SHORT REPORT: GRANULOMATOUS INFLAMMATORY RESPONSE TO RECOMBINANT Filarial PROTEINS OF Brugia SPECIES


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Abstract. The lymphatic inflammatory response in Brugia-infected jirds peaks early during primary infections and then decreases in severity as judged by the numbers of lymph thrombi present within these vessels. Antigen-specific hypersensitivity reactions in these animals was measured by a pulmonary granulomatous inflammatory response (PGRN) induced by somatic adult worm antigen (SAWA)-coated beads, and by cellular proliferative responses of renal lymph node cells. The kinetics of these responses temporally correspond to lymphatic lesion formation. The importance of any single antigen to the induction of this inflammatory response has not been elucidated. In this study, the PGRN was used to measure the cellular immune response to four recombinant filarial proteins during the course of a primary B. pahangi infection. These proteins were BpL4, glycoprotein (glutathione peroxidase) gp29, heat shock protein (hsp) 70, and filarial chitinase. All were fusion proteins of maltose-binding protein (MBP). Control beads included those coated with diethanolamine (DEA), SAWA, or MBP. The measurements of PGRN were made at 14, 28, 56, and >150 days postinfection (PI) in infected jirds, in jirds sensitized with SAWA, and in uninfected jirds. The secretory homolog of glutathione peroxidase gp29 was the only recombinant protein tested that induced a significantly greater PGRN (P < 0.05) than controls. This was seen at 28 days PI. These observations indicate that gp29 may be part of the worm antigen complex that induces an early inflammatory response, a response similar to that observed with SAWA. These studies indicate that this approach is useful in investigating the functional ability of specific proteins in the induction and down-regulation of immune-mediated inflammatory responses elicited by filarial parasites. Absence of a granulomatous response to the other recombinant proteins used may be related to the nature and sensitivity of the assay used or the character of recombinant proteins tested.

The life cycle of filarial nematodes is a complex process of growth, molting, and differentiation that occur within a suitable host involving diverse morphologic, physiologic, and antigenic modification. Identification of specific filarial molecules, particularly proteins, that induce protective or pathogenic immune responses during these infections has been a goal of innumerable investigations for decades. The majority of these studies have used serum from various patient groups and soluble extracts of Brugia spp. that have been separated electrophoretically or by chromatographic procedures. Other experiments have examined the in vitro cellular responses to chromatographically fractionated antigens. Although these studies have detected specific differences in responses to some separated proteins by clinically defined patient groups, they failed to identify molecules of specific importance to immune-mediated pathology. However, a purified filarial protein and its recombinant form, a homolog of γ-glutamyl transeptidase, has been shown to induce IgE antibody in vitro and to selectively recognize this antibody isotype in serum and bronchoalveolar fluids of patients with tropical pulmonary eosinophilia, suggesting a role for such antigen(s) in the induction of filarial pathogenesis. With the advent of recombinant DNA technologies, a growing number of proteins have been cloned and their putative biologic functions identified. A number of these have been examined for the immunologic activity using serum or tissue fractionated proteins. A number of these have been shown to induce protective resistance in animal models of this infection. However, the potential significance of recombinant molecules in the induction of pathologic responses has not been experimentally investigated.

The objective of these experiments was to examine the potential role of a few, well-defined recombinant filarial proteins in the induction of filarial mediate inflammatory responses. The Brugia pahangi-jird model of lymphatic filariasis was used for this purpose. In this system, primary infections of B. pahangi induce a granulomatous inflammatory response within and around lymphatic vessels that is subsequently down-regulated. This inflammatory response can be systemically measured by determining granuloma areas that form around Sepharose 4B (Sigma, St. Louis, MO) beads coated with parasite proteins and subsequently elaborized in the lungs. A differential induction of this granulomatous response in infected animals has been demonstrated using high-performance liquid chromatography fractions of soluble worm extracts. More recently, the kinetics of lymphoproliferative responses of cells from renal lymph nodes that drain lymphatics harboring parasites have been shown to mimic that of the pulmonary granulomatous inflammatory response (PGRN).

The four recombinant filarial proteins selected for use in this study were based primarily on their potential importance to the host-parasite relationship and their availability. These four proteins were Brugia glycoprotein gp29, BpL4, heat shock protein (hsp)70, and chitinase. Glycoprotein gp29 has been found on the surface of parasites and is released into culture medium. This molecule is highly conserved within the filariae and has been shown to be a glutathione peroxidase. It may be involved in the regulation of local immune and inflammatory responses to the parasites. BpL4 is a 15-kD segment of a glycoprotein, gp15/400, that has a repetitive molecular structure. It is found in all life cycle stages and is secreted in vitro. This protein is structurally related to the nematode polyallergins found in many nematode species, and has been suggested to be involved in modulation of the host immune response.
shock protein 70 is constitutively expressed in all filarial life cycle stages and appears to be a dominate immunogen in infected individuals that were examined for antibodies to this protein. Although no direct link between antibody responses to this protein and pathology have been established, the widespread response of patients to it suggests that it may induce inflammatory responses upon the death of adult parasites or microfilariae. The filarial chitinase cloned from *Brugia* has been suggested to be important in the immune mediated elimination of microfilariae, and has homology to a 43-kD protein that is recognized by immune sera of putatively immune patients. Similar protective immune reactions may be relevant to the induction of inflammatory events in the *Brugia*-jird model.

