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An update of Vaccine development against *Entamoeba histolytica*

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Abstract

Purpose: This review literature reveals designing of an effective vaccine against *Entamoeba.histolytica*, but due to some major limiting factors a successful vaccine has not been designed yet. The researches focusing on designing an ideal vaccine are summarized in this review literature. The major limiting factors leading to the failure of this particular vaccine are also discussed here

Methodology: This study is literature based focusing on recent advancement and contribution to vaccine designing. The epidemiology and pathogenesis of *Entamoeba histolytica* has been studied posing a major health threat. Some vaccines candidates and adjuvants have also been studied having promising results but require clinical trials.

Findings: The major finding in this review is less investment on tropical diseases and lack of long term immunity also needs to be elucidated. Various proteins associated with the chemical structure of *Entamoeba histolytica* are immunogenic and can be used as potential vaccine candidates. The interaction of *Entamoeba histolytica* with gut microbiota may result into new strains of the pathogen leading to evasion strategy of this particular parasite. In asymptomatic cases it colonizes the large intestine and sometimes spread to other soft organs of the body causing abscesses. *Entamoeba histolytica* can be transmitted through polluted water and food. The international transport of food materials may have a major role in *Entamoeba histolytica* transmission.

Recommendation: Further researches may be the base for an effective vaccine having long term immunological memory against *Entamoeba histolytica*. An effective vaccine may be effective in all age groups with no adverse effects. The improvement in hygiene and health care system may the possible ways of combating amoebiasis.

Keywords: *Parasitic infection, Immunization, Liver abscesses, Amoebiasis, Vaccine efficacy, Evasion, Vaccine candidates, Immunological memory*

Introduction

Entamoeba histolytica is a human enteric protozoan parasite causing amoebiasis and is globally the third leading cause of death (Huang, et al., 2020).Ameobiasis causes 100000 deaths annually (Tsukui & Nozaki, 2016). Recent studies show that 50 million cases and 55,000 to 100,000 deaths are globally reported due to amoebiasis infection each year and the infection rate is higher in developing countries (Cavazos & Knoll, 2020).*Entamoeba histolytica* is a global public health pathogen affecting up to 50 million people in developing countries (Kantor, et al., Entamoeba Histolytica: Updates in Clinical Manifestation, Pathogenesis, and Vaccine Development, 2018). It can be transmitted through contaminated water or food containing the cysts (Hemmati, Hooshmand, & Hosseini, 2015). As in developing countries the water purification system and food items are not managed properly leading to more chances of amoebiasis.

The developing countries are highly affected by *Entamoeba histlytica* due to their poor hygienic conditions and weak health care system (Shirley, Farr, Watanabe, & Moonah, 2018). Contaminated water and food sources transmit the cysts affecting the consumers. Malnutrition may also be an important factor leading to the susceptibility of infection (Hughes & Kelly, 2006). Immunocompromised individuals may be affected seriously by intestinal parasites (Hung, et al., 2008). It exists in two forms; in its active form called trophozoite performing the process of division leading to the process of pathogenesis whereas in its dormant form it exists as cyst survive for a long period of time in the environment under unfavorable conditions (Sehgal, Bhattacharya, & Bhattacharya, 1996). The trophozoite is the active form colonizing the large intestine causing pathogenesis and if first line of defense is crossed it enters into circulation spreading to other organs of the body resulting into abscesses and most commonly the liver is affected. The liver controls many physiological functions and these normal physiological functions of human body may be disturbed in case of liver disorders. The intestinal epithelium is the first line of defense avoiding adherence of the pathogen to the epithelial lining of the intestine (Pitman & Blumberg, 2000).

