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MAJOR ARTICLE

Entamoeba histolytica–Encoded Homolog of Macrophage Migration Inhibitory Factor Contributes to Mucosal Inflammation during Amebic Colitis

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Understanding the mechanisms by which *Entamoeba histolytica* drives gut inflammation is critical for the development of improved preventive and therapeutic strategies. *E. histolytica* encodes a homolog of the human cytokine macrophage migration inhibitory factor (MIF). Here, we investigated the role of *E. histolytica* MIF (*Eh*MIF) during infection. We found that the concentration of fecal *Eh*MIF correlated with the level of intestinal inflammation in persons with intestinal amebiasis. Mice treated with antibodies that specifically block *Eh*MIF had reduced chemokine expression and neutrophil infiltration in the mucosa. In addition to antibody-mediated neutralization, we used a genetic approach to test the effect of *Eh*MIF on mucosal inflammation. Mice infected with parasites overexpressing *Eh*MIF had increased chemokine expression, neutrophil influx, and mucosal damage. Together, these results uncover a specific parasite protein that increases mucosal inflammation, expands our knowledge of host–parasite interaction during amebic colitis, and highlights a potential immunomodulatory target.

Keywords. Chemokines; colitis; diarrhea; *Entamoeba histolytica*; host–parasite interaction; macrophage migration inhibitory factor; mucosal inflammation; neutrophils.

The mucosal surfaces of the nasal, intestinal, respiratory, and genitourinary tracts are the points of first contact for many protozoan parasites. Mucosal inflammation triggered by the interaction with these parasites plays a key role in human disease. However, the mechanisms by which parasites induce mucosal inflammation are incompletely understood.

Globally, diarrheal disease is second only to pneumonia as a leading cause of death in children under 5 years of age [1]. *Entamoeba histolytica* is a protozoan parasite that causes colitis, a leading cause of severe diarrhea in low-income countries. [2, 3]. *E. histolytica* infection is also a concern among returning travelers with infectious gastrointestinal disease: *E. histolytica* infection occurs at an estimated rate of 14 per 1000 returned unwell travelers [4]. Fulminant amebic colitis is an uncommon but life-threatening complication and is associated with high mortality and morbidity despite antimicrobial therapy, with case fatality rates ranging from 40% to 89% [5]. There is neither an effective vaccine nor have there been advancements in therapies for amebic colitis for over half a century, following the introduction of the nitroimidazole agents [6].

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Mucosal inflammation is a hallmark of amebic colitis, explaining why it is often misdiagnosed as inflammatory bowel disease [5]. While inflammatory cells represent a line of defense [7], there is significant evidence that the inflammatory response contributes to the tissue damage seen in amebic colitis [8]. During amebic colitis, neutrophils infiltrate the intestinal tract [9]. It has been known for decades that neutrophilic enzymes such as myeloperoxidase (MPO) generate oxygen-free radicals, which kill invading pathogens. Oxygen-free radicals are also responsible for collateral tissue damage during the inflammatory period [10]. There is a direct positive correlation between MPO activity in the colon and the extent of intestinal tract epithelial damage [10].

Neutrophil migration depends on chemokines produced by epithelial cells. Interleukin-8 (IL-8) is a potent neutrophil chemoattractant that contributes to mucosal inflammation in various infectious and inflammatory diseases. Persons with severe forms of amebic colitis have higher colonic tissue levels of IL-8 and neutrophils [11, 12]. Both IL-8 inhibition and neutrophil depletion resulted in less mucosal damage during *E. histolytica* infection in a mouse-human intestinal xenograft model [13, 14]. Neutralization of a crucial parasite mediator of host immunopathology may prevent or attenuate disease. However, key parasite mediators of mucosal neutrophil influx during amebic infection remain incompletely understood.

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine that is a critical upstream mediator of the innate immune response. MIF enhances the secretion of

inflammatory mediators, and there is a strong association between MIF and colitis [15–19]. *E. histolytica* encodes a homolog of the cytokine MIF. However, the effect of the *E. histolytica*–encoded MIF homolog on mucosal inflammation during infection is unknown. In the present study, we examined the role of *E. histolytica* MIF (*Eh*MIF) during infection using in vitro approaches, mouse model, and in persons with intestinal amebiasis. Taken together, the results of our study suggest that *Eh*MIF is a key contributor of parasite-induced mucosal inflammation.

METHODS

Study Approval

All animal studies were performed in compliance with the federal regulations set forth in the Animal Welfare Act, the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, and the guidelines of the University of Virginia Institutional Animal Care and Use Committee. All protocols for animal use were approved by the University of Virginia Institutional Animal Care and Use Committee. Use of serum and stool samples from human participants were approved by the Institutional Review Board at the University of Virginia, the Research and Ethical Research Review Committees of the International Center for Diarrheal Disease Research, Bangladesh, and the Research and Ethics Committee of the University of Venda, South Africa.

