Brief Definitive Report

Intravascular Filarial Parasites Elaborate Cyclooxygenase-derived Eicosanoids

By Leo X. Liu,* Charles N. Serhan,‡ and Peter F. Weller*

*From the *Charles A. Dana Research Institute and the Harvard-Thorndike Laboratory, Infectious Diseases Division, Department of Medicine, Beth Israel Hospital and Harvard Medical School; and the ‡Hematology Division, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115*

Summary

The nematode parasites that cause human lymphatic filariasis survive for long periods in their vascular habitats despite continual exposure to host cells. Since prostanoids formed from arachidonic acid can modulate interactions among platelets, leukocytes, and endothelial cells, we examined whether intravascular nematode parasites can elaborate prostanoids. Microfilariae of *Brugia malayi* utilize exogenous and endogenous arachidonic acid to generate and release two predominant prostanoids, prostacyclin and prostaglandin E_2. Filarial metabolism of host fatty acids to form these vasodilatory, antiaggregatory, and immunomodulatory eicosanoids provides a means by which these helminthic parasites may influence host immune and other cellular responses.

Lymphatic filariasis, a mosquito-borne parasitic disease that affects >100 million people worldwide, is caused by the intravascular nematodes *Wuchereria bancrofti*, *Brugia malayi*, and *B. timori*. As with other helminthic parasites that live for long periods in host organs (1), little is known about how filariae overcome host cellular responses to survive and cause chronic disease. Adult filarial worms inhabit lymphatic vessels, and their larval offspring, microfilariae, live in the bloodstream. Microfilariae are motile multicellular worms that repeatedly encounter host leukocytes, platelets, and vascular endothelial cells. Despite their size (~220 μm long by ~10 μm in diameter), microfilariae are not usually irreversibly trapped within small blood vessels, and neither platelets nor granulocytes adhere to microfilariae in vivo. In infected persons with detectable microfilaremia, lymphocytes respond poorly to parasite antigens, and levels of parasite-specific antibody are low (2). Since microfilariae represent the parasite stage ingested by mosquito vectors, survival of microfilariae is important not only for the individual infected human host, but also for the transmission of filarial infection to other humans.

Filarial parasites cannot produce polyunsaturated fatty acids de novo (3), but essential fatty acids are available to microfilariae in human plasma. Microfilariae avidly incorporate exogenous arachidonic acid into parasite phospholipids (4, 5), and can also form arachidonic acid from exogenous linoleic acid (6). Prostanoids, generated from arachidonic acid via the cyclooxygenase pathway in mammalian cells, exert a spectrum of potent paracrine actions that can modulate interactions among platelets, leukocytes, and endothelial cells (7). We therefore examined whether intravascular filarial parasites can transform arachidonic acid into prostanoids.

Materials and Methods

Isolation of Microfilariae. Microfilariae were obtained by saline peritoneal lavage of *B. malayi*–infected jirds and separated from jird peritoneal cells by passage over a Sephadex G-25 column equilibrated with RPMI 1640 (8) to yield >99% viable (motile) microfilariae. After purification, jird peritoneal cells were routinely undetectable in hemacytometer counts of 10^3 microfilariae, so $<10^3$ potentially contaminating jird cells, if any, were present per 10^6 microfilariae.

Biosynthesis and Release of Microfilarial Prostanoids. 10^6 microfilariae were incubated with 50 nM ^{3}H-arachidonic acid (201 Ci/mmol; Amersham Corp., Arlington Heights, IL) in 1 ml of RPMI 1640, pH 7.4, at 37°C for 30 min, after which media and parasites were separated by centrifugation. Lipids in incubation media and ultrasonically disrupted parasites were extracted with 2 vol of acidified ether (5), and resolved for prostanoids by reverse-phase HPLC using a 4.6-mm × 10-cm Microsorb C18 column (Rainin Instrument Co. Inc., Woburn, MA) and an on-line radioactive flow detector (Flo-1 β; Radiomatic Instruments & Chemical Co. Inc., Tampa, FL). The column was eluted with an isocratic acetonitrile/water (27:73 [vol/vol] 2 ml/min) mobile phase (9). Radiolabeled materials of interest were collected in 2-ml fractions and rechromatographed with a second HPLC solvent system consisting of a linear gradient (1 ml/min) from water/acetonitrile/TFA (60:40:0.0008 [vol/vol/vol]) to methanol/acetonitrile/TFA (60:40:0.002 [vol/vol/vol]) over 40 min (9).

