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Vaccination with intestinal tract antigens does not induce protective immunity in a permissive model of filariasis

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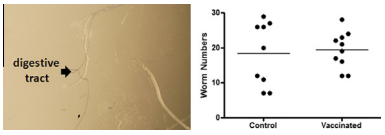
HIGHLIGHTS

- *L. sigmodontis* intestinal antigens (GutAg) exhibit qualities of hidden antigens.
- Infected mice develop allergy associated immune responses to GutAg.
- Vaccinated mice develop GutAg specific IgG1 and IgG2A antibodies.
- Mice vaccinated with GutAg are not protected from challenge infection.

GRAPHICAL ABSTRACT



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ABSTRACT

Antigens obtained from the intestinal tract of filarial nematodes have been proposed as potential safe and effective vaccine candidates. Because they may be 'hidden' from the immune response during natural infection, yet accessible by antibodies induced by vaccination, intestinal antigens may have a low potential for eliciting allergic responses when vaccinating previously infected individuals. Despite prior promising data, vaccination with intestinal antigens has yet to be tested in a permissive model of filariasis. In this study we investigated the efficacy of vaccination with filarial intestinal antigens in the permissive *Litomosoides sigmodontis* BALB/c model of filariasis, and we evaluated the extent to which these antigens are recognized by the immune system during and after infection. Infected BALB/c mice developed lower IgG antibody responses to soluble intestinal antigens (GutAg) than to soluble antigens of whole worms (LsAg). Similarly, GutAg induced less proliferation and less production of IL-4 and IFNγ from splenocytes of infected mice than LsAg. In contrast to these differences, active infection resulted in equivalent levels of circulating GutAg-specific IgE and LsAg-specific IgE levels. Consistent with this, basophil activation, as assessed by flow cytometric staining of intracellular basophil IL-4 expression, was equivalent in response to GutAg and LsAg. Vaccination with GutAg adsorbed to CpG/alum induced GutAg specific IgG1 and IgG2A production, with GutAg specific IgG titers greater than 5-fold higher than those measured in previously infected animals. Despite this response to GutAg vaccination, vaccinated mice harbored similar parasite burdens 8 weeks post infection when compared to non-vaccinated controls. These studies demonstrate that soluble antigens obtained from the intestinal tracts of *L. sigmodontis* have some qualities of 'hidden' antigens, but they still sensitize mice to allergic reactions and fail to protect against future infection when given as a vaccine adsorbed to alum/CPG.

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1. Introduction[1]

Filarial nematodes cause diseases such as African River Blindness and Elephantiasis, debilitating conditions that have severe economic, psychological, and social impacts on affected individuals (Babu et al., 2002, 2006, 2009). Similarly, zoonotic nematodes such as *Dirofilaria immitis* and *Haemonchus contortus* cause severe pathological sequelae in pets and livestock (Getachew et al., 2007; McCall et al., 2008). While there are medications to treat these infections, there are currently no vaccines to prevent them. Filarial vaccines could provide a cost effective tool for aiding in the control of these diseases.

Development of vaccines against parasitic filarial worms is complicated by two major issues. The first challenge is the lack of natural sterilizing immunity. In susceptible hosts, the immune responses that develop during infection are generally inadequate to clear the parasite. Indeed, filarial worms often survive for years within permissive hosts despite the development of robust antifilarial immune responses (McCall et al., 2008). Furthermore, in many models, these immune responses provide little or no protection against future infection (Denham et al., 1972, 1984; Klei et al., 1990; Kowalski and Ash, 1975).

The second major challenge to filaria vaccine development is the risk that vaccination would elicit allergic responses in exposed individuals. Like other helminths, filariae typically induce a type 2 shifted immune response with production of high levels of parasite specific IgE (Diemert et al., 2012; Maizels et al., 2004). Thus, a vaccine comprised of parasite antigen has the potential to induce allergic reactions in individuals that are currently or have previously been infected with a filarial worm (Diemert et al., 2012).

The use of "hidden" antigens, antigens which are not typically targeted by the immune system during the normal course of infection, could potentially overcome both of these obstacles. Interestingly, prior studies have suggested that intestinal tracts of adult nematodes may contain exactly such antigens. This concept has been best demonstrated with *Haemonchus contortus*, a nematode infection of sheep. Although infection elicits relatively low antibody levels to the intestinal tract of *H. contortus*, vaccination with intestinal antigens induces substantial protection against challenge infection (Cachat et al., 2010; Smith, 1993). Antibodies from vaccinated animals were shown to disrupt the parasite's hemoglobinase activity, suggesting that protection occurs by inhibiting parasite metabolic activity (Ekoja and Smith, 2010). In addition to the work carried out using fractions of intestinal antigens, vaccination with individual digestive tract antigens has also shown protection in many animal models of helminth infection (Acosta et al., 2008; Chlichlia et al., 2001, 2003; Dalton et al., 2003; Loukas et al., 2004; Pearson et al., 2009, 2010; Zhou et al., 2010).