Parasites and Cell Culture

Entamoeba histolytica strain HM1:IMSS trophozoites were grown at 37°C in TYI-S-33 medium. The human intestinal epithelial cell line Caco-2 (American Type Culture Collection) was grown in Dulbecco's Modified Eagle medium (Gibco). Cell lines tested negative for *Mycoplasma* (Lonza). Coculturing of epithelial cells with ameba were done at a ratio of 10:1 host cells to parasite in M199 medium [20]. IL-8 in cell culture supernatant was measured by enzyme-linked immunosorbent assay (ELISA; eBioscience). For overexpression of *Eh*MIF in *E. histolytica* trophozoites, the *Eh*MIF gene with no tag was cloned into the pKT3M expression vector [21] and confirmed by sequencing. Parasites were transfected by a previously described technique [22]. Transfectants were selected with 12 μg/ml G-418 (Gibco). *Eh*MIF protein overexpression was confirmed by immunoblot analysis using specific anti-*Eh*MIF antibodies [23]. Parasites transfected only with pKT3M expression vector were used as empty vector controls. Parasite growth was measured using CyQUANT Direct Cell Proliferation Assay kit (Invitrogen) according to the manufacturer's instructions. For amebic cytotoxicity assays, *E. histolytica* trophozoites were added to intestinal epithelial cell line monolayers in M199 as previously described [20]. Lactate dehydrogenase (LDH) levels in the supernatant were measured using Cyto Tox-ONE Homogeneous Membrane Integrity Assay (Promega) as directed. The maximum amount of LDH released was determined by the addition of Triton-X to intestinal epithelial cells alone. Percent cytotoxicity was calculated as: [(LDH release + E. histolytica) – (LDH – *E. histolytica*)] / [maximum LDH release]. Conditions were tested in triplicates. Each experiment was repeated at least 3 times, and representative experiments are shown.

Measurement of *Eh*MIF and Stool Myeloperoxidase

We developed an ELISA to measure *Eh*MIF levels, similar to a recently described method [24]. Corning 96-well high-protein-binding polystyrene plates were coated with 5 μg/ml rabbit polyclonal anti-*Eh*MIF [23] in phosphate-buffered saline (PBS) overnight and blocked for 1 hour with PBS containing 1% bovine serum albumin. Recombinant *Eh*MIF was used as a protein standard. Stool samples were incubated overnight at 4°C, then washed before the addition of biotinylated anti-*Eh*MIF at 0.25 μg/ml. After incubation and washing, avidin-conjugated horseradish peroxidase (eBioscience) was added, and detection was performed with 3,3′,5,5′-tetramethylbenzidine ELISA detection reagent (eBioscience). The sensitivity of the ELISA was 15.6 pg. There was no cross-reactivity to human MIF. *Eh*MIF concentrations were measured in deidentified diarrheal stool samples from 35 South African patients with intestinal amebiasis. Stool Myeloperoxidase (MPO) levels were measured by ELISA (ALPCO) according to the manufacturer's instructions [25].

Mice

Wild-type CBA/J mice were obtained from the Jackson Laboratory. Male mice were used at 10 weeks of age.

Parasite Infection

Infections were carried out via intracecal inoculation of mice with *E. histolytica* trophozoites [26]. A total of 5×10^5 trophozoites in 100 μl of TYI media were injected intracecally after laparotomy for antibody-mediated neutralization and *Eh*MIF overexpression studies. For antibody neutralization studies, 0.5 mg mouse anti-*Eh*MIF blocking antibodies were given by intraperitoneal injection 24 hours before and intracecally at the time of infection. Isotype antibodies given at the same dose, route, and timing were used as controls. Intracecal injection with media only was used as uninfected controls. No differences in the inflammatory markers and cytokine levels were observed among mice that did not received intracecal injection and those injected with media only and PBS. Mice were sacrificed 24 hours postinfection. TechLab E. histolytica–II kit was used to determine amebic antigen burden in cecal contents [26]. Cecal tissue lysates were prepared as in [27]. C-X-C motif (CXC) chemokine ligand 1 (CXCL1), CXC chemokine ligand 2 (CXCL2), and matrix metalloproteinase-3 (MMP3) levels in cecal lysates were measured by ELISA (R&D Systems). Myeloperoxidase activity in cecal tissue was determined using the same standard protocol as used in [28].

Human Samples

Serum sample concentrations of anti-*Eh*MIF antibodies were measured in 79 children (2–5 years old) from a well-characterized cohort in the endemic area of Mirpur within Dhaka, Bangladesh [23]. After serum sample collection, monthly stool samples were routinely obtained from all children and tested for *E. histolytica* using real-time polymerase chain reaction as previously published [29].

