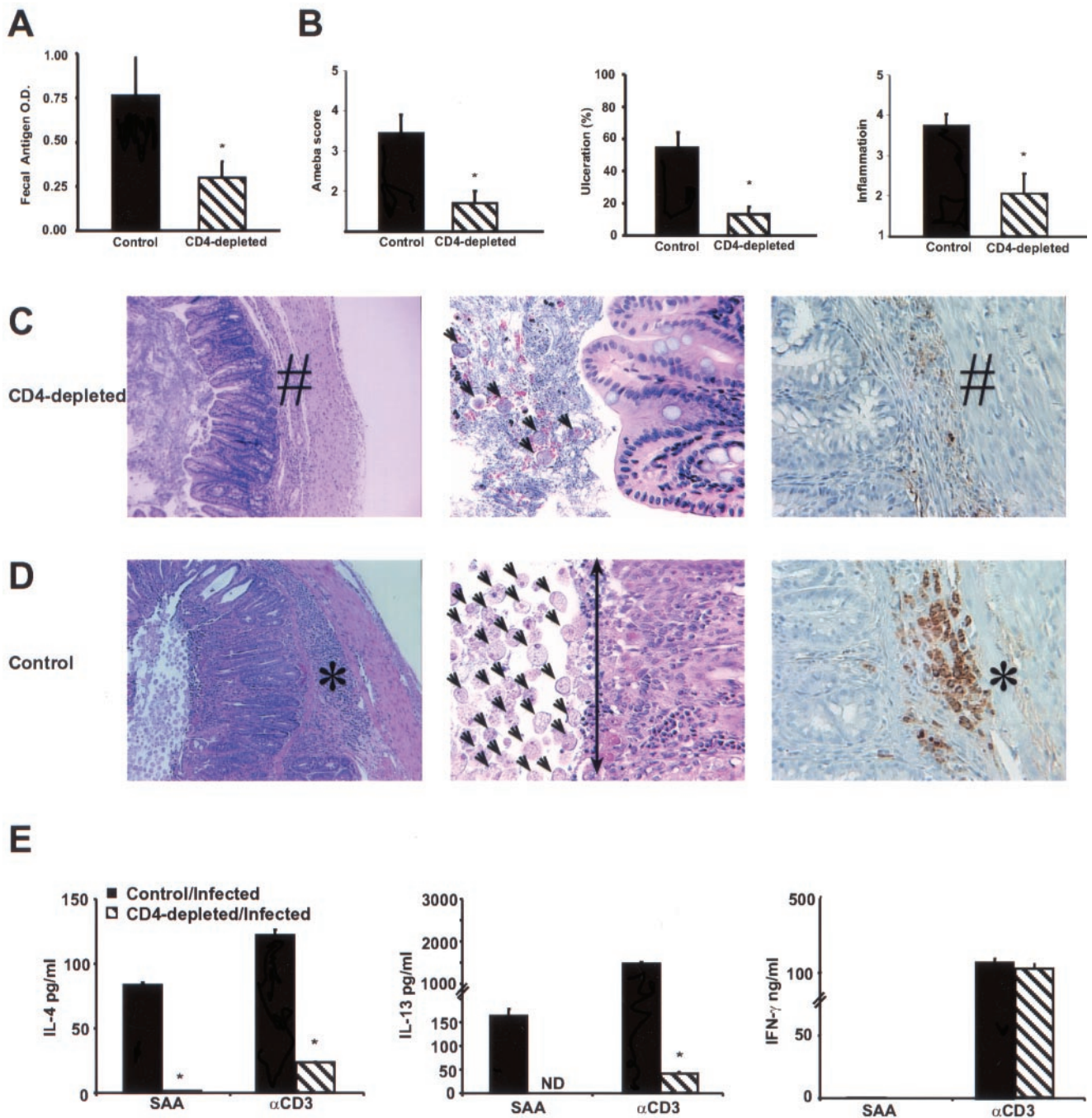


FIGURE 4. CD4⁺ T cell depletion diminished parasite burden and severity of amebic colitis. Mice were administered anti-CD4 or control Ab before and after challenge. CD4⁺ depletion was confirmed by FACS during wk 1 and 4 at time of sacrifice. **A**, Fecal Ag excretion was measured by ELISA during wk 2, 3, and 4 in control/infected and CD4-depleted/infected mice. Data are shown as mean fecal Ag OD \pm SE ($n \geq 4$ mice per group). *, $p = 0.05$. **B**, All ceca were fixed, stained with H&E, and scored in blinded fashion for numbers of ameba, extent of ulceration, and degree of inflammation as detailed in *Materials and Methods*. Data are shown as mean \pm SE from two experiments ($n = 8$ and 5 for control/infected and CD4-depleted/infected mice, respectively). *, $p < 0.05$. **C** and **D**, Representative histopathology of CD4-depleted/infected vs control/infected mice showed minimal submucosal inflammation (# vs *), fewer numbers of ameba (arrowheads), a nonulcerated epithelium (vs control/infected ulceration, arrow), and an absence of submucosal mast cells as identified by immunohistochemistry for *c-kit* (# vs *). Panels are shown (from left to right) at $\times 100$ (H&E), $\times 400$ (H&E), and $\times 400$. **E**, MLNs from control/infected and CD4-depleted/infected mice at 1 mo postinfection were stimulated with SAA or anti-CD3 and supernatants were measured for production of IL-4, IL-13, and IFN- γ by ELISA. Data represent mean \pm SE from supernatants pooled from at least two mice from each group. ND, Not detectable. *, $p < 0.0003$.

the resistance to initial infection with *E. histolytica* in the BALB/c and C57BL/56 strains cannot be solely attributed to robust innate macrophage activity given the persistence of resistance in IL-12, IFN- γ , and iNOS knockout animals.

The final issue is how this model relates to the human condition. Foremost, this mouse model of amebic colitis demonstrates compatible pathology with human disease (21). As for observations from humans that CD4⁺ T cells contribute to disease, early reports

of amebic colitis in patients receiving immunosuppression (44) suggest that in some patients CMI responses may be protective. However, the expected increases in invasive amebiasis among HIV-infected patients have not been realized (45) despite an up to 43% incidence of intestinal carriage in such individuals (46). It is an exciting and important possibility that certain individuals are predisposed to either acquiring infection or, based on divergent CD4⁺ T cell responses to the parasite once infection is acquired, developing inflammation and disease. It is hoped that through much needed field studies on the epidemiology of amebiasis in developing countries combined with future work to define the mechanism of immunopathogenesis in this model we can bring new insight into how to protect humans from this important worldwide disease.

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