Depression of Endothelium-Dependent Relaxation in Aorta From Rats With Brugia pahangi Lymphatic Filariasis

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A role for altered endothelial cell function is emerging in the pathogenesis of disease. We have previously demonstrated that Dirofilaria immitis, the canine heartworm, depresses endothelium-dependent responses and alters the mechanism of relaxation in the in vivo femoral artery of infected dogs. Exposure of rat aorta to the parasite or parasite-conditioned medium selectively depresses endothelium-dependent relaxation. D. immitis is closely related to the major human filarial pathogens. This study was designed to examine the effect of chronic infection with the filarial nematode Brugia pahangi on endothelium-mediated responses of the rat aorta in vitro. We tested the hypothesis that endothelium-dependent responses are depressed in the aorta from rats infected with B. pahangi. Rings of thoracic and abdominal aorta were suspended in muscle baths for measurement of isometric tension. Dose–response relations to norepinephrine, endothelium-dependent dilators (acetylcholine, histamine, and A23187), and nitroglycerin were done. In some experiments, inhibitors of cyclooxygenase (indomethacin and aspirin), guanylate cyclase (methylene blue), and nitric oxide formation (N-nitro-L-arginine methyl ester; L-NOARG) were used. No differences in vascular reactivity were detected in the thoracic aorta. In contrast, endothelium-dependent responses in abdominal aorta of Brugia-infected rats were significantly depressed when compared with control aorta from noninfected rats. Acetylcholine relaxation was further depressed by indomethacin and aspirin. After L-NOARG, acetylcholine relaxation in control abdominal aorta was completely abolished; however, in abdominal aorta of Brugia-infected rats, acetylcholine still caused relaxation. Methylene blue inhibited acetylcholine relaxation in both control and Brugia-infected abdominal aorta; however, relaxation in Brugia-infected aorta was significantly greater than control. This study demonstrates that endothelium-dependent relaxation can be altered by chronic experimental filarial infection in the absence of direct contact between the blood vessel and the parasite. The mechanism of relaxation in the Brugia-infected abdominal aorta appears to be altered when compared with control, suggesting that parasites are capable of modulating vascular reactivity by inducing changes in endothelial cell behavior. The mechanism may involve parasite-induced local inflammation or alterations in endothelial cell metabolism. Understanding how chronic experimental filarial infection alters vascular reactivity may enhance our understanding of the pathogenesis of human filariasis. (Circulation Research 1991;68:1703–1712)

Worldwide, approximately 300 million people and countless animals are infected with filarial parasites.1–5 Despite its prevalence, the pathogenesis of filariasis is poorly under-
*Dirofilaria immitis*, the canine heartworm, provides one of the few naturally occurring model systems available. The parasite is closely related to the major human filarial pathogens, and infected dogs provide a useful experimental model of filarial infections. We previously reported that *D. immitis* infection depresses endothelium-dependent relaxation and alters the mechanism of relaxation in the in vivo femoral artery of infected dogs. In addition, short-term exposure of rat aorta to either *D. immitis* or *D. immitis*-conditioned media specifically depresses endothelium-dependent relaxation. This effect involves a filarial product that is between 100 and 1,000 MW and may be a filarial arachidonic acid metabolite.

In lymphatic filariasis caused by *Wuchereria* and *Brugia* species, the adult parasites reside primarily in the lymphatic system. The adults are closely associated with lymphatic endothelial cells, while vascular endothelial cells are exposed to parasite products and microfilariae. However, in canine heartworm infection, the adult parasites are most commonly found in the vascular system, primarily the right heart and pulmonary arteries. Therefore, the adult parasites, circulating microfilariae, and parasite products are in proximity to vascular endothelial cells. On the basis of these findings with *D. immitis* and in view of the emerging role for altered endothelial cell behavior in the pathogenesis of disease, we postulated that the behavior of vascular endothelial cells may be altered in lymphatic filariasis. The purpose of this study was to examine the effect of chronic experimental filarial infection with *B. pahangi* on endothelial cell-mediated responses of the in vitro rat aorta. Experiments were designed to test the hypothesis that endothelium-dependent responses are depressed in aorta from rats infected with the filarial nematode *B. pahangi* when compared with control aorta from noninfected rats.

