

SCIENTIFIC REPORTS



OPEN

Interaction between parasite-encoded JAB1/CSN5 and macrophage migration inhibitory factor proteins attenuates its proinflammatory function

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Multiple protozoans produce homologs of the cytokine MIF which play a role in immune evasion, invasion and pathogenesis. However, how parasite-encoded MIF activity is controlled remains poorly understood. Cytokine activity can be inhibited by intracellular binding partners that are released in the extracellular space during cell death. We investigated the presence of an endogenous parasite protein that was capable of interacting and interfering with MIF activity. A screen for protein-protein interaction was performed using immunoaffinity purification of amebic cell lysate with specific anti-*Entamoeba histolytica* MIF (*EhMIF*) antibody followed by mass spectrometry analysis, which revealed an *E. histolytica*-produced JAB1 protein (*EhJAB1*) as a potential binding partner. JAB1 was found to be highly conserved in protozoans. Direct interaction between the *EhMIF* and *EhJAB1* was confirmed by several independent approaches with GST pull-down, co-immunoprecipitation, and Biolayer interferometry (BLI) assays. Furthermore, the C-terminal region outside the functional JAMM deneddylase motif was required for *EhMIF* binding, which was consistent with the top *in silico* predictions. In addition, *EhJAB1* binding blocked *EhMIF*-induced IL-8 production by human epithelial cells. We report the initial characterization of a parasite-encoded JAB1 and uncover a new binding partner for a protozoan-produced MIF protein, acting as a possible negative regulator of *EhMIF*.

Protozoan parasites represent a major threat to health and contribute significantly to morbidity and mortality worldwide. For example, *Entamoeba histolytica* is a protozoan parasite that causes colitis¹. Severe forms of amebic colitis are associated with high case fatality rates ranging from 40% to 89%². There is neither an effective vaccine nor have there been advancements in therapies for amebic colitis for over fifty years³. Therefore, there remains an ongoing need to find new drug and vaccine targets through a better understanding of parasite biology.

Macrophage migration inhibitory factor (MIF), one of the first cytokines to be discovered, is a pleiotropic inflammatory cytokine and a critical upstream mediator of innate immunity. Many of the inflammatory effects of MIF are mediated through direct binding to the CD74 cell surface receptor, causing the secretion of proinflammatory cytokines such as IL-8⁴⁻⁶. An increase in MIF expression contributes to excessive inflammation and immunopathology. Hence, MIF has been reported to have a role in the pathogenesis of several inflammatory diseases such as inflammatory bowel disease and rheumatoid arthritis^{7,8}. MIF proinflammatory properties also make it a crucial mediator in the immune response against a wide range of pathogens⁹⁻¹¹.

Counterintuitively, MIF homologs have been characterized in several pathogenic protozoans including *Entamoeba*, *Plasmodium*, *Toxoplasma*, and *Leishmania*. These protozoan MIF homologs have demonstrated similar proinflammatory activities to that of human MIF, and play a role in immune evasion, invasion and pathogenesis¹²⁻¹⁹. Despite the growing literature on protozoan-encoded MIF proinflammatory activity, very little is known about how it is regulated. Here, we uncover a parasite-encoded JAB1 (c-Jun activation domain binding protein 1),

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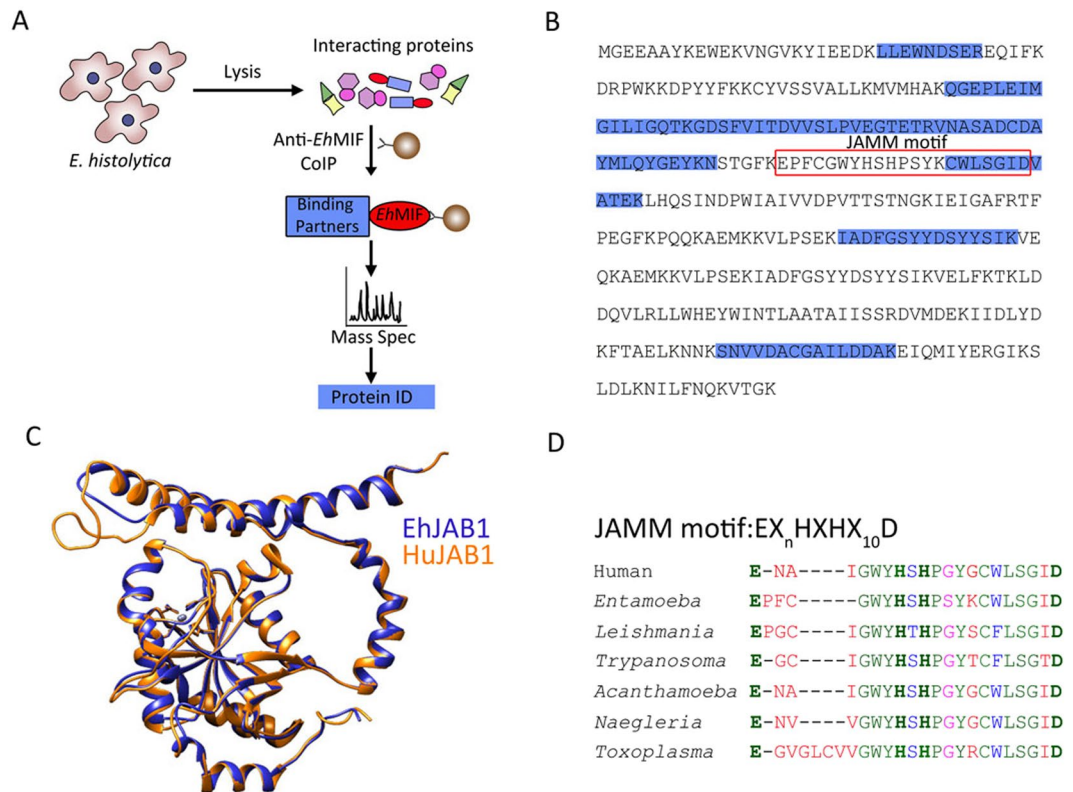


