Toxoplasma gondii Alters Eicosanoid Release by Human Mononuclear Phagocytes: Role of Leukotrienes in Interferon γ–induced Antitoxoplasma Activity

By Elenita C. Yong,* Emil Y. Chi,‡ and William R. Henderson, Jr.*

*From the Departments of *Medicine and ‡Pathology, University of Washington, Seattle, Washington 98195*

Summary

Toxoplasma gondii tachyzoites markedly alter the profile of eicosanoids released by human mononuclear phagocytes. Freshly isolated, 2-h adherent human monocytes release both cyclooxygenase (e.g., thromboxane [TX] B_2, prostaglandin [PG] E_2) and 5-lipoxygenase (e.g., leukotriene [LT] B_4, LTC_4) products of arachidonic acid metabolism after stimulation by the calcium ionophore A23187 or ingestion of opsonized zymosan particles or heat-killed *T. gondii*. However, after incubation with viable *T. gondii*, normal and chronic granulomatous disease monocytes release only the cyclooxygenase products TXB_2 and PGE_2 and fail to form LTB_4, LTC_4, or other 5-lipoxygenase products. Monocytes maintained in culture for 5 d lose this capacity to release TXB_2 and PGE_2 after incubation with *T. gondii*. *T. gondii* significantly inhibit calcium ionophore A23187–induced LTB_4 release by monocyte-derived macrophages; heat-killed organisms do not affect this calcium ionophore A23187–induced release of LTB_4. *T. gondii*–induced inhibition of LTB_4 release by calcium ionophore A23187–stimulated monocyte-derived macrophages is reversed by interferon (IFN)-γ treatment of the monolayers. LTB_4 induced extensive damage to the cellular membranes and cytoplasmic contents of the organisms as observed by transmission electron microscopy. Exogenous LTB_4 (10^{-6} M) induced intracellular killing of ingested *T. gondii* by non-IFN-γ–treated monocyte-derived macrophages. IFN-γ–induced antitoxoplasma activity in monocyte-derived macrophages was inhibited by the selective 5-lipoxygenase inhibitor zileuton but not by the cyclooxygenase inhibitor indomethacin. These findings suggest a novel role for 5-lipoxygenase arachidonic acid products in human macrophage IFN-γ–induced antitoxoplasma activity.

Human monocytes, but not monocyte-derived macrophages, are cytotoxic to *Toxoplasma gondii*, an obligate intracellular protozoal parasite (1–3). Monocytes possess a granule peroxidase identical to myeloperoxidase (MPO)[1] of neutrophils and respond to stimulation with a respiratory burst leading to the formation of superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) (4). Monocytes derived from peripheral blood and maintained as adherent cells in culture undergo a variety of biochemical and morphologic changes and differentiate into cells resembling tissue macrophages (5–7). During this transformation in culture, there is a progressive loss of the granule peroxidase (5, 8); after an initial rise at day 3, H_2O_2 release also greatly decreases (9). Treatment of monocyte-derived macrophages with IFN-γ results in their activation with a concomitant increase in H_2O_2 generation when stimulated (7, 10).

The capacity to generate oxygen radicals and the presence of MPO has been correlated with the antiprotozoal activity of phagocytes (2, 11, 12). Neutrophils and freshly isolated peroxidase-containing monocytes release large amounts of O_2^- and H_2O_2 after incubation with *T. gondii* or other protozoa (e.g., *Leishmania donovani* and *Trypanosoma cruzi*); these phagocytes rapidly destroy the ingested *T. gondii* (13). In contrast, by day 5 in culture, monocyte-derived macrophages fail to kill ingested protozoa, which proceed to replicate intracellularly (1, 2, 14). The failure of lysosomes to fuse with the *T. gondii*–containing phagosomes is a striking feature of this infection (15, 16). IFN-γ activates monocyte-derived macrophages to significantly increase their respiratory burst activity and augment their cytotoxic activity against such intracel-

[1] *Abbreviations used in this paper:* CGD, chronic granulomatous disease; HETE, hydroxyeicosatetraenoic acid; IFA, indirect fluorescence antibody; LT, leukotriene; MPO, myeloperoxidase; TX, thromboxane.

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/94/11/1637/12 $2.00
Volume 180 November 1994 1637–1648

lular pathogens as *T. gondii* (1–3) and *L. donovani* (14, 17). Chronic granulomatous disease (CGD) monocytes that fail to undergo a respiratory burst have a marked impairment in toxoplasmacidal and toxoplasmastatic activities (2). However, their toxoplasmastatic activity is augmented by IFN-γ treatment, suggesting activation of an oxygen-independent antiprotozoal system in these phagocytes (2).

As observed in human monocyte-derived macrophages, *T. gondii* inhibit respiratory burst activity and phagolysosomal fusion in resident murine peritoneal macrophages (18, 19). Furthermore, *T. gondii* alter the metabolism of arachidonic acid in murine peritoneal macrophages. Resident murine macrophages release the 5-lipoxygenase arachidonate products leukotriene (LT) B_4 and LTC_4 after incubation with the calcium ionophore A23187 and LTC_4 and LTD_4 after ingestion of opsonized zymosan particles (19). In contrast, *T. gondii* inhibit 5-lipoxygenase product release and shift arachidonic acid metabolism to monohydroxyeicosatetraenoic acid (HETE) (i.e., 11-, 12-, and 15-HETE) release by these macrophages. Viable *T. gondii* are required for this alteration in arachidonic acid metabolism since heat-killed *T. gondii* induce release of LTD_4 but not mono-HETEs from the murine macrophages (19).

We report here that human mononuclear phagocyte eicosanoid metabolism is altered by *T. gondii* tachyzoites. Whereas 5-lipoxygenase arachidonate products (LTB_4, LTC_4) are released by freshly isolated human peripheral blood monocytes incubated with soluble (i.e., the calcium ionophore A23187) or particulate (i.e., opsonized zymosan or heat-killed *T. gondii*) stimuli, viable *T. gondii* fail to induce 5-lipoxygenase product release by these cells. Furthermore, *T. gondii* inhibit LTB_4 release by calcium ionophore A23187–stimulated monocyte-derived macrophages; this inhibitory effect is reversed by IFN-γ. We also report that LTB_4 exerts potent cytotoxic activity against *T. gondii*. The selective 5-lipoxygenase inhibitor zileuton but not the cyclooxygenase inhibitor indomethacin blocks IFN-γ–induced antitoxoplasma activity in monocyte-derived macrophages. These data suggest that inhibition of macrophage LT release may be an important mechanism used by *T. gondii* in their evasion of host inflammatory responses.

Materials and Methods

Special Reagents. LTB_4, LTC_4, LTD_4, 5-HETE, 11-HETE, 12-HETE, 15-HETE, PGE_2, 6-keto-$PGF_{1\alpha}$, and thromboxane (TX) B_2 were obtained from Cayman Chemical Co., Inc. (Ann Arbor, MI). These eicosanoids were supplied in ethanol with dilutions made in PBS. ^{3}H-LTB_4, ^{3}H-LTC_4, ^{3}H-PGE_2, ^{3}H-6-keto-$PGF_{1\alpha}$, and ^{3}H-TXB_2 were obtained from New England Nuclear (Boston, MA). The cyclooxygenase inhibitor indomethacin was dissolved in ethanol at a concentration of 10^{-2} M and diluted with PBS. The selective 5-lipoxygenase inhibitor zileuton [*N*-(1-benzo{b}thien-2-ylethyl)-*N*-hydroxyurea] (20) was kindly provided by R. L. Bell (Abbott Laboratories, Abbott Park, IL). Zileuton was dissolved in DMSO and then diluted in PBS with a final concentration of 0.1% DMSO. The final pH of the indomethacin and zileuton solutions was 7.0. Recombinant human IFN-γ was generously provided by P. Trown (Hoffmann-La Roche, Nutley, NJ). IFN-γ activity was determined by the manufacturer by use of the WISH cell line (CCL 25; American Type Culture Collection [ATCC], Rockville MD)/vesicular stomatitis virus (VSV) cytopathic effect microtiter assay that was standardized with the National Institutes of Health (NIH) human IFN-γ reference standard Gg 23-901-530.

Human Subjects. Monocytes were isolated from the blood of normal human adult subjects and a patient with CGD. The subjects had not ingested acetylsalicylic acid or other nonsteroidal antiinflammatory drugs for 2 wk before the study; the CGD patient was not on IFN-γ prophylaxis. Each subject was negative for *T. gondii*–specific IgM and IgG antibodies by an indirect fluorescence antibody (IFA) test (Microbiological Research Corp., Bountiful, UT). As previously described (21), the test serum was incubated with *T. gondii* (RH strain) fixed on microscope slides. After reaction with either fluorescein-conjugated anti-human IgG (γ chain specific) or fluorescein-conjugated IgM (μ chain specific), the *T. gondii* were examined by fluorescence microscopy for the characteristic peripheral staining pattern. The absence of prior acute or chronic *T. gondii* infection was indicated by the lack of fluorescence at a 1:8 serum IgM-IFA titer and 1:16 serum IgG-IFA titer, respectively. Normal sera that had been heat inactivated at 56°C for 45 min were used at a 1:10 dilution (final concentration) in the LTB_4-*Toxoplasma* interaction studies.

