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Resistance of C57BL/6 Mice to Amoebiasis Is Mediated by Nonhemopoietic Cells but Requires Hemopoietic IL-10 Production

Shinjiro Hamano,*† Amon Asgharpour,* Suzanne E. Stroup,* Thomas A. Wynn,‡ Edward H. Leiter,*§ and Eric Houpt2*

Resistance to intestinal amoebiasis is mouse strain dependent. C57BL/6 (B6) mice clear *Entamoeba histolytica* within hours of challenge, whereas C3H and CBA strains are susceptible to infection and disease. In this study, we show using bone marrow (BM) chimeric mice that mouse strain-dependent resistance is mediated by nonhemopoietic cells; specifically, B6 BM → CBA recipients remained susceptible as measured by amoeba score and culture, whereas CBA BM → B6 recipients remained resistant. Interestingly, hemopoietic IL-10 was required for maintaining the resistance of B6 mice, in that B6 IL-10-deficient mice and IL-10−/− BM → wild-type recipients, but not IL-10+/+ BM → IL-10−/− recipients, exhibited higher amoeba scores than their wild-type controls. Additionally, C57BL/10 IL-10−/−/Rag2−/− mice exhibited diminished amoeba scores and culture rates vs IL-10−/− mice, indicating that lymphocytes potentiated the susceptibility of IL-10-deficient mice. We conclude that nonhemopoietic cells mediate the natural resistance to intestinal amoebiasis of B6 mice, yet this resistance depends on hemopoietic IL-10 activity. The Journal of Immunology, 2006, 177: 1208–1213.

*Entamoeba histolytica* is the agent of amoebic colitis and liver abscess and second only to malaria in global mortality due to protozoa (1). In addition to invasive disease, the parasite asymptptomatically colonizes the intestine of up to 11% of endemic populations (2, 3). The mechanisms of human resistance to colonization or invasive disease are poorly understood. One contributing factor is fecal IgA to the parasite, particularly to its galactose-inhibitable adherence lectin, which correlates with partial protection against infection in children and vaccinated mice (3, 4). Additionally, a protective role for IFN-γ, TNF-α, and NO on macrophages has been demonstrated in vitro (5). Yet TNF-α also contributes to amoeba-induced epithelial damage (6) and is chemotactic for amoeba (7), such that the role of proinflammatory cytokines may depend on the cellular context.

To define the host mechanisms of resistance to intestinal amoebiasis, we have used a mouse model of amoebic colitis that develops upon intrarectal inoculation of trophozoites (4, 8, 9). A majority of CBA or C3H (either TLR4 mutant or wild-type (WT))3 mice develop patent infections upon challenge. Early after infection, viable amoeba are seen predominantly at sites of epithelial breakdown and a robust inflammatory response ensues that is not typically protective, insofar as neutrophil-depleted or dexamethasone-treated C3H or CBA mice show increased infection rates and/or disease scores (9).

In contrast, several other inbred mouse strains such as C57BL/6 (B6) are highly resistant to the infection, exhibiting rapid clearance of trophozoites within hours of challenge. As this timing suggests, their resistance is innate and persists in B6 SCID mice (9). In contrast to susceptible mouse strains, the B6 response to amoeba histologically demonstrates a normal mucosa without inflammation or epithelial breakdown. Indeed, gene expression analysis suggests the absence of a response to amoeba in B6 mice (only 1 of 12,422 genes or expressed sequence tag was dysregulated between B6 sham- and B6 amoeba-challenged mice; data published in (9) and available at (https://genes.med.virginia.edu/public data/index.cgi)). Moreover, B6 resistance does not rely on an innate proinflammatory response in that IL-12p40-deficient, inducible NO synthase-deficient, phagocyte oxidase-deficient, MyD88-deficient, or neutrophil-depleted B6 mice remain resistant (8, 9).