Ameobiasis is a pathological condition caused by *Entamoeba histolytica* in developing countries with poor hygienic conditions, lack of clean water, low socioeconomic status, weak health care system and poor sanitation (Tharmaratnam, et al., 2020). Due to weak health care system and poor hygiene conditions, the ratio of amoebisasis is much higher in developing countries leading to more fatalities annually (Duc, et al., 2011). Four species of intestinal amoebae have been recognized including *E. histolytica*, *E. dispar*, *E. moshkovskii, and E. bangladeshi* (Leder & Weller, 2014). Among these four species *Entamoeba histolytica* is the one causing pathogenesis in human. Some major complications are caused by this particular pathogen including Protozoal diarrhea, dysentery and amoebic abscesses leading to other physiological complications (Mukhopadhyay, Saha, Sarkar, & Mukherjee, 2010). The normal physiological functions of human body organs may be affected.

Various therapies are applied against *Entamoeba histolytica*, but not proved completely effective as the drug resistance and adverse effects are of great concern. The drugs including metronidazole and nitromedazole are effective in combating amoebiasis but causing adverse side effects and sometimes drug resistance also occurs leading to drug therapy failure (Bansal, Malla, & Mahajan, 2006). Lactoferrin and Lactoferricin are also effective having synergistic effect with metronidazole (Sicairos, Lpez, Pichardo, & Garza, 2006). Experimental trial shows that melatonin reduces severity of amoebiasis by increasing leukophagocytosis resulting into greater amount of dead amoeba (Botelho, et al., 2011). In 2013 the total number of global

deaths reached 11300 ranking it as the fourth major cause of infection in the world (Cornick & Chadee, 2017). The amoebiasis is a public health problem causing more deaths globally due to the lack of vaccine as there is no approved vaccine against *Entamoeba histolytica* (Cavazos & Knoll, Entamoeba histolytica: Five facts about modeling a complex human disease in rodents, 2020). However, candidate vaccines designed having encouraging results. Gal/GalNAc lectin has been under clinical trial proved future vaccine candidates with promising results (Abhyanka, et al., 2017).

The vaccine developed against *Entamoeba histolytica* will be cost effective and safe with no side effects. The serine rich *Entamoeba histolytica* protein used as vaccine against this particular pathogen provided effective immunity with promising results (Lejeune, Rybicka, & Chadee, 2009).The Gal/GalNAc provided protection in mice against *Enatamoeba histolytica* through T cell dependent mechanism implicating the role of CD4+ and CD8+ cells (Guo, Barroso, Lyerly, PetriJr., & Houpt, 2011). An adjuvant containing TLR4 and TLR7/8 agonists used in the formulation of vaccine enhanced protection against *Entamoeba histolytica* by the production of intestinal IgA and plasma IgG2a/IgG1 and some cytokines (Abhyanka, et al., 2017). The nature of the acquired immunity has not been completely elucidated that poses a major limitation in developing vaccine against *Entamoeba histolytica* (Abhyankar, et al., 2018). The glycoproteins Ubiquitin Ub can elicit immune response leading to the production of Ig in immunized rabbits (Flores, et al., 2020). Transportation and emigration may be the possible ways of pathogen transmission to developed countries leading to the increase of infection in non-endemic areas (Kantor, et al., 2018). International trade may also be implicated in the transmission of *Entamoeba histolytica.*

Pathogenesis and Clinical Manifestations of *Entamoeba histolytica*

The invasiveness of *Entamoeba histolytica* in some individual is much complicated mechanism implicating several stages of pathogenesis like adherence of trophozoite to the target cell, lysis and phagocytosis of target cell (Sehgal, Bhattacharya, & Bhattacharya, 1996). The mucosa and sub mucosa are invaded and the trophozoites enter into the portal vein through blood circulation infecting the liver and other organs of the body (Mortimer & Chadee, 2010). It causes amoebic dysentery, colitis and liver abscesses in severe cases (Cornick & Chadee, 2017).

