Review Article

The Role of Lipopeptidophosphoglycan in the Immune Response to Entamoeba histolytica

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The sensing of Pathogen Associated Molecular Patterns (PAMPs) by innate immune receptors, such as Toll-like receptors (TLRs), is the first step in the inflammatory response to pathogens. Entamoeba histolytica, the etiological agent of amebiasis, has a surface molecule with the characteristics of a PAMP. This molecule, which was termed lipopeptidophosphoglycan (LPPG), is recognized through TLR2 and TLR4 and leads to the release of cytokines from human monocytes, macrophages, and dendritic cells; LPPG-activated dendritic cells have increased expression of costimulatory molecules. LPPG activates NKT cells in a CD1d-dependent manner, and this interaction limits amebic liver abscess development. LPPG also induces antibody production, and anti-LPPG antibodies prevent disease development in animal models of amebiasis. Because LPPG is recognized by both the innate and the adaptive immune system (it is a “Pamptigen”), it may be a good candidate to develop a vaccine against E. histolytica infection and an effective adjuvant.

1. Introduction

Amebiasis is a disease caused by Entamoeba histolytica, a parasite protozoan that infects humans and is responsible for 40,000 to 110,000 deaths per year [1]. Ten percent of infected persons exhibit clinical symptoms; 80% to 98% of these are intestinal, and 2% to 20% are extraintestinal. The clinical symptoms can range from a mild and nonspecific presentation (constipation alternated with diarrhea, pain in the lower abdomen, mild nausea during or after meals, and mild abdominal distension with pain in the right iliac fossa) to dysentery, fulminating colitis, and toxic megacolon. Less frequently, amebiasis can cause appendicitis and ameboma. Amebic liver abscess is the most frequent presentation of invasive extraintestinal amebiasis, but the lungs, heart, brain, skin, and genitals can also be affected [2].

Approximately 500 million people in the world are currently infected with E. histolytica [1]. The incidence of amebiasis has decreased significantly in recent years because of increased sanitation in many countries and the use of effective therapeutic agents. The World Health Organization and the Pan-American Health Organization recommend the treatment of all patients with confirmed E. histolytica infection, regardless of the presence of symptoms. The treatments of choice for asymptomatic intestinal amebiasis are the luminal amebicides paromomycin sulfate and diloxanide.
furoate. Symptomatic intestinal or extraintestinal infection is treated with metronidazole in combination with a luminal amebicide. Nitazoxanide is an effective luminal amebicide, and it is also effective for invasive amebiasis. Gastrointestinal complications, such as perforation, intestinal obstruction, and toxic megacolon, are treated with surgery. Most hepatic abscesses respond to metronidazole, but if they do not, they can be aspirated by puncture or treated with open surgery [3–5].

In spite of the effective therapeutic agents that are available for the treatment of amebiasis, it still constitutes a global health problem [6]. The prevalence of amebiasis varies from 1% in industrialized countries to 50%–80% in tropical countries [7–10].

2. Identification of Lipopeptidophosphoglycan

In the 1970s, amebiasis was the fourth most frequent infectious disease in Mexico, with an incidence of 118.9 per 10,000 inhabitants (almost 1500 times higher than the incidence in the United States in the same year) [11]. This situation prompted many researchers to study several aspects of this parasitic disease, including comparative studies of drugs for the treatment of acute amebic liver abscess [12] and various studies of seroepidemiology of amebiasis in adults [13–17].

