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Basophils help establish protective immunity induced by irradiated larval vaccination for filariasis☆

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ABSTRACT

Basophils are increasingly recognized as playing important roles in the immune response toward helminths. In this study, we evaluated the role of basophils in vaccine-mediated protection against filariae, tissue-invasive parasitic nematodes responsible for diseases such as elephantiasis and river blindness. Protective immunity and immunological responses were assessed in BALB/c mice vaccinated with irradiated L3 stage larvae and depleted of basophils with weekly injections of anti-CD200R3 antibody. Depletion of basophils after administration of the vaccination regimen but before challenge infection did not alter protective immunity. In contrast, basophil depletion initiated prior to vaccination and continued after challenge infection significantly attenuated the protective effect conferred by vaccination. Vaccine-induced cellular immune responses to parasite antigen were substantially decreased in basophil-depleted mice, with significant decreases in $CD4^+$ T-cell production of IL-4, IL-5, IL-10, and IFN-γ. Interestingly, skin mast cell numbers, which increased significantly after vaccination with irradiated L3 larvae, were unchanged after vaccination in basophil-depleted mice. These findings demonstrate that basophils help establish the immune responses responsible for irradiated L3 vaccine protection.

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1. Introduction

Filariae are vector-borne tissue-invasive parasitic nematodes. As the agents of diseases such as elephantiasis and river blindness, they cause significant pain and suffering worldwide. Lymphatic filariasis causes lymphedema in 15 million people and urogenital swelling in 25 million [1], and onchocerciasis is the 4th leading cause of preventable blindness [2]. While there are important ongoing efforts to control these diseases through mass drug administration, development of anti-filarial vaccines would greatly aid our ability to decrease the prevalence of these infections [3].

A significant obstacle to rational development of filaria vaccines is an incomplete understanding of the immunological mechanisms capable of eliminating these parasites. To date, one of the most effective approaches used to induce protective immunity in animal models of filariasis is vaccination with irradiated L3 larvae. First demonstrated effective in *Brugia malayi* infection of rhesus monkeys in 1969 [4], vaccination with radiation-attenuated L3 stage larvae has been shown to be effective in numerous animal models of filariasis [5–10]. Vaccination with irradiated larvae results in development of type 2 immune responses, with production of parasite-specific IgE, increased release of IL-4 and IL-5, and enhanced eosinophilia after infection [11–13].

Recently, basophils have become increasingly recognized as being important amplifiers of type 2 immune responses during helminth infections [14–16]. Basophils are circulating granulocytes that are major contributors of IL-4 and are primarily activated by cross-linking of IgE antibodies bound to their cell surface by high affinity IgE receptors [17]. By upregulating CD40L on their cell surface and releasing IL-4 upon activation, basophils are capable of both driving $CD4^+$ T-cells toward a Th2 phenotype and of triggering IgE isotype switching in B cells [18,19]. Basophils are also thought to amplify type 2 responses by release of both IL-13 and TSLP [20,21]. Basophils are a major source of IL-4 in patients infected with filariasis [22], and depletion of basophils during primary infection of mice infected with the rodent filaria *Litomosoides sigmodontis* results in decreased parasite-specific IgE and parasite antigen-driven IL-4 production from $CD4^+$ T-cells [23].

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In addition to amplifying type 2 immune responses, basophils can have important effector cell functions. Activation of basophils results in the immediate release of pre-formed inflammatory mediators such as histamine, leukotriene C4, and antimicrobial peptides, as well as subsequent release of several cytokines and chemokines [24].

To date, no studies have evaluated the role basophils may have in protective vaccine regimens for filariasis. While most studies demonstrate that basophils are not protective against primary helminth infections (reviewed in [14]), a recent study demonstrated that basophil-deficient mice exhibit impaired parasite clearance after secondary infection with the intestinal nematode *Nippostrongylus brasiliensis* [25].

The goal of this study was to assess whether basophils are important to establish the immune response to irradiated larval vaccination in filariasis. To test this, we assessed the protective efficacy of L3 vaccination against challenge infection in mice depleted of basophils at different timepoints. We utilized *L. sigmodontis*, a filariasis model in which parasites develop to maturity in immunocompetent BALB/c mice [26]. Our results demonstrate that basophils are necessary at time of immunization to establish the immune responses responsible for vaccine-mediated protective immunity.

2. Materials and methods

2.1. Mice and parasites

Female BALB/c mice (NCI Mouse Repository, Frederick, MD) were maintained at the Uniformed Services University (USU) animal facility. Experiments were performed with mice between 5 and 8 weeks of age under a protocol approved by the USU Institutional Animal Care and Use Committee. Infectious-stage L3 larvae from *L. sigmodontis* were isolated by lavage from the pleural cavity of four-day infected jirds (*Meriones unguiculatus*, obtained from TRS Laboratory Inc., Athens, GA) as previously described [27]. BALB/c mice were vaccinated with three weekly subcutaneous injections of 25 irradiated L3 larvae (450 Gy, cobalt 60 irradiator) in media (RPMI-1640, Mediatech, Herndon, VA). Two weeks after the last vaccination, mice were challenged by subcutaneous injection of 40 L3 larvae. Adult parasites were enumerated after physical extraction from the pleural cavity of mice that had been infected for 4 weeks.

