Intravascular Filarial Parasites Elaborate Cyclooxygenase-derived Eicosanoids

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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 E2. 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.

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 10^6 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 ^3H-arachidonic acid (201 Ci/mmoll; 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 x 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/isooctane/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 ^3H-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, 100 mM sodium phosphate, and 10 mM L-arginine.
penicillin G (100 U/ml), and streptomycin (100 µg/ml) at 37°C, 5% CO₂ 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-PGF₁₀ 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 ³H-arachidonic acid for 30 min. Lipids extracted from incubation media and from parasites were resolved by HPLC. Incubation media contained two major ³H-labeled products that coeluted with authentic prostaglandin E₂ (PGE₂) and 6-keto-PGF₁₀α, the stable hydrolysis product of prostacyclin (Fig. 1 A). These ³H-labeled products, when individually collected and analyzed with a second HPLC system, again coeluted with PGE₂ and 6-keto-PGF₁₀α (Fig. 1 B). TLC of lipid extracts further confirmed the formation of ³H-labeled PGE₂ and 6-keto-PGF₁₀α, with ~0.8% of total ³H-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 Arachidonic**

| Prostanoid | Released | Retained |
|------------|----------|----------|
| 6kPGF₁₀α  | 1,971 ± 511 | 97 ± 64  |
| PGE₂      | 801 ± 131  | 68 ± 45  |
| PGD₂      | 192 ± 56   | 76 ± 45  |
| PGF₁₀α    | 0         | 29 ± 15  |
| TXB₂      | 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 x 10⁷) after 24-h incubation contained 520 pg TXB₂, only 102 pg PGE₂, and no 6-keto-PGF₁₀α.

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 ³H-arachidonic acid, were prostacyclin and PGE₂ (Table I). A small amount of PGD₂, but no PGF₁₀α or thromboxane B₂ (the stable hydrolysis product of TXA₂), was detected in the incubation media. Only minimal amounts of prostanoids...
remained within microfilariae, as also found with microfilariae incubated with ³H-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 PGE₂ was visualized around the surface of microfilariae with specific anti-PGE₂ 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-PGF₁α antisera, and none were lethal to microfilariae (viability >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 PGE₂ are potent vasodilators (7); the release of these specific prostanoids by microfilariae might thereby ease their passage through small capillary vessels. PGD₂, detected in small amounts, also inhibits platelet aggregation and is a weak vasodilator (7). PGE₂ 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 PGE₂ 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.

We thank Dr. David L. Longworth for his preliminary studies; Andre Lopez, Alberto Iaia, and Janet Buhlmann for technical assistance; and Dr. Salvador Moncada (Wellcome Research Laboratories, Beckenham, Kent, UK) for the gift of BW755c. Jirgs infected with B. malayi were provided by a National Institute of Allergy and Infectious Diseases supply contract (AI-02642), U.S.-Japan Cooperative Medical Science Program.

This work was supported by grants AI-07061 (P.F. Weller) and AI-23826 (L.X. Liu) from the National Institutes of Health and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (830108). L.X. Liu is a recipient of a Charles A. King Trust Fellowship and a Young Investigator Matching Grant Award from the National Foundation for Infectious Diseases. C. N. Serhan is a Pew Scholar in the Biomedical Sciences. P. F. Weller is an Established Investigator of the American Heart Association.

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Received for publication 23 April 1990 and in revised form 11 June 1990.

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