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Crosstalk at the initial encounter: Interplay between host defense and ameba survival strategies

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Abstract

The host-parasite relationship is based on a series of interplays between host defense mechanisms and parasite survival strategies. Progress has been made in understanding the role of host immune response in amebiasis. While host cells elaborate diverse mechanisms for pathogen expulsion, amebae have also developed complex strategies to modulate host immune response and facilitate their own survival. This paper will give an overview of current research on the mutual interactions between host and *Entamoeba histolytica* in human and experimental amebiasis. Understanding this crosstalk is crucial for the effective design and implementation of new vaccines and drugs for this leading parasitic disease.

Introduction

Amebiasis, a disease caused by protozoan parasite *Entamoeba histolytica*, is estimated to result in 50 million cases of colitis and liver abscess and up to 100 thousand deaths each year (1). The majority of infections occur in areas of poor sanitation and nutrition. For example, a 40% annual incidence of *E. histolytica* infection was observed in a cohort of preschool children in Bangladesh (2); 80% of the children acquired infection at least once, and 53% of them got a repeat infection over the 4-year observation (3). It was reported in a serologic study that anti-*E. histolytica* antibodies were detected in 8.4% of the population in Mexico (4). *E. histolytica* infection resolves in most people with no or nonspecific symptoms; only approximately 10% to 20% of infected patients become symptomatic, manifesting in dysentery, chronic colitis, toxic megacolon, or extraintestinal disease (5).

During the establishment of infection, *E. histolytica* confronts a series of host innate defenses, including intestinal mucosa and epithelial barrier, phagocytes, granulocytes, and lytic serum components (6). While host cells elaborate diverse mechanisms for pathogen expulsion, amebae have also developed complex strategies to evade host defense and facilitate their own survival. Dissection of this crosstalk is essential for the development of novel immunotherapeutics for amebiasis. In this review, we summarize recent advances on the role of innate barriers and immune components involved in the host defense to *E. histolytica*; and the mechanisms by which *E. histolytica* evades immune clearance in human and experimental amebiasis.

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Interactions of host innate immune system with *E. histolytica*

Innate cell-mediated immunity to *E. histolytica*

The early stage (within 1–2 days) of ALA lesion is characterized by a dominant infiltration of polymorphonuclear leukocytes (PMNs) surrounding trophozoites. In later stage (after 3 days), lymphocytes, macrophages and epithelioid cells were recruited to the sites, associated with the formation of granuloma, which contribute to the confinement of invading trophozoites (7,8). Histopathological studies in intestinal amebiasis model also demonstrated the early recruitment and destruction of leukocytes following amebic invasion (9). The following will be focused on the crosstalk of invasive ameba with each individual cell type.

1. Neutrophil—Neutrophils are frequently observed as the earliest infiltrating cells during the acute phase of host response to amebic invasion (8,10). It is possible that they are recruited by the chemotactic activity of an amebic membrane-bound peptide (11,12) and chemokines secreted by epithelial cells exposed to *E. histolytica* (13). C5a and C3a fragments produced by activated complement on the parasite surface might also contribute to neutrophil chemotaxis (14). As the consequence of interaction with trophozoites, neutrophils are activated with the release of reactive oxygen species and anti-microbial peptides. Many in vitro studies have reported neutrophil amebicidal activity after stimulation by IFN- γ , TNF- α , LPS or amebic antigens (15,16). Depletion of neutrophils with anti-Gr-1 neutralizing antibodies resulted in exacerbated amebic hepatic (17,18) and intestinal (19,20) lesions in SCID (17), CBA (19) and BALB/c (18,20) mice, suggesting that these cells might play a protective role in early resistance to amebiasis. It is worth noting that these antibodies might recognize and deplete other granulocytes like eosinophils, which were found infiltrating and degranulating in some colon biopsies from amebic colitis patients (Haque R and Petri WA, unpublished work).