If the first line of defense is crossed, it may spread to body organs and the liver may be affected causing liver abscess (Orozco, Guarneros, Palomo, & Sánchez, 1983).The main organs of the body may be severely affected leading to death if not treated on time (Ramos, et al., 2007). Infection in human tissues can be established through lectins, a protein present in *Entamoeba histolytica* implicated in host parasite interaction and cytopathy (Téllez, et al., 2020). Surface galactose-*N*-acetyl-*D*-galactosamine inhibitable lectin (Gal-lectin) on the surface of trophozoite initiates the process of pathogenesis that binds to galactose and N-acetyl galactosamine residues of the mucin in colon (Chadee, Jr, Innes, & Ravdin, 1987). The Gal-lectin is immunogenic and may be potential vaccine candidate against *Entamoeba histolytica* (Singh, Walia, Kanwar, & Kennedy, 2016). However, some clinical trials are needed to elucidate its efficacy and safety. Some major proteolytic enzymes have been relevant to amoebic pathogenesis expressed by peptidase genes including cysteine, aspartic, serine and metallo peptidases (Baxt & Singh, 2008). The lysine and glutamic acid rich protein KERP1 may act as virulence factors causing liver abscess in *Entamoeba histolytica* infection (Rocca, et al., 2008).

Some enzymes and virulence factors associated with *Entamoeba histolytica* are under investigations enhancing the risk of amoebiasis (Kantor, et al., 2018). These enzymes and virulence factors initiate the pathogenesis. Adhesion and colonization to the mucus layer are primary events implicated in the pathogenesis followed by mucus depletion, epithelium disruption and spread of pathogen to the soft organs (Quach, Pierre, & Chadee, 2014). Research shows that mutation in major virulence factors including Gal/GalNAc lectin may affect the cytolysis and adhesion stages of *E.histolytica* (Gilchrist & Petri, 1999). These virulence factors may be of clinical significance in future therapies and vaccine designing against this particular pathogen (Mann, 2002). The major virulence factor of *Entamoeba histolytica* are the cysteine proteinases degrading the extracellular matrix in the intestine and circumvent the host immune response leading to the production of antibodies and activation of the complement system (Que & Reed, 2000). Cysteine proteinase is highly inflammatory and inflammation may be a protective response of the host tissues against pathogen (Angelo, 2004). Other virulence factors having role in evasion from host defense include amebapore, arginase, alcohol dehydrogenase, peroxiredoxin and lipopeptidophosphglycan (Quach, Pierre, & Chadee, 2014).The trophozoite spreading to the epithelial cells below the protective mucus layer trigger and phagocytosis of epithelial cells (Huston, Houpt, Mann, Hahn, & Jr, 2000). The galactose inhibitable lectin may have an important role in colonization and invasion of *Entamoeba histolytica* in the human gut (Ravdin, Abd-Alla, Welles, Reddy, & Jackson, 2003). Recent researches show that interaction of *Entamoeba histolytica* with gut microbiota may result into more virulent strains (SS, et al., 2012). The new strains may be the result of mutation leading to drug resistance and evasion mechanism of the parasite (Begum, Quach, & Chadee, 2015). Respiratory bursts of macrophages are disturbed and nitric oxide production is decreased. Neutrophil apoptosis may be established leading to the destruction of immune cells (Iles & Forman, 2002). There may be some other factors implicating the evasion strategy of *Entamoeba histolytica*.

The Human Immune System against *Entamoeba histolytica*

The intestinal mucosal layer is the first line of defense against pathogen preventing pathogen from adherence to the intestinal walls. If the first line of defense is crossed, intestinal immune response is initiated that is considered to be the secondary defense against *Entamoeba histolytica* (Tsukui & Nozaki, 2016). Innate immune response may be implicated in fighting against pathogen that can be recognized through innate receptors (Kumar, Kawai, & Akira, 2009). The pathogen is recognized through germline encoded pattern recognition receptors. A broad spectrum of microbial components can be recognized through Toll like receptors (Kawai & Akira, 2011). Serum antibodies are produced within seven days in amebic liver abscesses and persist for 10 years leading to a long term immunological memory (Dhawan, et al., 2019).