Figure 1. Characterization of *Eh*JAB1. (A) Schematic of procedure for identifying novel protein-protein interaction with co-immunoprecipitation followed by mass spectrometric analysis. (B) *E. histolytica* JAB1 (*Eh*JAB1) amino acid sequence. Peptides unique to the *Eh*JAB1 protein identified by mass spectrometry are highlighted (blue). JAMM motif (EX_nHXHX₁₀D) shown in red box. (C) Structural homology between Human JAB1 (HuJAB1) and *Eh*JAB1. HuJAB1 (orange) was superimposed with the predicted structure of *Eh*JAB1 (blue). (D) Multiple sequence alignment of the conserved JAMM motif of JAB1 from pathogenic parasites. Identical (green), conserved (blue), semi-conserved (pink), and non-conserved residues (red).

which is highly conserved throughout protozoan parasites, as a novel binding partner and potential regulator of the MIF homolog of *Entamoeba histolytica*.

Results

Identification and characterization of a parasite-produced JAB1. Co-immunoprecipitation (co-IP) of proteins followed by mass spectrometric identification is a standard approach for identifying novel protein-protein interactions^{20,21}. Parasite cell lysates were incubated with a specific antibody against *Entamoeba histolytica* MIF (*Eh*MIF) or IgG control antibody. The immunoprecipitates were subjected to mass spectrometry analysis. An *Entamoeba histolytica*-encoded JAB1 (*Eh*JAB1, EHI_050500) was identified as a potential *Eh*MIF binding partner (Fig. 1A,B). While a number of putative interacting proteins were co-precipitated with anti-*Eh*-MIF antibody, *Eh*JAB1 was selected for further analysis given: (i) *Eh*JAB1 was detected in the anti-*Eh*MIF co-IP sample and not detected in the control IP; (ii) *Eh*JAB1 was among the highest percent coverage (Supp. Fig. S1); and (iii) previous report of the intracellular interaction between human MIF and human JAB1 (HuJAB1)^{22–24}. *Eh*JAB1 gene consists of 957 base pairs with no intron and a GC content of 30%. The gene encodes for a protein of a predicted molecular weight of 36.5 kDa. *Eh*JAB1 has 39% identity and 61% similarity with HuJAB1 and appears structurally conserved (Figs 1C and S2). JAB1 is conserved in multiple parasites that cause disease in humans, all containing the MPN domain with the JAMM (JAB1/MPN/Mov34 metalloenzyme) motif (Fig. 1D). JAMM motif is a metalloprotease motif, consisting of the amino acid sequence EX_nHXHX₁₀D, present in the deubiquitinating enzymes RPN11 and JAB1²⁵. RPN11 was also found in these parasites (Supp. Fig. S3).

***Eh*MIF directly interacts with *Eh*JAB1.** We investigated whether the interaction between *Eh*MIF and *Eh*JAB1 was direct or indirect. First, we performed GST-pull-down assays (Fig. 2A). *Eh*MIF and *Eh*JAB1 fused to GST were expressed and purified (Fig. 2B). GST-*Eh*JAB1 immobilized on magnetic beads was incubated with *Eh*MIF. GST alone immobilized on magnetic beads and incubated with the same concentration of *Eh*MIF was used as control. As shown in Fig. 2C, the GST-*Eh*JAB1 fusion protein readily pulled down *Eh*MIF relative to the GST control, demonstrating direct interaction. In an independent approach, we used co-immunoprecipitation experiments to further verify direct interaction. *Eh*JAB1 immunoprecipitated with anti-*Eh*MIF antibody, but not