Several genes from *E. histolytica* were cloned, sequenced and expressed in an effort to identify new drug targets for this parasite, including the alcohol dehydrogenase gene (Ehadh3) [18], the ferredoxin oxidoreductase gene [19], the EhDEAD1 RNA helicase gene [20], and the Ehva2 gene (which encodes the B subunit of the vacular ATPase) [21]. Mechanisms of drug resistance in *E. histolytica* were also studied, and it was determined that the multidrug-resistant phenotype is regulated at the transcriptional level by the P-glycoprotein-like genes (EhPgp) 1 and 5 [22]. A protein complex (EhCPADH) was identified on the surface of *E. histolytica*. This complex is formed by a cysteine proteinase that digests gelatin, collagen type I, fibronectin and hemoglobin (EhCP112), and an adhesin (EhADH112), and is involved in adherence, phagocytosis, and cytolyis [23, 24]. Polypeptides derived from this complex were assessed as vaccine candidates, and it was demonstrated that they confer partial protection from amebic liver abscess in hamsters (*Mesocricetus auratus*) [25].

The role of the immune response in the pathogenesis of amebiasis was also studied; the early approaches demonstrated that serum from infected patients could neutralize the virulence of *E. histolytica* cultures [26] and that this serum could confer antiameba passive immunity in hamsters [27]. The importance of cellular immunity in the control of amebiasis was addressed in several studies, which demonstrated the ability of activated eosinophils to kill the parasite in vitro [28] and to protect from amebic liver abscess in vivo [29], and the killing of trophozoites by peritoneal macrophages in hamsters [30] and by activated T lymphocytes and macrophages in humans [31]. It was shown that patients cured from amebic liver abscess had specific T lymphocytes that killed trophozoites in vitro [31]. It was also demonstrated that molecules from *E. histolytica* were able modulate the host immune response. The supernatant fluid of axenically grown *E. histolytica* could inhibit chemotaxis and random mobility of human monocytes, without affecting the locomotion of neutrophils [32]. The effect was attributed to a monocyte locomotion inhibitory factor (MLIF), and physicochemical analysis revealed that MLIF is a heat-stable pentapeptide (Met-Gln-Cys-Asn-Ser) that inhibits locomotion of monocytes, respiratory burst of monocytes and neutrophils, and delayed hypersensitivity skin reactions to dinitrochlorobenzene in guinea pigs (*Cavia porcellus*) [33]. MLIF decreased the expression of macrophage inflammatory protein-1alpha (MIP-1alpha), MIP-1beta, and chemokine receptor CCR1 in a phorbol myristate acetate- (PMA-) stimulated human monocyte cell line, which suggests that the inhibition of monocyte locomotion could be attributed to downregulation of chemokines and chemokine receptors [34]. MLIF also decreased interleukin-1beta and increased IL-10 production by PMA-stimulated human CD4 T lymphocytes [35]. Immunization with a tetramer of MLIF around a lysine core completely protected gerbils (*Meriones unguiculatus*) against amebic liver abscess [36].

In 1969, Galanos et al. developed a new method for the extraction of bacterial lipopolysaccharide (LPS, Figure 1(a)) [37] and, in the following years, many of the chemical, biological, and immunological properties of the so-called endotoxin were determined [38–44]. LPS is a major structural component of the outer membrane of Gram-negative bacteria; it activates many cell types, induces inflammation, and produces fever and shock. We decided to determine if *E. histolytica* had a surface molecule with chemical and immunological properties similar to those of bacterial LPS. The use of a modified phenol-water extraction procedure on *E. histolytica* trophozoites yielded a molecule with 85% carbohydrate, 8% peptide, 2.5% lipid, and 1% phosphate, which was termed lipopeptidophosphoglycan (LPPG, Figure 1(c)) [45, 46]. The isolation and structural characterization of microbial molecules can lead to the identification of new drug targets and new antigens that are recognized by the immune system; some antigens are good candidates for vaccine development. LPPG was first identified as an antigen; antiameba IgG antibodies were detected in rats after intracereal inoculation of trophozoites [47], anti-LPPG IgA antibodies were found in colostrum of healthy volunteers [48], and antiameba plasma cells were found in peripheral blood of patients with amebic liver abscesses [49]. Monoclonal antiproteophosphoglycan antibodies were described by several groups [50–53]. However, as research in immunology progressed, LPPG was studied as a molecule that could be sensed not only by the adaptive immune system but also by the innate immune system.