Secretion Assay

Preparation of *E. histolytica*–secreted fractions were modified as described in [30]. Briefly, 1×10^7 trophozoites per mL were suspended in M199 media (Gibco) and incubated at 37°C for 2 hours. Cell-free supernatant representing the secreted fraction was collected for further analysis. Proteins in the secreted fraction was not due to cell stress or cell death, as only a minor portion of cells stained positive for Trypan blue (less than 5%), and no cellular actin was found by immunoblot analysis. For inhibition assays, trophozoites were incubated with pharmacological agents brefeldin A and probenicid [31].

Immunohistochemical Staining and Histopathological Examination

Mouse immunohistochemical staining was performed by the University of Virginia Biorepository and Tissue Research Facility. Staining was performed using the DAKO Autostainer Universal Staining System with specific antibody directed against *Eh*MIF at a dilution of 1:600. Mouse cecal tissue was fixed in Bouin's solution (Sigma) and stored in 70% ethanol. Tissue was stained with hematoxylin and eosin by the University of Virginia Research Histology Core. Histological scoring for inflammatory infiltration and epithelial cell damage was performed by 2 independent blinded scorers as previously described [32].

Mass Spectrometry

Proteins from the *E. histolytica*–secreted fraction were separated by gel electrophoresis. The section spanning 10–20 kilodaltons was then excised from the gel. The gel sample was submitted to the W. M. Keck Biomedical Mass Spectrometry Laboratory for mass spectrometry analysis.

Statistics

Statistical differences between 2 groups were determined using the Mann–Whitney *U* test or Student *t* test. Pearson's correlation was used for correlation analysis. Survival differences were analyzed by the log-rank test. A *P* value less than .05 was considered statistically significant.

RESULTS

Association of *Eh*MIF With Intestinal Inflammation in Persons With Intestinal Amebiasis

Given that inflammation is a characteristic feature of amebic colitis and *Eh*MIF is expressed during human infection, we tested in humans for a proinflammatory effect of *Eh*MIF: MPO is a major component of neutrophils, and the concentration of MPO in stool samples is a widely used marker of intestinal inflammation [25, 33]. We measured the concentrations of *Eh*MIF and MPO in the stools samples of persons with intestinal amebiasis and found a positive association between *Eh*MIF and intestinal inflammation by Pearson's correlation (n = 35, r = 0.41; *P* = .015 (Figure 1A and 1B). We concluded that the correlation of stool *Eh*MIF with MPO was consistent with a potential role for *Eh*MIF in colonic inflammation in humans with amebiasis.

*Eh*MIF Induces IL-8 Secretion From Human Intestinal Epithelial Cells

Cytokines such as IL-1β and MIF are secretory proteins that lack a signal peptide and therefore do not follow the classical

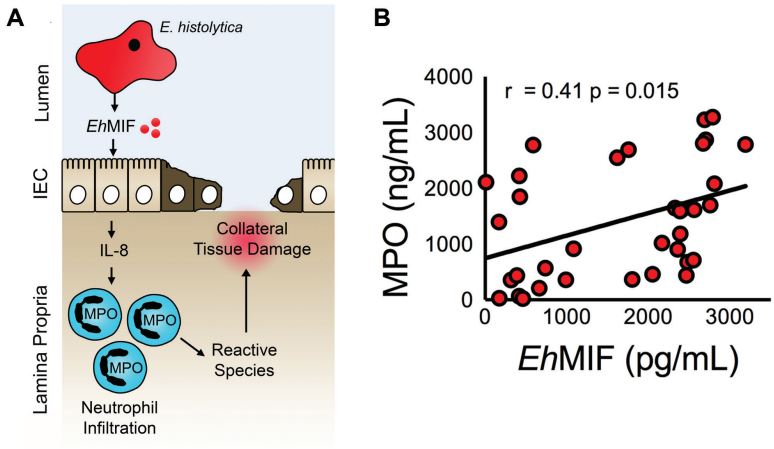

Figure 1. Association between *Eh*MIF and intestinal inflammation. *A*, Schematic of the hypothesis of how secreted *Eh*MIF promotes mucosal inflammation. *B*, Significant positive correlation between fecal *Eh*MIF levels and the MPO marker of intestinal inflammation in persons with amebiasis ($n = 35$). A *P* value < .05 was considered statistically significant. Abbreviations: *Eh*MIF, *Entamoeba histolytica* macrophage migration inhibitory factor; MPO, myeloperoxidase.