However, the above rationale was challenged by observations that neutrophils not only failed to destroy *E. histolytica*, but in fact were lysed or inactivated by virulent ameba (21). There are several mechanisms whereby ameba can interfere with the proper functioning of neutrophils (Table. 1). By disruption of NADPH oxidase activities, ameba could inhibit the respiratory burst of neutrophils and resist the oxidative stress (22–24). Peroxiredoxin, a 29 kDa surface protein conferring resistance to host reactive oxygen defences, was shown to be associated with the virulence of ameba (25–27). It was reported in a recent study that *E. histolytica* could induce neutrophil apoptosis, associated with ERK1/2 activation and mediated by NADPH oxidase-generated ROS (24). The destruction of neutrophils by *E. histolytica* results in the release of cytotoxic oxidase and lytic peptidases, leading to the host tissue damage.

The conflicting results on the role of neutrophils in amebiasis may be attributed to the context in host genetic background, parasite pathogenicity, and activation state of neutrophils. Experimental manipulations including challenge dose may also make difference for the results of the investigations.

2. Macrophage—The role of macrophages as effector cells during amebic infection has been documented in both animal models and patient studies. Like neutrophils, macrophages acquire amebicidal activity after in vitro stimulation with IFN- γ , TNF- α , or colony stimulating factor-1 (28–30). Different surface components of trophozoites were shown to be recognized by macrophages via TLR-2 and TLR-4 signaling (31). Upregulated TLR-2 expression was observed in macrophages exposed to Gal/GalNac lectin of *E. histolytica*, resulting in NF- κ B activation and proinflammatory cytokine production (32). Macrophages lacking TLR-2 and TLR-4 showed impaired response to *E. histolytica* lipopeptidophosphoglycan (LPPG), suggesting an essential role of pattern recognition for the macrophage response (31). As an important cytotoxicity mediator, nitric oxide (NO) was found to be capable of inhibiting cysteine proteinases and alcohol dehydrogenase 2, virulence factors of the parasite (33).

Inducible nitric oxide synthase (iNOS)-deficient mice were more susceptible to ALA and *E. histolytica*-induced hepatocytic apoptosis (34), suggesting that NO plays a critical role in host defense against amebiasis.

Despite the sensitivity of *E. histolytica* to NO-mediated cytotoxicity, a suppression of cell-mediated immunity with impaired macrophage function has frequently been observed in human and experimental amebiasis, suggesting that amebae have developed strategies to modulate macrophage responses. The amebic modulation is multifactorial. For instance, exposure to *E. histolytica* trophozoites or amebic components has resulted in suppressed respiratory burst ((ROI: H₂O₂, O₂⁻, OH[·]) (35) and reduced NO production (36) by macrophages. Inhibition of NO production was mediated via the competitive consumption of NOS substrate L-arginine by a putative arginase expressed in *E. histolytica*, which converts L-arginine into L-ornithine (37). A decrease in TNF- α secretion was also observed due to rapid degradation of c-fos and TNF- α transcripts by ameba (38); Secreted or whole soluble amebic proteins inhibited IFN- γ -induced MHC-II expression on macrophages, suggesting an impaired amebic antigen presentation capacity in these cells (39).

The amebic molecules and mechanisms involved in the subversion of macrophage function are still poorly understood. It is suggested that the mechanisms involved depend at least partially on prostaglandin E2 (PGE2), an immunoregulator produced by *E. histolytica* or macrophages exposed to amebic proteins (40,41). In vertebrates, PGE2 is synthesized by cyclooxygenase (COX). Expression of COX isoforms has been detected in *E. histolytica* trophozoites (40) as well as ameba-exposed macrophages (41). PGE2 elevates cAMP levels in macrophages, triggering the PKA pathway, which in turn inhibits the expression of Ia molecules, the release of Th1 cytokines, NADPH-mediated oxidative burst, as well as NO synthesis via PKC pathway (39,41). The COX inhibitor indomethacin partially restores Ia expression on the surface of macrophages (39). An immunosuppressor synthesized by ameba, Monocyte Locomotion Inhibitory Factor (MLIF), also contributes to the modulation of host immune responses. MLIF is a soluble pentapeptide with anti-inflammatory properties, inhibiting the production of NO (42) and pro-inflammatory chemokines (43) by human leukocytes. More recently, MLIF was found to disturb the balance of pro- and anti-inflammatory cytokines, specifically, inhibit IL-1 while favor IL-10 production by CD4⁺ T cells (44).