3. Sensing of Parasites by the Immune System

The relevance of adaptive immunity (whose main effectors are T and B lymphocytes) in protection against infections was well recognized in the last decades of the past century, while the role of neutrophils, monocytes, macrophages, and
Figure 1: (a) Lipopolysaccharide (LPS) from the Gram-negative bacterium *Escherichia coli* is the most potent activator of TLR4 [54]. (b) Alpha-galactosyl ceramide from the marine sponge *Agelas mauritanius* is presented via CD1d and activates NKT cells [55]. (c) Partial structure of lipopeptidophosphoglycan (LPPG) from *Entamoeba histolytica* of the HM1 : IMSS strain, which was originally isolated from a patient with liver abscess [56, 57]. The structure of the active phosphoinositol moiety of LPPG was characterized in [58].
other cells of the innate immune system was seen as a that of a “first line of defense” that contained infections until adaptive immunity was fully activated. It was known that LPS, a component of the outer membrane of Gram-negative bacteria, caused fever and shock in animal models and that it induced the secretion of proinflammatory cytokines by monocytes, macrophages, and epithelial cells. However, the receptor that sensed LPS remained elusive. Many phagocytic receptors on macrophages had been described, but none of these was responsible for the biological properties of LPS [59].

Two strains of mice, C3H/HeJ and C57BL/10ScCr, which were resistant to endotoxic shock, were identified [60, 61]. In 1998, the positional cloning of the affected locus in C3H/HeJ mice showed a point mutation in Toll-like receptor (TLR) 4, a previously orphan receptor, and C57BL/10ScCr mouse were found to lack TLR4 [62]. TLR4 is a member of a family of proteins that share a signaling domain (TIR) with IL-1 receptor and are related to the Toll proteins of the fruit fly, *Drosophila*. Toll was described in 1988 as a transmembrane protein that is required for establishing the embryonic dorsal-ventral pattern in flies [63]. In 1996, Toll was shown to be critical for the antifungal response in *Drosophila* [64], and it was suspected that the human homologues of Toll, which were described in 1998 [65], would be relevant for the immune response in humans. Indeed, it was found that a constitutively active mutant of human TLR4, transfected into a monocytic cell line, could induce the activation of NF-kappaB and the expression of the proinflammatory cytokines IL-1, IL-6, and IL-8 as well as the expression of the costimulatory molecule CD80 (B7.1), which is required for the activation of naive T lymphocytes [66]. After the establishment of TLR4 as the main sensor for LPS in mice, it was immediately suggested that other members of the Toll family in mammals could also serve as sensors for microbial molecules. Many molecules of bacteria, viruses, and fungi, and others that are found during viral replication, have been identified as agonists of mammalian TLRs (Table 1) [67, 68]. The study of TLRs and other innate receptors has established innate immunity not only as a first line of defense against infections but also as a critical component of the immune system that induces and regulates the adaptive response [69].

The molecules that are sensed by TLRs are widely distributed among groups of microorganisms, and they are essential for the metabolism or the structural integrity of the microbe, so they are highly conserved in evolution. These molecules were termed pathogen-associated molecular patterns (PAMPs) [70], although their expression is not restricted to pathogens, and this term is used widely to this day. Several molecules from protozoan and helminth parasites were also identified as PAMPs [71] (Table 2). In protozoan parasites, many surface molecules are linked to glycosylphosphatidylinositol (GPI), which is inserted in the plasma membrane. GPI-anchored molecules include lipophosphoglycan (LPG) and LPPG; they are essential for survival and virulence of the parasite, and they are likely the major macromolecules on the trophozoite surface [72]. In *Leishmania*, LPG is involved in intestinal adhesion and resistance to insect hydrolases; LPG-deficient strains are unable to survive in their vector. *Leishmania* LPG gains phosphosaccharide domains as procyclic promastigotes in the vector midgut differentiate to infectious metacyclic promastigotes; this structural change in LPG mediates attachment from vector midgut and acquisition of complement resistance [73]. LPG also induces the production of nitric oxide and proinflammatory cytokines by macrophages in the host [74]. LPG from metacyclic promastigotes is a more effective activator of TLR2 in NK cells than LPG from procyclic promastigotes [75].

