CHAPTER V

DISCUSSION AND CONCLUSION

Lymphatic filariasis represents a broad spectrum of clinical and parasitological symptoms. There is considerable debate about the underlying etiology and the pathologic mechanisms for the clinical manifestations. Host immune responses stimulated by parasite antigens are thought to contribute to the development of disease (Freedman., 1998). However, immune responses to adult worm extracts are strongly associated with the patient's infection status. While patients with chronic manifestations display the higher levels of antifilarial immunity (Baird *et al.*, 2002), asymptomatic patients with microfilariaemia (Ag+/Mf+) show cellular hyporesponsiveness. However, specific parasite factors that trigger the immune responses associated with disease development in these individuals have not yet been identified.

It has been thought that *Wolbachia* play an important role in the pathogenesis of filariasis with respect to their potential to activate intense innate inflammatory responses (Taylor *et al.*, 2000; Brattig *et al.*, 2001, 2004; Saint Andre *et al.*, 2002), and adaptive immune responses (Punkosdy *et al.*, 2003). The recent study in cytokine profiles against *D. immitis* recombinant *Wolbachia* Surface Protein (rWSP) shows pronounced IFN-γ response, a Th1-related cytokine, but not Th2-related cytokines (Brattig *et al.*, 2004). Therefore, *Wolbachia*-mediated adaptive immune responses are characterized by Th1-type responses, as characteristics of exposure to pathogenic bacteria. In lymphatic filariasis, a state of immunosuppression is characterized by an expression of anti-filarial specific IgG4, and marked Th2 cytokine responses (Kwan-Lim *et al.*, 1990; Ottesen, 1992). In

another word, immunity against *Wolbachia* does not seem to be present as specific immunosuppression.

In this study, we found that a major antibody response to the rWSP in the *W. bancrofti*-infected people is primarily anti-rWSP specific IgG1 and IgG3, but not IgG2 and IgG4 antibodies. This finding was consistently observed in characterization of humoral immune responses to *D. immitis* rWSP (Brattig *et al.*, 2004), and a non-structural *Wolbachia* protein (Fischer *et al.*, 2002). Similarly, finding characterized by total IgG responses against rWSP in another population with lymphatic filariasis has been also reported (Punkosdy *et al.*, 2003).

The observation that active infection with *W. bancrofti* could diminish the immune response to unrelated antigens (Nookala *et al.*, 2004) may apply to the characteristic of the humoral immune response to rWSP in lymphatic filariasis. In this study, we also found that amicrofilaraemia patients (Ag+/Mf-) were significantly more likely to mount anti-WSP IgG3 antibody than microfilaraemia patients (Ag+/Mf+). However, there is no inverse correlation between anti-WSP IgG3 levels and levels of microfilaria in blood circulation. Further study on the WSP-specific T-cell or B-cell clone in asymptomatic microfilaraemic patients will define finer aspects of an immune response against the endobacteria in lymphatic filariasis.

Consistent with previous studies (Pundosky *et al.*, 2003), some of endemic normals were also positive for anti-WSP IgG subclasses. There are at least three hypotheses that may explain the results. The first hypothesis, and perhaps the most difficult to test, is that some degree of cross-reactivity between WSP and unidentified bacterial antigens exists in human populations in areas where the infection is endemic. We consider this hypothesis unlikely given lack of an association between WSP and other

bacterial antigens and the observation that WSP epitopes recognized by endemic normals in this study do not have significant homology to non-*Wolbachia* antigens when compared by BLAST search (data not shown). However, carefully controlled experiments would be needed to rule this hypothesis out.

Alternatively, exposure to other *Wolbachia*-containing filarial nematodes may elicit an anti-WSP antibody response (Bazzocchi *et al.*, 2000; Simon *et al.*, 2003). However, there is no other filarial species in this area. Finally, a third hypothesis is that *Wolbachia* of filarial worms does not represent the only means of human exposure to *Wolbachia* antigen(s). The WSP epitopes primarily recognize by patients with lymphedema and hydrocele are concentrated at the amino- and carboxy-terminal ends of the protein, while the first 13 amino acids of the epitope that are primarily recognized (Jiggins *et al.*, 2002), are located in the second transmembrane domain (Jiggins *et al.*, 2002). Interestingly, mathematical predictions based on the ratio of synonymous and nonsynonymous amino acid substitutions suggest that the transmembrane regions of WSP are not under positive selective pressure in either arthropod or nematode *Wolbachia* (Jiggins *et al.*, 2002). These results suggest that the transmembrane regions of WSP are likely to have the greatest degree of sequence conservation between different strains of *Wolbachia*. In addition to filarial worms, *Wolbachia* bacteria also reside in a number of other invertebrates distributed throughout the world that are known to have contact with humans (Jeyaprakash *et al.*, 2000; Werren and Windsor, 2000). Although it has been reported that human exposure to *Wolbachia* antigens from arthropods does not occur (Zimmer., 2001), this hypothesis has not been empirically tested. In light of the observation that anti-WSP IgG can be detected in human subjects in areas where the infection is not endemic, this hypothesis deserves further consideration.