endoplasmic reticulum–to-Golgi pathway of secretion. Human MIF is constitutively expressed, accumulated in the cytoplasm, and secreted by a nonclassical pathway involving an ATP-binding cassette (ABC) transporter [31, 34]. Similar to human MIF, *Eh*MIF lacks a signal peptide and ABC transporters can be found in *E. histolytica* [35]. We used mass spectrometry to further confirm the protein expression of *Eh*MIF (Supplemental Figure 1). We investigated whether *Eh*MIF is secreted and found it present in secreted fractions by ELISA and immunoblot (Figure 2A). We further investigated the effect of transport inhibitors on *Eh*MIF secretion. We found that *Eh*MIF secretion was not inhibited by brefeldin A, an inhibitor of the classical secretory pathway. Probenicid, an inhibitor of nonclassical protein export, blocked *Eh*MIF secretion (Figure 2A). These findings suggest that *Eh*MIF shared with other MIF homologs secretion by a nonclassical pathway. In our study, the maximum inhibition achieved was approximately 50%. This raises the possibility of other secretion pathways for *Eh*MIF.

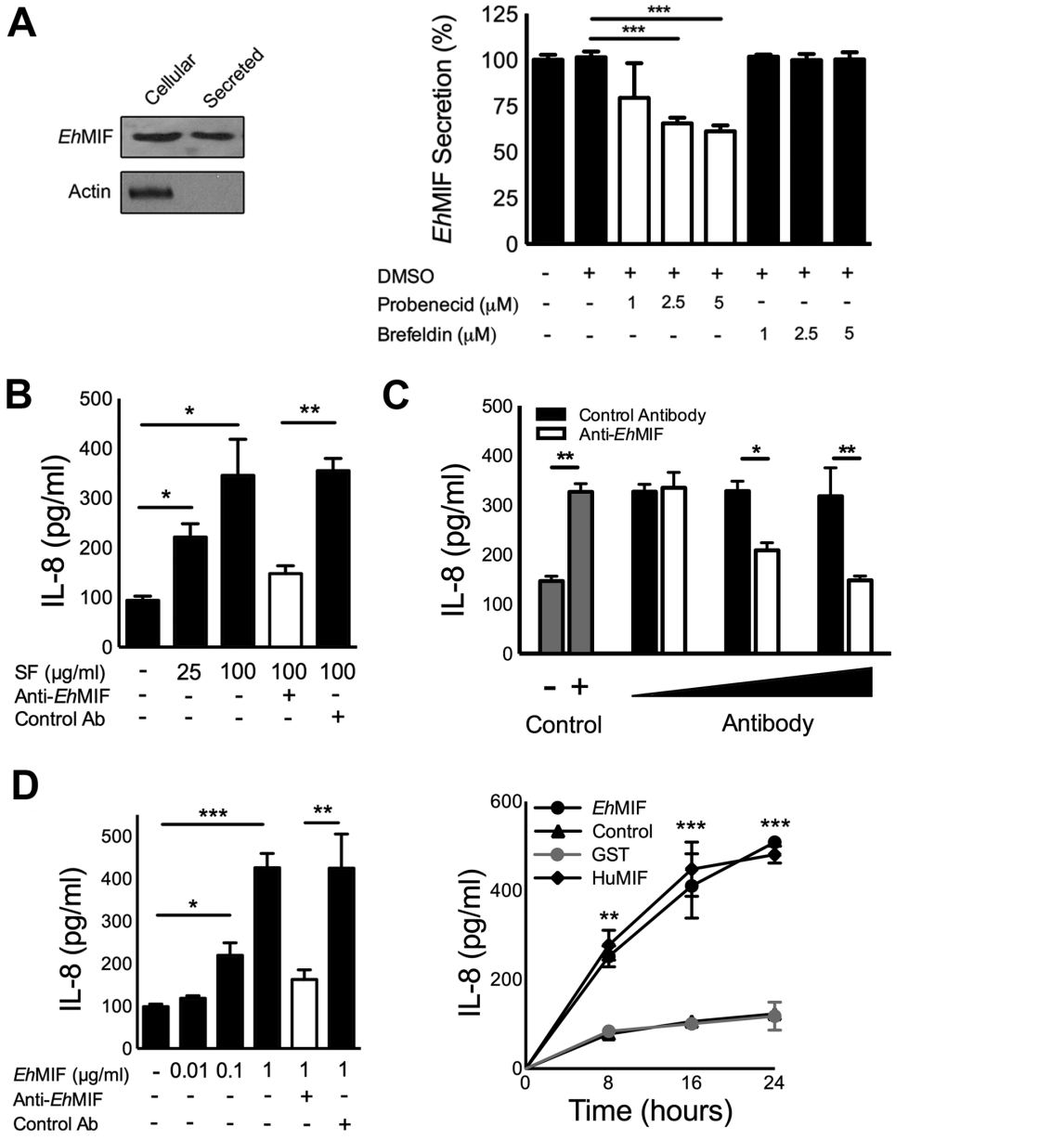

Figure 2. *Eh*MIF induces IL-8 production from human intestinal epithelial cells. *A*, Secretion of *Eh*MIF by amebic trophozoites. Immunoblot analyses of the cell lysate and secreted fractions of *E. histolytica* using anti-*Eh*MIF antibodies. Actin detection serves as negative controls for cell lysis. *Eh*MIF ELISA of *E. histolytica* secreted fractions. *Eh*MIF secretion is not inhibited by the classical pathway inhibitor brefeldin A. Probenicid, an inhibitor of nonclassical protein export, blocked *Eh*MIF secretion. *B*, Anti-*Eh*MIF antibodies blocked *E. histolytica* secretory fraction-induced IL-8 production by colonic epithelial cells (Caco-2 cells). *C*, *E. histolytica* parasites cocultured with IECs in the presence of antibodies. *D*, *Eh*MIF stimulates IL-8 production in a dose- and time-dependent manner. Data represent mean and SD of triplicates from 1 experiment and are representative of 3 independent experiments. $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$. Abbreviations: *Eh*MIF, *Entamoeba histolytica* macrophage migration inhibitory factor; ELISA, enzyme-linked immunosorbent assay; IECs, intestinal epithelial cells; IL-8, interleukin-8; SF, secretory fraction.