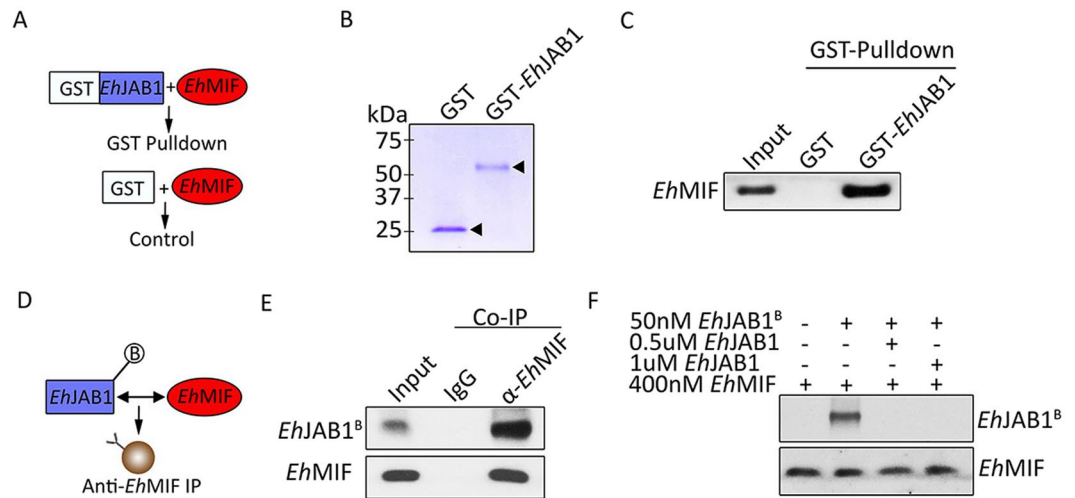


Figure 2. Direct interaction between *EhMIF* and *EhJAB1*. **(A)** Schematic of GST pull-down assay. **(B)** Purified recombinant GST and GST-*EhJAB1* proteins (arrowhead) used in the GST pull-down assay were separated by SDS/PAGE and stained with Coomassie Blue. **(C)** Interaction between *EhMIF* and *EhJAB1* by GST pull-down assay. GST-*EhJAB1* and GST control were mixed with *EhMIF*. Input (10%) and pull-down material were separated by SDS/PAGE, and *EhMIF* was detected by immunoblot analysis using anti-*EhMIF* antibody. **(D)** Schematic diagram of co-immunoprecipitation assay. **(E,F)** Co-immunoprecipitation of *EhMIF* and biotinylated *EhJAB1* (*EhJAB1*^B) mixture using specific anti-*EhMIF* antibody bound beads. *EhJAB1*^B was detected by immunoblot analysis using goat anti-biotin HRP conjugated antibody.

with the IgG antibody control (Fig. 2D–F). Together, these findings suggest a direct binding between *EhMIF* and *EhJAB1* proteins.

High-affinity binding between *EhMIF* and *EhJAB1* proteins. Bi-layer interferometry (BLI) is a useful technique for measuring interactions between proteins in real time²⁶. We determined the equilibrium dissociation constant for *EhMIF* binding to *EhJAB1* using BLI. GST-tagged *EhJAB1* was coupled to the surface of anti-GST antibody coated BLI sensors, followed by binding measurements in different concentrations of *EhMIF*. Analysis revealed a dissociation constant of K_D of 3.86×10^{-8} M (Fig. 3A). GST alone coupled to anti-GST antibody coated BLI sensors was used as control. BLI measurements demonstrate that *EhMIF* did not bind to the GST control, K_D not applicable (Fig. 3B). These findings suggest *EhMIF* binds to *EhJAB1* within the range considered biologically relevant^{27,28}.

***EhJAB1* interacts with *EhMIF* via its C-terminal domain.** We investigated the binding domain of *EhJAB1* responsible for interacting with *EhMIF*. First, an *in silico* approach using pyDockWEB was applied to assess the binding of *EhJAB1* to *EhMIF*. pyDockWEB allows the best rigid-body docking orientations generated by pyDock scoring function, which consists of electrostatics, desolvation energy and limited van der Waals contribution²⁹. The C-terminal domain of *EhJAB1* was revealed to be the *EhMIF* binding region in all top ten predictions (Figs 4A and S6). Next, we further examine the region of *EhJAB1* that is responsible for the formation of the complex with *EhMIF* using deletion mutant analysis. GST-tagged full-length *EhJAB1* and various GST-tagged deletion mutants of *EhJAB1*, with either N-terminal, MPN, JAMM or C-terminal domain deletion (Fig. 4B,C), were immobilized on magnetic beads and incubated with *EhMIF* protein. *EhMIF* specifically interacted with GST-*EhJAB1*, except for the mutant lacking the C-terminal domain. Interaction was regained when the 187–246 amino acid sequence of the C-terminal was restored (Fig. 4D). These results indicate binding of *EhJAB1* to *EhMIF* is mediated through the C-terminal region outside of the MPN domain.