This study demonstrates that endothelium-dependent relaxation of the abdominal aorta is specifically depressed in this model of lymphatic filariasis. The results suggest that the interaction between filarial parasites, inflammatory cells, and endothelial cells may be important in the pathogenesis of filariasis, including possibly human elephantiasis.

**Materials and Methods**

**Experimental Model of Lymphatic Filariasis**

There are several experimental hosts for *B. pahangi*, including the Mongolian jird, nude mouse, ferret, PD4 hamster, and inbred strains of rat (Lewis, August, and PVG/C). These hosts show different susceptibility to infection and manifest a variety of pathological abnormalities. We used male Lewis rats because 1) vascular responses in the rat are well characterized; 2) within 8 months of inoculation, 95% of male Lewis rats inoculated with third-stage larvae of *B. pahangi* develop patent (microfilariae-positive) infections with a preponderance of adult parasites in the periaortic lymphatics; 3) blood samples to test for microfilariae are easily obtained from rats; and 4) inoculated Lewis rats develop reasonably similar infection intensities.

Infected rats were obtained from Dr. John McCall through National Institutes of Health Supply Contract AI-02642, US-Japan Cooperative Medical Science Program. One hundred male Lewis rats (8–10 weeks old; Harlan Sprague Dawley Inc., Indianapolis, Ind.) were transported to the University of Georgia, Athens. Fifty of the rats were inoculated with a subcutaneous injection of 75–100 third stage larvae of *B. pahangi*; the other rats served as noninfected controls. Larvae of *B. pahangi* were obtained from *Aedes aegypti* mosquitoes infected 10–12 days previously by feeding on infected jirds. After inoculation, all of the rats were transported to Michigan State University, East Lansing, and housed in the University Animal Care Facilities.

Experiments were done on 49 infected and 47 age-matched male Lewis rats that were 9–18 months postinoculation at the time of the experiments. To minimize any effect of age, infected and control rats were studied on the same day or the same protocol was repeated on 2 successive days. To ensure that inoculated rats had patent infections, blood samples were examined for microfilariae. One inoculated rat without evidence of microfilariae was eliminated from the study.

**Isolated Vessel Studies**

Experiments were done on 2-mm rings of rat thoracic and abdominal aorta. Aortas were removed immediately after deep anesthesia was obtained with an overdose of pentobarbital. Aortas were immediately placed in cold physiological saline solution (millimolar composition: NaCl 130, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 15, glucose 5.5, and EDTA 0.03). The surrounding tissue was removed, and the rings were suspended horizontally in muscle baths (10 or 50 ml) filled with warm physiological saline solution (37°C) and aerated with 95% O₂–5% CO₂. Care was taken not to disrupt the intima. The ring was connected by silk sutures to both a stationary rod in the muscle bath and a force transducer (Grass Instrument Co., Quincy, Mass.). Changes in tension were continuously recorded on a polygraph (Grass model 7D). The rings were stretched to 2 g passive tension and equilibrated for 90 minutes. To determine optimum passive tension, length–tension curves were generated (in thoracic and abdominal aorta of control and *Brugia*-infected rats). These curves demonstrated that 2 g passive tension is in the optimal range of the length–tension relation for all vessels studied.

**Experimental Groups**

Four groups of aortic rings were studied: control thoracic aorta, control abdominal aorta, *Brugia*-infected thoracic aorta (referred to as *Brugia* thoracic
aorta), and *Brugia*-infected abdominal aorta (Brugia abdominal aorta).

**Experimental Protocols**

Dose–response relations to the vasoconstrictor norepinephrine, endothelium-dependent vasodilators (acetylcholine, histamine, and A23187),16–18 and the endothelium-independent vasodilator nitroglycerin16–19 were determined in rings of thoracic and abdominal aorta from control and *Brugia*-infected rats. In some experiments, relaxation responses were examined in the presence and absence of inhibitors of cyclooxygenase (indomethacin and aspirin), guanylate cyclase (methylene blue), or nitric oxide formation (N-nitro-L-arginine methyl ester [L-NOARG]). In additional experiments using *Brugia* abdominal aorta, constrictor responses to norepinephrine, serotonin (5-HT), and prostaglandin E\(_2\) (PG\(_E2\)) were examined in the presence and absence of inhibitors of cyclooxygenase (indomethacin or aspirin).

