

Immunoglobulin G Antibodies against the Endosymbionts of Filarial Nematodes (*Wolbachia*) in Patients with Pulmonary Dirofilariasis

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The dog parasite *Dirofilaria immitis* can infect humans. Patients with pulmonary dirofilariasis were tested for immunoglobulin G (IgG) antibodies against the surface protein of *Wolbachia*, the bacterial endosymbiont of *D. immitis*. These patients showed significantly higher IgG titers than healthy individuals from areas in which *D. immitis* was endemic as well as areas in which it was not endemic. Titration of anti-*Wolbachia* surface protein IgG could become useful for diagnostic applications.

Dirofilaria immitis is the causative agent of heartworm disease in dogs and cats. The disease is found worldwide in subtropical and temperate areas (8). In dogs and cats, the parasite develops to the adult stage in the pulmonary arteries and in the right cardiac chambers (6, 10). The infection is transmitted by several species of mosquitoes that are frequently able to bite both humans and animals. People living in areas of endemicity are thus at risk of infection. Humans are, however, “dead-end hosts,” since larvae do not normally develop into the adult stage in humans. Human pulmonary dirofilariasis develops when the nematode dies, embolizes and travels to the lung, and lodges in a small branch of the pulmonary artery. In these cases, chest radiography shows well-circumscribed, noncalcified or calcified nodules (5, 9, 12).

In areas in which heartworm infection in dogs is endemic, clinically healthy people are frequently found positive for antibodies against *D. immitis* antigens. For example, Prieto et al. (14) recently recorded seroprevalence values ranging from 26 to 37% in three areas of southern Europe. The high percentage of seroprevalence in healthy people in areas of endemicity hampers the serological diagnosis of pulmonary dirofilariasis. Two methods have been proposed for the experimental diagnosis of this disease; they are based on the use of recombinant or native *D. immitis* proteins (13, 18). However, neither method allows clear-cut distinction between healthy humans from areas of endemicity and patients with pulmonary nodules. Serological studies have also shown different antibody profiles in humans: immunoglobulin G (IgG), IgM, and predominantly IgE antibodies against *D. immitis* antigens were detectable in healthy individuals, while in patients with pulmonary lesions, the IgE response was not observed (7, 17). Evaluation of immunoglobulin profiles could aid in diagnosis. However, the IgG, IgM, and IgE responses in healthy individuals vary throughout the year (7, 11).

Filarial nematodes, including *D. immitis*, harbor obligate,

intracellular, gram-negative bacteria belonging to the genus *Wolbachia* (*Rickettsiales*). *Wolbachia* is a stable and abundant component of the body of filarial nematodes (1, 2). It was recently shown that *Wolbachia* surface protein (WSP) induces a specific IgG response in cats infected with *D. immitis* (3) and in monkeys infected with lymphatic filariae (15). In addition, *Wolbachia* appears to play a role in the immunopathogenesis of filarial diseases (4, 16).

So far, all studies which have shown a specific antibody response against *Wolbachia* proteins have been performed with natural hosts of filarial nematodes, with hosts in which the parasite can develop to the adult stage, or after inoculation of hundreds of infective larvae (e.g., see reference 3). Whether an antibody response against *Wolbachia* develops in dead-end hosts under natural conditions (such as for *D. immitis* in humans) is not known. The aim of this study was to investigate the IgG response against a *Wolbachia* protein in humans living in areas in which dog heartworm disease is endemic.

Forty-two serum samples from humans were assigned to the following groups. Group 1 (G1) contains 10 serum samples from patients with pulmonary nodules due to *D. immitis* infection (these samples were kindly supplied by Patrick Lammie, Centers for Disease Control and Prevention, Atlanta Ga.; diagnosis was made by biopsy sampling). Group 2 (G2) contains 18 serum samples from clinically healthy humans living in areas in which heartworm infection is endemic (Po River Valley, northern Italy: 10 samples; Colombian Amazonia, South America: 8 samples) and previously found by an enzyme-linked immunosorbent assay (ELISA) to be IgG positive for *D. immitis* by use of both somatic and excretory or secretory antigens from adult nematodes (14, 19). Group 3 (G3) contains 14 serum samples from healthy humans living in a mountainous area of the province of Salamanca, Spain, where *D. immitis* infection in dogs and mosquitoes has not been recorded; these donors were found by the ELISA to be seronegative for *D. immitis* infection.

The WSP of *D. immitis*, produced in recombinant form and purified as described by Bazzocchi et al. (3), was used as an antigen in an ELISA for the detection of IgG in the 42 serum samples. The ELISA was performed as described by Perera et al. (13), with minor modifications. Briefly, microplate wells

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TABLE 1. ELISA results for recombinant WSP in human sera

Group	OD		SD
	Avg	Range	
G1	0.87	0.58–1.35	0.26
G2	0.35	0.26–0.58	0.11
G3	0.03	0.20–0.39	0.07

were coated with 0.8 µg of recombinant WSP; serum samples were analyzed at a 1:30 dilution, and anti-human peroxidase-conjugated IgG was diluted 1:4,000. The optical density (OD) was measured at 492 nm. The cutoff (0.5) was the OD arithmetical average plus three standard deviations for the 14 serum samples from the clinically healthy blood donors living in the *D. immitis*-free area.

The ELISA results are reported in Table 1. All of the serum samples from patients with pulmonary nodules due to *D. immitis* (G1) showed high ODs that were consistently above the cutoff. Serum samples from healthy donors found serologically positive for *D. immitis* (G2) and from donors living in areas of nonendemicity (G3) had significantly lower ODs (one-way analysis of variance; *F*, 49.04; *P*, <0.0001; degrees of freedom, 39).

Our results show that the IgG response against the WSP of *D. immitis* is consistently detectable only in patients with pulmonary nodules due to the parasite. In healthy blood donors from areas in which *D. immitis* is endemic and who have IgG against somatic and excretory or secretory antigens of adult parasites, the IgG levels against WSP are lower. Only in 3 cases out of 14 were the IgG titers in this group above the cutoff of our ELISA. This result suggests that the surface protein of *Wolbachia* endosymbionts stimulates the host immune system only after the death of preadult worms in the small branches of pulmonary arteries, or at least when the development of *D. immitis* has progressed to a stage at which nematode death can lead to the release of a sufficient amount of bacteria. In any case, our results provide further evidence for the immunological role of *Wolbachia* in filarial infection, with special reference to humans, and also show that IgG titers are related in some way to the clinical status of the patient.

Our results may suggest an interesting method for the serodiagnosis of pulmonary dirofilariasis. Differential diagnosis (e.g., to exclude pulmonary cancer) requires surgical biopsy, and even a reliable serological assay would not necessarily exclude the need for histological analysis. However, a reliable serological test for pulmonary dirofilariasis would aid in the final diagnosis, allowing better treatment of patients and better planning of invasive diagnostic procedures, such as thoracoscopy. Based on our results, titration of anti-WSP IgG appears to be a very promising tool for the diagnosis of pulmonary dirofilariasis, possibly in combination with titration of IgE against *D. immitis* antigens. Of course, examination of additional serum samples from healthy individuals, from patients

with pulmonary nodules, and from patients with different parasitoses is needed before an anti-WSP assay can be proposed as a test for the diagnosis of human dirofilariasis.

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