***EhJAB1* inhibits *EhMIF* interaction with the CD74 receptor and *EhMIF*-induced cytokine production.** CD74 is a cell surface receptor for human MIF, which mediates many of its inflammatory effects³⁰. Several parasite MIF homologs, including *EhMIF*, were previously shown to interact with the CD74 receptor^{13,17,18,31}. Here, we were able to reproduce this finding and found that *EhMIF*-CD74 interaction was blocked by preincubating *EhMIF* with *EhJAB1* (Fig. 5A). The epithelial surfaces of the skin, nasal, intestinal, respiratory, and genitourinary tracts are the first points of contact for many protozoans. Epithelial cells express CD74 and are a rich source of IL-8³². Stimulation of IL-8 production has been the most reproducible activity of protozoan MIF homologs. *EhMIF* was recently shown to stimulate IL-8 secretion from human intestinal epithelial cells¹⁹. IL-8 is a potent neutrophil chemoattractant that contributes to inflammation in various infectious and inflammatory diseases. We proceeded to examine if *EhJAB1* binding to *EhMIF* attenuates its proinflammatory function using two different colonic epithelial cell lines. *EhJAB1* blocked *EhMIF*-induced IL-8 secretion by HCT116 and Caco2 colonic cells (Fig. 5B,C). These data indicate that when bound to *EhJAB1*, *EhMIF* is no longer capable of carrying out its inflammatory functions.

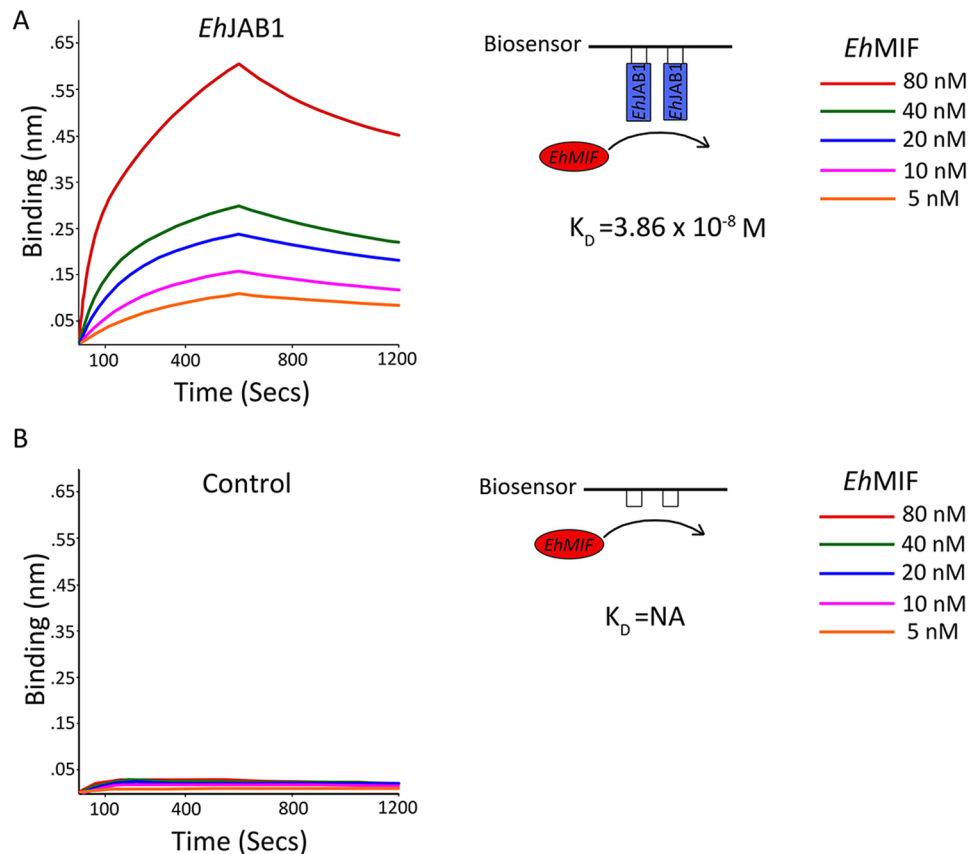


Figure 3. Characterization of *EhMIF* binding to *EhJAB1* using the Biolayer interferometry. **(A)** Representative binding and dissociation curves for *EhMIF* binding to *EhJAB1*. Anti-GST antibody-coated biosensors were loaded with GST-*EhJAB1*. Sensors were placed into solutions with *EhMIF*, concentrations range from 5 to 80 nM (association analysis: 0 to 600 secs). Subsequently, the sensors were transferred to buffer without *EhMIF* for dissociation analysis (from 600 to 1200 secs). Analysis revealed a dissociation constant of K_D of 3.86×10^{-8} M. **(B)** Biosensors loaded with GST only were used as controls.

Discussion

Parasites can exploit the host inflammatory response to promote tissue invasion¹. *EhMIF*-stimulated human intestinal epithelial cells secrete IL-8, a potent promoter of inflammation¹⁹. One of the downstream effects of *E. histolytica* MIF-induced inflammation is an increase in matrix metalloproteinases (MMPs) production, which was shown recently to promote *E. histolytica* tissue invasion in human colon^{19,33}. That said, parasites are likely to have developed mechanisms to regulate their MIF actions especially in situations where they fail to evade the host inflammatory response induced by producing such a molecule.