Monocytes and Monocyte-derived Macrophages. As previously described for monocyte isolation (7), venous blood was collected in 0.5% K_2 EDTA, diluted 1:1 (vol/vol) with Ca^{2+}/Mg^{2+}-free PBS, centrifuged at 125 *g* for 20 min at 4°C, and the platelet-containing supernatants discarded. After washing in Ca^{2+}/Mg^{2+}-free PBS, the cell pellets were resuspended in Ca^{2+}/Mg^{2+}-free PBS containing 0.3 mM Na_2EDTA and underlayered with Histopaque 1077 (Sigma Chemical Co., St. Louis, MO) at a ratio of 1.5:1 (cell suspension/Histopaque; vol/vol) before centrifugation at 800 *g* for 25 min at 23°C (22). The mononuclear cells located in the Ca^{2+}/Mg^{2+}-free PBS:Histopaque interface were collected, washed twice with Ca^{2+}/Mg^{2+}-free PBS, and resuspended in RPMI 1640 media buffered with 10 mM Hepes (Whittaker M.A. Bioproducts, Walkersville, MD) and supplemented with 2 mM L-glutamine, 25 U/ml penicillin G, and 25 μg/ml streptomycin (GIBCO BRL, Gaithersburg, MD). At a concentration of 3×10^6 monocytes per milliliter, the cells were added to sterile 60 × 15 mm tissue culture plates (Falcon 3801 Primaria; Becton Dickinson & Co., Mountain View, CA) and allowed to adhere for 2 h at 37°C in 5% CO_2/95% air with humidity. The plates were washed with sterile PBS to remove nonadherent cells. Adherent monocytes were either used immediately or maintained for 5 d in culture to obtain monocyte-derived macrophages, with RPMI media containing 10–15% autologous serum changed daily. Some monolayers were stained with Diffquik (Difco Laboratories, Inc., Detroit, MI) for evaluation of cell morphology or benzidine dihydrochloride to detect peroxidase (23). In some studies, the media contained 100 U/ml recombinant human IFN-γ. All buffers and media were prepared with sterile nonpyrogenic water (Baxter Healthcare Corp., Deerfield, IL) and were filtered before use with 0.22 μm filter units (Corning Inc., Corning, NY). All glassware had been heated for 24 h at 170°C to destroy endotoxin.

T. gondii. The *T. gondii* RH strain was maintained by intraperitoneal passage in BALB/c mice (21). After lavage with Ca^{2+}/Mg^{2+}-free PBS, the peritoneal fluid was filtered through a 3-μm polycarbonate filter (Nucleopore Corp., Pleasanton, CA) to remove leukocytes. The *T. gondii* were centrifuged at 1,000 *g* for 15 min at 4°C, washed twice with Ca^{2+}/Mg^{2+}-free PBS, and resuspended in PBS before use. The organisms were >95% viable as assessed by trypan blue exclusion (24). In selected studies, *T. gondii* were killed by heating at 100°C for 5 min.

Eicosanoid Studies. The monocytes or day 5 monocyte-derived

macrophages were washed six to eight times with warm sterile Tyrode's buffer before addition of the reaction components in a final volume of 3 ml Tyrode's buffer. The cell monolayers were incubated at 37°C in 5% CO_2/95% air with the calcium ionophore A23187 (Sigma Chemical Co.; 2.5 μM) for 20 min, or opsonized zymosan particles (ICN Biomedicals, Inc., Costa Mesa, CA; 1 mg/ml) for 90 min, or *T. gondii* (viable or heat killed) at a ratio of 1:5 (mononuclear phagocyte:organism) for 30 min at 37°C. In some studies, the monolayers after incubation with *T. gondii* for 90 min were then stimulated with the calcium ionophore A23187 (2.5 μM) for 20 min. Zymosan was opsonized by incubation for 15 min at 37°C with 50% normal human serum from an individual negative for *T. gondii*–specific IgG and IgM antibodies (i.e., IgG-IFA titer <1:16 and IgM IFA titer <1:8). The zymosan particles were washed free of unbound serum components before addition to the monolayers. At the completion of the incubation periods, the reaction mixtures were collected, centrifuged at 250 *g* for 10 min at 4°C, and the supernatants stored at −70°C until assayed for eicosanoids by HPLC or RIA.

The reaction mixture supernatants underwent solid phase extraction with 1 ml octadecyl Baker-10 columns (J. T. Baker Chemical Co., Phillipsburg, NJ) (25). The eluants were evaporated to dryness under nitrogen, resuspended in methanol, and centrifuged at 3000 *g* for 5 min at 4°C. The clear supernatant underwent reverse-phase HPLC on a 5-μm particle size 2.1 ×200 mm C_{18} microbore column (Hypersil ODS; Hewlett-Packard Co., Palo Alto, CA) with use of methanol/water/acetic acid (75:25:0.01, vol/vol/vol), pH 4.7, at a flow rate of 1 ml/min. A liquid chromatograph with diode array detector (models 1090 and 1040; Hewlett-Packard Co.) were used to monitor eluting peaks at 235 and 270 nm with rapid UV spectral scanning for identification of compounds that cochromatographed with authentic LT and HETE standards (26). The mean retention times for these compounds are as follows: LTB_4, 2.4 min; LTC_4, 3.7 min; 15-HETE, 4.4 min; 11-HETE, 4.7 min; 12-HETE, 4.9 min; LTD_4, 5.3 min; and 5-HETE, 6.0 min. The limits of detection of lipoxygenase products by reverse-phase HPLC are as follows: LTB_4, 25 ng; LTC_4, 50 ng; LTD_4, 50 ng; 5-HETE, 30 ng; 11-HETE, 65 ng; 12-HETE, 65 ng; and 15-HETE, 65 ng.

LTB_4, LTC_4, PGE_2, and the respective stable hydrolysis products of PGI_2, TXA_2, 6-keto-$PGF_{1\alpha}$, and TXB_2 were assayed in the reaction mixture supernatants by RIA. Each RIA was performed in duplicate according to standard protocols. Unlabeled and ^3H-labeled synthetic eicosanoid standards were obtained from Cayman Chemical Co. and New England Nuclear, respectively.

Rabbit antisera against LTB_4 and LTC_4 were kindly provided by Drs. Robert W. Egan and John L. Humes (Merck Research Laboratories, Rahway, NJ) (27). The LTB_4 antiserum had a sensitivity of 10 pg per 0.1-ml sample and the following cross-reactivities at B/B_o 50%: 5(*S*), 12(*R*)-LTB_4, 100%; 5(*S*), 12(*R*)-6-*trans*-LTB_4, 6%; 5(*S*), 12(*S*)-6-*trans*-LTB_4, 0%; 5(*S*), 12(*S*)-LTB_4, 0.2%; 5(*S*), 12(*S*)-6, 10-*trans*-8,14-*cis*-di-HETE, <7.0%; 20-OH-LTB_4, 2.0%; 20-COOH-LTB_4, 0%; 5-HETE, 0.2%; LTC_4, 0.02%; LTD_4, 0.04%; LTE_4, 0.15%, LTF_4, 0%; and arachidonic acid, 0%. The LTC_4 antiserum had a sensitivity of 20 pg per 0.1-ml sample and the following cross-reactivities at B/B_o 50%: (5*S*, 6*R*)-LTC_4, 100%; LTD_4, 43%; 11-*trans*-LTD_4, 47%; LTE_4, 6%; LTF_4, 50%; (5*R*, 6*R*)-LTC_4, 100%; (5*S*, 6*S*)-LTC_4, 1.7%; (5*R*, 5*S*)-LTC_4, 0.7%; LTC_4-sulfone, 10%; LTD_4-sulfone, 7%; LTE_4-sulfone, 0.4%; LTF_4-sulfone, 1.0%; and LTB_4, (5*S*, 6*R*)-7,8,9,10,11,12, 14,15-octahydro-LTC_4, PGE_2, $PGF_{2\alpha}$, glutathione, 5-HETE, and arachidonic acid, each <0.2%.

The PGE_2, 6-keto-$PGF_{1\alpha}$, and TXB_2 antisera were produced in rabbits in our laboratory (28, 29) by the method of Jaffe and Behrman (30). The PGE_2 antiserum at a dilution of 1:6,000 had a sensitivity of 10 pg per 0.1-ml sample and the following cross-reactivities at B/B_o 50%: PGE_2, 100%; PGD_2, 0.8%; PGE_1, 9.3%; 6-keto-PGE_2, 2.0%; $PGF_{1\alpha}$, 0.3%; 6-keto-$PGF_{1\alpha}$, 2.2%; and $PGF_{2\alpha}$, 1.0%. The 6-keto-$PGF_{1\alpha}$ antiserum at a dilution of 1:6,000 had a sensitivity of 10 pg per 0.1-ml sample and the following cross-reactivities at B/B_o 50%: 6-keto-$PGF_{1\alpha}$, 100%; PGD_2, 0.42%; PGE_1, 0.1%; PGE_2, 0.11%, 6-keto-PGE_2, 2.5%, $PGF_{2\alpha}$, 1.43%; and $PGF_{1\alpha}$, 0.77%. The TXB_2 antiserum at a dilution of 1:100,000 had a sensitivity of 1 pg per 0.1-ml sample and the following cross-reactivities at B/B_o 50%: TXB_2, 100%; PGD_2, 0.53%; $PGF_{2\alpha}$, <0.2%; and 6-keto-PGE_2, $PGF_{1\alpha}$, and 6-keto-$PGF_{1\alpha}$, each <0.02%.

Morphologic Studies. *T. gondii* (2×10^7) were incubated for 30 min at 37°C in PBS in the absence or presence of LTB_4 at the concentrations indicated in the legends to Figs. 4 and 5. The preparations were then centrifuged at 1,000 *g* for 15 min before fixation. In studies to assess antitoxoplasma activity of LTB_4, human monocyte–derived macrophages incubated in the absence of IFN-γ for 5 d were challenged with *T. gondii* (1:5 ratio, macrophage:organism) for 30 min at 37°C, washed three to five times in Ca^{2+}/Mg^{2+}-free PBS, and incubated in the absence or presence of 10^{-6} M LTB_4 in RPMI media containing 10% autologous serum for 60 min at 37°C. The preparations were washed again in Ca^{2+}/Mg^{2+}-free PBS and incubated in RPMI media with 10% autologous serum for an additional 23 h at 37°C in 5% CO_2/95% air with humidity. The samples were fixed in 4% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, for 2 h. The samples were prepared for transmission electron microscopy, with thin sections placed on a 200-mesh grid coated with 1% Parlodion (Mallinckrodt Inc., St. Louis, MO) and examined on an electron microscope (model 100B; JEOL Ltd., Tokyo, Japan) at 60 kV as described previously (31).