The attachment of *Entamoeba histolytica* to the colonic mucin layer leads to the establishment of infection (Tsukui & Nozaki, 2016). In case of weak immune response the severity infection is enhanced and the pathogen spread to other organs of the body leading to fatality (Fonseca, et al., 2018). The tropphozoites colonizes the epithelial surface and causes extensive destruction of the tissue and components of the host's extracellular matrix implicating some virulence factors like amebopore, hydrolytic enzymes and cysteine proteases. (Kelsall & Ravdin, 1993). The mucin layer are the physical barrier and consider to be the first line of defense and the first line of defense is crossed then secondary immune response takes place implicating the production of antibodies like mucosal immunoglobulins (Lamm, 1998). The mucosal Immunoglobulin (Ig) play an important role in gut defense mechanism against pathogen and homeostasis may be maintained through these antibodies. The intestinal

homeostasis may be maintained by the production of mucosal immunoglobulin in the human gut providing the first line of defense against *Entamoeba histolytica* (Lamm,1997). These antibodies having role in preventing pathogens from mucosal surfaces and may be considered as natural immunity.

The carbohydrate recognition domain of the Gal /GalNA lectin is attached to intestinal epithelial cells via toll-like receptor (TLR)-2/4 activating NFκB and various types of inflammatory cytokines are produced including IL-1β, IL-6, IL-8, IL-12, IFN-γ, and TNF-α (Tsukui & Nozaki, 2016). Intestinal Epithelial Cells express pathogen recognition receptors through which pathogen can be recognized leading to the production of various types of inflammatory cytokines initiating the process of inflammation. IFN-γ produced by peripheral mononuclear cells may clear the infection and protect from reinfection of *Entamoeba histolytica* (Tsukui & Nozaki, 2016). This will be a part of adaptive immunity that is specific in its action preventing reinfection with the same pathogen (Kowalczyk, et al., 2016). Adaptive immunity may lead to the formation of immunological memory.

Vaccines and adjuvants

An ideal vaccine should have long term immunological memory, high safety value and easy rout of its administration leading to child health improvement and combating amebic colitis and liver abscesses (Ivory & Chadee, 2007). Theoretically a vaccine can prevent the intestinal infection of *Entameoba histolytica*, but due to some major challenges an ideal vaccine has not been approved yet (Sim & Jr, 2002). For enhancing the immunogenicity of vaccine, an adjuvant can be used. Freund's adjuvant can be used for amoebiasis vaccine development leading to a strong immune response (Jr & Ravdin, 1991). Some other adjuvants can also be used in designing an effective vaccine against this particular pathogen, but needs to be further investigated elucidating their adverse effects (Israeli, Levin, Blank, & Shoenfeld, 2009). Studies show that galactose lectin may be a protective antigen having strong immune response.

The recent research shows that lectins may be the potential vaccine candidates against infections caused by virus, protozoa and bacteria (Téllez, et al., 2020). Gal/GalNAc lectin from *E. histolytica* is also an important future vaccine candidate with promising results in ALA and intestinal amoebiasis (Singh, Walia, Kanwar, & Kennedy, 2016). Gal lectin is an immunogenic and has the most promising results and may be a future vaccine candidate (Gaucher & Chadee, 2003).

Cholera toxin B subunit is immunogenic and shows less toxicity in human and may be used as adjuvant to elicit mucosal Th2 immune response (Quach, Pierre, & Chadee, The future for vaccine development against Entamoeba histolytica, 2014). Cytosine guanine oligodeoxynucleotides (CpG-ODNs) can be used as an effective adjuvant eliciting Th1 mucosal immune response (Ivory, Keller, & Chadee, 2006). The discovery of trained immunity may also play an important role in the elimination of infection, but this trained immunization mechanism needs further clinical trials (Netea, et al., 2016). The innate immune cells may enhance responsiveness of innate immune cells against pathogens.Trained immunity involving some epigenetic changes in cell physiology and gene expression without permanent genetic changes.