The hypothesis that a PGRN to recombinant filarial protein is initiated and subsequently down-regulated during infection was tested by comparing the kinetics of this response around the recombinant filarial proteins gp29, BpL4, chitinase, hsp70, and maltose-binding protein (MBP)–coated beads and to those of somatic adult worm antigen (SAWA) embolized in the lungs. The methods used to couple the beads were essentially followed as previously described. To confirm that these proteins were bound to the beads, protein determination on beads coated with these proteins was performed with Bio-Rad (Hercules, CA) protein detection assay. The absorbance values indicated that similar amounts of recombinant proteins and MBP were coupled to the surface of all coated beads. Beads coated with diethanolamine (DEA) showed no protein binding.

Two experiments were conducted using this assay. The significance of differences in granulomas was tested by using one-way analysis of variance and all pairwise multiple comparisons were made using the Student-Newman-Keuls method and/or the method of Dunn. The differences between the groups were considered significant when the *P* values were < 0.05.

In the first experiment, groups of five jirds at 30, 60 and 150 days post-infection (PI) were used. Uninfected, age-matched jirds served as negative controls. Groups of these jirds were inoculated with beads coated with gp29, BpL4, SAWA, or DEA. All animals were maintained according to the guidelines of the Division of Laboratory Animal Medical Ethics guidelines (School of Veterinary Medicine, Louisiana State University) and the National Institutes of Health guidelines in the *Guides for the Care and Use of Laboratory Animals*. In the first experiment, response to SAWA reached maximum at 30 days PI and began to decrease at 60 days PI, and were not significantly different from DEA controls at 150 days PI (Figure 1). The response to gp29 was significantly greater than in the controls (*P* < 0.05) at 30 days PI, a time point when the fourth-stage larvae (L4) begin to molt and mature to L5 (Figure 1). No significant response to BpL4 was observed.

In a second set of experiments, the kinetics of the PGRN was further tested at 14 and 28 days PI, which correspond to the early developmental stages of the infection and at 56 days PI, a point when the microfilariae begin to appear in circulation. Jirds were inoculated with beads coated with BpL4, gp29, hsp70, chitinase, or the fusion partner of these recombinants, MBP. The SAWA– and DEA-coated beads were inoculated into infected (n = 5/group) and uninfected jirds (n = 5/group) at these time points and served as controls. Beads coated with different recombinant proteins, SAWA, or DEA were also inoculated into groups of jirds (n = 5/group) presensitized intraperitoneally with a single inoculation of 100 mg of SAWA in phosphate-buffered saline 14 days prior to the necropsy.

The results of this second experiment are similar to those of experiment 1 (Figure 2). Of the four filarial recombinants tested, only gp29 induced a significant inflammatory response, and this was again seen only at 28 days PI, a time point following L3 and L4 development, yet prior to the maturation of adult worms. The kinetics of the response to gp29 is different from that of SAWA in that a significant response was not seen early during the infection at 14 days PI. However, gp29 gene expression has been demonstrated...
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Figure 2. Sizes of pulmonary granulomatous inflammatory responses (in mm²) around beads coated with recombinant proteins BpL4, gp29, chitinase, and heat shock protein (hsp)70, maltose-binding protein (MBP), soluble adult worm antigen (SAWA), and diethanolamine (DEA) three days after embolization in the lungs of Brugia pahangi-infected, uninfected, and SAWA-sensitized jirds at 14, 28, and 56 days postinfection (DPI). Bars show the mean ± SD. *P < 0.05, significantly different from others in the group.

in all stages of parasite development. It can be speculated that a sufficient exposure of the host to the expressed protein does not occur until after 14 days PI. A significant response to the recombinant proteins including gp29 was not seen in the SAWA sensitized animals, suggesting that the difference in the magnitude of the response induced by recombinant gp29 and SAWA in the PGRN may be related to the differences in the number of cells responding to a single protein compared with the number that recognize the many immunogenic molecules present in SAWA.

Initial studies indicate that the lymphatic granulomatous lesions are immune mediated, and correlate with down-regulation of the PGRNs. Both the PGRN and lymphatic lesions have been shown to be a cell-mediated response. The phenotypic character of these responses has not been clearly defined but is mediated by cells and not by serum, and may be associated with the production of specific cytokines. The results presented here indicate that gp29 is capable of eliciting a similar immune response that induces this type of lesion. Antibody responses to gp29 have been detected early during infection of cats with B. pahangi and of monkeys with Wuchereria bancrofti. However, the isotypic character of these antibodies is not known and cellular responses of animals or human patients to this protein have not been described. The potential reasons for the absence of detectable cellular responses to other filarial recombinant molecules in this animal model are unclear and numerous. It is possible that these proteins do not induce the immune effectors required for this response. Nonetheless, it can be hypothesized that filariasis patients showing signs of chronic lymphatic inflammation would respond to proteins such as gp29 in cell proliferation assays and induce the production of inflammatory cytokines that may exacerbate the disease.

At this juncture, it is important to consider that the immune response elicited by the recombinant filarial proteins may differ from those elicited by the native proteins derived from the surface or secretory component of the parasite. Interestingly, certain mouse B and T lymphocytes that are reactive to the recombinant protein gp15/400 of B. malayi are completely unresponsive to the native glycosylated molecule. The complexity of immune responses that can be elicited by crude recombinant proteins and those that are purified from bacterial products clearly may be different. Measurement of cellular responses using uncleaved recombinant proteins and those that are involved in granulomatous inflammation in Brugia-infected jirds, and further screening of several defined recombinants proteins with different expression vectors is warranted.

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REFERENCES


2. Lammie PJ, Eberhard ML, Lowrie RC, 1990. Differential humoral and cellular immunoreactivity to saline-and detergent-