The recognition of WSP by the human immune system and the association between antibody responses to WSP and chronic filarial disease raise the question of whether *Wolbachia* may play a causative role in the development of filarial disease. Because *Wolbachia* bacteria are located inside the filarial worm, it is likely that *Wolbachia* antigens will come into contact with components of the host immune system only if they are released following worm death. Interestingly, a critical factor in the development of chronic pathology seems to be the death of the adult worm. Therefore, release of *Wolbachia* following worm death would put these bacteria in a potential environment in which *Wolbachia*-specific immune responses may trigger the initial events in the development of chronic filarial disease.

Furthermore, we found that patients with chronic infection were significantly more likely to mount anti-WSP IgG1 antibody than endemic normals (**Figure 6**). Therefore, anti-rWSP IgG1 antibody may be a useful indicator of immunopathologic development in lymphatic filariasis. One other factor that has repeatedly been suggested to play an important role in the progression of filarial disease is lymphatic damage caused by secondary bacterial infections. Recurrent bacterial infections that manifest as acute dermatolymphangioadenitis have been shown to contribute to the development of chronic lymphedema (Dreyer *et al.*, 1999). In addition, patients with lymphedema have been shown to display heightened immune reactivity to bacterial antigens (especially streptolysin O) compared to infection-matched individuals without disease (Baird *et al.*, 2002). This raises the question of the possible association between anti-*Wolbachia* immune responses and immune responses directed at other bacteria. In previous study (Punkosdy *et al.*, 2003), they found there was no difference in levels of serum antibody to various bacterial antigens between anti-WSP^{+} and anti-WSP^{-} women with lymphedema. Therefore, we believe that anti-WSP reactivity is not caused by cross-reaction with other

bacterial infections that occur commonly among persons with lymphedema. Instead, it is likely that *Wolbachia* bacteria are recognized by the host immune system during the initial pathologic events following worm death and that secondary bacterial infections contribute to the progression of disease development only after these events lead to lymphatic stasis and an inability to clear invading organisms. To the extent that filarial pathology is associated with a shift in host response from a Th2- to a Th1-type immune response, an interesting hypothesis is that immune reactivity to *Wolbachia* may trigger this shift in host response to a Th1-like immune response to both filarial and nonfilarial antigens. This heightened inflammatory reactivity to bacterial antigens then may be associated with increased lymphatic damage and skin pathology as disease progresses.

In the absence of longitudinal data, it is unclear whether any of the anti-WSP individuals had ever mounted antibody responses to WSP. Longitudinal data from humans and monkeys (Punkosdy *et al.*, 2001) demonstrate the importance of longitudinal data in analyzing antibody responses to WSP. In both cases, peaks in anti-WSP IgG were temporally associated with the onset of clinically apparent disease, and detectable levels of anti-WSP IgG antibody were transient.

However, longitudinal specimens from humans who develop filarial disease are difficult to obtain, and as a result, it may be impossible to determine the true patterns of antibody responses to WSP by using only cross-sectional data. An alternative approach may be to assay for cell-mediated immune responses to WSP. A critical component of the cell-mediated immune system is the production of memory T cells that can be stimulated with antigens in vitro to mount immune responses similar to those which they would mount in vivo. Considering the importance of T cells in the production of an effective antibody response (i.e., switching of the constant region of the heavy chain to produce IgG isotype antibodies), individuals who mount antibody responses to WSP would be expected

to also display cell-mediated immunity to WSP. Consistent with our previous results, WSP could stimulate inflammatory-type immune responses that may serve as a potential trigger for the development of disease. The elevated antibody reactivity to *Wolbachia* antigens in patients with lymphatic filariasis observed in this study and others (Punkosdy *et al.*, 2003) provides further support for a role for *Wolbachia*- mediated immune responses in disease pathogenesis. Further studies to determine whether *Wolbachia* may play a causative role in the development of filarial disease should focus on the localized immune responses to *Wolbachia* following worm death. Analysis of histologic specimens collected following worm death may help determine whether *Wolbachia* organisms are released following worm death and whether *Wolbachia* organisms or their antigens come into contact with components of the human immune system.