3. NK cell and NKT cell—NK cells and NKT cells are known to play an important role in host defense by production of IFN- γ and cytolytic peptides after activation. NK cells are detectable early during infection in ALA mouse models (8,45). Elevated cytotoxic activity of NK cells was found in mice infected with pathogenic ameba compared to those infected with non-pathogenic strains (46), suggesting the involvement of NK cell-mediated defense in this model. One recent study reported the gender-dependent differences regarding to the control of ALA in C57BL/6 mice. Compared to susceptible male mice, increased IFN- γ and functional NKT cells were found in females and thought to be crucial for the enhanced resistance seen in females (45).

4. Mast cell—The essential role of mast cells in host control of infection has been shown in animal models infected with various bacterial and parasitic pathogens (47–49). In these models, mast cell deficient W/W^v mice exhibit delayed or failed clearance of pathogens and resistance is restored by mast cell reconstitution. Activated mast cells can kill pathogens by release of toxic proteases and mediators; cytokines produced by mast cells (ie. IL-6, TNF- α) can recruit phagocytes and influence lymphocytic development and functions; mast cells also mediate IgE-associated acquired immunity that contributes to type I immune responses (47). However, mast cell activation is not always associated with pathogen resistance and can correlate with the severity of inflammation and failed pathogen clearance (49–50).

Compared to bacterial and other parasitic pathogens, limited information is available regarding the mast cell-mediated immune response in amebiasis. Our previous study has reported an increased mast cell infiltration associated with substantially up-regulated mast cell protease genes in infected mouse ceca at 10 weeks post challenge (51). However, the fundamental question that whether mast cells contribute to parasite clearance or play a pathologic role in tissue damage remains unanswered.

Complement-mediated lysis of *E. histolytica*

After trophozoites penetrate the epithelial layer they presumably come into contact with host plasma components, such that soluble factors that can potentially destroy invading parasites or target them for killing by effector cells. The alternative and classic pathways of complement provide defense against the invading parasites in blood stream. The activation of the alternative pathway is initiated at least in part via cleavage of C3 and C5 by the parasite's 56-kDa neutral cysteine proteinase (52). C3a and C5a are strong chemoattractants involved in the recruitment of inflammatory cells and activation of phagocytic cells, all of which may contribute to parasite expulsion or tissue damage (53). The cysteine proteinase of *E. histolytica*, which is capable of cleaving C3 and C5, may degrade the anaphylatoxins C3a and C5a to evade host immunity (53). Moreover, the Gal/GalNac lectin heavy subunit of *E. histolytica* inhibits the assembly of C8 and C9 into the C5b-9 membrane attack complex, thereby preventing complement-mediated lysis of the parasite (54).

Interactions of intestinal epithelial cells with *E. histolytica*

Defining the role of intestinal epithelial cells (IECs) in parasitic infection is an exciting and rapidly expanding area. Increasing evidence indicates that IECs serve as the effectors of mucosal immune system, responding to pathogens by secretion of proinflammatory mediators and acting as antigen presenting cells (55–57). Coculture of epithelial cell lines with *E. histolytica* trophozoites resulted in increased production of TNF- α , IL-1 α , IL-6, IL-8, GRO α , and GM-CSF by the epithelial cells (57). Similar results were observed in the SCID mouse-human intestinal xenograft (SCID-Hu-INT) model, where *E. histolytica* infection resulted in elevated production of IL-1 β and IL-8 by the human intestinal xenograft (56). This ameba-induced epithelial inflammatory cascade was at least in part dependent on the signaling through NF- κ B (56). However the role of NF- κ B in amebiasis is not entirely clear. Chadee's group (58) has reported that amebic proteins could actually inhibit NF- κ B activation in IEC primed by macrophage secretions. This protective effect on IEC was mediated by the stress protein Hsp, known to be upregulated by many stimuli and to promote cell survival (59). Finally, it remains unclear whether NF- κ B activation and other defensive functions are mediated by pattern recognition receptors like TLRs. It is generally thought that normal epithelium has very low expression of PRRs which results in a tolerant mucosal response to commensal antigens; Whereas under pathological conditions, the expression of PRRs is upregulated, leading to amplified immune response with consequent elimination of pathogens and probable mucosal destruction.

