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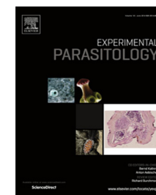

Intranasal coadministration of Cholera toxin with amoeba lysates modulates the secretion of IgA and IgG antibodies, production of cytokines and expression of pIgR in the nasal cavity of mice in the model of *Naegleria fowleri* meningoencephalitis

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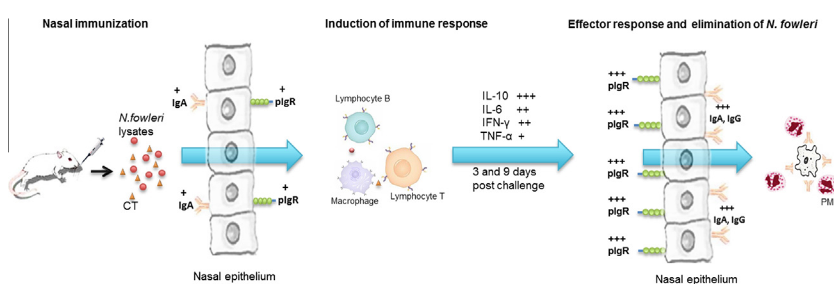
HIGHLIGHTS

- CT as adjuvant confers up to 100% of protection against *N. fowleri* infection.
- IgA e IgG antibodies interact specifically with *N. fowleri* in the nasal lumen of mice.
- Immunization with CT plus lysates modulate the expression of pIgR and cytokines.
- Nasal immunization favors the elimination of trophozoites of *N. fowleri* by PMN.

GRAPHICAL ABSTRACT



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ABSTRACT

The nasal mucosa is the first contact with antigens to induce IgA response. The role of this site has rarely been studied. We have shown than intranasal administration with *Naegleria fowleri* lysates plus Cholera toxin (CT) increased the protection (survival up to 100%) against *N. fowleri* infection in mice and apparently antibodies IgA and IgG together with polymorphonuclear (PMN) cells avoid the attachment of *N. fowleri* to apical side of the nasal epithelium. We also observed that nasal immunization resulted in the induction of antigen-specific IgG subclasses (IgG1 and IgG2a) in nasal washes at days 3 and 9 after the challenge and IgA and IgG in the nasal cavity, compared to healthy and infected mice. We found that immunization with both treatments, *N. fowleri* lysates plus CT or CT alone, increased the expression of the genes for alpha chain, its receptor (pIgR), and it also increased the expression of the corresponding proteins evidenced by the ~65 and ~74 kDa bands, respectively. Since the production of pIgR, IgA and IgG antibodies, is up-regulated by some factors, we analyzed the expression of genes for IL-10, IL-6, IFN-γ, TNF-α and IL-1β by using RT-PCR of nasal passages. Immunization resulted in an increased expression of IL-10, IL-6, and IFN-γ cytokines. We also aimed to examine the possible influences of immunization and challenge on the production of inflammatory cytokines (TNF-α and IL-1β). We observed that the stimulus of immunization inhibits the production of TNF-α compared to the infected group where the infection without immunization causes an increase in it. Thus, it is possible that the coexistence of selected cytokines produced by our immunization model may provide a highly effective immunological

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environment for the production of IgA, IgG and pIgR as well as a strong activation of the PMN in mucosal effector tissue such as nasal passages.

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1. Introduction

Within the genus *Naegleria*, comprised of free-living ameboflagellates that exist in diverse habitats worldwide, only *Naegleria fowleri* is associated with human disease (Marciano-Cabral, 1988, 2009; Visvesvara et al., 2007). This species is the etiologic agent of primary amebic meningoencephalitis. The amoeba attacks the body through the nasal cavity and invades the brain, causing a rapidly progressing disease that usually results in death within 3–7 days. Most *N. fowleri* infections occur in children and young individuals who have recently swum in warm fresh water (Yoder et al., 2012). The experimental *N. fowleri* infection in mice closely resembles human meningoencephalitis. Previous studies performed by Rojas-Hernandez et al. (2004a,b) have shown that nasal antibodies (IgA e IgG) are induced followed by intranasal immunization with *N. fowleri* lysates plus Cholera toxin. Such mucosal antibodies levels seem to protect against *N. fowleri* infection increasing survival up to 100% in mice challenged with a lethal dose of the amoeba. In the same work, intranasal immunization with CT alone conferred a protection of 60%. The high protection rates achieved by administration of CT alone, as well as the fact that mice which survived *N. fowleri* infection and which had been treated with the adjuvant alone, did not elicit significant antibody responses to *N. fowleri* suggesting that CT can stimulate innate immune mechanisms. Accordingly, it has been reported that CT administration alone confers a protective adjuvant effect against influenza (Matsuo et al., 2000). This supports the notion that CT can stimulate innate immunity. These results indicate that both acquired and innate immune systems may play a role in the host defenses against *N. fowleri* infection.