**Vasoconstrictors.** Dose–response relations to norepinephrine (–10.2 to –6.2 log M, bath concentration) were done. The concentration of norepinephrine necessary to elicit 80% to 90% maximum norepinephrine (–7 log M) constriction was used to preconstrict the rings.

In paired experiments, indomethacin (50 µM) was placed in the bath 30 minutes before any experimental intervention.12,13,17,18,20 Comparisons were made between control and *Brugia* with and without indomethacin. Unexpectedly, indomethacin (50 µM) inhibited norepinephrine constriction in the abdominal aorta. Therefore, additional experiments were done in the *Brugia* abdominal aorta. Maximum constriction to norepinephrine (–6.52 log M) with or without indomethacin (10 or 50 µM) or aspirin (50 µg/ml) was determined. Constriction responses to serotonin (–4.52 log M) with or without indomethacin (10 µM) and PG\(_E2\) (–4.68 log M) with or without indomethacin (10 µM) or aspirin (50 µg/ml) were also evaluated.

**Vasodilators.** For vasodilator studies, all vessels were preconstricted with norepinephrine (–7.0 log M), allowed to reach a steady-state constriction, and then responses to vasodilators were determined. All experiments with endothelium-dependent vasodilators were done with endothelial cells intact, with the exception of four experiments using abdominal aorta from *Brugia*-infected rats, for which endothelial cells were removed by gently rubbing the intimal (luminal) surface with jewelers’ forceps.

Dose–response relations to acetylcholine (–10 to –4.52 log M) were done in thoracic and abdominal aorta of *Brugia* and control rats. In paired experiments, indomethacin (50 µM) was placed in the bath 30 minutes before any experimental intervention.12,13,17,18,20 Comparisons were made between control and *Brugia* with and without indomethacin. Unexpectedly, indomethacin (50 µM) inhibited acetylcholine relaxation in the *Brugia* abdominal aorta. Therefore, additional experiments were done. Relaxation responses to acetylcholine (–8 and –7.52 log M) were determined with and without indomethacin (10 or 50 µM) or aspirin (50 µg/ml). These concentrations were used because they reproducibly caused relaxation responses that were significantly different from maximum relaxation.

In additional experiments L-NOARG was used to inhibit nitric oxide formation.21 Twelve minutes after L-NOARG (–4.82 log M) was added,21 the vessel was preconstricted with norepinephrine, and dose–response relations to acetylcholine were determined. Two minutes after addition of the highest concentration of acetylcholine, L-arginine (–3.82 log M) was added and relaxation responses were examined at 15 minutes.20 Data from experiments with L-NOARG are expressed as percent of maximum relaxation.

In paired experiments, maximum acetylcholine relaxation responses of the abdominal aorta were also evaluated in the presence and absence of methylene blue (10 µM).18,19 To assess acetylcholine relaxation (before the addition of methylene blue), the vessel was preconstricted and the response to acetylcholine (–5 log M) was evaluated. Only vessels with relaxation responses of greater than 45% were used. The vessels were then washed and reequilibrated, and methylene blue was added. Methylene blue caused a constriction of up to 2 g, which returned to control after approximately 60 minutes. Sixty minutes after the addition of methylene blue, dose–response relations to acetylcholine were done. Data are expressed as percent of maximum relaxation with or without methylene blue.

In one series of experiments, endothelial cells were removed from the abdominal aorta of *Brugia*-infected rats, and acetylcholine (–5 log M) relaxation was evaluated. These experiments were done to ensure that acetylcholine relaxation is endothelium-dependent in the *Brugia* abdominal aorta.

Dose–response relations to the calcium ionophore A23187 (–9.72 to –5.24 log M) and histamine (–8 to –4 log M) were also done in both the thoracic and abdominal aorta of control and *Brugia*-infected rats. In preliminary experiments (n=4), thrombin, substance P, and ATP resulted in neither consistent nor dose-dependent relaxation in the abdominal aorta of control or infected rats. However, these vessels did relax to acetylcholine. Therefore, further experiments with these agents were not pursued.

Dose–response relations to the endothelium-independent vasodilator nitroglycerin (–9.59 to –6.11 log M) were determined in both the thoracic and abdominal aorta. Nitroglycerin was used to determine if vascular smooth muscle function and the guanylate cyclase/cGMP system were affected by infection with *Brugia*. A single dose of nitroglycerin (–6.11 log M) was also given at the end of each experiment with endothelium-dependent vasodilators.