The distribution of morphologic changes induced in the *T. gondii* after incubation with LTB_4 was determined by morphometric analysis as described previously (31, 32). The organisms were classified as exhibiting either no cytotoxic changes or one of the following cytotoxic characteristics: (*a*) intracellular vacuole formation; (*b*) extrusion of cytoplasm into the space between the inner and outer surface membrane units; (*c*) extracellular release of cytoplasmic material; or (*d*) surface membrane vesiculation and disruption. 10 randomly selected fields in each grid were photographed at a magnification of 7,000 for morphometric analysis.

The role of cyclooxygenase versus 5-lipoxygenase arachidonic acid products in macrophage antitoxoplasma activity was also assessed. Monocyte-derived macrophages adherent to 22-mm square glass coverslips were incubated in RPMI media containing 10% autologous serum and IFN-γ (100 U/ml) for 5 d in 5% CO_2/95% air with humidity. After washing, the monolayers were incubated with freshly isolated *T. gondii* (1:5 ratio, macrophage:organism) in media alone or media containing either the cyclooxygenase inhibitor indomethacin (10^{-6} M) (33) or the 5-lipoxygenase inhibitor zileuton (10^{-6} M) (20, 34) for 30 min at 37°C. Noningested organisms were then removed by washing, and the cell monolayers were either fixed with 5% formalin (time 0) or incubated in RPMI media containing 10% autologous serum in the absence or presence of indomethacin or zileuton for an additional 6 h before fixation in 5% formalin. After staining with Giemsa, the number of intracellular *T. gondii* per macrophage was assessed by light microscopy at a magnification of 400 by use of a Whipple micrometer disc (Bausch & Lomb Inc., Rochester, NY). To confirm that zileuton is a selective inhibitor of the 5-lipoxygenase pathway under the conditions of these experiments, IFN-γ–treated

monocyte-derived macrophages after incubation with 10^{-6} M zileuton for 30 min were stimulated with the calcium ionophore A23187 (10 μM) for 20 min at 37°C. The supernatants of the reaction mixtures were assayed for the 5-lipoxygenase product LTB_4 and the cyclooxygenase product TXB_2 by RIA as described above.

Statistical Analysis. The data are reported as the mean ± SE of the combined experiments. Differences were analyzed for significance ($p < 0.05$) by Student's two-tailed t test for independent means.

Results

Monocytes. Greater than 95% of freshly isolated (2-h adherent) normal human monocytes were peroxidase positive and had the characteristic morphology of monocytes by light microscopic evaluation of Diffquik-stained preparations. Human monocytes incubated in buffer alone for 90 min released low levels (<33 pg per 10^5 monocytes) of each of the following eicosanoids: LTB_4 and LTC_4 (Figs. 1 *A* and 2) and TXB_2, PGE_2, and 6-keto-$PGF_{1\alpha}$ (Fig. 2); 5-, 11-, 12-, and 15-HETE were not detected in these monocyte supernatants. 63% of the total eicosanoids released by these control monocytes were cyclooxygenase products (Fig. 3). *T. gondii* incubated in buffer alone for 90 min release PGE_2 (73.4 pg per 10^6 organisms in 90 min as we have previously reported [21]) but do not generate mono-HETEs or 5-lipoxygenase products.

When human monocytes were incubated with *T. gondii* (1:5 ratio, monocyte:organism), release of TXB_2 and PGE_2 was augmented 15.5-fold ($p < 0.02$) and 9-fold ($p < 0.05$), respectively, compared with control (Fig. 2). TXB_2 was the predominant eicosanoid released by the *T. gondii*–stimulated monocytes (356 pg TXB_2 per 10^5 monocytes, Fig. 2; 56.6% of total eicosanoids assayed, Fig. 3). *T. gondii* did not stimulate a significant release of LTB_4, LTC_4 (Figs. 1 *B* and 2), or 6-keto-$PGF_{1\alpha}$ (Fig. 2), or 5-, 11-, 12-, or 15-HETE (not shown) above that by monocytes alone. Similarly, freshly isolated CGD monocytes failed to release LTB_4 or LTC_4 (Fig. 1 *C*) but did generate TXB_2 (mean 399 pg per 10^5 cells, $n = 2$) and PGE_2 (mean 227 pg per 10^5 cells, $n = 2$) after incubation with *T. gondii*. 88.7 (Fig. 3) and 91.8% of eicosanoids released by *T. gondii*–stimulated normal and CGD monocytes, respectively were cyclooxygenase products.

In contrast, heat-killed *T. gondii* significantly increased LTB_4 ($p < 0.05$), TXB_2 ($p < 0.02$), and PGE_2 ($p < 0.05$) release above control (Fig. 2). Approximately equal amounts

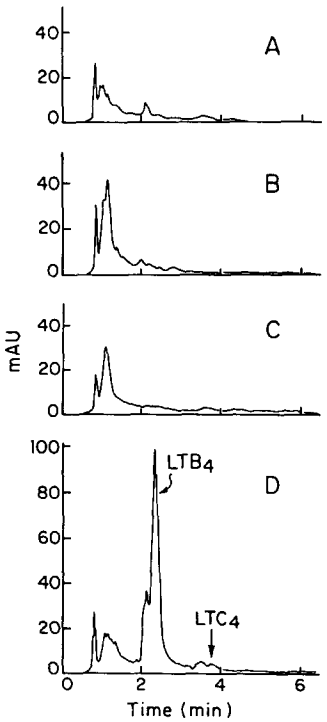


Figure 1. Effect of *T. gondii* on human monocyte eicosanoid release. Freshly isolated normal (*A*, *B*, and *D*) and CGD (*C*) monocytes after adherence to plastic for 2 h were incubated at 37°C in the absence (*A*) or presence (*B* and *C*) of *T. gondii* tachyzoites (1:5 ratio; monocyte/organism) for 90 min or with 2.5 μM of the calcium ionophore A23187 (*D*) for 20 min. The supernatants underwent reverse-phase HPLC with UV absorbance measured at 270 nm as described in Materials and Methods.

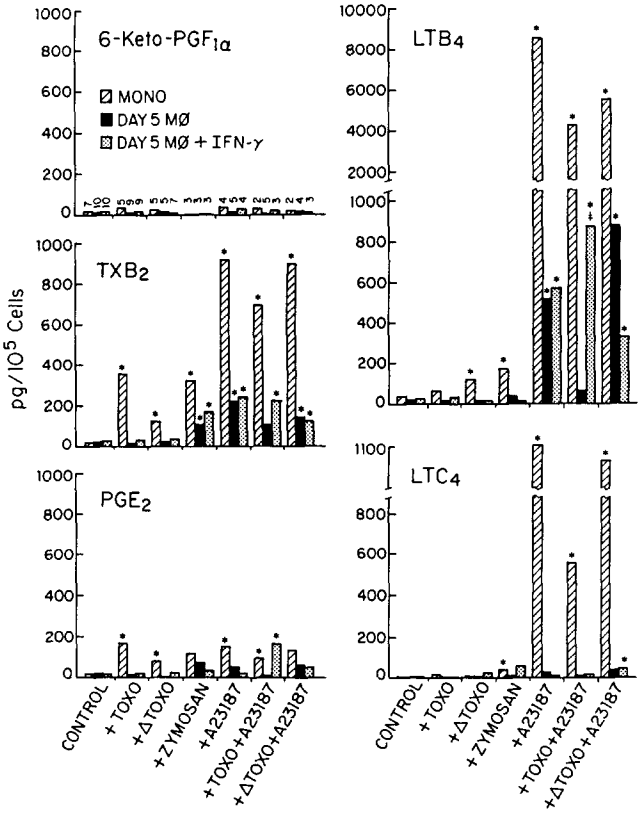


Figure 2. Effect of *T. gondii* on eicosanoid release by human mononuclear phagocytes. Normal monocytes (*MONO*) after adherence to plastic for 2 h and monocyte-derived macrophages cultured for 5 d in the absence (*Day 5 Mϕ*) or presence of 100 U/ml recombinant human IFN-γ (*Day 5 Mϕ* + IFN-γ) were incubated at 37°C for 90 min in the absence (*CONTROL*), or presence of viable or heat-killed *T. gondii* (*Δ TOXO*; 1:5 ratio), or opsonized zymosan particles (1 mg/ml), or for 20 min with the calcium ionophore A23187 (2.5 μM). In some studies, the phagocytes were incubated at 37°C for 90 min with either viable or heat-killed *T. gondii* followed by incubation for an additional 20 min with the calcium ionophore A23187 (2.5 μM). Cyclooxygenase (i.e., PGE_2, 6-keto-$PGF_{1\alpha}$, and TXB_2) and 5-lipoxygenase (i.e., LTB_4 and LTC_4) arachidonate products were assayed by RIA as described in Materials and Methods. The number of studies performed for each condition is shown within the 6-keto-$PGF_{1\alpha}$ graph. $*p < 0.05$ compared with control (i.e., monocytes, day-5 monocyte-derived macrophages, or IFN-γ–treated day-5 monocyte-derived macrophages incubated in buffer alone). $\ddagger p < 0.05$ compared with non-IFN-γ–treated day-5 monocyte-derived macrophages.