In the field of filariasis, vaccination with intestinal antigens has only been tried with the mouse model of *D. immitis* infection. Although dogs infected with *D. immitis* were shown to produce only low level antibody responses to *D. immitis* intestinal antigens, presumably because these antigens are sequestered within the worm (McGonigle et al., 2001), vaccination of mice with *D. immitis* intestinal antigens induced high titer antibody responses to these antigens and resulted in increased protection against challenge infection (McGonigle et al., 2001). While exciting, this study was limited by the use of *D. immitis* challenge into mice. *D. immitis* survives for only a short period of time in mice and has never been shown to complete its lifecycle in this host (Abraham et al., 1988; Zielke, 1977). For this reason, it is not clear if the protection garnered through vaccination was simply the result of an accelerated immune response to *D. immitis*, or if a truly novel protective immune response was produced through vaccination.

The purpose of this study was to investigate the vaccine potential of filarial intestinal antigens using the permissive *Litomosoides sigmodontis* BALB/c model of infection, the only murine model of filariasis in which infective-stage L3 larvae develop into adults that release microfilariae in fully immunocompetent mice (Hoffmann et al., 2000). *L. sigmodontis* is a good model for investigating vaccine approaches for filariasis because the immune responses which develop in infected mice closely mimic those observed in humans (Babayan et al., 2003; Hoffmann et al., 2000; Taylor et al., 2005). Additionally, enumeration of adult worms is reliably complete as over 95% of the adult worms can be recovered from the pleural cavity.

Specific goals of this study were to 1) determine the extent to which intestinal antigens of *L. sigmodontis* (GutAg) are immunologically "hidden" during infection of mice, and 2) to test whether vaccination with GutAg confers protection against challenge infection .

2. Materials and methods

2.1. Infection with L. sigmodontis

Infectious larvae of *L. sigmodontis* were obtained by performing a pleural lavage on jirds 4 days after exposure to mites harboring infectious stage larvae as previously described (Hubner et al., 2009). Briefly, Jirds were euthanized with CO_2, the peritoneal cavity was opened, and a small incision (0.5 cm) made on the ventral aspect of the diaphragm. A transfer pipette was used to flush the pleural cavity repeatedly with 10 ml warmed (37 °C) RPMI (Mediatech Inc., Manassas, VA, USA). L3 larvae were then counted using a dissecting microscope, aspirated with a 20 μl micropipettor, and placed into a syringe. 40 L3 in 100 μl of RPMI were injected subcutaneously with a 22 gage needle into the nape of the neck of 5–8 week old female BALB/c mice.

2.2. Production of soluble antigen extracts from whole worms (LsAg) and worm intestines (GutAg)

Adult female worms were obtained from the pleural and peritoneal cavities of infected jirds. These were separated and maintained in worm culture media until dissection, up to four days using 12 well culture plates (5 worms in 3 ml/well). Worm culture medium consisted of RPMI (Mediatech Cat 15-040-CV), 1% glucose (Sigma–Aldrich Co. St. Louis, MO, USA), 100 I.U/ml Penicillin (Mediatech Inc., USA), 100 μg/ml streptomycin (Mediatech Inc., USA), and 25 mM HEPES (Mediatech Inc., USA).

Worm dissections were carried out in PBS in a Petri dish using fine tipped forceps and a dissecting microscope. Two sets of forceps were used to grasp the cuticle. Pressure was applied in opposing directions causing the cuticle to tear. The cuticle was then slowly peeled away from the internal structures. The intestinal tract was then separated from the two uterine tubes and placed in ice-cold PBS. The intestines were stored at −20 °C. Soluble GutAg (GutAg) and soluble antigen from adult worms (LsAg) were prepared by placing purified intestinal tracts or whole worms (male and female) into "D" tubes from BioPulverizer System 1 (MP Biomedicals Cat 6750–200) and run on a FastPrep-24 system (MP Biomedicals) at 4.0 M/s^2 for 20 s. After mechanical homogenization, samples were centrifuged at 16,100*g* for 1 min, supernatant saved, and the pellet resuspended in another 1 ml PBS. The fast-prep run, centrifugation, and removal of supernatant was repeated on the resuspended pellet. The saved supernatants were then combined, centrifuged at 15,000*g* for 5 min, and the final supernatant re-

[1] GutAg- Soluble fraction of antigens obtained from the digestive tract of adult *L. sigmodontis*LsAg- Soluble fraction of antigens obtained from adult *L. sigmodontis*PI-Post Infection.