**Pathology**

Isolated aortic rings were fixed with 10% buffered formalin, processed by standard histological techniques, and stained with hematoxylin and eosin.
Light microscopy was used to verify the presence of endothelial cells.

Drugs and Chemicals

Acetylcholine, A23187, aspirin, histamine, indomethacin, L-NOARG, L-arginine, methylene blue, serotonin, and PGF$_{2a}$ were obtained from Sigma Chemical Co., St. Louis; norepinephrine (Levolphed) was from Winthrop Pharmaceuticals, New York; and nitroglycerin (Nitro-Stat) was from Parke-Davis, Morris Plains, N.J. Indomethacin and aspirin were mixed 1:4 by weight with sodium carbonate in distilled water. Drug solutions were made fresh daily and diluted to the appropriate concentrations.

Statistical Analysis

Data are expressed as mean±SEM with $p<0.05$ taken as the criterion of statistical significance. Relaxation responses are expressed as percent relaxation from norepinephrine preconstriction (0% relaxation). Data were analyzed by one-way or mixed-design analysis of variance and least significant difference. ED$_{50}$s were determined for individual experiments, and then means for groups were determined. For each group, $n$ represents the number of animals studied.

Results

Norepinephrine

Norepinephrine caused a dose-dependent constriction in all vessels (Figure 1A). No differences were detected between control ($n=11$) and Brugia ($n=12$) thoracic aorta or control ($n=7$) and Brugia ($n=9$) abdominal aorta. However, in both groups of rats, ED$_{50}$ concentrations were significantly less in the abdominal aorta (control thoracic aorta $-7.80$ versus abdominal aorta $-7.08$; Brugia thoracic aorta $-7.85$ versus abdominal aorta $-7.14$ log M; $p<0.05$). There was no difference in the concentration of norepinephrine necessary to elicit 80–90% maximum norepinephrine constriction ($-7$ log M).

Norepinephrine Plus Indomethacin

Indomethacin did not significantly alter norepinephrine constriction in thoracic aorta. However, in the abdominal aorta of both Brugia-infected and control rats, indomethacin decreased maximum constriction and shifted the dose–response relations to the right (Figure 1A). Norepinephrine constriction was significantly depressed by indomethacin in control (seven of eight doses studied; $p<0.05$) and Brugia (six of eight doses) rings. No differences in ED$_{50}$ concentrations were detected (control $-7.22$ versus control plus indomethacin $-7.64$; Brugia $-6.80$ versus Brugia plus indomethacin $-7.74$ log M; NS).

Figure 1B shows the effect of cyclooxygenase inhibition on maximum constrictor responses to norepinephrine, 5-HT, and PGF$_{2a}$ in Brugia abdominal aorta. Indomethacin (10 μM [n=7] and 50 μM [n=7]) and aspirin (50 μg/ml [n=6]) significantly decreased maximum norepinephrine constriction ($p<0.05$). However, cyclooxygenase inhibition did not alter constriction to 5-HT (n=5) or PGF$_{2a}$ (n=5).

Acetylcholine

In experiments in which endothelial cells were intact, acetylcholine caused a dose-dependent relaxation in all groups studied (Figure 2). No differences were detected in the thoracic aorta (Figure 2A). When compared with control, acetylcholine relaxation was significantly depressed in Brugia abdominal aorta.
aorta (Figure 2B). There were no differences in maximum relaxation or ED$_{50}$ concentration (control $-7.34$ versus Brugia $-7.39$ log M; NS). Not unexpectedly, removal of endothelial cells from Brugia abdominal aorta eliminated acetylcholine relaxation.

**Acetylcholine Plus Indomethacin**

In the thoracic aorta, indomethacin did not significantly alter acetylcholine relaxation. However, indomethacin significantly depressed acetylcholine relaxation in Brugia abdominal aorta (Figure 3 [n=7]) but did not affect the ED$_{50}$ concentration. Indomethacin shifted the dose–response relation down and to the right. In contrast, in control abdominal aorta, indomethacin did not significantly depress maximum relaxation (control 73±13% versus control+indomethacin 46.2±15%; NS [n=5]) or ED$_{50}$ concentration ($-6.79$ versus $-5.95$ log M; NS) but did significantly decrease relaxation at three of 12 doses studied ($-7.52$, $-7.00$, and $-5.52$ log M).