Natural resistance to experimental amebiasis in murine models

Hamano et al. (60) recently demonstrated that the resistance to intestinal amebiasis of the C57BL/6 strain was conferred by non-hemopoietic cells, suggesting that the epithelial response is critical. Notably, there was a requirement for hemopoietic IL-10 in this natural resistance, as IL-10 deficient mice or mice whose hemopoietic cells were deficient in IL-10 became susceptible to infection. Since IL-10 deficient mice manifest abundant defects in their epithelium a two-step protection model was proposed from these results, whereby the epithelium mediates the initial response (and protection requires IL-10), followed by acute inflammation, whereby either response can exhibit protective or deleterious immunity.

Interactions of host adaptive immune system with *E. histolytica*

Humoral immune response to *E. histolytica*

Humoral immune responses to *E. histolytica* have been characterized for years. Seroepidemiologic studies indicate that 81–100% of patients with invasive amebiasis develop specific circulatory antibodies within 7 days of infection (61). Our earlier prospective study on a cohort of pre-school children in Dhaka, Bangladesh has demonstrated that mucosal IgA response directed at the carbohydrate recognition domain (CRD) of the parasite Gal/GalNAc lectin is linked to protection from both infection and disease (2,3) (Figure 2). Epitope-specific mouse monoclonal antibodies against the Gal/GalNAc lectin have been shown to inhibit adherence of ameba to target cells in vitro and prevent colonization (62,63). In contrast to mucosal IgA, serum IgA has not been correlated to protection. A higher lectin-specific IgG level was found in ALA and intestinal amebiasis patients as compared with asymptomatic controls (61). In our study, we observed an increased frequency of new *E. histolytica* infections in children with serum IgG antibodies to the lectin (64). These findings suggested that systemic anti-lectin antibody response might not offer direct protection from amebiasis. Furthermore, passive transfer of serum from vaccinated mice has not transferred protection from intestinal infection (unpublished work, A. Asgharpour and E. Houpt).

In recent years progress has been made in identification of possible vaccine candidates, the necessary routes of vaccination, and the immune correlates required for protection against amebiasis. Different proteins associated with amebic virulence have been proposed as candidate vaccine targets (reviewed in 65). The purified native Gal/GalNAc lectin has been demonstrated to be efficacious to protect gerbils and mice from amebic liver abscess (66) and intestinal infection (67), respectively. Protection from amebic colitis was associated with a positive fecal anti-lectin IgA response (67). Vaccination with the recombinant lectin fragment (LecA) elicited a substantial IgA response to the antigen, but the degree of protection was weaker compared to those vaccinated with native lectin (67).

T cell-mediated immune response to *E. histolytica*

The induction of effective immunity against *Entamoeba* must consider the activation of antigen-specific T cells which lead to secretion of appropriate cytokines and direct cytotoxicity to ameba. In our recent cohort study in Dhaka, amebic antigen stimulated IFN- γ production by PBMCs was associated with protection from future *E. histolytica* caused diarrhea (68), suggesting a potentially important role for Th1 cell-mediated protection (Figure 3). Furthermore, case reports have suggested that invasive amebiasis is exacerbated following interventions that suppress cell-mediated immunity, such as steroid therapy and splenectomy (69,70). However, in an experimental amebic colitis model, CD4⁺ T cells have been suggested to mediate pathogenesis (51) instead of protection, therefore the pattern of CD4⁺ T cell response may be critical. Namely, an increase in mast cell activation and infiltration was associated with elevated Th2 cytokines in the colitis model at 10 weeks post challenge, suggesting the involvement of mast cells and a Th2 response in the progression of amebic colitis during the chronic phase (51).

Growing evidence has suggested that during amebic infection T cells appear to be hyporesponsive to mitogen or antigen-stimulated proliferation (71). In vitro amebic components have favored Th2 cytokine production, with suppression of the Th1 cytokines (72). The amebic molecules conferring this suppression remain to be determined, but perhaps include MLIF activity (44). The Th1 to Th2 shift during amebic infection might result in a retarded T cell response directed to amebic antigen. Considering that ameba have mitogenic effects on lymphocytes, presumably nonspecific polyclonal activation may also disrupt antigen-specific anti-amebic immunity mediated by Th1 cells.