Because an innate proinflammatory response appeared irrelevant for B6 clearance, we embarked upon the hypothesis that B6 resistance owes to the strain’s capacity to maintain intestinal homeostasis in the face of amoeba challenge. We examined the role of IL-10, because IL-10 regulates the host immune response to gut flora (10–12) and prevents diverse lymphoid and epithelial defects (13, 14). Through this work we find that B6 resistance to intestinal *E. histolytica* infection occurs at the nonhemopoietic level, yet that the protective capacity of this compartment is maintained via hemopoietic IL-10.

Materials and Methods

**Mice**

CBA/J, C57BL/6, C57BL/6 IL-10−/−, and C57BL/6 (CD45.1+) mice were purchased from The Jackson Laboratory or obtained from the research colony of E. Leiter (also at The Jackson Laboratory). C57BL/10 mice were purchased from Taconic Farms. C57BL/10 IL-10−/− and IL-10−/−/Rag2−/− mice were generated as described previously (15) and obtained from the National Institutes of Allergy and Infectious Diseases Taconic...
colony (T. Wynn, Bethesda, MD). Animals were maintained under specific pathogen-free conditions at the University of Virginia and were challenged at 6–12 wk of age. The Institutional Animal Care and Use Committee approved all protocols.

Parasites and intracecal inoculation

Trophozoites for intracecal injections were originally derived from laboratory strain HM1:IMSS (American Type Culture Collection) that were sequentially passed in vivo through the mouse cecum. Cecal contents were cultured in trypsin-yeast-iron (TYI-S-33) medium supplemented with 25 U/ml penicillin and 25 mg/ml streptomycin until trophozoite growth was axenic as confirmed by the absence of bacterial growth on Trypticase soy agar with 5% sheep blood (BD Biosciences). For all intracecal inoculations, axenic trophozoites were grown to the log phase and counted with a hemacytometer, and 2 x 10^6 trophozoites in 150 μl were injected thrice intraceally after laparotomy as described (8).

Bone marrow (BM) chimera

Female CBA/J, C57BL/6 IL-10^{-/-} (CD45.2), C57BL/6 IL-10^{-/-} (CD45.1), and C57BL/6 IL-10^{-/-} mice (CD45.2) were used for BM chimera mice. Recipient mice were total body irradiated (1100–1300 rad from a 137Cs source) and reconstituted i.v. within 6 h with BM cells prepared (for syngeneic chimeras 1 x 10^5 cells were transferred, whereas for allo- genetic chimeras 2 x 10^5 cells were transferred) from donor femurs and tibia and then rested for 7–8 wk. For allogeneic BM chimeras, BM cells were pretreated with rabbit antimouse T cell antiserum (Thy 1, CL2005; Cedarlane Laboratories) and rabbit complement (CL3051; Cedarlane Laboratories) to prevent graft-vs-host disease. During weeks 0–3, cimetic mice were given trimethoprim/sulfamethoxazole (5.00/86.4 mg)-supplemented water. Eight groups were generated. For the mouse-strain chimera experiment: CBA/J to CBA/J, CBA/J to C57BL/6, C57BL/6 to CBA, and C57BL/6 to C57BL/6; for the IL-10{-/-} chimera experiment: IL-10^{-/-} (CD45.1) to IL-10^{-/-} (CD45.2), IL-10^{-/-} (CD45.1) to IL-10^{-/-} (CD45.2), IL-10^{-/-} (CD45.1), and IL-10^{-/-} (CD45.2). More than 90% engraftment of hematopoietic cells from donor origin was confirmed on peripheral blood leukocytes at 6 wk postreconstitution, and on splenocytes and mesenteric lymph node (MLN) cells upon sacrifice by FACS using conjugated Abs to H-2Kb (AF6-88.5) and H-2K{sup}b (36-75) for the allogeneic chimeras and CD45.1 (A20) and CD45.2 (104) for the IL-10{-/-} chimera experiment (Abs obtained from BD Biosciences).