For TLC resolution of radiolabeled lipid extracts, silica gel TLC plates (LK5D; Whatman Inc., Clifton, NJ) were developed with the organic phase of ethyl acetate/isoctane/glacial acetic acid/H_2O (110:50:20:100 [vol/vol/vol]) (10) and scanned with a TLC radiation detector (model RS; Radiomatic Instruments & Chemical Co. Inc.). Migrations of microfilarial ^{3}H-labeled prostanoids were compared with cochromatographed lipid standards.

To assess microfilarial prostanoid biosynthesis in the absence of exogenous arachidonic acid, microfilariae (10^6) were incubated in 1 ml of RPMI 1640, pH 7.4, supplemented with 10 mM Hepes,

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penicillin G (100 U/ml), and streptomycin (100 μg/ml) at 37°C, 5% CO_2 for 24 h, after which microfilarial viability (uptake of acridine orange–ethidium bromide [11]) was ≥98%. Prostanoids were measured by RIA using prostanoid class-specific antisera (Advanced Magnetics, Cambridge, MA). Released prostanoids were assayed directly in unextracted incubation media, while retained products in parasites were extracted (5) and resolved by TLC to remove crossreacting materials before RIA (10). In some experiments, microfilariae were incubated with pharmacologic inhibitors of arachidonic acid metabolism, and 6-keto-$PGF_{1\alpha}$ in the incubation media was quantitated by RIA. These inhibitors included indomethacin (100 μM), compound BW755c (100 μM), and 5,8,11,14-eicosatetraynoic acid (ETYA; 20 μM).

Results and Discussion

To investigate parasite metabolism of exogenous arachidonic acid, *B. malayi* microfilariae were incubated with ^{3}H-arachidonic acid for 30 min. Lipids extracted from incubation media and from parasites were resolved by HPLC. Incubation media contained two major ^{3}H-labeled products that coeluted with authentic prostaglandin E_2 (PGE_2) and 6-keto-$PGF_{1\alpha}$, the stable hydrolysis product of prostacyclin (Fig. 1 *A*). These ^{3}H-labeled products, when individually collected and analyzed with a second HPLC system, again coeluted with PGE_2 and 6-keto-$PGF_{1\alpha}$ (Fig. 1 *B*). TLC of lipid extracts further confirmed the formation of ^{3}H-labeled PGE_2 and 6-keto-$PGF_{1\alpha}$, with ~0.8% of total ^{3}H-arachidonic acid converted to each of these radiolabeled products (data not shown). These radiolabeled products were not formed by heat-killed microfilariae, and only small amounts were formed by microfilariae incubated at 4°C (data not shown).

Table I. *Biosynthesis and Release of Microfilarial Prostanoids from Endogenous Arachidonate*

Prostanoid	Prostanoid production	
	Released	Retained
	pg/10^6 microfilariae	
6kPGF$_{1\alpha}$	1,971 ± 511	97 ± 64
PGE_2	801 ± 131	68 ± 45
PGD_2	192 ± 56	76 ± 45
$PGF_{2\alpha}$	0	29 ± 15
TXB_2	0	0

Values shown are means ± SEM (n = 3–6). Immunoreactive material was not detected in control media incubated without parasites. Media from jird peritoneal cells (8 × 10^7) after 24-h incubation contained 520 pg TXB_2, only 102 pg PGE_2, and no 6-keto-$PGF_{1\alpha}$.

The utilization of endogenous stores of arachidonate for the formation and release of prostanoids by parasites was evaluated by RIA, after incubating microfilariae in serum-free media for 24 h. The predominant prostanoids formed by microfilariae from endogenous arachidonic acid, as from exogenous ^{3}H-arachidonic acid, were prostacyclin and PGE_2 (Table 1). A small amount of PGD_2, but no $PGF_{2\alpha}$ or thromboxane B_2 (the stable hydrolysis product of TXA_2), was detected in the incubation media. Only minimal amounts of prostanoids