2.2. Basophil depletion

For in vivo basophil depletion, Ba103, a rat monoclonal IgG2b antibody that recognizes CD200R3, was obtained as described before [28] and injected weekly into mice at a concentration of 50 μg i.p. Although CD200R3 is present on both basophils and mast cells, prior studies have shown that administration of Ba103 results in almost complete depletion of basophils without affecting skin or peritoneum mast cell numbers [23,28]. Control mice were given i.p. injections of rat IgG2b isotype control antibody (BD Biosciences, San Jose, CA).

2.3. Flow cytometric detection of basophils and eosinophils

Whole blood (100 μl) was aliquoted in 5 ml polyprolylene round-bottom tubes (BD Falcon). Red blood cells were lysed and leukocytes fixed with a whole blood lysing reagent kit (Beckman Coulter, Galway, Ireland). Cells were washed twice with 2 ml of phosphate-buffered saline (PBS, Mediatech) and centrifuged at $500 \times g$ for 5 min. Supernatants were aspirated and cells resuspended in 100 μL of 1% BSA/PBS followed by incubation at 4 °C for 1 h. Cells were stained with anti-IgE FITC (R35-72), anti-CD4 PerCP (RM4-5) and anti-B220 PerCP (RA3-6B2) to identify basophils; or SiglecF PE (E50-2440), CD45 FITC (30-F11) and CD11c APC (HL3) to identify eosinophils. All the antibodies were purchased from BD Pharmingen. Cells were washed and resuspended in 200 μL of PBS for analysis using a BD LSR II Optical Bench flow cytometer.

2.4. L. sigmodontis antigen (LsAg)

Soluble LsAg was made from adult male and female *L. sigmodontis* parasites as previously described [23]. Although there are no L3 stage parasites used in the production of LsAg, antibody and cellular immune responses induced by L3 stage parasites are reactive to LsAg [29].

2.5. Parasite specific IgE ELISA

Blood was collected from mice by cardiac puncture and analyzed for LsAg-specific IgE by colorimetric ELISA as previously described [23].

2.6. Cytokine quantification and proliferation assays

Splenocytes were resuspended in ACK Lysing buffer (Quality Biological, Inc., Gaithersburg, MD) to lyse red blood cells. Cells were washed and then resuspended in Iscove's Dulbecco modified medium (Mediatech) supplemented with 10% fetal calf serum (Valley Biomedical, Winchester, VA), 1% L-glutamine (Mediatech), 1% insulin–transferrin–selenium medium (Invitrogen Inc., Carlsbad, CA) and 80 μg/ml gentamicin (Quality Biological, Inc.). $CD4^+$ cells and $CD11c^+$ cells were isolated from splenocytes by magnetic cell sorting (Miltenyi Biotec, Auburn, CA). $CD4^+$ cells were plated at 2×10^6 cells/ml along with 2×10^5 dendritic cells/ml isolated from naïve mice. Cells were stimulated with 20 μg/ml LsAg or 5 μg/ml α-CD3 (eBioscience, San Diego, CA). After 3 days, supernatants were collected and assayed for IL-4, IL-5, IL-10 and IFN-γ using ELISA kits (eBioscience). $CD4^+$ cell proliferation was quantified by chemiluminescence after 3 day culture utilizing a BrdU incorporation immunoassay (Roche Applied Science, Mannheim, Germany).

2.7. Skin histology

Two weeks after vaccination, mice were euthanized and a piece of skin in the area of injections removed from each mouse. Skin samples were fixed in 10% formalin (Sigma–Aldrich). Hematoxylin–eosin and Toluidine-blue stained slices were observed for eosinophils and mast-cells, respectively, by a pathologist (B.M) blinded to the intervention group.

2.8. Statistical analysis

Statistical analyses were performed with GraphPad Prism 4 statistics software (GraphPad Software, San Diego, CA). Differences between groups were performed by Kruskal–Wallis Test followed by Dunn's Multiple Comparison Test. p-values < 0.05 were considered significant. Data are expressed as mean value ± SEM. All experiments were conducted twice with a minimum of four mice per group for each trial. Figures include combined data from both trials for each experiment.

3. Results

3.1. Basophil depletion after vaccination of mice with irradiated L. sigmodontis larvae does not reduce protection against L. sigmodontis challenge

Vaccination of mice with subcutaneous injection of irradiated *L. sigmodontis* larvae typically confers >70% protection against