Non-immune host defenses and *E. histolytica*

1. Mucin

As the major constituent of the intestinal mucus layer, mucin forms the first line of host defense against invasion of *E. histolytica* (73). Mucin is a glycoprotein synthesized by goblet cells and submucosal glands, containing 80% carbohydrate by mass as O-linked polysaccharide chains. This makes it a high affinity ligand for the Gal/GalNac lectin of *E. histolytica*, potentially competing for trophozoite attachment to the underlying epithelium (74). Previous work has shown that invading trophozoites secrete cysteine proteases and glycosidases which help disrupt of the mucus barrier and epithelial tight junctions (75). *E. histolytica* trophozoites also secrete a heat stable factor that causes mucous hypersecretion from goblet cells, resulting in mucin depletion (76). The disrupted mucus layer does not inhibit amebic adherence to target epithelial cells as effectively as does the native polymer, indicative of a loss of protective function.

The potential capacity for synthetic alterations of mucins might add resistance to degradation. Although the chemical alterations of colonic mucins in response to *E. histolytica* have not been addressed, changes in the glycosylation patterns of rat mucin have been observed during *Nippostrongylus brasiliensis* infection (77). These transient alterations in sialylated oligosaccharides have been directly related to parasite expulsion.

2. Host bacterial flora and intestinal motility

Accumulating studies have suggested that gut resident flora appear to be more than just a food source for ameba. The interactions between resident bacteria and *E. histolytica* may be critical determinants for host defense or parasite virulence.

A number of studies have shown that *E. histolytica* trophozoites enhanced their virulence (78,79) and modulated surface antigens (80,81) in response to co-cultured bacteria. Certain strains of bacteria have been shown to reduce the virulence of the trophozoite in vitro, which is associated with a downregulated level of the Gal/GalNac lectin light subunit (82). Although the mechanisms by which commensal bacteria could compromise the virulence of *E. histolytica* remain hypothetical, it was suggested that a glycosidase produced by intestinal bacteria could degrade the adherence lectin, the key virulent factor of *E. histolytica* (83). Bacteria could also regulate the invasiveness of trophozoites by conditioning the flora profile which favors encystation of *E. histolytica*; the levels of short-chain fatty acids produced by resident bacterial flora may be involved in signaling encystation (84).

In addition, intestinal motility may also contribute to the host defense against amebic invasion. It was reported that immune-dependent increase in intestinal smooth muscle function might contribute to the eradication of nematode parasites (85); likewise, expulsion of *Giardia lamblia* in mice was accompanied by small-intestinal hypermotility, which appeared to depend on the adaptive immune response against the parasite (86). Understanding the mechanisms behind the immunomodulation of intestinal motility may also impact on other enteric infections or inflammatory bowel disease, in which motility disturbances often occur.

Conclusions

Exciting progress has been made in understanding the way in which ameba interacts with the host defense mechanisms. Despite these intense investigations, we have only just begun to ask the fundamental and intricate questions. To name but a few: What are the determinants for invasive and noninvasive infection? What is the role of inflammation seen in the initial phases of amebic infection? What are the signals for the initiation of amebic invasion process? With the growing understanding of amebic genomes and virulence mechanisms, future studies

should focus on what molecules and cellular processes ameba elaborates to manipulate host defense and determine the outcome of infection. A better understanding of the parasite-host interactions will help the design of effective vaccines delivered in such a way as to induce long-lasting immunity without induction of pathology.