Pathology and scoring of amoebic colitis

Mice were sacrificed and each cecum longitudinally bisected. One-half of the cecum was placed in Holland’s fixative, cut into three to five equal cross sections, paraffin embedded, and 4-μm sections were stained with H&E. Histopathology was scored blindly for each mouse as described previously (8). Briefly, numbers of histologically visible amoeba were scored 0–5 (0, none; 1, present but 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, virtualy complete occupation of the lumen by ameba). Degree of inflammation was scored 0–5 (0, normal; 1, mucosal hyperplasia; 2, spotty infiltration of inflammatory cells not involving the entire thickness of the mucosa; 3, marked increase in inflammatory cells involving full thickness of mucosa; 4, marked increase in inflammatory cells involving full thickness of mucosa; 5, virtualy complete occupation of the mucosa by ameba). Degree of inflammation was scored 0–5 (0, normal; 1, mucosal hyperplasia; 2, spotty infiltration of inflammatory cells not involving the entire thickness of the mucosa; 3, marked increase in inflammatory cells involving full thickness of mucosa; 4, marked increase in inflammatory cells of mucosa and submucosa, with tissue architecture in tact; 5, virtualy complete occupation of mucosa by ameba). Degree of inflammation was scored 0–5 (0, normal; 1, mucosal hyperplasia; 2, spotty infiltration of inflammatory cells not involving the entire thickness of the mucosa; 3, marked increase in inflammatory cells involving full thickness of mucosa; 4, marked increase in inflammatory cells involving full thickness of mucosa; 5, virtualy complete occupation of the mucosa by ameba). Degree of inflammation was scored 0–5 (0, normal; 1, mucosal hyperplasia; 2, spotty infiltration of inflammatory cells not involving the entire thickness of the mucosa; 3, marked increase in inflammatory cells involving full thickness of mucosa; 4, marked increase in inflammatory cells involving full thickness of mucosa; 5, virtualy complete occupation of the mucosa by ameba).

IL-10 is required for resistance to intestinal amoebiasis in C57BL/6 mice

These results suggested that the strain-dependent phenotype of resistance or susceptibility is governed by nonhemopoietic cells. We next examined the outcome of infection in IL-10^{-/-} mice on the resistant C57BL/6 background, because these mice acquire abundant defects in their epithelium (13, 14, 21–23). When sacrificed at 9–10 days postchallenge, B6 IL-10{-/-} deficient mice exhibited a significantly higher amoeba score, inflammation score, and culture-positive rate (15 and 0 of 9 ceca were culture positive, respectively; p = NS). Likewise, B6→CBA chimeras exhibited the susceptibility of CBA→CBA mice according to amoeba score, inflammation score, and culture-positive rate (13 of 14 and 6 of 7 ceca were culture positive, respectively; p = NS).

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IL-10−/− developed acutely (Fig. 2C). Notably, the susceptibility of B6 IL-10−/− mice appeared to be distinct from that of CBA mice in terms of a higher inflammation:amoeba score ratio (2.70 ± 0.59 vs 1.07 ± 0.18 in B6 IL-10−/− and CBA/J, respectively; n = 22 and 11; p < 0.05). Furthermore, B6 IL-10−/− mice appeared to ultimately clear the parasite (0 of 8 C57BL/6 IL-10−/− mice were infected by histology or culture after 14 days) in contrast to the chronic nonhealing infection of C3H or CBA mice (9). These results suggested that IL-10 functions to maintain C57BL/6 resistance to the early establishment of infection.