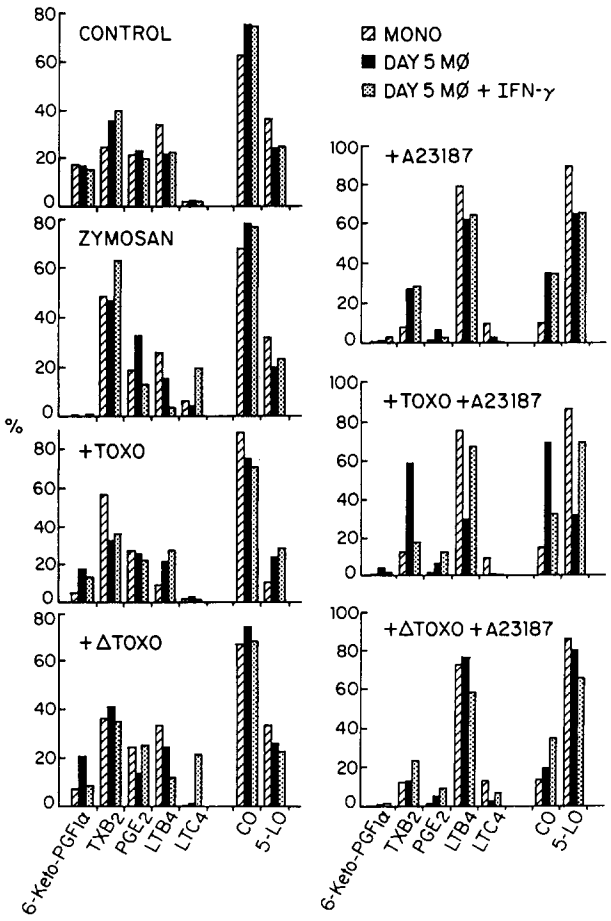


Figure 3. Effect of *T. gondii* on the profile of cyclooxygenase and 5-lipoxygenase arachidonate products released by human mononuclear phagocytes. The percentage of each cyclooxygenase (*CO*) (i.e., PGE_2, 6-keto-$PGF_{1\alpha}$, and TXB_2) and 5-lipoxygenase (*5-LO*) (i.e., LTB_4 and LTC_4) product of the total eicosanoids assayed for each experimental condition described in Fig. 2 is shown.

of LTB_4 (116 pg per 10^5 cells) and TXB_2 (126 pg per 10^5 cells) were released by monocytes incubated with heat-killed *T. gondii* (Fig. 2). Opsonized zymosan induced LTB_4 ($p < 0.001$), LTC_4 ($p < 0.05$), and TXB_2 ($p = 0.0001$) release above background levels (Fig. 2). As in *T. gondii*–stimulated cells, cyclooxygenase products were the principal eicosanoids released by heat-killed *T. gondii*– and zymosan-stimulated monocytes (67 and 68%, respectively of total eicosanoids assayed, Fig. 3).

The soluble stimulus calcium ionophore A23187 induced both a marked increase in the total amount and a change in profile of eicosanoids released by the human monocytes compared with that induced by *T. gondii* or zymosan particles. Calcium ionophore A23187–induced eicosanoid release was 17.0-, 30.4-, and 16.1-fold greater than that stimulated by freshly isolated *T. gondii*, heat-killed *T. gondii*, and zymosan respectively (Fig. 2). 5-Lipoxygenase products constituted 89.5% of the assayed arachidonate metabolites produced by calcium ionophore A23187–stimulated cells with LTB_4 (79.2% of total eicosanoids assayed), the predominant eicosanoid released (Figs. 1 *D*, 2, and 3). Incubation of the monocytes with either viable or heat-killed *T. gondii* before calcium ionophore A23187 stimulation did not alter significantly the amount of LTB_4 or other eicosanoids released compared with that released by monocytes incubated with calcium ionophore A23187 alone (Figs. 2 and 3).

Monocyte-derived Macrophages. Human peripheral blood monocytes were maintained in culture for 5 d, during which period they lose their endogenous peroxidase activity (7, 9). *T. gondii* failed to stimulate the release of TXB_2, PGE_2, 6-keto-$PGF_{1\alpha}$, LTB_4, LTC_4 (Fig. 2), or mono-HETEs (not shown) by the day 5–adherent monocyte-derived macrophages compared with controls. Although the total amount of eicosanoids released by zymosan- and calcium ionophore A23187–stimulated monocyte-derived macrophages decreased by 2.7- and 12.7-fold, respectively, compared with freshly isolated monocytes, these cultured cells remained capable of eicosanoid release (Fig. 2). Compared with unstimulated cultured cells, TXB_2 release by the day 5–adherent cells was increased 4.1-fold ($p < 0.02$) by incubation with zymosan. As with freshly isolated monocytes, cyclooxygenase products (80.2% of eicosanoids assayed) were the predominant arachidonate metabolites released by the zymosan-stimulated monocyte-derived macrophages (Fig. 3).

The calcium ionophore A23187 increased day 5 monocyte-derived macrophage release of TXB_2 by 8.1-fold ($p < 0.05$) and LTB_4 by 30.6-fold ($p < 0.001$) compared with controls (Fig. 2). LTB_4 (61.8% of assayed eicosanoids) was the predominant arachidonate product released by calcium ionophore A23187–stimulated monocyte-derived macrophages (Fig. 3). Prior incubation of the monocyte-derived macrophages with viable *T. gondii* before calcium ionophore A23187 stimulation significantly inhibited the ionophore-induced release of 5-lipoxygenase products by these cells. An 8.7-fold reduction ($p < 0.02$) in the calcium ionophore A23187–stimulated

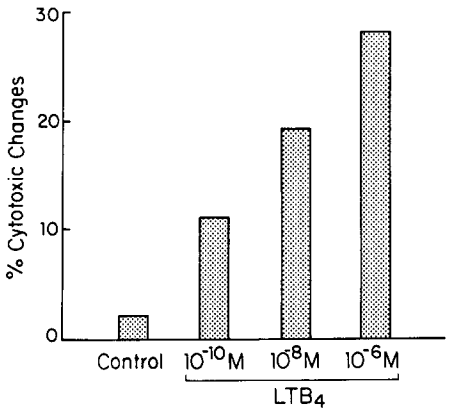
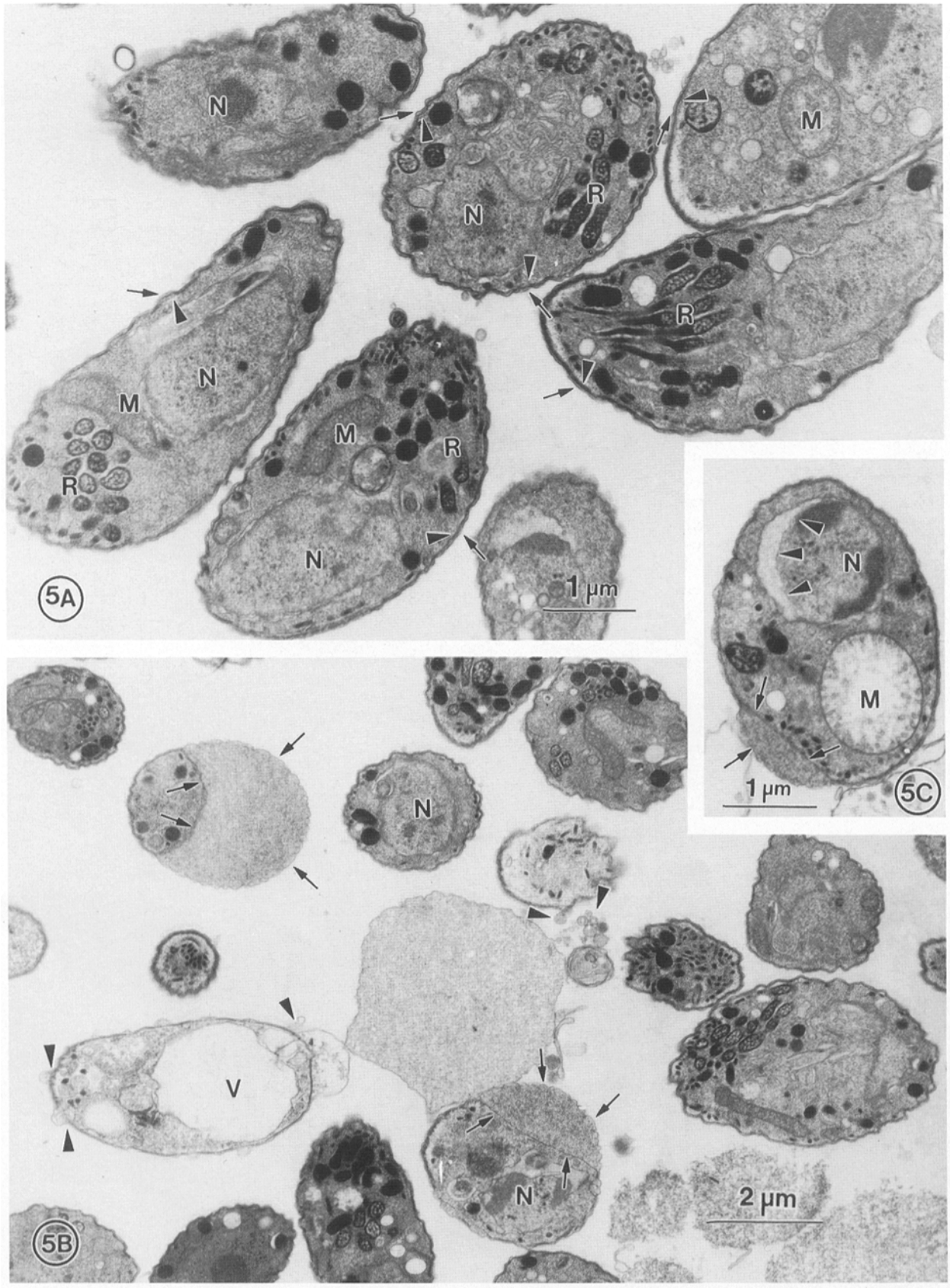


Figure 4. Morphometric analysis of the cytotoxic effect of LTB_4 against *T. gondii*. *T. gondii* tachyzoites were incubated in PBS in the absence (control) or presence of LTB_4 (10^{-10} - 10^{-6} M) at 37°C for 30 min. The samples were prepared for transmission electron microscopy, and the percentage distribution of *T. gondii* cytotoxic changes was determined by morphometric analysis as described in Materials and Methods. 1,157 *T. gondii* were randomly selected and examined for these studies.