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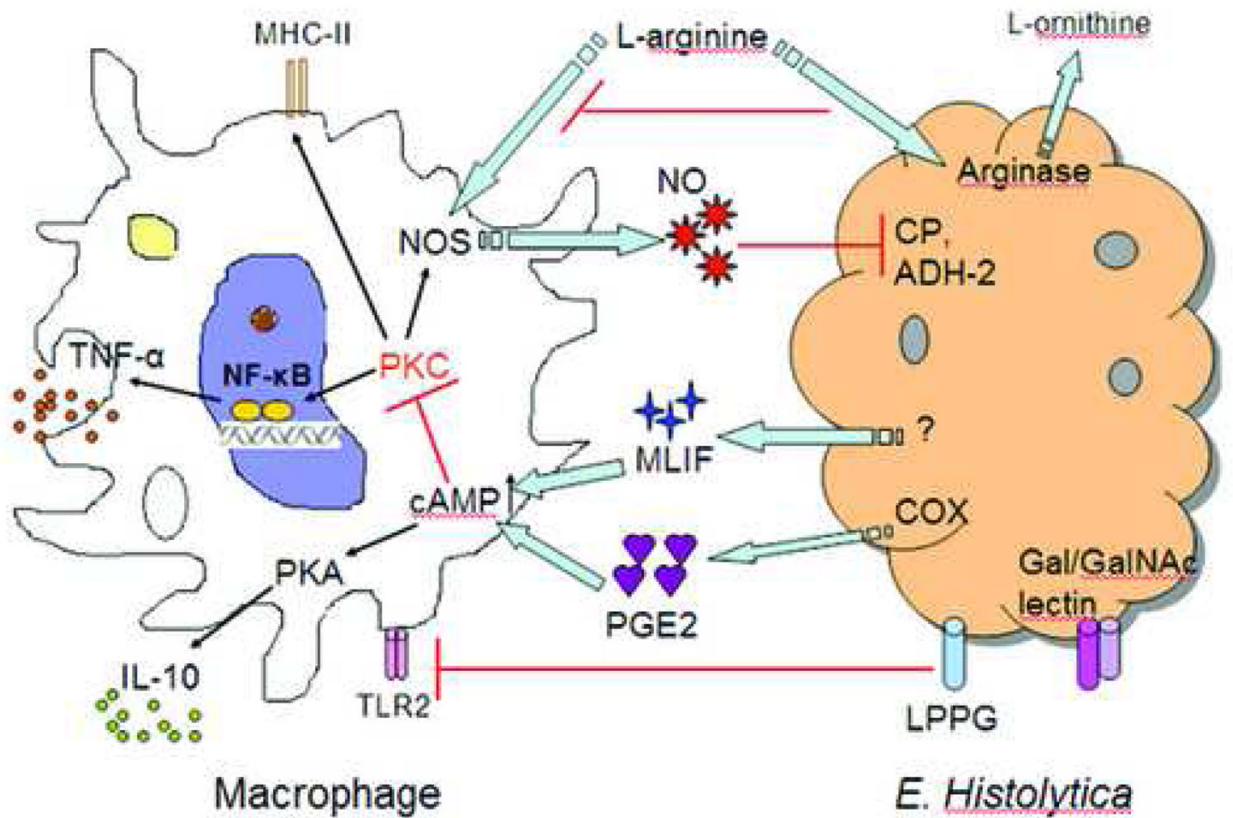
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