Identifying a binding partner that inhibits MIF function provides insight into how MIF's actions are regulated²⁷. In this study, a proteomics approach was used to identify parasite-encoded protein interaction partners of a homolog of MIF from *Entamoeba histolytica*. We found that *EhJAB1* protein interacts with *EhMIF*. This interaction was validated by multiple independent approaches with GST-pulldowns, co-immunoprecipitation, *in silico* experiments and Biolayer interferometry.

JAB1, also known as COP9 signalosome subunit 5 (CSN5), is well characterized in non-parasitic eukaryotes³⁴. It constitutes the catalytic center of the large multi-protein COP9 signalosome complex, since it harbors the JAMM/MPN⁺ metalloprotease motif^{35–37}. It carries out a Zn dependent reaction called deneddylation, equivalent to de-ubiquitylation, where it removes a ubiquitin (Ub) like protein Nedd8 from the Cullin-RING E3 Ub-ligases (CRL)^{35,38,39}. Regulation of CRLs, such as the Skp/Cullin/F-box (SCF) complex, by CSN mediated deneddylation is critical for proper cell division, cell cycle control and DNA damage response^{40,41}. Here, we report the initial characterization of a parasite-encoded JAB1. Given parasites including *E. histolytica* express cullins and Nedd8 proteins^{42–45}, it is plausible that JAB1 in parasites regulate cullins by deneddylation and modulate ubiquitin proteasomal system (UPS) activity, however, further studies to confirm this are needed. Surprisingly, we did not find a JAB1/CSN5 homolog in *Plasmodium* genome. However, the incomplete genome assembly and annotation in *Plasmodium spp.* could limit our *in-silico* analysis and explain why JAB1 was not identified in *Plasmodium spp.*

JAB1 function is not limited to CSN dependent deneddylation. JAB1 is also stable and functional in a free, monomeric, CSN independent form^{46–48}. JAB1 monomers are catalytically inactive, but bind to certain proteins and alter their activity^{34,48}. We showed that the JAMM catalytic motif of *EhJAB1* was not required for its interaction with *EhMIF*, which is consistent with the previous report on mammalian proteins²³. The dissociation

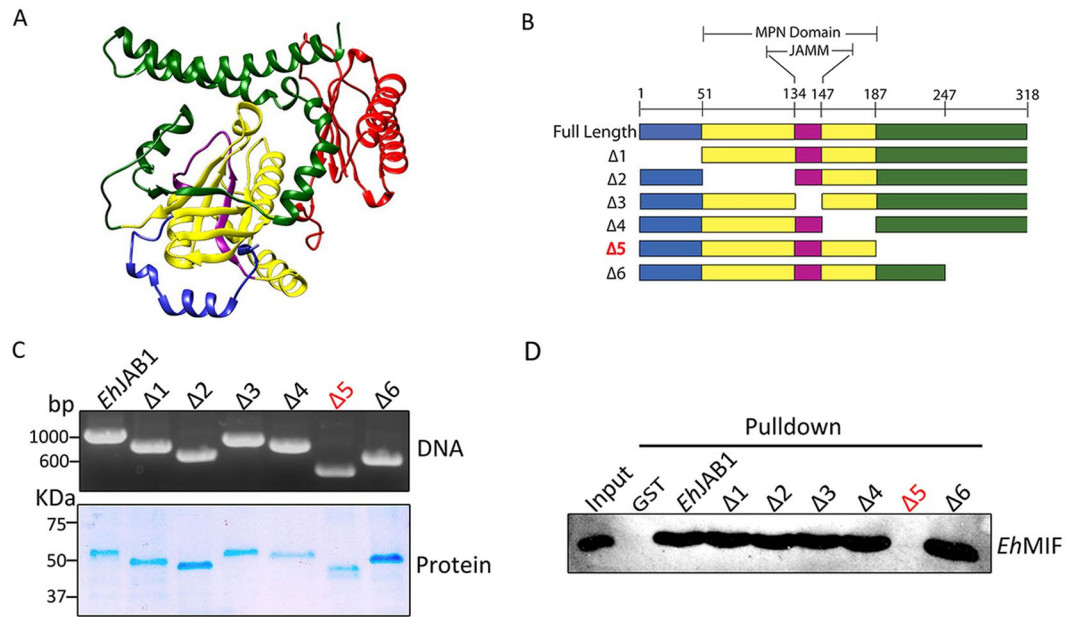


Figure 4. Mapping the *EhMIF*-interacting domain of *EhJAB1*. **(A)** Predicted interaction between *EhMIF* and *EhJAB1*. *EhMIF* (red) and *EhJAB1* N-terminal (blue), MPN domain (yellow), JAMM motif (purple) and C-terminal (green). **(B)** Schematic representations of full-length and deletion mutants of *EhJAB1*. $\Delta 1$ (deletion of 3–50 aa), $\Delta 2$ (deletion of 51–133 aa), $\Delta 3$ (deletion of 134–147 aa), $\Delta 4$ (deletion of 148–187 aa), $\Delta 5$ (deletion of 187–318 aa), $\Delta 6$ (deletion of 247–318 aa). **(C)** PCR amplicons and protein expression of GST-tagged full-length *EhJAB1* and GST-tagged deletion mutants of *EhJAB1*. **(D)** Interactions of *EhMIF* with deletion mutants were analyzed by immunoblotting.

constant for *EhJAB1*-*EhMIF* complex was well within the range considered physiologically relevant, suggesting a biologically significant interaction^{27,28}.