*Eh*MIF was tested for its ability to induce IL-8 using a cellular approach, given that epithelial cells are the first host cells to encounter secretory products released by enteric parasites. It has long been hypothesized that *E. histolytica*, by means of soluble mediators, stimulates chemokine production from host cells [36]. The *E. histolytica* secretory protein fraction was previously shown to induce IL-8 production by Caco-2 human colonic epithelial cells [37]. We were able to reproduce this finding and found that IL-8 production was inhibited by antibodies that blocked *Eh*MIF (Figure 2B). The rabbit anti-*Eh*MIF antibodies used for these experiments did not cross-react with human MIF (Supplemental Figure 2A). In addition, anti-*Eh*MIF antibodies inhibited the IL-8 secretion induced by coculturing intestinal epithelial cells (IECs) with *E. histolytica* parasites (Figure 2C). We also determined the effect of endotoxin-free recombinant *Eh*MIF (<1 pg LPS/μg protein) on IECs. Recombinant glutathione S-transferase, an irrelevant protein, was used as a negative control and human MIF as a positive control. *Eh*MIF induced IL-8 production in a time- and dose-dependent manner, and treatment with anti-*Eh*MIF inhibited *Eh*MIF-induced IL-8 production (Figure 2D). These data indicate that *Eh*MIF was a cause of IL-8 secretion by *E. histolytica.*

Anti-*Eh*MIF Antibody Treatment Reduces Mucosal Inflammation

We further investigated in an amebic mouse model the role of *Eh*MIF on chemokine secretion and mucosal inflammation. CXCL1/KC and CXCL2/MIP-2 are mouse homologs of human IL-8 and are key chemokines in neutrophil recruitment and inflammation. Host MIF was shown to induce mouse IL-8 homolog production from alveolar epithelial cells [38, 39]. Mice infected with *E. histolytica* had elevated levels of CXCL1 and CXCL2 (Figure 3B), in keeping with previous studies [28]. We found that mice treated with mouse anti-*Eh*MIF-blocking antibodies had reduced CXCL1 (Figure 3B). Neutrophil MPO activity, an indicator of neutrophil infiltration [6, 32, 40], was significantly lower in anti-*Eh*MIF-treated mice compared with controls (Figure 3C). The reduction of neutrophil infiltration by anti-*Eh*MIF antibodies was consistent with its effect on chemokine production. These anti-*Eh*MIF antibodies did not cross-react with mouse MIF (Supplemental Figure 2B). We concluded that anti-*Eh*MIF blocked neutrophil recruitment to the gut in the mouse model of amebic colitis. In a previous study, anti-*Eh*MIF antibodies were detected in the serum samples of children living in an endemic area [23]. We tested whether anti-*Eh*MIF was associated with protection from amebiasis. Children in the top 50th percentile for anti-*Eh*MIF serum immunoglobulin G had a significantly higher probability of survival free of *E. histolytica* infection, compared with children in the lower 50th percentile (Supplemental Figure 3). This finding supports the hypothesis that anti-*Eh*MIF antibodies have a protective role.