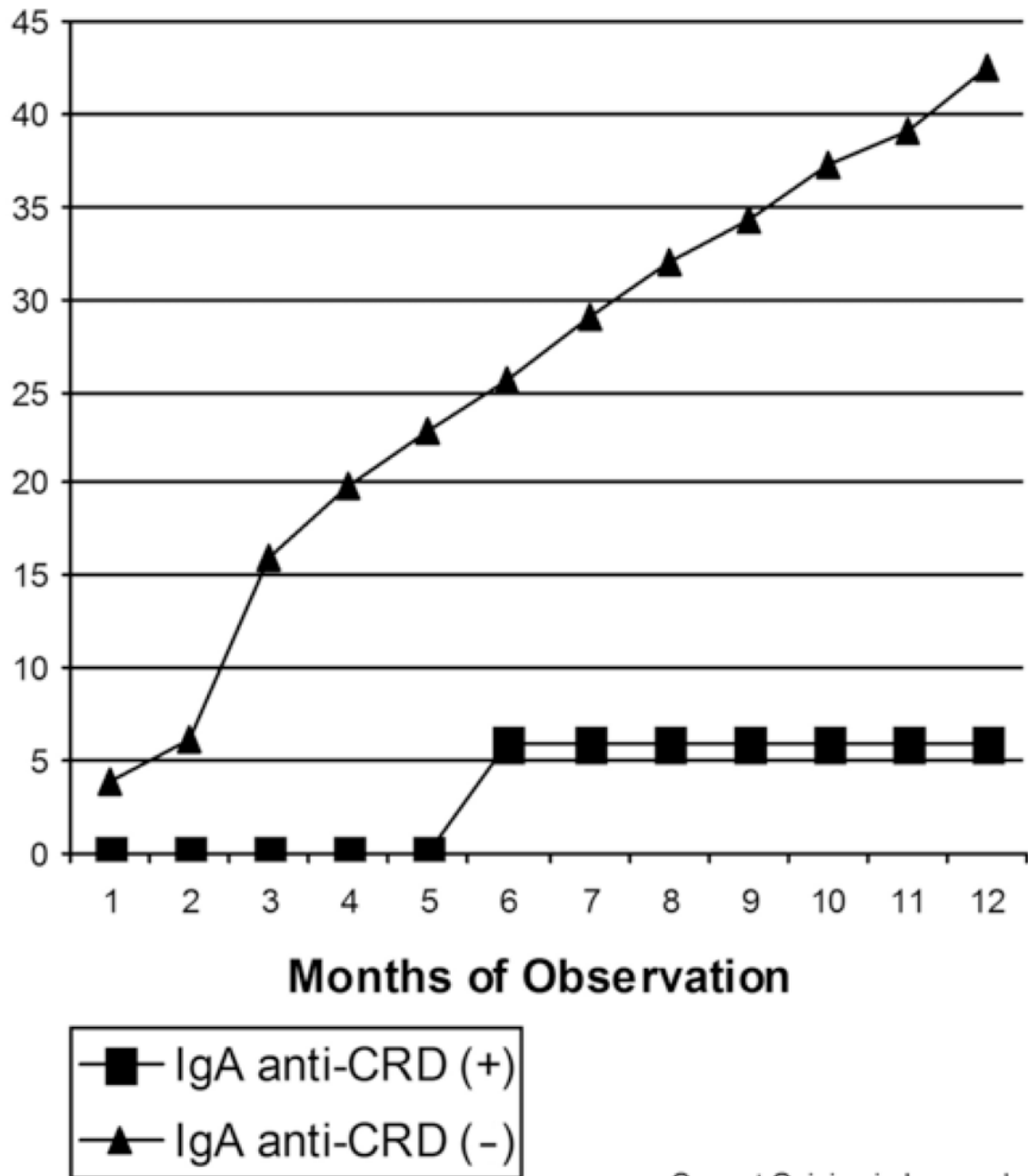
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Current Opinion in Immunology

