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Review

Amoebiasis vaccine development: A snapshot on *E. histolytica* with emphasis on perspectives of Gal/GalNAc lectin

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ABSTRACT

Amoebiasis/amebiasis is a gastrointestinal infection caused by an enteric dwelling protozoan, *Entamoeba histolytica*. The disease is endemic in the developing world and is transmitted mainly via the faecal-oral route (e.g., in water or food) and may or may not be symptomatic. This disease of socio-economic importance worldwide involves parasite adherence and cytolysis of human cells followed by invasion that is mediated by galactose-binding (Gal/GalNAc) surface lectin. Disruption of the mucus layer leads to invasive intestinal and extraintestinal infection. Gal-lectin based vaccinations have conferred protection in various animal models against *E. histolytica* infections. Keeping in view the pivotal role of Gal/GalNAc lectin in amoebiasis vaccine development, its regulation, genomic view of the parasite involving gene conversion in lectin gene families, current knowledge about involvement of Gal/GalNAc lectin in adherence, pathogenicity, signalling, encystment, generating host immune response, and in turn protozoa escape strategies, and finally its role as effective vaccine candidate has been described. This review will help researchers to explore pathogenesis mechanism along with genomic studies and will also provide a framework for future amoebiasis vaccine development studies.

Contents

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1. Introduction

Lectins are ubiquitous proteins of non-immune origin which agglutinate cells or precipitate glycoconjugates through sugar specific binding sites and play an important role as recognition molecules in cell–cell or cell-matrix interactions [1,2]. They can interact with internal sugars of complex oligosaccharide sequences or the terminal, non-reducing residues in complex saccharides and are thus termed as endolectins or exolectins, respectively [3].

Lectins are reported from plants, animals, fungi, bacteria, protozoa and viruses, and they play pivotal roles in different organisms [4]. Most of the studies regarding biological properties of lectins have been focused on plant lectins for decades; however microbial lectins have received a great interest among the scientists in the past few years. Owing to unique carbohydrate binding specificities, mushroom lectins [5] and algal lectins [6] have been endowed with various biomedical applications like antitumor, antiviral, antiproliferative, immunestimulating etc. Lectins from microfungi have diverse applications in various fields involving cytochemical and histochemical methods as well as in glycobiology research [7]. Amongst microfungi, high incidence of lectins have been reported from aspergilli [8–10], penicilli [11,12] and *Fusarium* species [13]. Lectin from *Aspergillus nidulans* possesses immunomodulatory and therapeutic potential against ulcerative colitis [14]. A wide variety of microbial lectins possess mitogenic potential and upon interaction with cell receptors triggers multitude of reactions which ultimately results in cell proliferation [15].

Lectin mediated cellular-recognition process plays a role in attachment of pathogenic yeast to host cell surface and in yeast flocculation [16]. Symbiotic association of fungal lectin with algae and cyanobacteria in lichens is facilitated by lectin mediated cell–cell interaction [17]. Several parasitic protozoans such as *Entamoeba histolytica* [18], *Giardia lamblia* [19], *Cryptosporidium parvum* [20], *Trichomonas vaginalis* [21] etc. possess lectins which mediate parasite adherence to host cell. Among the protozoa, the most thoroughly studied is *Entamoeba histolytica* lectin which has affinity for D-galactose and *N*-acetyl-D-galactosamine [22]. Gal/GalNAc lectins in *E. histolytica* play a role in disease pathogenesis [18]. *E. histolytica* infection causes amoebiasis and the disease could be prevented by targeting Gal/GalNAc lectin which is most promising vaccine candidate molecule. The present article provides background on amoebiasis with insight into the regulation of Gal/GalNAc lectin with current understanding of protozoa genome; role of Gal/GalNAc lectin in various fields, and also highlights the areas that could shed crucial insight into Gal/GalNAc lectin based vaccine development.

2. *Entamoeba histolytica*: etiological agent of amoebiasis

Entamoeba histolytica (*Eh*) is the most prevalent intestinal pathogen worldwide responsible for leading parasitic disease among developing countries. Infection by *E. histolytica* causes amoebiasis/amebiasis. Amoebiasis is endemic in many less-developed countries [23] and travel to endemic countries is a risk of developing an *E. histolytica* infection in travellers [24]. Asymptomatic infections occur in 90% of individuals, whereas remaining 10% develop symptomatic infections, around 50 million people develop severe amoebiasis, and 50,000–1,00,000 deaths occur annually due to amoebiasis [25]. This etiological agent thus adds to disease burden in the developing world.

Transmission of cyst (immature form with single nucleus and differentiate into mature with four nuclei) is mainly through faecal-oral route by contaminated water or food and its subsequent excystation into trophozoite (single nucleus) in the intestinal epithelium causes human infection [26]. The basis of amoebiasis is parasite destruction of host tissue, and its clinical manifestations include invasive intestinal amoebiasis, amoebic colitis, amoebic abscesses and amoeboma. Invasive amoebiasis can be intestinal or extraintestinal based on site of infection. Invasion of intestinal lining causes amoebic colitis and further access to afferent circulation that spread into the liver can cause amoebic liver abscess (ALA) or abscess in other soft organs [27,28]. Amoeboma is a rare manifestation of intestinal amoebiasis in which there is formation of annular chronic granulation resulting in large local lesion of the bowel in response to infecting amoeba [29].

3. Regulation of Gal/GalNAc lectin

Molecular basis of amoebiais depicts involvement of various cell surface and secreted molecules and molecules involved in phagocytosis and cell surface associated signalling [30]. An important hallmark of amoebiasis is contact-dependent cytolysis of host cells by *E. histolytica*; thus, adherence mediated by Gal/GalNAc lectin binding to host mucin oligosaccharides and colonization of mucus epithelial layer is a pivotal step [31]. Amoebapores (proteins forming oligomeric pores in target cell membrane), cysteine proteases and Gal/GalNAc lectin are key virulence factors involved in the pathogenesis. The Gal/GalNAc lectin is a heterodimeric, cell surface molecule (Fig. 1) composed of a transmembrane heavy (Hgl, 170-kDa) subunit linked by disulfide bond to GPI-anchored light (Lgl, 31/35-kDa) subunit and non-covalently associated with an intermediate (Igl, 150 kDa) subunit [32]. Amino terminal (1209 residues) and carbohydrate recognition domain (aa 898–998) of Hgl are extracellular, whereas its carboxy terminal (41 aa) comprise cytoplasmic domain which interacts with actin [33].

The association of Gal/GalNAc lectin subunits with membrane microdomain (lipid rafts) regulates their assembly and function [34]. Lipid rafts are tightly packed, cholesterol- and sphingolipid-rich membrane microdomains, involved in organizing trafficking and signalling pathways in a variety of cell types. They suggested a model of *E. histolytica* Gal/GalNAc lectin assembly in which GPI-anchored Igl reside in raft-like domain, whereas Hgl-Lgl dimer resides in different sub-membrane compartments. Upon cholesterol loading (activation event), it stimulates Hgl-Lgl dimer recruitment into raft-like domain and leads to co-localisation of the three subunits which might act as a positive regulator of Gal/GalNAc lectin function. Thus, insight into the molecular mechanism involved in transforming lectin into functional adhesion provides insight into pathogenicity of this enteric pathogen [34]. Co-localization of lectin subunits in lipid rafts occurs upon exposure to host ligands and might act as an initial step in signalling pathway involving PIP_2 and calcium pathway [35]. These rafts thus mediate initial parasite attachment to host epithelial layer.