Overexpression of *Eh*MIF Enhances Mucosal Inflammation

In addition to antibody-mediated neutralization, we used a genetic approach to test the effect of *Eh*MIF on mucosal

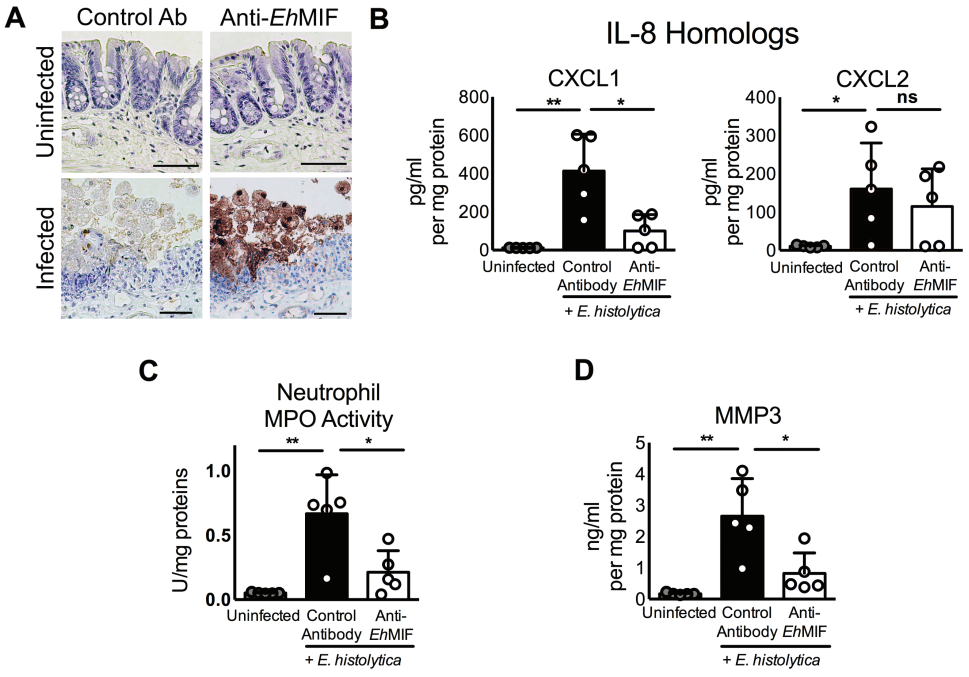

Figure 3. Anti-*Eh*MIF antibody treatment reduces *E. histolytica*–induced inflammation. *A*, Immunohistochemical stain showing *Eh*MIF protein expression (brown) and interaction with host during infection. Scale bars, 50μm. *B-D*, Mice treated with anti-*Eh*MIF antibodies had reduced intestinal tissue levels of CXCL1 chemokine, neutrophil infiltration, and MMP-3. Data represent mean and SD ($n = 5$ mice per group). $^{*}P < .05$; $^{**}P < .01$. Abbreviations: CXCL1, C-X-C motif chemokine ligand 1; *Eh*MIF, *Entamoeba histolytica* macrophage migration inhibitory factor; MMP-3, matrix metalloproteinase 3; ns, not significant.

inflammation. We generated *E. histolytica* trophozoites that overexpress *Eh*MIF (Figure 4A and 4B), given that *Eh*MIF is a soluble secreted nontoxic protein, and gene overexpression can be technically accomplished in amebic strains that are adapted for virulence in the mouse model [22, 41, 42]. Mice infected with parasites overexpressing *Eh*MIF showed increased chemokine production, mucosal inflammation, and pathology compared with parasites transfected with the empty vector (Figure 4C, 5A–D). No significant differences in parasite antigen load were observed between mice infected with parasites overexpressing *Eh*MIF and controls postchallenge. This was also true for groups given isotype antibody control or anti-*Eh*MIF antibody, indicating that the 2 groups were exposed to the same levels of *E. histolytica* antigens. In addition, parasites overexpressing *Eh*MIF did not exhibit any growth or cytotoxicity difference compared with controls (Figure 4D). These data indicate that overexpression of *Eh*MIF increased intestinal inflammation and damage.

*Eh*MIF and Matrix Metalloproteinases Expression

Matrix metalloproteinases (MMPs) are enzymes capable of degrading extracellular matrix proteins. MMPs are expressed in all infections with protozoan parasites [43]. Matrix metalloproteinase 1 (MMP-1) and matrix metalloproteinase 3 (MMP-3) genes were among the most overexpressed genes in persons suffering from intestinal amebiasis [9]. In the human colon explant model, MMP-3 was shown to play a central role in amebic colitis, and inhibition of MMP activity blocked colonic mucosa invasion by *E. histolytica* [44]. We found that mice given anti-*Eh*MIF antibodies had reduced MMP-3 mucosal levels, and parasites overexpressing *Eh*MIF generated higher MMP-3 production during infection compared with controls (Figure 4D and Figure 5C). Proinflammatory cytokines, including human MIF have shown to stimulate the expression of MMPs [45]. However, in our hands, recombinant *Eh*MIF failed to directly induce MMP-3 production from intestinal epithelial cells and fibroblasts in vitro. These findings suggest that MMP3 elevation might be due to *Eh*MIF-induced mucosal inflammation and not a direct effect of *Eh*MIF on host cell MMP-3 secretion.