N
N
R
M
R
N
M
R
M
R
N
1 μm
5A
N
M
1 μm
5C
N
V
N
2 μm
5B

release of LTB_4 by these cells was observed (Fig. 2); this level of LTB_4 was not significantly different from that released by day 5 cells alone. In contrast, prior incubation of the monocyte-derived macrophages with heat-killed *T. gondii* did not affect the calcium ionophore A23187–induced release of eicosanoids by these monolayers (Figs. 2 and 3).

IFN-γ–treated Monocyte-derived Macrophages. Monocytes were maintained in culture for 5 d in the presence of IFN-γ (100 U/ml). IFN-γ treatment did not significantly alter the release of either cyclooxygenase or 5-lipoxygenase products by monocyte-derived macrophages incubated with *T. gondii* (viable or heat killed), zymosan, or the calcium ionophore A23187 alone compared with similarly stimulated cells incubated in the absence of IFN-γ (Fig. 2). However, IFN-γ restored the ability of *T. gondii*–infected monocyte-derived macrophages to release LTB_4 after stimulation with the calcium ionophore A23187. Calcium ionophore A23187–stimulated release of LTB_4 by *T. gondii*–infected monocyte-derived macrophages was augmented 14.5-fold ($p < 0.02$) in IFN-γ–treated cells compared with cells incubated in the absence of IFN-γ (Fig. 2). LTB_4 constituted 67.3% of eicosanoids released by the IFN-γ–supplemented monolayers stimulated with calcium ionophore A23187 after prior incubation with *T. gondii* (Fig. 3).

Since (*a*) *T. gondii* fail to stimulate either LTB_4 or LTC_4 release by human mononuclear phagocytes, (*b*) IFN-γ reverses *T. gondii*–induced suppression of LTB_4 generation in monocyte-derived macrophages, and (*c*) IFN-γ stimulates macrophage toxoplasmacidal activity (1–3), we examined whether 5-lipoxygenase arachidonic acid products exert cytotoxic activity against these organisms. As determined by morphometric analysis of transmission electron micrographs, LTB_4 (10^{-10}–10^{-8} M) induced cytotoxic changes in the *T. gondii* (Fig. 4). 28.1% of the tachyzoites showed evidence of cytotoxic injury after incubation with 10^{-6} M LTB_4 in PBS for 30 min compared with 2% of control organisms incubated in buffer either alone (Fig. 4) or containing 0.5% ethanol (vehicle control, not shown). LTB_4-induced antitoxoplasma activity was not significantly affected by the presence of serum; 24.9% of *T. gondii* tachyzoites ($n = 169$) exhibited cytotoxic changes after incubation for 30 min in PBS containing 10^{-6} M LTB_4 and 10% heat-inactivated normal serum that was negative for *Toxoplasma*-specific IgG and IgM antibodies. LTB_4-mediated cytotoxic changes included surface membrane vesiculation, extravasation of cytoplasmic contents into a space between the inner and outer surface membrane units, and cytoplasmic vacuolization in the *T. gondii* (Fig. 5).

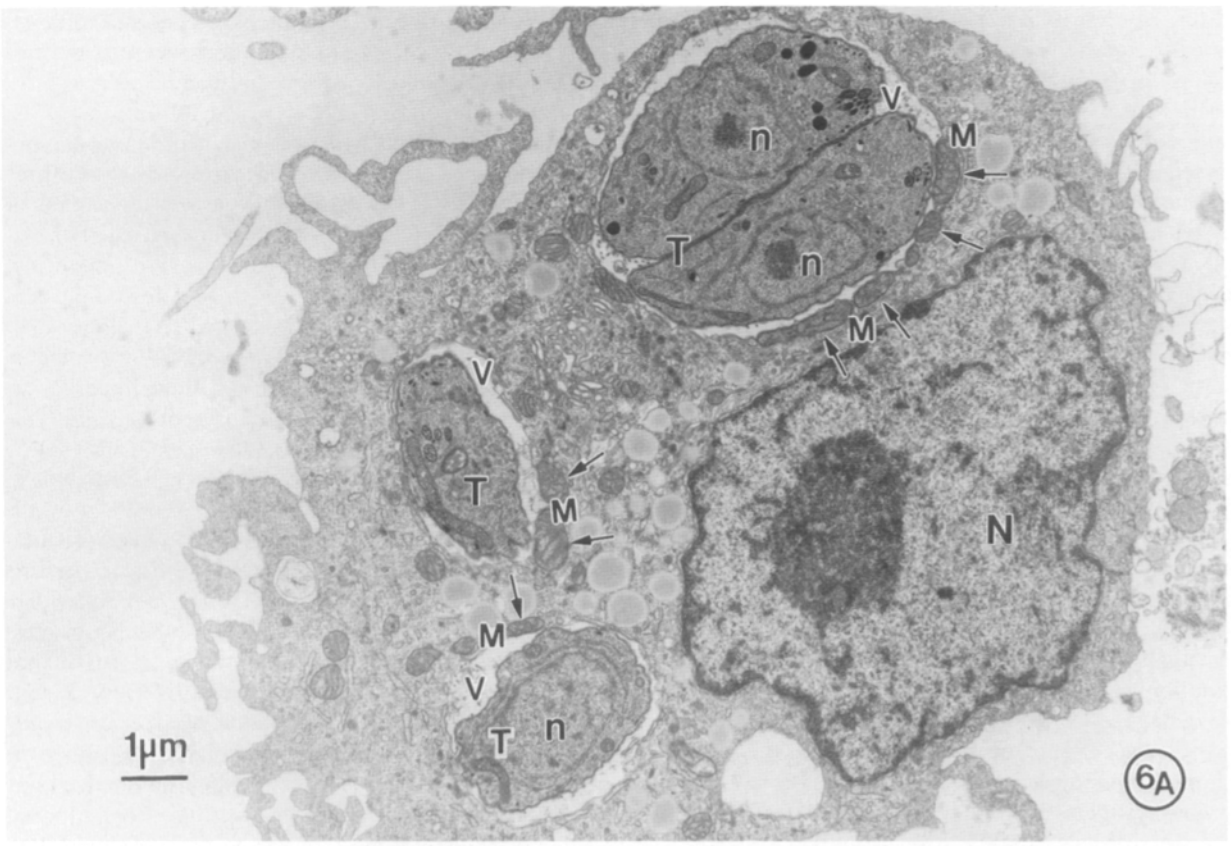
LTB_4 also promoted intracellular killing of ingested *T. gondii* tachyzoites by day 5 monocyte-derived macrophages (Fig. 6). Non-IFN-γ–treated monocyte-derived macrophages after incubation with *T. gondii* for 30 min were then washed to remove noningested organisms and incubated in the absence or presence of LTB_4 (10^{-6} M) for 60 min at 37°C. After washing, the monolayers were maintained in media containing 10% autologous serum and examined by transmission electron microscopy at 24 h. Whereas replication of normal-appearing *T. gondii* in monocyte-derived macrophages was observed 24 h after ingestion of organisms (Fig. 6 *A*), *T. gondii* replication was inhibited by incubation of the cells after ingestion of organisms with 10^{-6} M LTB_4 (Fig. 6 *B*). The phagocytic vacuoles in the LTB_4-treated cells contained damaged *T. gondii* of irregular shape and altered internal organelle structure. Close association of macrophage mitochondria with the surface of parasite vacuoles observed in *T. gondii*–infected cells (Fig. 6 *A*) was not present in cells after LTB_4 treatment (Fig. 6 *B*).