Plasmid GPI-anchored molecules are required for the induction of proinflammatory responses, which promote pathogenesis [76]. However, activation of innate and adaptive immune responses is necessary to control parasite growth and frequent *tlr4* polymorphisms predispose African children to severe malaria [77]. Therefore, it is proposed that overactivation or deregulation of the inflammatory response is the cause of the pathological condition [78, 79]. Several mucin-like GPI-anchored glycoproteins have been isolated from the *Trypanosoma cruzi* surface. A *T. cruzi* trans-sialidase adds sialic acid residues to these molecules, which are required for survival and infectivity [72, 80]. GPI-anchored molecules purified from *T. cruzi* trypomastigotes signal through TLR2 and induce the production of IL-12, tumor necrosis factor (TNF)-alpha and nitric oxide by murine macrophages [81]; signaling through TLR2 synergizes with TLR9 and is crucial to control the infection [82]. Tc52 is a soluble molecule that is released by *T. cruzi* during parasitemia, and it activates macrophages and dendritic cells via TLR2 [83]. GPI-anchored molecules isolated from *Toxoplasma* activate TLR4, while glycan cores and phospholipid moieties from these molecules activate both TLR2 and TLR4 [84]. Lisosphatidylserine-containing lipids from

| TLR | Microbial ligand | Source               |
|-----|------------------|----------------------|
| TLR1/TLR2 | Tricacyl lipopeptides | Bacteria             |
|      | Lipoarabinomannan  | Mycobacteria          |
| TLR2 | Peptidoglycan     | Bacteria              |
|      | Porins            | Gram-negative bacteria|
|      | Lipoteichoic acid | Gram-positive bacteria|
|      | Zymosan           | Fungi                 |
| TLR2/TLR6 | Diacyl lipopeptides | Mycoplasma           |
| TLR3 | dsRNA             | Virus                 |
| TLR4 | LPS               | Gram-negative bacteria|
|      | Porins            | Gram-negative bacteria|
| TLR5 | Flagellin         | Bacteria              |
| TLR7 | ssRNA             | Virus                 |
| TLR8 | ssRNA             | Virus                 |
| TLR9 | CpG DNA           | Bacteria, virus       |
| TLR11 | —                 | Uropathogenic bacteria|

![Table 1: Some TLR agonists from bacteria, viruses, and fungi (modified from [67, 68]).](modified from [67, 68]).
Table 2: TLR agonists from protozoan and helminth parasites.