Susceptibility of IL-10−/− mice is associated with a proinflammatory response

Because of the abundance of both lymphoid and epithelial intestinal defects that have been described in IL-10-deficient mice, to begin to understand the mechanism of IL-10−/− susceptibility we characterized the intestinal environment after E. histolytica challenge by microarray. Total RNA was prepared from three IL-10−/− and five IL-10+/+ cecae 18 h postchallenge and hybridized to Affymetrix MgU74Av2 arrays. Of 7264 genes, 399 were recorded as differentially expressed as detailed in Materials and Methods. Patterns of gene ontology were queried among the differentially expressed genes and indicated that “immune response” was the most statistically overrepresented gene ontology function in IL-10−/− mice (FDR = 0.0025), whereas “regulation of cellular process” was most overrepresented in IL-10+/+ mice (FDR = 0.02). Most (209 of 399) of the differentially expressed genes were not amoeba-specific, and were common to the identical sham-challenge cecal tissue comparison (three IL-10−/− and three IL-10+/+ cecae 18 h after sham challenge; Table I). Overall, the profile of the amoeba or sham-infected IL-10−/− cecae suggested a mixed inflammatory environment with up-regulation of several Ag presentation-related genes, certain innate immune genes and chemokines, as well as the anti-inflammatory gene PAP.

Hemopoietic cells are a required source of protective IL-10

Although we were seeking to find in IL-10−/− ceca the loss of canonical nonhemopoietic cell transcripts, the microarray analysis did not clearly incriminate a single cellular subset in the susceptibility defect of IL-10−/− mice. We therefore broadly examined the cellular anatomy of IL-10 production via BM chimeras. IL-10−/− → IL-10+/+ BM chimeras were generated, and chimerism was confirmed using CD45 markers in PBMCs, MLN cells, and splenocytes (Fig. 3A). Early susceptibility was examined at 2–3 days postchallenge. As expected, a lower amoeba score, inflammation score, and culture-positive rate (4 of 15 vs 6 of 7; p < 0.05) was observed in IL-10−/− → IL-10+/+ vs IL-10−/− → IL-10−/− chimeras (Fig. 3, B and C). Notably, the culture-positive rate at this early time point is relatively high in the resistant B6 IL-10+/+ → IL-10+/+ controls, therefore we feel the quantitative amoeba and inflammation scores are more meaningful for early events. The comparison of IL-10+/+ → IL-10+/+ vs IL-10−/− → IL-10−/− mice demonstrated that hemopoietic IL-10 was required for resistance (IL-10−/− → IL-10+/+ mice exhibited statistically higher amoeba score, inflammation score, and culture-positive rate (13 of 20 vs 4 of 15; p < 0.05)). Additionally, hemopoietic IL-10 was
largely sufficient for resistance, because IL-10\(^{+/−}\) → IL-10\(^{-/-}\) mice exhibited a statistically lower amoeba score and inflammation score vs IL-10\(^{-/-}\) → IL-10\(^{-/-}\) mice (culture-positive rate 8 of 15 vs 6 of 7, respectively; \(p = 0.19\)).

Because hemopoietic IL-10 played a role in maintaining the resistant state of C57BL/6 mice, we sought to examine the role of CD25\(^{+}\) T regulatory cells in this strain. We depleted the CD25\(^{+}\) proportion of CD4\(^{+}\) cells prepared from donor femur and tibia. Engraftment of >90% donor hemopoietic cells were confirmed by FACS for CD45 Ags at 6 wk posttransfer on PBMCs and upon sacrifice on splenocytes (\(A; n = 4\) group, one representative mouse per group shown) and MLN. At 7 wk posttransfer, mice were challenged intracecally with 2 × 10\(^{5}\) E. histolytica trophozoites and sacrificed after 2–3 days for evaluation of infection according to histologic amoeba score (\(B\)) and inflammation (\(C\)). *, \(p < 0.05\); \(n = 15, 20, 15,\) and 7 for the four groups indicated left to right on the x-axis. Data represent two independent experiments and are shown as mean ± SE. D. Representative histology from the ceca of BM chimeras demonstrated benign histology in IL-10\(^{+/-}\) → IL-10\(^{-/-}\) mice, mucosal hyperplasia with amoeba in IL-10\(^{-/-}\) → IL-10\(^{-/-}\) mice, mild mucosal hyperplasia in IL-10\(^{+/-}\) → IL-10\(^{-/-}\) mice, and severe inflammation and ulceration in IL-10\(^{-/-}\) → IL-10\(^{-/-}\) mice. H&E, ×200.