DISCUSSION

Mucosal inflammation resulting from infection with *E. histolytica* is a hallmark of amebic colitis. In this study, we examined the role of the cytokine MIF homolog of *E. histolytica* in mucosal inflammation. We found a positive correlation

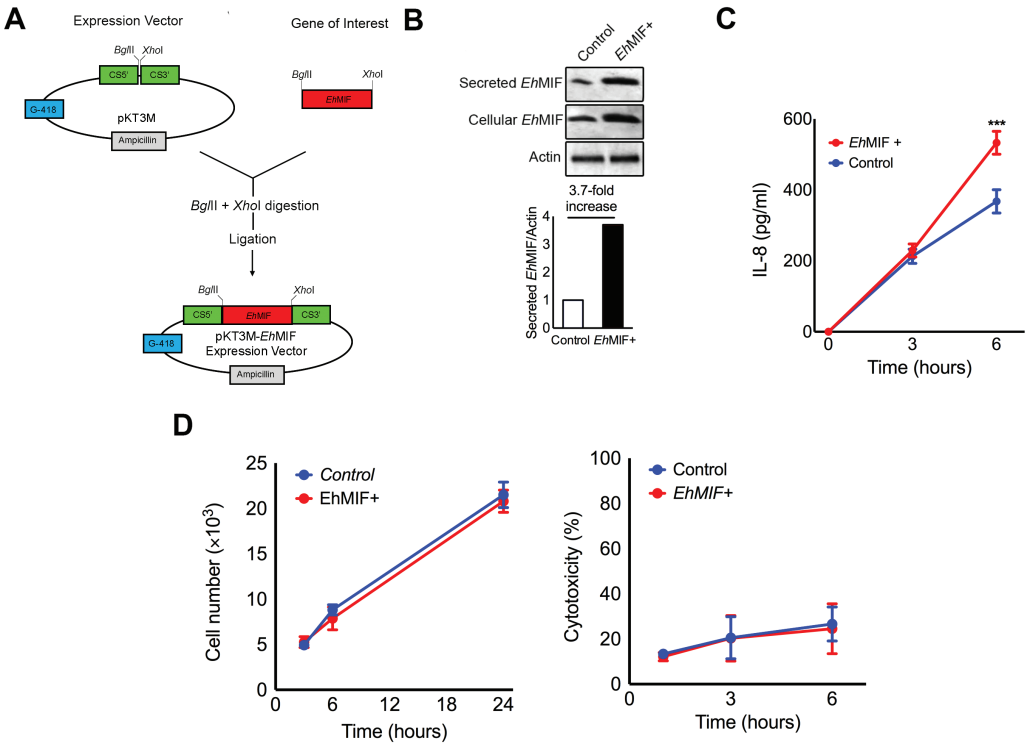

Figure 4. *Eh*MIF overexpression by *E. histolytica* parasites. *A*, Schematic for the preparation of the pKT3M-*Eh*MIF expression vector. *Eh*MIF gene (EHI7A_051880). CS5': CS promoter (EHI_024230). CS3': CS UTR (EHI_024230). G-418 and ampicillin resistance genes. *B*, *Eh*MIF expression assessed by immunoblot analysis. Actin was used as a loading control. Quantification of secreted *Eh*MIF bands relative to actin by densitometry. *C*, IECs cocultured with *E. histolytica* parasites overexpressing *Eh*MIF (*Eh*MIF$^+$) compared to empty vector controls. *D*, No difference in parasite growth or parasite-induced cytotoxicity between *Eh*MIF$^+$ parasites and WT parasite controls with empty vector. Data represent mean and SD of triplicates from 1 experiment and are representative of 3 independent experiments. A P value < .05 was considered statistically significant. *** $P < .001$. Abbreviations: *Eh*MIF, *Entamoeba histolytica* macrophage migration inhibitory factor; IECs, intestinal epithelial cells; WT, wild-type.