| TLR   | Parasite ligand                                                                 | Source                                      | Reference |
|-------|--------------------------------------------------------------------------------|---------------------------------------------|-----------|
| TLR2  | Lipopeptidophosphoglycan                                                       | Entamoeba histolytica (trophozoite)         | [85]      |
|       | Glycosylphosphatidylinositol                                                   | Plasmodium falciparum (merozoite)          | [76]      |
|       | Glycosylphosphatidylinositol                                                   | Toxoplasma gondii (tachyzoite)             | [84]      |
|       | Glycoinositol phospholipid                                                     | Plasmodium falciparum (merozoite)          | [76]      |
|       | Lysophosphatidylserine                                                         | Schistosoma mansoni (egg and adult worm)   | [87]      |
|       | Lipophosphoglycan                                                              | Leishmania major (promastigote)             | [74]      |
|       | Glycosylphosphatidylinositol with unsaturated alkyl-glycerol                   | Trypanosoma cruzi (trypomastigote)         | [81]      |
|       | Tc52                                                                           | Trypanosoma cruzi (epimastigote)           | [83]      |
| TLR2/TLR6 | Glycosylphosphatidylinositol                                             | Plasmodium falciparum (schizont)          | [78]      |
| TLR4  | Lipopeptidophosphoglycan                                                       | Entamoeba histolytica (trophozoite)         | [85]      |
|       | Glycosylphosphatidylinositol with ceramides                                   | Trypanosoma cruzi (epimastigote)           | [88]      |
|       | Glycosylphosphatidylinositol                                                   | Plasmodium falciparum (merozoite)          | [76]      |
|       | Glycoinositol phospholipid                                                     | Toxoplasma gondii (tachyzoite)             | [84]      |
|       | Phosphorylcholine                                                              | Filarial nematode                          | [89]      |
|       | Lacto-N-fucopentaose III                                                       | Schistosoma mansoni                         | [90]      |
| TLR9  | Hemozoin                                                                       | Plasmodium falciparum                      | [91]      |
|       | DNA                                                                            | Trypanosoma brucei                         | [92]      |
|       |                                                                               | Trypanosoma cruzi                          | [92]      |
|       |                                                                               | Leishmania major                            | [93]      |
|       |                                                                               | Entamoeba histolytica                       | [94]      |
| TLR11 | Profilin-like molecule                                                         | Toxoplasma gondii                          | [95]      |

Schistosoma mansoni induce the maturation of dendritic cells that prime Th2 and regulatory T cell responses, which favor the establishment of chronic infections with little tissue damage [87].

4. Function of LPPG as a PAMP and Role of Inflammation in the Pathogenesis of Amebiasis

The similarities in chemical structure between LPS and LPPG (Figures 1(a) and 1(c)), and the presence of a GPI anchor in LPPG, suggested that LPPG might be a PAMP. This would explain how the innate immune system senses the presence of E. histolytica, an event that is necessary for the orchestration of the inflammatory response in amebiasis. Studies from our laboratory demonstrated that LPPG is recognized through TLR2 and TLR4. Human embryonic kidney- (HEK-) 293 cells were rendered LPPG responsive through overexpression of TLR2 or TLR4/MD2. Coexpression of CD14 enhanced LPPG signal transmission through TLR2 and TLR4. The interaction of LPPG with TLR2 and TLR4 resulted in activation of NF-kappaB and release of IL-8, IL-10, IL-12p40, and TNF-alpha from human monocytes [85, 96]. Human macrophages and dendritic cells internalize LPPG. As shown by colocalization of LPPG with late endosomes marked with fluorescein isothiocyanate–dextran and LAMP-1, the internalization process involves intracellular traffic from the cell membrane to late endosomes. LPPG-activated dendritic cells have increased expression of costimulatory molecules CD80, CD86, and CD40 and produce TNF-alpha, IL-8, and IL-12 [97]. These results show that LPPG activates antigen-presenting cells and reaches intracellular compartments that are involved in antigen presentation. Responsiveness of mouse macrophages lacking TLR2 expression (TLR2−/−) or functional TLR4 (C3H/HeJ) to LPPG challenge was impaired, while macrophages from C3H/HeJ/TLR2−/− mice were unresponsive. In contrast to wild-type and TLR2−/− mice, which succumbed to LPPG-induced shock, C3H/HeJ mice were resistant [85]. All these results clearly establish that LPPG is a PAMP from E. histolytica that induces the activation of innate immunity.