Intracellular molecules released from damaged cells can have profound effects on the immune response. There is growing evidence that not all of these immunomodulatory molecules are pro-inflammatory, and interestingly, some have immunosuppressive or regulatory effects. These anti-inflammatory actions occur either directly or indirectly. For example, in the case of human MIF, an endogenous binding partner that is released in the extracellular space during cell death interacts and negatively interferes with MIF activity^{27,49–51}. JAB1 is an intracellular protein²², which is consistent with our mass spectrometric analysis of the parasite cytosolic fraction; while MIF homologs, such as *EhMIF* are secreted proteins^{18,19}. *E. histolytica* parasite has developed a number of mechanisms to evade the host immune response^{1,52}. However, amebic parasites could become damaged if they fail to evade the inflammatory response triggered by *EhMIF*. It would be reasonable to speculate that the free monomeric *EhJAB1* released from injured cells into the extracellular environment would then form complexes with *EhMIF*, preventing interaction with the host receptor CD74 and reducing *EhMIF*-induced inflammation, as a negative feedback mechanism. In addition, given the structural and functional similarity between human and *E. histolytica* MIF and JAB1 proteins, we do postulate that *EhJAB1* could also interact with human MIF. It would be interesting to determine whether an *EhJAB1*/human MIF interaction functions to evade the host immune response in future studies.

In conclusion, our data suggest that *E. histolytica* homolog of JAB1 interacts with the cytokine *EhMIF* and negatively regulates its pro-inflammatory function. Our study also generates the hypothesis that targeting *EhMIF* and its interaction with *EhJAB1* may disrupt the parasite's ability to exploit the host immune response and ultimately serve as a therapeutic target against this devastating parasitic disease.

Methods

Plasmids, cloning and PCR. The *EhJAB1* gene, codon optimized for expression in *E. coli* BL21(DE3) cells, was cloned within pDEST15 vector. Deletion constructs were prepared by inverse PCR on the pDEST15-full length JAB1 clone using the primers listed in the Supp. Fig. S4. A schematic of the mutation strategy is provided in the Supp. Fig. S5. Clones were screened by PCR across the gene boundaries within the vector followed by confirmation with sequencing. The *EhJAB1* gene was amplified from the pDEST15 vector with primers carrying 5'BamHI and 3'XhoI sites and sub-cloned within BamHI and XhoI sites of pGEX-4T1 vector to utilize the thrombin site for cleaving off the GST tag from the GST-*EhJAB1* protein. Also, the previously described codon optimized *EhMIF* gene cloned within pJexpress414 vector (DNA2.0)¹⁷ was used in this study.

Protein expression and purification. Protein expression of the recombinant *EhMIF* and *EhJAB1* was done following the previously described protocol¹⁷ except that the induction with isopropyl β -D-thiogalactoside (IPTG) was done for 18 hours at 15 °C. Cells were pelleted and lysed in CelLytic™ B Cell Lysis Reagent (Sigma) at room temperature for 15 minutes and lysate was collected following 30 min spin at maximum speed at 4 °C.