Figure 3B shows that acetylcholine ($-8$ and $-7.52$ log M) relaxation of Brugia abdominal aorta is significantly depressed by indomethacin (10 μM [n=5] and 50 μM [n=7]) and aspirin (50 μg/ml [ASA]).
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**FIGURE 4. Effect of N-nitro-L-arginine methyl ester (NOARG) and methylene blue (MB) on acetylcholine relaxation in control and Brugia abdominal aorta.** Panel A: The effect of 15 μM NOARG on acetylcholine (ACH) relaxation in paired rings of control (n=6) and Brugia (n=9) abdominal aorta preconstricted with norepinephrine (−7 log M). In both control and Brugia, ACH relaxation was significantly depressed by NOARG and restored by L-arginine (+LARG, 150 μM). After NOARG, Brugia relaxation was significantly greater than control (*p<0.05). Relaxation without NOARG and after LARG were not different. Panel B: The effect of methylene blue (MB; 10 μM) on ACH relaxation in paired rings of control (n=6) and Brugia (n=6) abdominal aorta preconstricted with norepinephrine (−7 log M). In both control and Brugia, ACH relaxation was significantly depressed by MB (*p<0.05). After MB, ACH relaxation was significantly greater in Brugia abdominal aorta when compared with control (tp<0.05).

*Brugia* preparations, there was a biphasic response after l-NOARG, low concentrations of acetylcholine caused constriction (2% to 16%), while higher concentrations caused relaxation. In two of nine preparations, acetylcholine caused only relaxation after l-NOARG. l-Arginine (−3.82 log M) caused a gradual relaxation response in all preparations after l-NOARG (Figure 4A). No significant differences were detected after l-arginine.

**Acetylcholine Plus Methylene Blue**

Methylene blue enhanced norepinephrine constriction in all preparations. In both control and *Brugia* abdominal aorta, methylene blue significantly depressed maximum acetylcholine relaxation (Figure 4B; p<0.05). However, after methylene blue, acetylcholine relaxation in *Brugia* abdominal aorta was significantly greater than control (Control 20.5±4.1% versus *Brugia* 38.7±8.6%; p<0.05 [n=6]). There was no difference in maximum acetylcholine relaxation without methylene blue (Figure 4B).

**Histamine**

Histamine caused a dose-dependent relaxation in both abdominal and thoracic aorta (Figure 5). No differences were detected in the thoracic aorta (Figure 5A). However, in *Brugia* abdominal aorta, maximum relaxation was depressed and the dose–response relation shifted down and to the right (Figure 5B). In *Brugia* rats, the ED<sub>50</sub> concentration was significantly less in thoracic aorta (thoracic aorta −5.53 versus abdominal aorta −4.57 log M; p<0.05). A23187

The calcium ionophore A23187 caused dose-dependent relaxation in the thoracic and abdominal
Nitroglycerin caused a dose-dependent relaxation in all groups (Figure 7). No differences in nitroglycerin relaxation were detected in either the thoracic or abdominal aorta. There were no differences in ED$_{50}$ concentrations between groups; however, in *Brugia* vessels, the ED$_{50}$ concentration was significantly less in the abdominal aorta (thoracic aorta $-6.7$ versus abdominal aorta $-7.7$ log M; $p<0.05$).

**Pathology**

After the experimental protocol was completed, rings from the abdominal and thoracic aorta from both infected and noninfected rats were examined for the presence of endothelial cells. Light microscopy verified that the intima was not disrupted by the experimental protocols.

**Discussion**

This study demonstrates for the first time that endothelium-dependent relaxation is specifically altered by chronic lymphatic filarial infection. Endothelium-dependent relaxation to acetylcholine, A23187, and histamine is depressed in the abdominal aorta of rats with chronic *B. pahangi* infection when compared with control. This suggests that chronic infection with the filarial nematode *B. pahangi* alters endothelium-dependent vascular responses in the abdominal aorta. The changes in vascular reactivity are selective and affect only endothelium-dependent relaxation. When compared with control, endothelium-dependent relaxation is depressed and the mediators of relaxation are altered. A non–nitric oxide (NO) relaxing factor, perhaps a cyclooxygenase product, may be responsible, in part, for endothelium-dependent relaxation in the *Brugia* abdominal aorta.