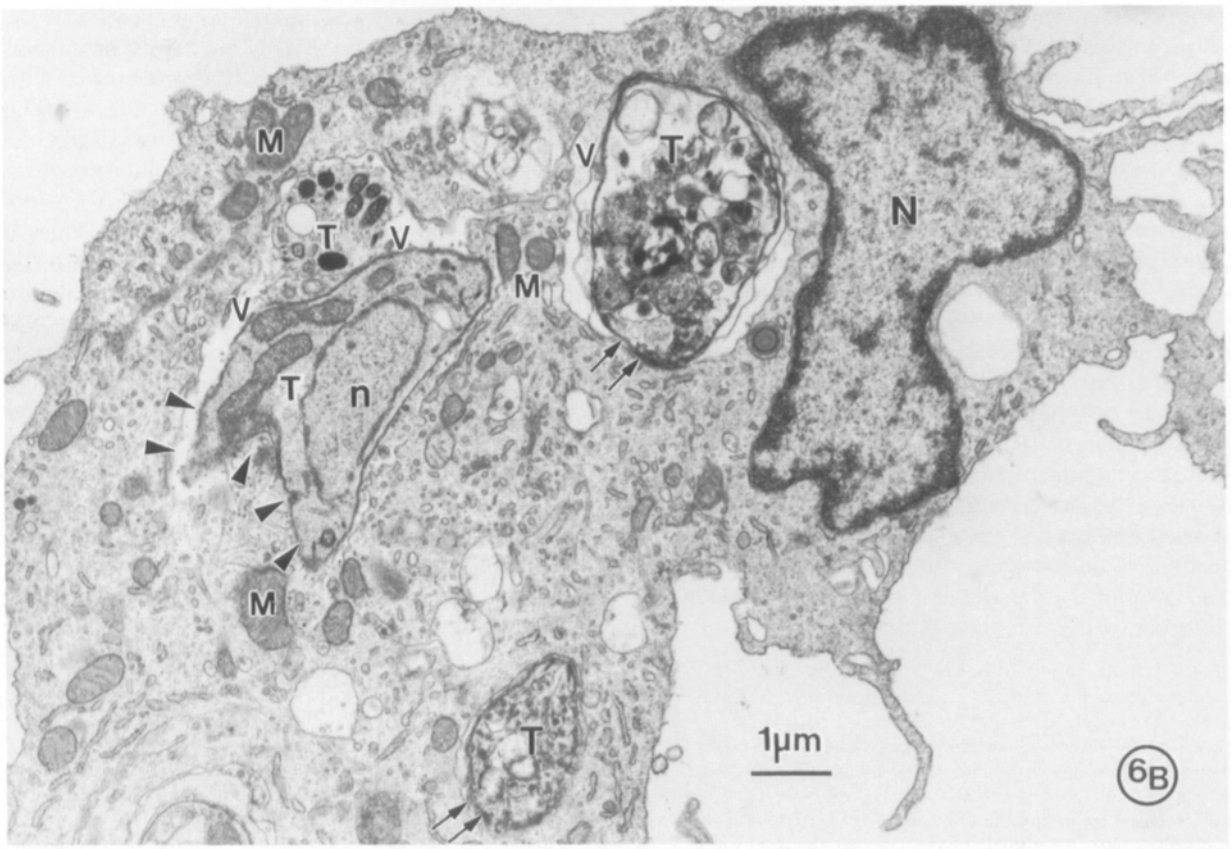
The role of cyclooxygenase versus 5-lipoxygenase products in IFN-γ–induced macrophage antitoxoplasma activity was next examined. IFN-γ–treated day-5 monocyte-derived macrophages were incubated with *T. gondii* in the absence or presence of either the cyclooxygenase inhibitor indomethacin (33) or the 5-lipoxygenase inhibitor zileuton (20, 34) for 30 min at 37°C. After washing, the incubations were either terminated by formalin fixation of the monolayers (0 h) or continued in media alone or media containing indomethacin or zileuton for an additional 6 h before fixation. IFN-γ–treated day-5 monocyte-derived macrophages exerted antitoxoplasma activity (Fig. 7). There was no significant increase in the number of *T. gondii* present in the IFN-γ–treated macrophages at 6 h (1.6 ± 0.3 *T. gondii* per macrophage) compared with 0 h (1.1 ± 0.2 *T. gondii* per macrophage, $p = 0.3028$) (Fig. 7). IFN-γ–induced macrophage antitoxoplasma activity was not significantly affected by cyclooxygenase inhibition by indomethacin (10^{-6} M). In contrast, the number of *T. gondii* per macrophage was increased 6.6-fold (6.6 ± 0.8 *T. gondii* per macrophage, $p = 0.0028$) at 6 h compared with 0 h (1.0 ± 1 *T. gondii* per macrophage) in the monolayers treated with zileuton. 10^{-6} M zileuton inhibited LTB_4 release by 91% but did not significantly affect TXB_2 release in IFN-γ–treated monocyte-derived macrophages stimulated with the calcium ionophore A23187, confirming that zileuton is a selective 5-lipoxygenase pathway inhibitor in these studies.

Discussion

We report here that *T. gondii* tachyzoites markedly alter eicosanoid metabolism in human mononuclear phagocytes. In particular, 5-lipoxygenase arachidonic acid product release

Figure 5. Ultrastructure of LTB_4-induced *T. gondii* cytotoxicity. (*A*) *T. gondii* tachyzoites were incubated at 37°C for 30 min in PBS. The nucleus (*N*), mitochondria (*M*), rhoptries (*R*), and other intracellular structures of the crescent-shaped control *T. gondii* are of normal appearance. The surface structure of the organisms consists of intact outer (*arrows*) and inner (*arrowheads*) membrane units. Bar, 1 μm. (*B* and *C*) The *T. gondii* were incubated at 37°C for 30 min in PBS with LTB_4 (10^{-6} M). (*B*) LTB_4 induced surface membrane vesiculation (*arrowheads*). Extravasation (*arrows*) of cytoplasmic contents through the inner parasite membrane caused bulging of the outer parasite membrane. Intracellular vacuolation (*V*) is observed. Bar, 2 μm. (*C*) Swelling of the membrane (*arrowheads*) surrounding the nucleus (*N*) and cytoplasmic leakage into the space (*arrows*) between the inner and outer surface membrane units are seen in *T. gondii* after incubation with LTB_4. Swelling of a mitochondrion (*M*) is also observed. Bar, 1 μm.


V
n
M
T
n
M
V
T
M
N
M
V
T n
1μm
6A


M
V
T
T
V
M
V
T
n
N
M
T
1μm
6B

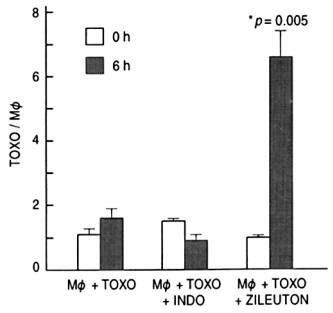


Figure 7. Effect of cyclooxygenase and 5-lipoxygenase inhibition on IFN-γ–mediated toxoplasmacidal activity. Monocyte-derived macrophages (*Mϕ*) cultured for 5 d in the presence of IFN-γ (100 U/ml) were incubated with *T. gondii* (*TOXO*; 1:5 ratio, macrophage/organism) in media alone or with 10^{-6} M indomethacin (*INDO*) or 10^{-6} M zileuton for 30 min at 37°C. After washing, the cell monolayers were either fixed immediately in 5% formalin (0 h) or incubated in the absence or presence of either indomethacin or zileuton for an additional 6 h before formalin fixation. The number of intracellular *T. gondii* per macrophage was determined by light microscopic examination of Giemsa-stained preparations. The data represent the mean ± SE of three experiments. Probability values for the difference from macrophage + *T. gondii* alone are shown where significant ($p < 0.05$).

by these cells is suppressed by viable *T. gondii*. We found that LTB_4 exerts cytotoxic activity against *T. gondii* tachyzoites promoting intracellular killing of these organisms in monocyte-derived macrophages. Furthermore, the inhibition of IFN-γ–induced macrophage antitoxoplasma activity by selective inhibition of 5-lipoxygenase suggests an important role for 5-lipoxygenase products in the cytotoxic activity of these mononuclear phagocytes against *T. gondii*.

Our data indicate that, in metabolism of arachidonic acid by human monocytes, the profile of products released is dependent on the nature of the specific stimulus. Both cyclooxygenase (i.e., TXB_2, PGE_2) and 5-lipoxygenase (i.e., LTB_4, LTC_4) products of arachidonic acid metabolism are released by normal human monocytes when stimulated by either particulate (i.e., opsonized zymosan particles, heat-killed *T. gondii*) or soluble (i.e., calcium ionophore A23187) stimuli. However, the monocytes (in comparison to nonstimulated control cells) fail to release significant amounts of 5-lipoxygenase products after ingestion of viable *T. gondii*. Possible explanations for this finding include the following: (*a*) LTs formed by *T. gondii*–stimulated normal human monocytes are degraded by MPO–H_2O_2–halide system oxidants or hydroxyl radicals (OH·) released by the phagocytes (25); and (*b*) *T. gondii* do not activate the 5-lipoxygenase pathway of arachidonic acid metabolism. Our findings suggest the second mechanism. CGD monocytes, which are unable to respond to stimulation with a respiratory burst and thus cannot oxidatively degrade LTs, also failed to release 5-lipoxygenase products after incubation with *T. gondii*. As in normal monocytes, only cyclooxygenase pathway activation occurs in *T. gondii*–stimulated CGD monocytes.

Cyclooxygenase arachidonate products were the predominant eicosanoids released by monocytes ingesting viable *T. gondii* or opsonized zymosan (88.7 and 68.0% of total eicosanoids, respectively). TXB_2 was the predominant eicosanoid released by zymosan-stimulated human monocytes, in agreement with previous studies (35). Similarly, we found that the monocytes after interaction with *T. gondii* primarily release TXB_2. We recently reported that human platelets are cytotoxic to *T. gondii* tachyzoites, and TX release is important in this platelet-mediated toxoplasmacidal activity (21). Surface membrane vesiculation and cytoplasmic lysis in the *T. gondii* are prominent ultrastructural features of TXA_2-induced injury (21). It is possible that TX generation by freshly isolated monocytes may contribute to the previously demonstrated (2) cytotoxic effect of these cells against *T. gondii*.

In monocytes, the soluble stimulus calcium ionophore A23187 primarily induced the release of 5-lipoxygenase arachidonate products (89.5% of total eicosanoids assayed), in agreement with previous studies (35–38). LTB_4 was the major product of calcium ionophore A23187–stimulated monocytes, with a ratio of $TXB_2/PGE_2/LTB_4/LTC_4$ of 6:1:54:7 observed. The calcium ionophore A23187 also markedly increased (i.e., up to 30.4-fold) the total amount of eicosanoids released by the monocytes compared with that induced by the particulate stimuli. Prior incubation of the cells with viable *T. gondii* did not significantly alter this pattern, with a ratio of $TXB_2/PGE_2/LTB_4/LTC_4$ of 7:1:43:6 observed. These data indicate that *T. gondii* do not inhibit activation of the 5-lipoxygenase pathway by a second stimulus in freshly isolated monocytes.