Fig. 1. Modulation of macrophage functions by *E. histolytica*

The killing of *E. histolytica* trophozoites by macrophages is mainly mediated by nitric oxide, derived from L-arginine by nitric oxide synthase (NOS). NO could inhibit amebic cysteine proteinases (CP) and alcohol dehydrogenase 2 (ADH2), the critical enzymes conferring virulence of *E. histolytica*. The arginase activity was detected in *E. histolytica*, which putatively converts L-arginine into L-ornithine, in turn limiting NO production by macrophage NOS. Prostaglandin E2 (PGE2) is an immunoregulatory molecule produced by cyclooxygenase (COX) in ameba or ameba-exposed macrophages. By activating cAMP-PKA pathway, PGE2 suppress macrophage effector functions by inhibiting PKC-mediated MHC-II expression and TNF- α production, while favoring IL-10 production. Monocyte Locomotion Inhibitory Factor (MLIF) produced by ameba may suppress macrophage functions in a manner similar to PGE2. Amebic lipophosphoglycan (LPG) might downregulate TLR2 expression on macrophages, thus control the effector mechanisms triggered through TLR2 signaling.



Current Opinion in Immunology

Fig 2. IgA anti-CRD is Associated with Immunity to *E. histolytica* Infection

Children with fecal IgA antibodies against the Gal/GalNAc lectin carbohydrate recognition domain (IgA anti-CRD (+); n = 81) had a lower incidence of new intestinal *E. histolytica* infection compared to children lacking this response (IgA anti-CRD (-); n=149). The two groups were statistically significantly different ($p \leq 0.04$) at every time point. The average duration of protection was 437 days (95% CI 346–528 days). (Haque et al. 2002, 2006).

Kaplan-Meier survival curves

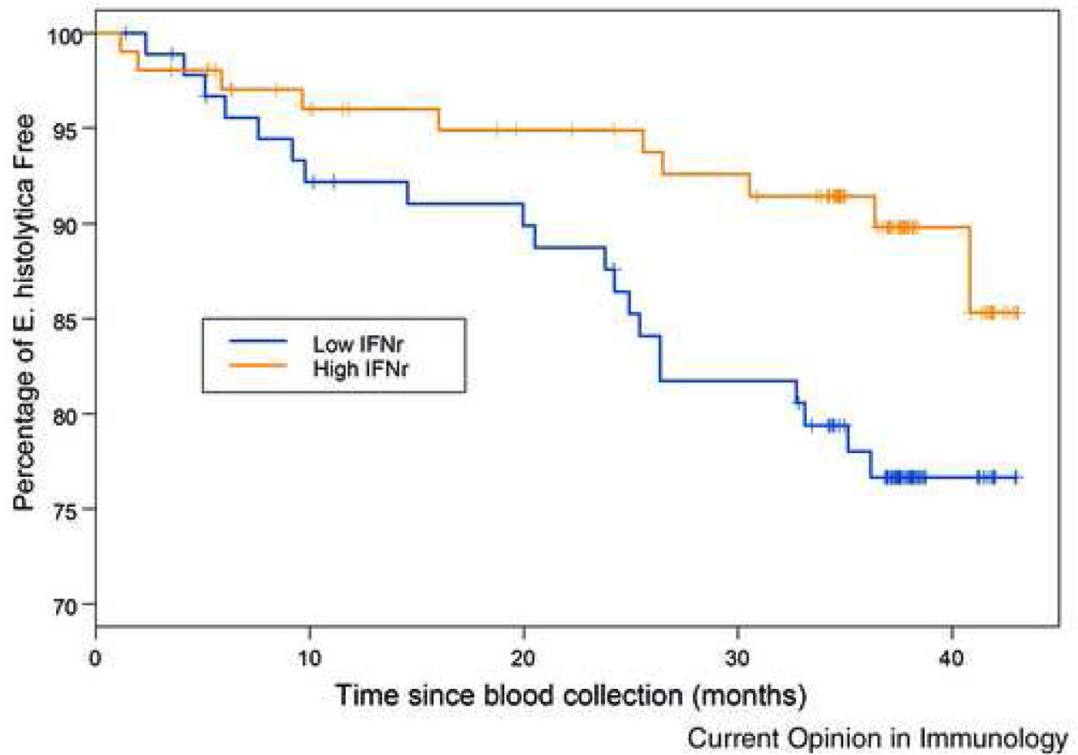


Fig. 3. High Levels of IFN- γ Predict Increased Survival Free of *E. histolytica* Diarrhea

PBMCs were stimulated with soluble amebic extract and children grouped by IFN- γ production in response to SAE stimulation. Children were then followed for 44 months and incidence of *E. histolytica* diarrhea measured. Upper line and lower line indicate children with and without interferon gamma response above the median for all children (580 pg/ml) respectively. The two lines are significantly different: Logrank test p-value=0.03. n = 92 for the low IFN- γ , and n = 103 for high IFN- γ groups (Haque et al 2006).

Table 1

<i>E. histolytica</i> components	Possible impact on neutrophils	reference
Live trophozoites	Induce apoptosis of neutrophils	24
<i>E. histolytica</i> iron-containing superoxide dismutase	Detoxify reactive oxygen species (ROS) by forming H ₂ O ₂	23
NADPH:flavin oxidoreductase (Eh34)	Detoxify ROS by forming H ₂ O ₂	23
Peroxiredoxin (Eh29)	Remove H ₂ O ₂	25–27