Endothelial cells are important controllers of vascular smooth muscle tone in vivo and in vitro.$^{16,22–24}$ In response to a variety of physiological and pharmacological stimuli, endothelial cells release a variety of vasoactive agents, both dilators and constrictors.$^{22}$ The endothelium-derived relaxing factor (EDRF) first described by Furchgott and Zawadzki has been identified as NO formed in endothelial cells from the terminal guanidino nitrogen of L-arginine.$^{23,25–27}$ Endothelial cells are known to release prostacyclin, adenosine, and NO. However, in the majority of
arteries from normal animals, including rat aorta, it is EDRF/NO, not prostacyclin or adenosine, that is responsible for the relaxation. Indeed, prostacyclin was ruled out early as the endothelium-derived vasodilator responsible for relaxation to acetylcholine. This does not, however, rule out a role for non-EDRF/NO dilators/contrstrictors in the pathogenesis of disease. Disease can alter endothelial cell arachidonic acid metabolism. Acetylcholine relaxation is depressed by endothelium-derived thromboxane in diabetic rabbit aorta. In dogs with experimental congestive heart failure, acetylcholine relaxation of the in vivo femoral artery is depressed when compared with control and is restored by indomethacin, again suggesting a role for constrictor metabolites of arachidonic acid. In this study, indomethacin and aspirin specifically depress acetylcholine relaxation of Brugia abdominal aorta. This suggests that arachidonic acid metabolites are important in relaxation of Brugia abdominal aorta. It appears that arachidonic acid metabolism is altered in Brugia abdominal aorta, and this could be explained by upregulation of cyclooxygenase.

Structural analogues of L-arginine are the most specific inhibitors of EDRF/NO currently available. Inhibition by these analogues is specifically reversed by L-arginine. Complete inhibition of acetylcholine relaxation by L-NOARG (and restoration with L-arginine) suggests that the response in control abdominal aorta is mediated entirely by EDRF/NO. However, acetylcholine relaxation persists in Brugia abdominal aorta after L-NOARG, suggesting that EDRF/NO is responsible for part, but not all, of the response in this vessel. Further support for this concept comes from the methylene blue experiments. After methylene blue, acetylcholine causes twice the relaxation in Brugia abdominal aorta, when compared with control. Therefore, it is likely that at least two endothelium-derived vasodilators, perhaps EDRF/NO and an arachidonic acid metabolite, are important in endothelium-dependent relaxation of the Brugia abdominal aorta.

In this study only endothelium-dependent responses were depressed; non-endothelium-dependent vascular responses were not different in infected and control rats. Therefore, it is unlikely that the changes observed in Brugia abdominal aorta are the result of a primary alteration of vascular smooth muscle function. Nitroglycerin is a well known activator of guanylate cyclase, yet in Brugia abdominal aorta, nitroglycerin relaxation is not different from control, suggesting that the guanylate cyclase/cGMP system is not altered. Likewise, norepinephrine constriction is not different from control, suggesting that adenylate cyclase/cAMP is not involved. Thus, the data do not support a role for primary changes in vascular smooth muscle function in the alteration of endothelium-dependent relaxation seen in Brugia abdominal aorta.

The present study does not address how chronic filarial infection alters endothelial cell-dependent relaxation in the abdominal aorta. Unlike previous reports, in our experiments there was no direct contact between the blood vessel and the parasite. Since isolated blood vessels are not exposed to the parasite, acute exposure to pharmacologically active factors released by the parasite is not likely involved. The adult filariae do not circulate in the vascular system; consequently, direct endothelial cell contact and subsequent alterations or damage cannot explain our results. Although the microfilariae circulate in the vasculature, they are not involved in the depression of endothelium-dependent relaxation seen in vivo and in vitro with D. immitis. The adult B. pahangi reside in the abdominal lymphatics and are therefore closely associated with the vasculature. It is reasonable to suppose that the proximity of the lymphatics and the vasculature could be involved in some of the changes we see. Direct extension of filarial-induced inflammation or chronic exposure to parasitic products could alter the behavior of vascular endothelial cells. Inflammatory cells, including mast cells and basophils, can alter endothelial cell-dependent responses by chronic release of vasoactive substances and consequent receptor down-regulation. Alternatively, acute stimulation of these cells in the muscle bath, by both receptor-mediated agents and the calcium ionophore, could lead to release of compounds that modulate vascular reactivity.