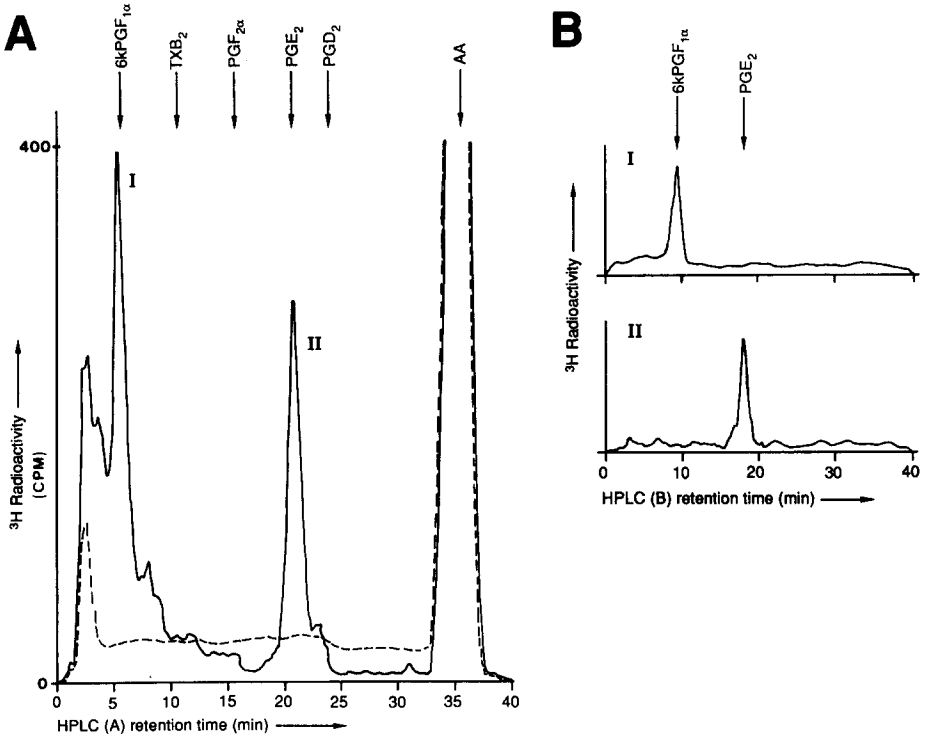

Figure 1. ^{3}H-labeled prostanoids generated by *B. malayi* microfilariae from exogenous ^{3}H-arachidonic acid. (*A*) Parasite-derived [^{3}H]-labeled lipids extracted from incubation media (*solid line*) and microfilariae (*dotted line*), resolved by HPLC as described in Materials and Methods. (*B*) Analysis of ^{3}H-labeled products collected from peaks labeled I and II in (*A*), and individually resolved by a second HPLC system, as described in Materials and Methods. Retention times of 6-keto-$PGF_{1\alpha}$ (6kPGF$_{1\alpha}$), thromboxane B_2 (TXB_2), $PGF_{2\alpha}$, PGE_2, PGD_2, and arachidonic acid (AA) standards are indicated (*arrows*). In control experiments, isolated jird peritoneal cells (10^7), incubated with ^{3}H-arachidonic acid, as for microfilariae, produced no detectable ^{3}H-labeled prostanoids.

remained within microfilariae, as also found with microfilariae incubated with ^{3}H-arachidonic acid (Fig. 1 *A*), indicating that newly formed parasite eicosanoids were released and not stored. Further evidence of parasite prostanoid release has been obtained using fluorescence immunocytochemistry, in which PGE_2 was visualized around the surface of microfilariae with specific anti-PGE_2 antiserum (Liu, L.X., J.E. Buhlman, and P.F. Weller, unpublished results).

Several pharmacologic inhibitors of mammalian arachidonic acid metabolism were evaluated to determine their impact on microfilarial prostanoid biosynthesis: indomethacin, BW755c (a pyrazoline antioxidant), and ETYA (the polyacetylenic analogue of arachidonic acid) (7). Microfilarial prostacyclin production was inhibited 95% by indomethacin (100 μM), 97% by BW755c (100 μM), and 92% by ETYA (20 μM) (means of three experiments), in comparison with incubations without inhibitors. None of the inhibitors cross-reacted with 6-keto-$PGF_{1\alpha}$ antisera, and none were lethal to microfilariae (viability $\geq$83% for all experiments).