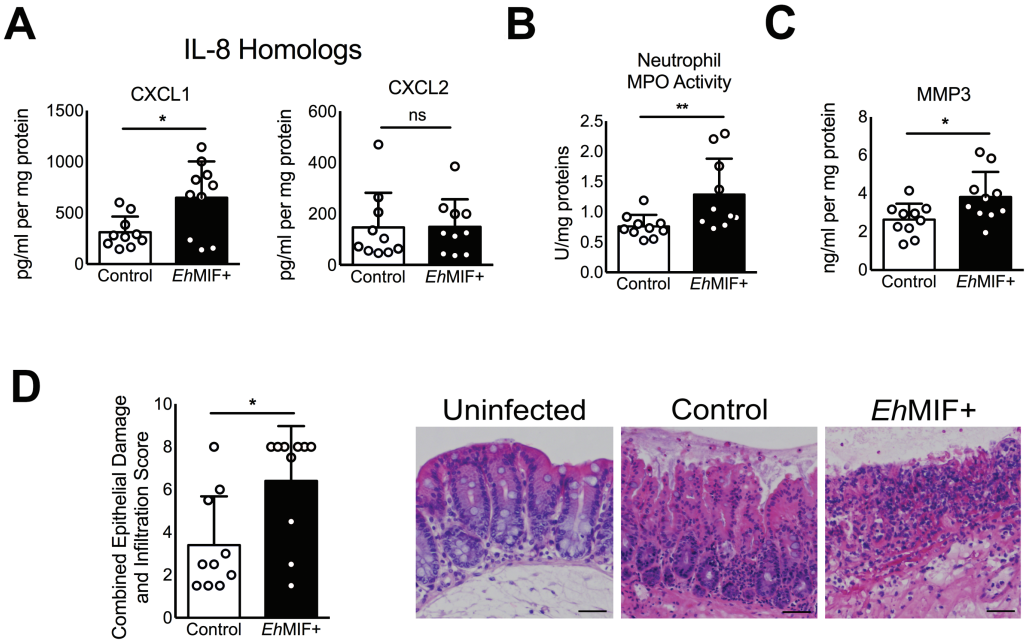

Figure 5. *Eh*MIF overexpression increases inflammation. *A–C*, Increased CXCL1, neutrophil influx, and MMP-3 tissue levels in mice infected with *Eh*MIF+ parasites compared with controls. *D*, Representative H&E-stained images and combined epithelial damage and infiltration scores. Scale bars, 100μm. Data represent mean and SD (n = 10 mice per group). *$P < .05$; **$P < .01$; ***$P < .001$. Abbreviations: CXCL1, C-X-C motif chemokine ligand 1; *Eh*MIF, *Entamoeba histolytica* macrophage migration inhibitory factor; H&E, hematoxylin and eosin; MMP-3, matrix metalloproteinase 3; ns, not significant

between *Eh*MIF levels and intestinal inflammation in infected persons. Using cellular and mouse models, we demonstrated that *Eh*MIF induces chemokine secretion from intestinal epithelial cells, resulting in neutrophil influx. These findings implicate *Eh*MIF as a causal factor of mucosal inflammation during infection.

Severe forms of amebic colitis are associated with both high mortality and morbidity. Antibiotics alone are often not enough to treat disease, and surgical removal of the inflamed colon may not prevent death [5]. Metronidazole is the antibiotic of choice for treating amebic colitis. In preclinical mouse models, metronidazole was shown to be very effective at killing ameba but had little effect on *E. histolytica*–induced mucosal inflammation [6, 46]. Adjunctive anti-inflammatory strategies may be needed to improve the clinical outcome of amebic colitis. Neutralization of a parasite mediator of host inflammation such as *Eh*MIF may attenuate disease. However, further studies are needed to determine whether the combination of metronidazole and anti-*Eh*MIF antibodies is superior to metronidazole alone for treatment of severe amebic colitis.

Mucosal inflammation also plays a key role in other human protozoan infections. Mucosal leishmaniasis is a destructive disease caused by the protozoan parasite *Leishmania*. Neutrophil recruitment and an exaggerated inflammatory response perpetuates the disease in mucosal leishmaniasis [47].*Trichomonas vaginalis* causes the most prevalent nonviral sexually transmitted infection worldwide. Vaginitis is characterized by infiltration of the vaginal mucosa with neutrophils, which contributes to the symptoms of vaginal discharge [48]. Toxoplasmosis is a parasitic disease, caused by *Toxoplasma gondii*, which can infect the brain, eye, and the developing fetus. The parasite first enters through the intestine and induces recruitment of neutrophils to the site of intestinal infection that was recently shown to facilitate the spread of infection [49]. Similar to *E. histolytica*, these parasites encode their own MIF homolog. It is possible that these parasite MIF homologs are contributing to the mucosal influx of neutrophils during their respective infections.

A recent study found that the *Plasmodium*-encoded MIF, through its proinflammatory properties, interfered with the development of immunological memory by inducing the development of short-lived effector cells rather than memory cells. This rendered the host susceptible to reinfection by the parasite [50]. This finding could help explain why antibodies against *Eh*MIF were associated with protection from reinfection. Additional studies, however, are needed to validate our preliminary findings.

In conclusion, we identified *Eh*MIF as a specific amebic mediator of host chemokine expression, neutrophil infiltration, and mucosal immunopathology during infection. Intestinal amebiasis remains a major global health problem, especially in children living in low-income countries. With no vaccine and only a single drug class to treat this devastating disease, *Eh*MIF may represent a promising immunotherapeutic target to prevent or attenuate amebic disease.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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