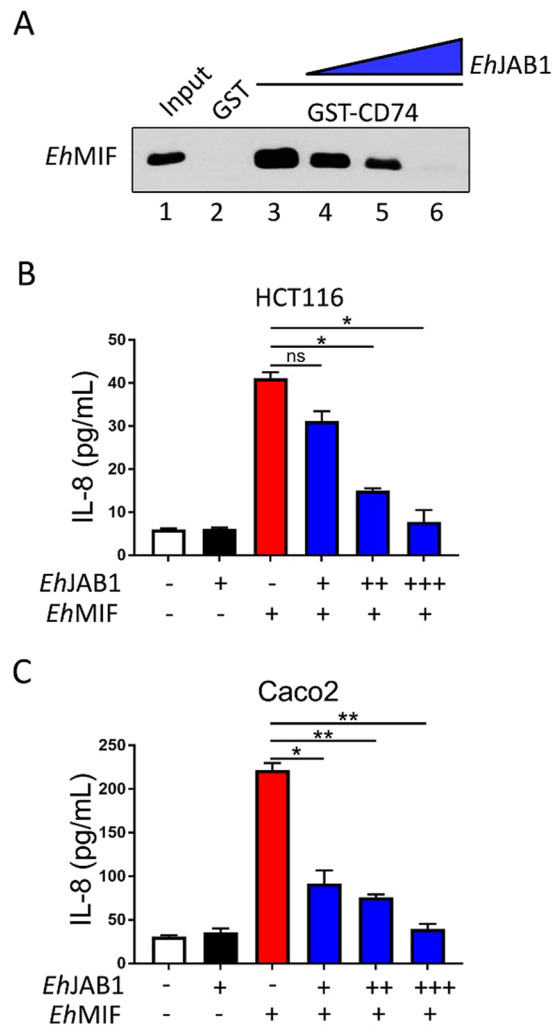


Figure 5. Effect of *EhJAB1* binding on *EhMIF* activity. **(A)** *EhJAB1* inhibits *EhMIF* interaction with the human MIF receptor CD74 by GST pull-down competition assay. Input (lane 1) and GST control (lane 2). *EhMIF* was preincubated with or without *EhJAB1* at increasing doses before mixing with GST-CD74 (lanes 3–6). **(B,C)** *EhJAB1* inhibits *EhMIF*-induced IL-8 production in a dose-dependent manner. Human colonic epithelial cells HCT116 and Caco2 were incubated with *EhMIF* with or without *EhJAB1* at increasing doses. Culture supernatants were collected after 8 h and IL-8 was quantified by ELISA. Data represent mean and SD of triplicates from 1 experiment and are representative of 3 independent experiments. * $P < 0.01$; ** $P < 0.001$. ns, not significant.

The purification of GST-fusion protein and His-tagged fusion protein was done as previously described¹⁷ using glutathione-sepharose (GE-Healthcare) and Ni-NTA agarose beads (Qiagen) respectively. Polymyxin B (Sigma) was used in the purification procedures for the removal of endotoxin. On-column cleavage of the GST-*EhJAB1* protein was done with the Thrombin Cleavage Capture Kit (Millipore) that utilizes biotinylated thrombin for its streptavidin agarose based removal.

GST-Pulldown Assay. GST pull down assays were done using the MagneGST™ Protein Purification System kit (Promega). Bacterial lysate expressing GST fused- full length or deletion mutant *EhJAB1* protein, diluted in MagneGST binding/wash buffer to a concentration 500 ng/ml in 500 μ l, was mixed with 25 μ l magnetic beads and was incubated overnight at 4 °C with rotation. Beads were washed 3 times with 500 μ l binding/wash buffer followed by incubation with 1 μ g purified *EhMIF* at 4 °C for overnight. The GST-CD74 pulldown assays were performed as previously described^{17,31}. Briefly, *EhMIF* protein was pre-incubated with or without 1X, 2X or 5X concentration of *EhJAB1* protein for 30 minutes in 500 μ l binding/wash buffer prior to incubation with the GST-CD74 bound magnetic beads at 4 °C for overnight. The bound complexes were eluted in 40 μ l elution buffer after washing the beads 5 times with 500 μ l binding/wash buffer. In a control experiment equivalent amount of GST protein was used in place of GST-*EhJAB1* or GST-CD74 proteins. Twenty five percent of the eluted samples were assayed by immunoblots.

Immunoprecipitation. Approximately 1.25×10^7 trophozoites of *E. histolytica*, grown at 37 °C in TYI-S-33 medium, were harvested in 3 ml non-denaturing lysis buffer (20 mM Tris HCl pH 8, 137 mM NaCl, 1% TritonX-100, 2 mM EDTA, 1x Protease inhibitor freshly added). Lysis was done with sonication followed by centrifugation at high speed for 10 minutes at 4 °C. Immunoprecipitations were performed with anti-*EhMIF* rabbit serum¹⁷ and rabbit IgG (Santa Cruz Biotechnology). For each, 100 µl Dynabeads™ Protein A (Invitrogen) was chemically conjugated with 10 µg antibodies using 5 mM Bis[Sulfosuccinimidyl] suberate or BS³ (Thermo Scientific) following the manufacturer's manual. Antibody conjugated beads were added to 500 µl amebic lysate and incubated overnight at 4 °C with rotation. Following binding, beads were washed 5 times with 300 µl wash buffer (10 mM Tris HCl pH 7.4, 150 mM NaCl, 1% TritonX-100, 1 mM EDTA, 1x Protease inhibitor freshly added). The bound complexes were eluted in 40 µl 1x Laemmli sample buffer (Bio-Rad) by boiling. Co-precipitated endogenous proteins were analyzed by Mass-Spectrometry. For direct binding experiment, tag free *EhJAB1* protein was biotinylated using the EZ-Link[®] Sulfo-NHS-LC-Biotinylation kit (Thermo Scientific). Immunoprecipitation was performed with 50 µl dynabeads bound to 10 µg anti-*EhMIF* rabbit serum or rabbit IgG. For each set, 1.5 µg of *EhMIF* mixed with 500 ng biotinylated *EhJAB1* protein was incubated with the antibody coated beads in 300 µl binding buffer (as described above) for overnight at 4 °C with rotation. Wash and elution of the bound complexes were done the same way as above. The immunoprecipitation was repeated in the presence of 5 µg and 10 µg non-biotinylated tag free *EhJAB1* protein. Twenty-five percent of the eluted samples were assayed by immunoblots.