Tripp et al. (39) have reported that when murine peritoneal macrophages are maintained in tissue culture, there is a progressive decrease in the capacity of these cells to metabolize arachidonic acid. By 4 d in culture, they noted a minimum release of both cyclooxygenase (i.e., 6-keto-$PGE_{1\alpha}$, PGE_2, TXB_2) and 5-lipoxygenase (i.e., LTC_4) products by these macrophages. Similarly, we observed that as human monocytes differentiate in culture to monocyte-derived macrophages, there is a marked reduction in the ability of these cells to release eicosanoids after incubation with either soluble or particulate stimuli. In general, 5-lipoxygenase product release was inhibited to a greater extent than cyclooxygenase product

Figure 6. Effect of LTB_4 on intracellular killing of *T. gondii* by human monocyte–derived macrophages. Non-IFN-γ–treated day-5 monocyte-derived macrophages were incubated with *T. gondii* (1:5 ratio, monocyte-derived macrophage/organism) for 30 min at 37°C, washed to remove noningested organisms, and then incubated in the absence (*A*) or presence (*B*) of 10^{-6} M LTB_4 and 10% autologous serum that was negative for *Toxoplasma*-specific IgG and IgM antibodies for 60 min at 37°C. After washing in Ca^{2+}/Mg^{2+}-free PBS, the cells were incubated for an additional 23 h in RPMI media containing 10% autologous serum before fixation and examination by transmission electron microscopy. (*A*) In this representative *T. gondii*-infected monocyte-derived macrophage that was not treated with LTB_4, the nucleus (*N*) is of normal appearance and vacuoles (*V*) containing ingested *T. gondii* (*T*) are seen. Replication of organisms within a vacuole is observed. The crescent-shaped organisms contain a centrally located nucleus (*n*). Mitochondria (*M*) of the macrophage are located close to the surface (*arrows*) of the parasite vacuole. Bar, 1 μm. (*B*) After incubation with LTB_4, *T. gondii*–infected monocyte-derived macrophages contain phagocytic vacuoles (*V*) surrounding disrupted organisms. *T. gondii* (*T*) of irregular shape (*arrowheads*) and loss of normal internal organelle structure are seen. Close association of macrophage mitochondria (*M*) with the surface of the parasite vacuoles is not observed in these preparations after LTB_4 treatment. Bar, 1 μm.

release in the monocyte-derived macrophages. Monolayers maintained in culture for 5 d produced 12.7-fold less total eicosanoids after calcium ionophore A23187 stimulation than comparably activated freshly isolated monocytes. LTB_4 and LTC_4 release was decreased 16.3- and 44.4-fold, respectively (17.6-fold decrease in 5-lipoxygenase products), whereas TXB_2 and PGE_2 release were decreased 4.1- and 2.8-fold, respectively (3.8-fold decrease in cyclooxygenase products) in the calcium ionophore A23187–stimulated day 5 monolayers. Release of LTB_4 and TXB_2 by calcium ionophore A23187–stimulated day 5 monolayer cells was still significantly greater than that of unstimulated controls, however. In zymosan-stimulated monocyte-derived macrophages, a 2.7-fold decrease in total eicosanoids (4.4-fold decrease in 5-lipoxygenase products and 2.3-fold decrease in cyclooxygenase products) released compared with zymosan-activated monocytes was observed.

In day 5 monolayers incubated with *T. gondii*, eicosanoid release was decreased to a greater extent than in cells incubated with zymosan particles. Although freshly isolated monocytes release both TXB_2 and PGE_2 after ingestion of *T. gondii*, monocytes maintained in culture for 5 d are not stimulated by *T. gondii* to release these cyclooxygenase products. Compared with freshly isolated monocytes, total eicosanoid release fell ninefold in monocyte-derived macrophages incubated with *T. gondii*, with a 15.5- and 9.5-fold reduction in release of TXB_2 and PGE_2, respectively. Furthermore, infection of the day-5 monocyte-derived macrophages by *T. gondii* prevented 5-lipoxygenase pathway activation by a second stimulus, the calcium ionophore A23187. Failure of *T. gondii*–infected monocyte-derived macrophages to release eicosanoids (e.g., TXB_2 and LTB_4) that exert toxoplasmacidal activity may be important in the inability of human monocyte–derived macrophages to kill these parasites.

We have previously demonstrated that *T. gondii* cell membranes are disrupted by the cyclooxygenase product TXA_2 (generated by platelet microsomal fractions) (21) and by the linoleic acid metabolite 13-hydroxyoctadecadienoic acid (31) but not by the 12-lipoxygenase product 12-HETE released by *T. gondii*–stimulated human platelets (26). As shown in this report, the 5-lipoxygenase arachidonate product LTB_4 also exerts potent cytotoxic activity against *T. gondii* tachyzoites. LTB_4 induced surface membrane vesiculation, leakage of cytoplasmic contents into the space between the inner and outer surface membrane units, and intracytoplasmic vacuolation in *T. gondii*. Comparable cytotoxic changes occur in *T. gondii* after incubation with 10^{-6} M LTC_4 for 30 min at 37°C (Chi, E. Y., and Henderson, W. R., Jr., unpublished observations). We have also found that incubation of *T. gondii*–infected non-IFN-γ–treated monocyte-derived macrophages with LTB_4 (10^{-6} M) for 30 min results in intracellular killing of the pathogens in contrast to intracellular replication of the *T. gondii* and disruption of the infected macrophages incubated in the absence of LTB_4. LTB_4 is a potent chemoattractant for human neutrophils and monocytes in vitro and in vivo (40, 41). *T. gondii*–induced inhibition of mononuclear phagocyte LTB_4 release could be an important antiinflammatory mechanism used by the parasites to diminish the influx of leukocytes into the tissue sites of *T. gondii* infection.

We also examined the effect of IFN-γ activation of the human mononuclear phagocytes on the metabolism of arachidonic acid by these cells. Prior studies have shown that activation of murine peritoneal macrophages results in an alteration in both the release of arachidonic acid and profile of generated eicosanoids that is dependent on whether the cells are activated in vivo or in vitro and the specific stimulus triggering eicosanoid release.

In vivo activation of murine peritoneal macrophages by intraperitoneal injection of *Corynebacterium parvum* greatly reduces release of PGI_2 (42–44), PGE_2 (43, 44), HETEs (44), and LTC_4 (44) after incubation of cells with zymosan (43, 44), phorbol myristate acetate (43), or PGH_2 (42). However, conservation of TXA_2 synthesis occurs in murine peritoneal macrophages activated in vivo only by treatment with either *C. parvum* (44) or *Listeria monocytogenes* (45). For example, TXA_2 synthesis in *L. monocytogenes*–activated macrophages after zymosan stimulation is not significantly different from resident peritoneal macrophages, whereas total release of eicosanoids by the elicited cells is decreased by 94% compared with resident macrophages after incubation with zymosan (45). In contrast, in vitro activation of murine peritoneal macrophages by IFN-γ results in diminished release of thromboxane (46) as well as PGI_2 (46), PGE_2 (46), LTB_4 (47), and LTC_4 (47) after zymosan stimulation compared with resident cells without IFN-γ treatment. Tripp et al. (45) have proposed that there is a different response to IFN-γ of monocytes migrating to the site of *L. monocytogenes* infection compared with that of resident macrophages, which accounts for TXA_2 synthesis to be conserved in the in vivo–activated mononuclear phagocyte cell populations but downregulated in the in vitro IFN-γ–treated cells.

The nature of the stimulus triggering eicosanoid synthesis also affects the profile of cyclooxygenase and 5-lipoxygenase products released by activated macrophages in comparison to resident cells. As described above, IFN-γ treatment of murine peritoneal macrophages inhibits zymosan-induced release of both arachidonic acid (46) and all cyclooxygenase (i.e., PGE_2, TXB_2, PGI_2) and 5-lipoxygenase (i.e., LTB_4, LTC_4) arachidonate products. However, while arachidonic acid and LTC_4 release is decreased in calcium ionophore A23187–stimulated IFN-γ–activated murine peritoneal macrophages, TXA_2 release is unaffected, and PGE_2 and PGI_2 release is augmented compared with resident cells (46). Furthermore, total arachidonic acid release is increased in these IFN-γ–treated cells after PMA stimulation, which also increases the release of PGE_2 and PGI_2 (46).

Conflicting reports on the effects of IFN-γ on human monocyte eicosanoid release have been published. In one study (48), IFN-γ (500 U/ml)-treatment of monocytes produced decreased release of PGE_2, TXB_2, $PGF_{2\alpha}$, and LTC_4 after concanavalin A stimulation. However, in monocytes treated with IFN-γ at a concentration of 10 U/ml and then stimulated with LPS, different effects on eicosanoid release have been observed. Nichols and Garrison (49) reported that such

treatment increases PGE_2 release without affecting TXB_2 release by these cells. In contrast, Browning and Ribolini (50) found that PGE_2 release was decreased and TXB_2 release was increased in monocytes pretreated with IFN-γ before LPS stimulation.

We found that incubation of human monocytes with IFN-γ (100 U/ml) for 5 d in culture did not significantly affect the release of either cyclooxygenase or 5-lipoxygenase products by the monocyte-derived macrophages after incubation with one of the following stimuli: zymosan, the calcium ionophore A23187, or heat-killed *T. gondii*. However, IFN-γ restored the ability of *T. gondii*–infected monocyte-derived macrophages to release LTB_4 after calcium ionophore A23187 stimulation. The inhibition of IFN-γ–mediated toxoplasmacidal activity by the selective 5-lipoxygenase inhibitor zileuton suggests that 5-lipoxygenase arachidonic acid metabolites play an important role in the mediation of this cytotoxic activity.