On chronic exposure, parasite products could alter endothelial cell behavior and affect endothelium-dependent relaxation. D. immitis releases a small, stable, biologically active factor that depresses endothelial cell-dependent relaxation and alters the metabolic pathways involved in relaxation. Acute exposure of rat aorta to D. immitis depresses endothelial cell–dependent relaxation to acetylcholine, carbachol, and A23187 but not to nitroglycerin. In vitro exposure to D. immitis mimics the effect of D. immitis on endothelial cell–dependent responses in vivo. Chronic exposure of the aorta may result in downregulation of endothelial cell or vascular smooth muscle receptors. However, since relaxation to A23187 is also depressed in the abdominal aorta, a direct effect of either B. pahangi or parasite-induced inflammatory cells on endothelial cell metabolism is more likely.

Clinical symptoms of human filarial diseases are extremely variable and dependent on a complex interaction between the host and the parasites. A role for altered vascular function in the pathogenesis of lymphatic filariasis has not been examined. It is likely that lymphatic endothelial cells play an important role in control of lymphatic tone; however, this has been little explored. Lymphatic endothelial cells are morphologically abnormal in experimental Brugia filariasis. Circulating filarial factors could alter endothelial cell function in both the vascular and lymphatic systems, and indirect evidence supports...
this hypothesis. In a study by Tani et al., adult heartworms were transplanted into the peritoneal cavity of dogs; serial lymphographic studies demonstrated subsequent alteration of lymphatic function and anatomy. These authors suggested the mechanism is due to a systemic response, not obstruction, and that "substances of the body of filarial worms" were involved. Further support comes from clinical cases of severe filarial elephantiasis. Surgical treatment involves removal of large numbers of adult parasites, anastomosis of the affected lymphatic to the ipsilateral venous system, and excision of excess edematous tissue. Surgical treatment results in initial improvement of the lymphedema; however, lymphedema often recurs within 1 year. Furthermore, limb swelling has been associated with *O. volvulus*, a non-lymphatic-dwelling filarial nematode associated with river blindness and skin lesions. Interactions between filarial parasites, parasite products, and lymphatic and/or vascular endothelial cells will likely be important in the pathogenesis of all these diseases.

An interesting and unexpected finding of this study is that norepinephrine constriction is depressed by indomethacin in *Brugia* and control abdominal aorta. Constriction in the thoracic aorta is not affected. In *Brugia* abdominal aorta, norepinephrine constriction is depressed by two concentrations of indomethacin and aspirin. However, since neither 5-HT nor PGE_{2α} constriction is depressed by cyclooxygenase inhibitors, it is likely that norepinephrine constriction is specifically depressed by cyclooxygenase inhibitors in the abdominal aorta of Lewis rats. To our knowledge, vasoactive responses of abdominal aorta have not been previously reported. Indomethacin inhibits constriction responses to norepinephrine, as well as to angiotensin II, histamine, and 5-HT, in the superior mesenteric vasculature of the rat. Prostaglandin E_{2} restored the constrictor responses to all agents. The authors state that although the depressant effect of indomethacin on all constrictors tested suggested a nonspecific effect of the drug on vascular smooth muscle, restoration of constriction by prostaglandin E_{2} is compatible with an effect on prostaglandin synthesis. Norepinephrine causes a dose-dependent release of the potent vasodilator prostacyclin from thoracic aorta of Sprague-Dawley rats. It is likely the prostacyclin is predominantly of endothelial cell origin. Therefore, inhibition of cyclooxygenase in this preparation would be expected to increase, not decrease, norepinephrine constriction. Indeed, several groups have demonstrated in other vascular tissues, enhanced adrenergic constriction after endothelial cell removal. Neither the mechanism, nor the homeostatic advantage, of selective depression of norepinephrine constriction in the abdominal, but not thoracic aorta, of Lewis rats is known. Further studies will be necessary to determine if this is related to the inbred strain of rats used in this study, the age of the animals studied, or something peculiar to the abdominal aorta.

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