The interaction of gut microbiome and innate immune cells may play an effective role in future therapies and vaccine design (Negi, Das, Pahari, Nadeem, & Agrewala, 2019). The serine rich protein of *Entamoeba histolytica* is an also a successful vaccine candidate against *Entamoeba histolytica* and showed protection in gerbils against ALA (Jr., Tian, Koester, & Li, 1995).

Complete function of SREHP has not been elucidated yet, but in vitro studies show that it acts as adhesion and chemoattractants for Entamoeba histolytica trophozoites (Jr, Becker, Jenkins, Foster, & Li, 1990). This potential vaccine may be passed through clinical trials to show its efficacy and effectiveness.

Important Vaccine candidates

Some important proteins identified as vaccine candidates against *Entamoeba histolytica* including heparin sulfate binding Protein (HSBP), and the 30kDa protein (CBP30) of this particular pathogen have been under clinical trial implicated in designing an effective vaccine against *Entamoeba histolytica*. HSBPs play an important role in adherence to host heparin protein and may be used in designing potential vaccine against this particular pathogen (Kaur, Khurana, Saikia, & Dubey, 2013). These vaccine candidates may undergo clinical trials to prove its efficacy and safety.

Challenges in Designing Vaccine against *Entamoeba histolytica*

The vaccines prepared against *Entamoeba histolytica* lacks long term immunological memory that is considered to be the hallmark of a successful vaccine. Several vaccines are under clinical trial, but the major challenge is failure of eliciting long term immune response (Kantor, et al., 2018). Long term immunological memory is crucial to prevent infection in children and adults (Ahmed & Gray, 1996). The duration of antibodies must be prolonged preventing infection. Another limiting factor in vaccine development is less investment in neglected tropical diseases (NTDs) affecting developing countries (Bethony, et al., 2011). The prevalence of these tropical diseases has been considerably increased since last few decades affecting low income countries due to the lack of an effective vaccine and poor hygienic conditions. The efficacy of Gal Lectin based intra nasal synthetic peptide vaccine was determined in an experiment releasing xenic *Entamoeba histolytica* trophozoites into the small bowel of baboons resulting in antibody production followed by infection (Alla, et al., 2012). Adverse effects and short term immunological memory are the major hurdles in vaccine development.

Combating Amoebiasis and Vaccine approval

The candidate vaccines should be thoroughly tested in human before it is licensed for general use. Some major socioeconomic aspects should be taken into consideration while developing vaccines. Several researches are going on developing an effective vaccine that will lead to the eradication of amoebiasis in the future. Awareness and literacy rate may be increased in developing countries combating the rate of amoebiasis. Food and water resources must be maintained hygienically breaking the chain of transmission of *Entamoeba histolytica* (Ximénez, et al., 2011). International transport of food material should be handled hygienically avoiding the transmission process. Health care facilities may also play an important role in combating amoebiasis (Nath, Ghosh, Singha, & Paul, 2015). On time effective treatment can reduce the rate of amoebiasis (Shirley, Farr, Watanabe, & Moonah, 2018).

Conclusion

An effective vaccine has not been licensed against *Entamoeba histolytica* yet, but clinical trials on various vaccine candidates have been going with promising results. The major limiting factor is short term immunological memory and researches are going on to produce an ideal vaccine with long term immunological memory that is the hallmark of an effective vaccine. Another limiting factor may be less investment on tropical diseases leading to higher number of deaths in developing countries.

Future Planning and Recommendations

The developing countries are mostly affected by amoebiasis due to their unhygienic water and food sources. Awareness and improvement of hygienic conditions can combat amoebiasis. The food materials transported internationally may transmit the pathogens and monitoring of food materials may break the chain of transmission. An ideal vaccine with long term immunological memory is expected to be prepared in future.

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