On the other hand (Jarillo-Luna et al., 2008), indicate that intranasal immunization with amoebic lysates plus Cry1Ac as adjuvant causes an increase in areas with metaplasia in the olfactory epithelium, allowing the secretion of IgA. As a result, IgA antibodies were found interacting with trophozoites in the nasal lumen, and there was a marked increase of IgA in the metaplasic epithelium. They suggest that the IgA response increased induced in the nasal mucosa by immunization probably impedes both amoebic adhesion and subsequent invasion of the parasite to the nasal epithelium.

In another study, using Knockout mice, we suggested that STAT6-dependent humoral immunity plays a critical role in the protection against *Naegleria* infection (Carrasco-Yepez et al., 2010). In this work, we proposed that immune humoral response is crucial to induce vaccine protection against *N. fowleri* meningoencephalitis and that the adjuvant Cry1Ac activity could also induce antibody response.

The specific antibody response against *N. fowleri* in the lumen of mice immunized with *N. fowleri* lysates plus CT or Cry1Ac is closely related with the IgA transport from the lamina propria to the lumen. It has been proposed dimeric IgA, with an incorporated J chain, is the predominant antibody isotype expressed by B cells in the mucosal lamina propria. It is actively transported by the epithelial polymeric Ig receptor (pIgR) and released into mucosal secretions as secretory IgA (SIgA), a complex of dimeric IgA with a bound secretory component (the extracellular domain of the pIgR) (Phalipon and Corthesy, 2003). Luminal SIgA is believed to interfere with pathogen adherence to mucosal epithelial cells, a process called immune exclusion (Pilette et al., 2001).

Furthermore, it has been described that intranasal immunization with CT induced increased in the production of IgA and IgG nasal, IgG systemic, IL-4, and IL-10 and increase protection (Foss and Murtaugh, 1999; Sheoran et al., 2002; Tsuji et al., 2004). But, there are no report which describes in an *in vivo* model the role of pIgR and the host cytokine profile during pathogenesis and protection of *N. fowleri* meningoencephalitis.

In this work immunization with CT alone or coadministered with lysates of *N. fowleri* modulates the gene expression pattern of some cytokines which probably are involved in the IgA, IgG antibodies secretion, polymeric immunoglobulin receptor (pIgR) regulation, as well as the interaction in the lumen of the nasal cavity of mice the IgA and IgG antibodies and polymorphonuclear cells with trophozoites of *N. fowleri.*

2. Materials and methods

2.1. Animals

All protocols were approved by the Animal Care and Use Committee of the Superior Medicine School. All mice used in this study were 8–12 weeks of age and weighing 25–30 g. They were handled in accordance with Mexican federal regulations for animal experimentation and care (NOM-062-ZOO-1999, Ministry of Agriculture, Mexico City, Mexico) and approved by the Institutional Animal Care and Use Committee. The maintenance of amoebic virulence and obtaining of the antigens were performed according to a method described in a previous work (Rojas-Hernandez et al., 2004a,b).

2.2. Immunization and challenge with live amoebae

Immunization scheme was performed according to a method described in the same paper (Rojas-Hernandez et al., 2004a,b). Each experimental group contained 18 animals, to which four antigen doses were applied on days 1, 7, 14 and 21. The antigens applied to each group were (i) amoebic lysates (100 μg of protein) plus 2 μg of Cholera toxin (CT; Sigma–Aldrich), and (iii) 2 μg of CT alone. Control mice received 30 μL of PBS. After the last immunization mice were challenged with 5×10^4 live virulent *N. fowleri* trophozoites in 30 μL of PBS, which was administered intranasally. All mice were killed by decapitation 24 h post-challenge. For Western blot and real time PCR assays, pooled cell suspensions from nasal passages were obtained as described previously (Hiroi et al., 1998).

2.3. Nasal fluid collection and antibodies levels

Nasal fluids were collected and anti-*N. fowleri* antibodies level in mucosal samples were determined by an indirect enzyme-linked immunosorbent assay (ELISA) for all our experimental groups following the methodology described by Rojas-Hernandez et al. (2004a,b). IgA, IgG1 and IgG2a were determined using100 μL of goat anti-mouse antiimmunoglobulin anti-IgA (ZymedLaboratories, San Francisco, Calif.), or horseradish peroxidase-labeled goat anti-mouse IgG1 or IgG2a secondary antibodies (Zymed Laboratories). The absorbance at 492 nm (A_{492}) was measured in a Multiscan Ascent (Thermo Labsystems) microplatereader.

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