High Levels of Serum Thromboxane B2 Are Generated during Human Pulmonary Dirofilariosis

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The canine parasite Dirofilaria immitis can infect humans. Patients with pulmonary dirofilariosis develop significantly higher thromboxane B2 levels than healthy individuals living in areas where dirofilariosis is endemic and in areas where dirofilariosis is not endemic. The possible role of Wolbachia bacteria in the appearance of this eicosanoid is discussed.

Heartworm disease caused by Dirofilaria immitis in dogs and cats is distributed in temperate, subtropical, and tropical areas worldwide (7). The infection is transmitted by several mosquito species, most of which are able to feed on both animal reservoirs and humans. Thus, in areas of endemicity, people are at risk of infection. Human dirofilariosis is radiologically characterized by a solitary pulmonary calcified or noncalcified nodule (5, 15). These nodules appear when a migrating immature worm is trapped in a branch of the pulmonary artery and dies, causing coagulation necrosis with fibrosis and infiltration of inflammatory cells (8, 16). Wolbachia symbiont bacteria are a stable and abundant component of the bodies of many filarial species (2). In human D. immitis infections, high levels of immunoglobulin G (IgG) antibodies against the Wolbachia surface protein (WSP) have consistently been detected in patients with pulmonary dirofilariosis, while in healthy donors living in areas of endemicity, the levels of IgG anti-WSP are much lower (18). In addition, Wolbachia bacteria play an important role in the immunopathogenesis of both human filarial diseases (3, 19) and animal dirofilariosis (9). Eicosanoids are lipid mediators that regulate different physiological processes and modulate inflammatory and immunological responses in mammals (4). The effects of some of these eicosanoids antagonize the effects of others. Specifically, the thromboxanes (Txs) constrict blood vessels, suppress cyclic AMP, and promote platelet aggregation, while leukotrienes (LTs) are related to vascular permeability, chemotaxis, and polymorphonuclear leukocyte activation (17). Some of these lipid mediators have been observed in lymphatic filariae (10, 11, 13) and Onchocerca volvulus (4). Clinical studies have demonstrated high concentrations of TxB2 in the plasma of patients with septic and endotoxic shock (1) and in patients with chronic obstructive pulmonary disease (6).

The aim of this study was to investigate the levels of eicosanoids in the sera of patients with pulmonary dirofilariosis, a disease characterized by the inflammatory obstruction of pulmonary arteries and a disease in which the Wolbachia symbiont bacteria stimulate the immune systems of infected individuals. Eighty human serum samples were divided into three groups. Group 1 (G1; n = 10) comprised serum samples from patients diagnosed with pulmonary dirofilariosis caused by D. immitis (these samples were kindly provided by Patrick Lammie from the Centers for Disease Control and Prevention, Atlanta, Ga.). These samples have high levels of specific IgG against WSP, as demonstrated previously (18). Group 2 (G2; n = 40) comprised serum samples from healthy individuals living in zones of endemicity in Spain and Italy with a positive serology (by enzyme-linked immunosorbent assay [ELISA]) for D. immitis antigens but without symptoms (healthy donors from areas of endemicity). Group 3 (G3; n = 30) comprised serum samples from healthy individuals living in an area of nonendemicity in Spain, where D. immitis infection in dogs and mosquitoes has not been recorded; these serum samples had a negative serology (by ELISA) for D. immitis and Wolbachia antigens (healthy controls). The levels of TxB2 and LT B4 in serum samples were analyzed by commercial ELISAs (R&D Systems). Briefly, serum samples were tested at 1:100 and 1:2 dilutions, respectively, for TxB2 and LT B4. The optical densities (ODs) at 405 nm were measured in an Easy Reader (Bio-Rad). The conversion of ODs to μg/ml was carried out by following the instructions of the manufacturers. The intra- and interassay precisions (coefficient of variation [CV]) ranged from 3.6 to 1.6% and from 7.7 to 6.2%, respectively, for TxB2. For LT B4, the CVs were 6.0 to 5.9% and 15.7 to 5.0%, respectively. The nonparametric Kruskal-Wallis test was used for multiple comparisons of the immunologic data. A significant difference was defined as a P value of <0.5, for a confidence level of 95%. For the paired samples, the Dunn test was used. In this case, a P value of <0.5 was considered significantly different.

The results are shown in Fig. 1. TxB2 levels were significantly higher in G1 than in G2 and G3 (P < 0.001). Significant differences in TxB2 levels were also observed between G2 and G3 (P < 0.001). Moderate LT B4 levels were observed only in healthy donors from areas of endemicity and not in individuals with pulmonary nodules or in healthy controls. Significant differences in LT B4 levels were detected between G2 and G3 and between G2 and G1 (P < 0.05) but not between G1 and G3.
Humans are not an adequate host for *D. immitis*. In fact, most infections are caused by larvae inoculated by infected mosquitoes. The positive serology for *D. immitis* antigens observed in these individuals reflects an active or recent infection, but only some infected individuals develop pulmonary nodules (14). Prostaglandin E2 has been detected in the microfilariae of *Wuchereria bancrofti* and *Brugia malayi* (12) and in *Onchocerca volvulus* adult worms (4). Its presence is related to the survival of parasites in immunocompetent hosts, due to the capacity of this eicosanoid to stimulate the Th2-type anti-inflammatory response. This is consistent with the detection, in our work, of this eicosanoid to stimulate the Th2-type anti-inflammatory response. This is consistent with the detection, in our work, of this eicosanoid to stimulate the Th2-type anti-inflammatory response. This is consistent with the detection, in our work, of this eicosanoid to stimulate the Th2-type anti-inflammatory response.

![Graph](http://cvi.asm.org/)

**FIG. 1.** Levels of TxB2 and LTB4 in serum samples from individuals in G1, G2, and G3.

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REFERENCES