In humans, the pathogenesis of E. histolytica requires adhesion of trophozoites to the host cells, phagocytosis of host cells and bacteria by trophozoites, and tissue destruction by amebic enzymes and by enzymes released from lysed neutrophils. The adhesion of trophozoites to host cells is required for tissue invasion; this adhesion is mediated, in part, by a galactose/N-acetylgalactosamine (Gal/GalNAc-) binding lectin, which is also cytotoxic and confers protection from complement. Other important adhesins are a 220 kDa cell surface protein, a 112 kDa adhesin (EhADH112), and
a surface LPG [23, 98–100]. Phagocytosis is regulated by adhesins and by signaling pathways that control cytoskeleton structure and vesicular traffic. A phagosome-associated transmembrane kinase (PATMK) binds to phosphatidylinerine on host cells and initiates their phagocytosis by trophozoites [101]. Trophozoites cause damage of host cells and extracellular matrix through the action of amebapore, amebic phospholipases, and proteolytic enzymes (cytostane endopeptidases, cysteine proteinase, acid and neutral proteinases, collagensases, histolysin, amebapain, cathepsin B) [102–108]. Neutrophils are the first cells that infiltrate the necrotic lesions caused by E. histolytica in the intestine and liver [109–113], where they are killed by trophozoites. The enzymes and reactive oxygen species released from these neutrophils increase tissue damage, and in this context, LPPG could be seen as a virulence factor that promotes tissue invasion by causing inflammatory damage to host cells. However, the role of inflammation in amebiasis is still controversial [114]; in susceptible animals (hamsters and gerbils) inflammation is related to host cell lysis and facilitates the spreading of trophozoites [115], while in resistant animals (mice, guinea pigs), inflammatory cells protect the host by killing trophozoites [116, 117].

E. histolytica genomic DNA is recognized by TLR9 and induces the production of TNF-alpha by a macrophage cell line [94], and Gal/GalNAc-binding lectin activates NF-kappaB and mitogen-activated protein (MAP) kinases in macrophages. These transcription factors increase the expression of several genes, including TLR2 [118]. It is likely that genomic DNA and Gal/GalNAc-binding lectin, along with LPPG, contribute to the initiation of inflammation in response to E. histolytica.

Silencing of the expression of E. histolytica GPI-anchored molecules by antisense RNA-mediated inhibition of their biosynthetic pathways suppresses endocytosis, adhesion, and proliferation of the trophozoites [119]. Specific blockade of LPG and LPPG by monoclonal antibody EH5 reduces intestinal inflammation and tissue damage in a severe combined immunodeficient (SCID) mouse model of intestinal amebiasis with human intestine xenograft [120]. EH5 also prevents liver abscess development in an SCID mouse model [52] and E. histolytica adhesion and cytotoxicity to a hamster cell line [101]. These results suggest that LPPG is a virulence factor of E. histolytica. Mirelman and colleagues found that a nonvirulent E. histolytica strain had reduced expression of LPG and LPPG; they also found no LPG and a modified LPPG (with a higher negative charge and different lengths of oligosaccharide chains) in the low-virulence strain Rahman and in the nonpathogenic Entamoeba dispar [121].

Recently, a role for LPPG in protection against invasive amebiasis was shown. The chemical structure of LPPG has some similarities with alpha-galactosyl ceramide, a known activator of NK cells [55] (Figures 1(b) and 1(c)). NK cells share many surface receptors with natural killer (NK) cells and, like conventional T cells, express T cell receptors that are generated by somatic DNA rearrangement. However, most NK cells express semi-invariant T cell receptors, consisting of Vα14-Jα11/8.2 chains in mouse and Vα24-Jα11/8.2 chains in humans [122]. This limited repertoire, conserved between individuals and presumably selected by evolution, is more closely related to the pattern-recognition receptors of innate immunity than to the highly diverse receptors of adaptive immunity. NKT cells recognize glycolipid antigens presented by nonpolymorphic CD1d molecules; these glycolipids can be endogenous, like lysosomal isoglobotrihexosyl ceramide [123], and exogenous, like glycosyl ceramides from Gram-negative, LPS-negative Sphingomonas capsulata [124]. In a mouse model, it was demonstrated that NKT cells play a central role in the control of amebic liver abscess caused by E. histolytica. Specific activation of NKT cells by alpha-galactosyl ceramide or LPPG induced significant protection, while CD1d−/− mice suffered from severe abscess development [58]. The phosphoinositol moiety of LPPG was shown to induce interferon- (IFN-) gamma but not IL-4 secretion in NKT cells. NKT cell activation was dependent on the presence of CD1d and simultaneous TLR receptor signaling, as indicated by the absence of IFN-gamma secretion in antigen-presenting cells from TLR2- or TLR6-deficient mice [58]. These results suggest that NKT cell activation by LPPG is important to limit amebic liver abscess development and may help to explain why the vast majority of E. histolytica-infected individuals do not develop invasive amebiasis.