(Figure 3) Resistance to intestinal amoebiasis in C57BL/6 mice is maintained by hemopoietic IL-10 production. Recipient B6 IL-10\(^{-/-}\) or IL-10\(^{-/-}\) mice were total-body irradiated (1100 rad from a \(^{60}\)Co source) and reconstituted i.v. within 6 h with 1 × 10\(^{7}\) cells prepared from donor femur and tibia. Engraftment of >90% donor hemopoietic cells were confirmed by FACS for CD45 Ags at 6 wk posttransfer on PBMCs and upon sacrifice on splenocytes (\(A; n = 4\) group, one representative mouse per group shown) and MLN. At 7 wk posttransfer, mice were challenged intracecally with 2 × 10\(^{5}\) E. histolytica trophozoites and sacrificed after 2–3 days for evaluation of infection according to histologic amoeba score (\(B\)) and inflammation (\(C\)). *, \(p < 0.05\); \(n = 15, 20, 15,\) and 7 for the four groups indicated left to right on the x-axis. Data represent two independent experiments and are shown as mean ± SE. D. Representative histology from the ceca of BM chimeras demonstrated benign histology in IL-10\(^{+/-}\) → IL-10\(^{-/-}\) mice, mucosal hyperplasia with amoeba in IL-10\(^{-/-}\) → IL-10\(^{-/-}\) mice, mild mucosal hyperplasia in IL-10\(^{+/-}\) → IL-10\(^{-/-}\) mice, and severe inflammation and ulceration in IL-10\(^{-/-}\) → IL-10\(^{-/-}\) mice. H&E, ×200.

Table 1. Upregulated immune response genes in the ceca of C57BL/6 IL-10\(^{-/-}\) mice 18 h after E. histolytica challenge

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Affymetrix Probe Set</th>
<th>IL-10(^{-/-}) (signal intensity)</th>
<th>IL-10(^{-/-}) (signal intensity)</th>
<th>Fold Change</th>
<th>(p) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2-Ab1</td>
<td>Histocompatibility 2, class II</td>
<td>100998_at</td>
<td>992</td>
<td>126</td>
<td>7.9</td>
<td>0.010</td>
</tr>
<tr>
<td>Pax</td>
<td>Pancreatitis-associated protein</td>
<td>161800_f_at</td>
<td>34958</td>
<td>4784</td>
<td>7.3</td>
<td>0.014</td>
</tr>
<tr>
<td>Cdkn1a</td>
<td>Cyclin-dependent kinase inhibitor 1A (P21)</td>
<td>98067_at</td>
<td>315</td>
<td>65</td>
<td>4.9</td>
<td>0.032</td>
</tr>
<tr>
<td>Reg3γ</td>
<td>Regenerating islet-derived 3 γ</td>
<td>162187_f_at</td>
<td>1799</td>
<td>382</td>
<td>4.7</td>
<td>0.023</td>
</tr>
<tr>
<td>Pomb9</td>
<td>Proteosome subunit β type 9†</td>
<td>93085_at</td>
<td>569</td>
<td>124</td>
<td>4.6</td>
<td>0.000</td>
</tr>
<tr>
<td>Cd74</td>
<td>Ia-associated invariant chain</td>
<td>101054_at</td>
<td>3722</td>
<td>874</td>
<td>4.3</td>
<td>0.026</td>
</tr>
<tr>
<td>PGLYRP1</td>
<td>Peptidoglycan recognition protein 1</td>
<td>162475_f_at</td>
<td>4608</td>
<td>1659</td>
<td>2.8</td>
<td>0.003</td>
</tr>
<tr>
<td>OTUB1</td>
<td>OTU domain, ubiquitin aldehyde binding 1‡</td>
<td>94336_at</td>
<td>65</td>
<td>24</td>
<td>2.7</td>
<td>0.006</td>
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<tr>
<td>C4a</td>
<td>Complement component 4</td>
<td>103033_at</td>
<td>156</td>
<td>65</td>
<td>2.4</td>
<td>0.027</td>
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<tr>
<td>Cxcl12</td>
<td>Stromal cell-derived factor 1</td>
<td>160511_at</td>
<td>136</td>
<td>60</td>
<td>2.3</td>
<td>0.049</td>
</tr>
<tr>
<td>lrf8</td>
<td>IFN regulatory factor 8</td>
<td>98002_at</td>
<td>880</td>
<td>395</td>
<td>2.2</td>
<td>0.011</td>
</tr>
<tr>
<td>B2m</td>
<td>β2 microglobulin</td>
<td>93088_at</td>
<td>14690</td>
<td>7496</td>
<td>2.0</td>
<td>0.002</td>
</tr>
</tbody>
</table>