Microfilariae of *B. malayi*, therefore, utilized both exogenous and endogenous arachidonic acid to generate cyclooxygenase-derived eicosanoids. Microfilariae elaborated prostanoids in an apparently constitutive manner, not dependent on exogenous stimulants. Furthermore, prostanoids formed by microfilariae were released into the medium surrounding the parasites. Parasite elaboration of prostacyclin, the most potent natural inhibitor of platelet aggregation known (7), may inhibit thrombus formation on microfilarial surfaces. Conversely, thromboxane, which enhances platelet aggregation and vasoconstriction (7), was not formed by the parasite. Both prostacyclin and PGE_2 are potent vasodilators (7); the release of these specific prostanoids by microfilariae might thereby ease their passage through small capillary vessels. PGD_2, detected in small amounts, also inhibits platelet aggregation and is a weak vasodilator (7). PGE_2 has immunosuppressive and antiinflammatory effects, including inhibition of granulocyte and monocyte/macrophage functions, inhibition of T lymphocyte activation and lymphokine production, and induction of B lymphocyte unresponsiveness (12–14). Parasite-derived PGE_2 may thus contribute to the cellular and humoral immune defects observed in infected individuals with microfilaremia (2). The local release in vivo of these parasite-derived lipid mediators provides a mechanism for microfilariae to modulate cellular responses of contiguous human vascular cells. The capacity of filarial nematodes to metabolically transform host fatty acids into biologically active eicosanoids, a capability that may be shared by cestode (15, 16) and trematode (17, 18) parasites, may constitute a survival strategy developed by these helminthic parasites in their adaptation to parasitism.

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Address correspondence to Leo X. Liu, Beth Israel Hospital, 330 Brookline Avenue, Boston, MA 02215.

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References

1. David, J.R. 1989. Host-parasite interface: immune invasion. *In* Tropical and Geographic Medicine. Second edition. K.S. Warren, A.A.F. Mahmoud, editors. McGraw-Hill Inc., New York. 117–123.
2. Philipp, M., T.B. Davis, N. Storey, and C.K. Carlow. 1988. Immunity in filariasis: perspectives for vaccine development. *Annu. Rev. Microbiol.* 42:685.
3. Frayha, G.J., and J.D. Smyth. 1983. Lipid metabolism in parasitic helminths. *Adv. Parasitol.* 22:309.
4. Longworth, D.L., D.W. Foster, A.M. Dvorak, and P.F. Weller. 1985. Incorporation of arachidonic acid by microfilariae of *Brugia malayi*. *J. Infect. Dis.* 152:1317.
5. Longworth, D.L., D.C. King, and P.F. Weller. 1987. Rapid uptake and esterification of arachidonic acid and other fatty acids by microfilariae of *Brugia malayi*. *Mol. Biochem. Parasitol.* 23:275.
6. Liu, L.X., P.F. Weller. 1989. *Brugia malayi*: microfilarial polyunsaturated fatty acid composition and formation. *Exp. Parasitol.* 69:198.
7. Willis, A.L. 1987. CRC Handbook of Eicosanoids: Prostaglandins and Related Lipids. Vol. 1 and 2. CRC Press, Inc., Boca Raton.
8. Taylor, D.W., J.M. Goddard, and J.E. McMahon. 1984. Isolation and purification of microfilariae from nodules of *Onchocerca*

volvulus. *Trans. R. Soc. Trop. Med. Hyg.* 78:707.
9. Powell, W.S. 1985. High-pressure liquid chromatography of arachidonic acid metabolites. *Adv. Prostaglandin Thromboxane Leukotriene Res.* 15:53.
10. Salmon, J.A., and R.J. Flower. 1982. Extraction and thin-layer chromatography of arachidonic acid metabolites. *Methods Enzymol.* 86:477.
11. Mishell, B.B., and S.M. Shiigi. 1980. Selected Methods in Cellular Immunology. W.H. Freeman, San Francisco. 21–22.
12. Goodwin, J.S. 1985. Prostaglandins and Immunity. Martinus Nijhoff, Hingham. 209 pp.
13. Plescia, O.J., and S. Racis. 1988. Prostaglandins as physiological immunoregulators. *Prog. Allergy.* 44:153.
14. Schad, V., and R.P. Phipps. 1989. Prostaglandin E_2-dependent induction of B cell unresponsiveness. *J. Immunol.* 143:2127.
15. Leid, R.W., and L.A. McConnell. 1982. PGE_2 generation and release by the larval stage of the cestode, *Taenia taeniaformis*. *Prostaglandins Leukotrienes Med.* 11:317.
16. Leid, R.W., and L.A. McConnell. 1983. Thromboxane A_2 generation of the larval cestode, *Taenia taeniaformis*. *Clin. Immunol. Immunopath.* 28:67.
17. Fusco, A.C., B. Salafsky, and M.B. Kevin. 1985. *Schistosoma mansoni*: eicosanoid production by cercariae. *Exp. Parasitol.* 59:44.
18. Fusco, A.C., B. Salafsky, and K. Delbrook. 1986. *Schistosoma mansoni*: production of cercarial eicosanoids as correlates of penetration and transformation. *J. Parasitol.* 72:397.