Mass Spectrometry. The entire eluted fraction from anti-*EhMIF* and rabbit IgG immunoprecipitations were subjected to electrophoresis on SDS polyacrylamide gel. Proteins were separated via SDS-PAGE for a length of 1 cm⁵³. About 1 cm × 1 cm section of the gel, spanning all of the resolved proteins in each well, were excised. The gel samples were submitted to the W. M. Keck Biomedical Mass Spectrometry Laboratory for mass spectrometry analysis.

Immunoblotting. Protein samples obtained from GST-pulldown and immunoprecipitation experiments were resolved by SDS-PAGE followed by transfer onto polyvinylidene difluoride membranes (Millipore). The membranes were incubated overnight with primary antibodies at 4 °C. For *EhMIF* protein detection, rabbit anti-*EhMIF* antibody was used followed by anti-rabbit IgG HRP conjugate (Sigma) secondary antibody. For biotinylated *EhJAB1*, goat anti-biotin HRP conjugated antibody (Cell Signaling Technology) was used. Enhanced chemiluminescence (Thermo scientific) based substrates were used to detect antibody conjugated peroxidase activity.

IL8 secretion assay by ELISA. The human colonic epithelial cells (HCT116 & Caco2, American Type Culture Collection) with densities 10^6 cells/ml were cultured in 48 well plate (Corning) with 100 µL complete media (Dulbecco's Modified Eagle Medium, Gibco) for 12 hours followed by gentle washing and incubation with 100 µl serum free media for 12 hours. After washing the plates with 200 µl media, cells were treated with 0.5 µg/ml *EhMIF* in presence or absence of 0.5, 1 and 2.5 µg/ml *EhJAB1* protein for 8 hours. IL-8 in cell culture supernatant was measured by enzyme linked immunosorbent assay (ELISA, eBioscience).

Binding kinetics using BLI assay. The binding affinities between GST-*EhJAB1* and *EhMIF* proteins were measured using the Blitz System (Octet[®] Red 96 system, ForteBio). Briefly, Anti-GST Dip and Read™ Biosensors (ForteBio) were hydrated for 10 minutes in the sample dilution buffer (1x DPBS, 0.1% BSA, 0.02% Tween 20) followed by 3 cycles of priming and neutralization, 20 seconds each, in regeneration buffer (10 mM Glycine, pH 1.7) and the sample dilution buffer, respectively. Next, baseline stabilization of the primed biosensors was done in the dilution buffer for 5 minutes. Then, 10 µg GST-JAB1 or GST proteins diluted in sample dilution buffer was loaded onto the biosensors for 5 minutes. After washing the loaded biosensors in sample dilution buffer for 5 minutes, they were exposed for 10 minutes to 5, 2-fold dilution series of *EhMIF* protein starting at 80 nM concentration. Post-binding dissociation of *EhMIF* was done for 10 minutes in the sample dilution buffer. Binding affinities (K_D) were calculated using the Blitz system software (ForteBio).

Bioinformatics. Orthologues of JAMM/MPN⁺ motif of the *EhJAB1* protein from different protozoan parasites were aligned by Multiple Sequence Comparison by Log Expectation (MUSCLE) software⁵⁴. 3D structure of *EhJAB1* was constructed by Protein Homology/Analogy Recognition Engine v 2.0 (PHYRE2)⁵⁵. The predicted *EhJAB1* structure was then compared with that of the Human JAB1 protein using the UCSF Chimera software v. 1.10.2. The protein-protein interaction between *EhJAB1* and *EhMIF* was examined and potential docking interfaces were predicted using pyDockWeb that applies a rigid body protein-protein docking prediction model by electrostatic and desolvation scoring²⁹. The predictions were viewed using the UCSF Chimera software v. 1.10.2.

Statistical Tests. Statistical differences were determined using ANOVA followed by Dunnett's post-hoc test. A p value less than 0.05 was considered statistically significant.

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Acknowledgements

We thank William Petri, John Shannon, Bhupal Ban and Daniel Conrad for helpful advice; and the University of Virginia Molecular Interactions and Mass Spectrometry Research Facilities. This work was supported by the National Institutes of Health (NIH) R01AI026649-27S1, K08AI119181, and the Robert Wood Johnson Foundation–Harold Amos Medical Faculty Development Program Award.

Author Contributions

S.G., L.A.L., L.F., A.B. and S.M. conducted experiments. S.G. and S.M. wrote the paper.

Additional Information

Supplementary information accompanies this paper at <https://doi.org/10.1038/s41598-018-28625-1>.

Competing Interests: The authors declare no competing interests.

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