* Four other histocompatibility 2 class II transcripts were also up-regulated in IL-10\(^{-/-}\) mice (98035_g_at, 94285_at, 93092_at, 97541_f_at).
† Proteosome subunit β type 8 (102791_at) was also up-regulated in IL-10\(^{-/-}\) mice.
‡ Bold genes were those up-regulated only in amoeba-challenged IL-10\(^{-/-}\) mice, all others were up-regulated in both amoeba- and sham-challenged IL-10\(^{-/-}\) mice (vs WT controls).
hemopoietic IL-10 (i.e., IL-10<sup>−/−</sup> → IL-10<sup>+/+</sup> mice showed a statistically lower amoeba score and inflammation score vs IL-10<sup>−/−</sup> → IL-10<sup>−/−</sup> mice).

**Lymphocytes partly contribute to susceptible phenotype of IL-10<sup>−/−</sup> mice**

T lymphocytes are important targets of IL-10 activity in many models. Therefore, we sought to examine the requirement for lymphocytes in the susceptibility of IL-10<sup>−/−</sup> mice. We examined amoebic infection in Rag2<sup>−/−</sup> × IL-10<sup>−/−</sup> mice, which were available on the C57BL/10 background. C57BL/10 Rag2<sup>−/−</sup> × IL-10<sup>−/−</sup> exhibited diminished susceptibility vs C57BL/10 IL-10<sup>−/−</sup> mice according to amoeba score (Fig. 4A) or culture-positive rate (double knockout (KO) 5 of 25 vs 7 of 12 KO; p < 0.05). The effect of lymphocyte deficiency was modest, and inflammation score was not statistically altered (Fig. 4B), perhaps because the underlying strain susceptibility of C57BL/10 IL-10<sup>−/−</sup> was less than that of C57BL/6 IL-10<sup>−/−</sup> mice (data not shown). However, these results suggested that lymphocytes partially contributed to the susceptible state of IL-10-deficient mice.

**Discussion**

This work shows that the inherent resistance of C57BL/6 mice to amoebic infection is governed by nonhemopoietic cells yet can be altered by the hemopoietic compartment, specifically needing hemopoietic IL-10 for maintenance. These findings may be a clue to the variable resistance to colonization or invasive disease observed in humans. In the steady state, some individuals may exhibit a nonhemopoietic predisposition to susceptibility. Others are endowed with a resistant phenotype, which can be lost with hemopoietically derived alterations.

A protective role for IL-10 is at first counterintuitive given several systemic infectious disease models such as *Listeria monocytogenes*, *Toxoplasma gondii*, and *Trichinella spiralis* infection (24–26), where IL-10 dampsens protective immunity but prevents immunopathology. Interestingly, many gastrointestinal infections behave differently, and IL-10-deficient mice exhibit worse infection and disease burdens with *Trichuris muris* and *Helicobacter hepaticus* (27, 28). Disease in the latter model owes to the failure to generate a protective T regulatory cell population (29) and is manifest after 20 days during the phase of adaptive immunity. In this amoebiasis model, by contrast, the effect of IL-10 is on the immediate phase, because B6 IL-10-dependent mice resist amoebic infection within hours (9).

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Disclosures
The authors have no financial conflict of interest.

References