5. LPPG as a Molecule That Is Sensed by Both the Innate and the Adaptive Immune Systems

Molecules that are recognized by receptors of both innate and adaptive immune systems are, in general, highly immunogenic; we have referred to these molecules as "Pamptigens" [125, 126]. Some examples of molecules that present this dual recognition include porins, profilin, polysaccharide A, yellow fever vaccine, and respiratory syncytial virus vaccine. Salmonella typhi porins are recognized by TLR2 and TLR4 [126], and they induce high antibody titers that persist during the whole lifetime of mice [127]. Toxoplasma gondii profilin, a TLR11 agonist, is an immunodominant antigen in the CD4+ T cell response to the pathogen [128]. Bacteroides fragilis polysaccharide A activates CD4+ T cells by a mechanism that depends on TLR2 signaling and antigen presentation by the MHCIi pathway [129]. Live attenuated yellow fever vaccine 17D, one of the most effective vaccines available, activates TLR2, 7, 8, and 9 and induces antigen-specific CD8+ T cells [130]. Poor TLR signaling by a formalin-inactivated respiratory syncytial virus vaccine led to the induction of low-affinity antibodies and to the failure of the vaccine to protect immunized children [131].

Molecules that are recognized by innate and adaptive receptors of the immune system are also effective adjuvants. Innate immunity participates in the induction and regulation of adaptive responses; without adjuvants, molecules that are recognized by adaptive receptors but not by innate receptors fail to elicit antibody or T cell responses. Antigen recognition alone is not sufficient to activate adaptive immune responses, and innate signals are required to indicate the microbial origin of the antigen; adjuvants provide this signal by activating innate immune receptors [132, 133].
LPPG signals through TLR2 and TLR4, and it induces the production of IFN-gamma (a cytokine that activates macrophages and increases cytotoxic T cell responses) by NKT cells. LPPG is also an antigen, because anti-LPPG antibodies have been detected in animal models and in patients with amebiasis. The mechanism that leads to the production of these antibodies has not been determined, but it is probably influenced by the innate signaling of LPPG on dendritic cells and B cells.

6. Concluding Remarks

The study of *Entamoeba histolytica* was initially motivated by the high morbidity and mortality of amebiasis, and in our group, this research led to the identification of LPPG, one of the first PAMPs described in parasites, and a promising vaccine candidate and potential adjuvant. The incidence and severity of amebiasis has declined, because of improved sanitation and effective treatments, but this disease is still a health problem in many parts of the world. The development of a vaccine that effectively protects against *E. histolytica* infection would have a positive impact on global health.

**Abbreviations**

Gal/GalNAc: Galactose/N-acetylgalactosamine
GPI: Glycosylphosphatidylinositol
IFN: Interferon
IL: Interleukin
MAP kinases: Mitogen-activated protein kinases
LAMP: Lysosome-associated membrane glycoprotein
LPG: Lipophosphoglycan
LPPG: Lipopeptidophosphoglycan
LPS: Lipopolysaccharide
MHCI: Major histocompatibility complex class I
MIP: Macrophage inflammatory protein
MLIF: Monocyte locomotion inhibitory factor
NK: Natural killer
PAMP: Pathogen associated molecular pattern
PATMK: Phagosome-associated transmembrane kinase
PMA: Phorbol myristate acetate
SCID: Severe combined immunodeficiency
TLR: Toll-like receptor
TNF: Tumor necrosis factor.

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