In summary, these data indicate that *T. gondii* tachyzoites fail to activate the 5-lipoxygenase pathway in human monocytes and suppress activation of this pathway in monocyte-derived macrophages. This inhibition of mononuclear phagocyte 5-lipoxygenase product release may be important for the survival of these pathogens in vivo.

We thank Gertrude Chiang, Dong Nguyen, and Y. Z. Tien for excellent technical assistance and Rachel Norris for typing this manuscript.

This work was supported by National Institutes of Health grants AI-17758, AI-34578, and AI-23713.

Address correspondence to Dr. William R. Henderson, Jr., Department of Medicine, Mailstop SJ-10, University of Washington, Seattle, WA 98185.

Received for publication 17 May 1994 and in revised form 20 July 1994.

References

1. Nathan, C.F., H.W. Murray, M.E. Wiebe, and B.Y. Rubin. 1983. Identification of interferon-γ as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. *J. Exp. Med.* 158:670.
2. Murray, H.W., B.Y. Rubin, S.M. Carriero, A.M. Harris, and E.A. Jaffee. 1985. Human mononuclear phagocyte antiprotozoal mechanisms: oxygen-dependent vs. oxygen-independent activity against intracellular *Toxoplasma gondii*. *J. Immunol.* 134:1982.
3. Murray, H.W., D. Scavuzzo, J.L. Jacobs, M.H. Kaplan, D.M. Libby, J. Schindler, and R.B. Roberts. 1987. In vitro and in vivo activation of human mononuclear phagocytes by interferon-γ. Studies with normal and AIDS monocytes. *J. Immunol.* 138:2457.
4. Klebanoff, S.J. 1988. Phagocytic cells: products of oxygen metabolism. *In* Inflammation: Basic Principles and Clinical Correlates. J.I. Gallin, I.M. Goldstein, and R. Snyderman, editors. Raven Press, New York. 391–444.
5. Bainton, D.F., and D.W. Golde. 1978. Differentiation of macrophages from normal human bone marrow in liquid culture. Electron microscopy and cytochemistry. *J. Clin. Invest.* 61:1555.
6. van der Meer, J.W.M., R.H.J. Beelen, D.M. Fluitsma, and R. van Furth. 1979. Ultrastructure of mononuclear phagocytes developing in liquid bone marrow cultures. A study on peroxidatic activity. *J. Exp. Med.* 149:17.
7. Neill, M.A., W.R. Henderson, and S.J. Klebanoff. 1985. Oxidative degradation of leukotriene C_4 by human monocytes and monocyte-derived macrophages. *J. Exp. Med.* 162:1634.
8. Breton-Gorius, J., J. Guichard, W. Vainchenker, and J.L. Vilde. 1980. Ultrastructural and cytochemical changes induced by short and prolonged culture of human monocytes. *J. Reticuloendothel. Soc.* 27:289.
9. Nakagawara, A., C.F. Nathan, and Z.A. Cohn. 1981. Hydrogen peroxide metabolism in human monocytes during differentiation in vitro. *J. Clin. Invest.* 68:1243.
10. Nakagawara, A., N.M. DeSantis, N. Nogueira, and C.F. Nathan. 1982. Lymphokines enhance the capacity of human monocytes to secrete reactive oxygen intermediates. *J. Clin. Invest.* 70:1042.
11. Murray, H.W., C.W. Juangbhanich, C.F. Nathan, and Z.A. Cohn. 1979. Macrophage oxygen-dependent antimicrobial activity. II. The role of oxygen intermediates. *J. Exp. Med.* 150:950.
12. Locksley, R.M., C.S. Nelson, J.E. Fankhauser, and S.J. Klebanoff. 1987. Loss of granule myeloperoxidase during *in vitro* culture of human monocytes correlates with decay in antiprotozoa activity. *Am. J. Trop. Med. Hyg.* 36:541.
13. Wilson, C.B., and J.S. Remington. 1979. Activity of human blood leukocytes against *Toxoplasma gondii*. *J. Infect. Dis.* 140:890.
14. Murray, H.W., and D.M. Cartelli. 1983. Killing of intracellular *Leishmania donovani* by human mononuclear phagocytes. Evidence for oxygen-dependent and -independent leishmanicidal activity. *J. Clin. Invest.* 72:32.
15. Jones, T.C., and J.G. Hirsch. 1972. The interaction between *Toxoplasma gondii* and mammalian cells. II. The absence of lysosomal fusion with phagocytic vacuoles containing living parasites. *J. Exp. Med.* 136:1173.
16. Joiner, K.A., S.A. Fuhrman, H.M. Miettinen, L.H. Kasper, and I. Mellman. 1990. *Toxoplasma gondii*: fusion competence of parasitophorous vacuoles in Fc receptor–transfected fibroblasts. *Science (Wash. DC).* 249:641.
17. Murray, H.W., B.Y. Rubin, and C.D. Rothermel. 1983. Killing of intracellular *Leishmania donovani* by lymphokine-stimulated human mononuclear phagocytes. Evidence that interferon-γ

is the activating lymphokine. *J. Clin. Invest.* 72:1506.
18. Locksley, R.M., and S.J. Klebanoff. 1983. Oxygen-dependent microbicidal systems of phagocytes against intracellular protozoa. *J. Cell Biochem.* 22:173.
19. Locksley, R.M., J. Fankhauser, and W.R. Henderson. 1985. Alteration of leukotriene release by macrophages ingesting *Toxoplasma gondii*. *Proc. Natl. Acad. Sci. USA.* 82:6922.
20. Carter, G.W., P.R. Young, D.H. Albert, J. Bouska, R. Dyer, R.L. Bell, J.B. Summers, and D.W. Brooks. 1991. 5-Lipoxygenase inhibitory activity of zileuton. *J. Pharmacol. Exp. Ther.* 256:929.
21. Yong, E.C., E.Y. Chi, T.R. Fritsche, and W.R. Henderson, Jr. 1991. Human platelet-mediated cytotoxicity against *Toxoplasma gondii*: role of thromboxane. *J. Exp. Med.* 173:65.
22. Boyum, A. 1976. Isolation of lymphocytes, granulocytes and macrophages. *Scand. J. Immunol.* 5(Suppl.):9.
23. Kaplow, L.S. 1965. Simplified myeloperoxidase stain using benzidine dihydrochloride. *Blood.* 26:215.
24. Anderson, S.E., S.C. Bautista, and J.S. Remington. 1976. Specific antibody-dependent killing of *Toxoplasma gondii* by normal macrophages. *Clin. Exp. Immunol.* 26:375.
25. Henderson, W.R., and S.J. Klebanoff. 1983. Leukotriene production and inactivation by normal, chronic granulomatous disease and myeloperoxidase-deficient neutrophils. *J. Biol. Chem.* 258:13522.
26. Henderson, W.R., Jr., M. Rashed, E.C. Yong, T.R. Fritsche, and G.K. Chiang. 1992. *Toxoplasma gondii* stimulates the release of 13- and 9-hydroxyoctadecadienoic acids by human platelets. *Biochemistry.* 31:5356.
27. Rokach, J., E.C. Hayes, Y. Girard, D.L. Lombardo, A.L. Maycock, A.S. Rosenthal, R.N. Young, R.F. Zamboni, and H.J. Zweerink. 1984. The development of sensitive and specific radioimmunoassays for leukotrienes. *Prostaglandins Leukot. Med.* 13:21.
28. Geissler, F.T., and W.R. Henderson. 1988. Inability of aflatoxin B_1 to stimulate arachidonic acid metabolism in human polymorphonuclear and mononuclear leukocytes. *Carcinogenesis.* 9:1135.
29. Geissler, F.T., F.B. Kuzan, E.M. Faustman, and W.R. Henderson, Jr. 1989. Lipid mediator production by postimplantation rat embryos *in vitro*. *Prostaglandins.* 38:145.
30. Jaffe, B.M., and H.R. Behrman. 1974. Prostaglandins E, A and F. *In* Methods of Hormone Radioimmunoassay. B.M. Jaffe and H.R. Behrman, editors. Academic Press, Inc., New York. 19–34.
31. Henderson, W.R., Jr., and E.Y. Chi. 1992. Cytotoxic activity of 13-hydroxyoctadecadienoic acid against *Toxoplasma gondii*. *Parasitology.* 105:343.
32. Weibel, E.R. 1979. Stereological Methods Vol. 1: Practical Methods for Biological Morphometry. Academic Press, London. 101–161.
33. Flower, R.J. 1974. Drugs which inhibit prostaglandin biosynthesis. *Pharmacol. Rev.* 26:33.
34. Israel, E., R. Dermarkarian, M. Rosenberg, R. Sperling, G. Taylor, P. Rubin, and J.M. Drazen. 1990. The effects of a 5-lipoxygenase inhibitor on asthma induced by cold, dry air. *N. Engl. J. Med.* 323:1740.
35. Pawlowski, N.A., G. Kaplan, A.L. Hamill, Z.A. Cohn, and W.A. Scott. 1983. Arachidonic acid metabolism by human monocytes. Studies with platelet-depleted cultures. *J. Exp. Med.* 158:393.
36. Goldyne, M.E., G.F. Burrish, P. Poubelle, and P. Borgeat. 1984. Arachidonic acid metabolism among human mononuclear leukocytes: lipoxygenase-related pathways. *J. Biol. Chem.* 259:8815.
37. Williams, J.D., J.K. Czop, and K.F. Austen. 1984. Release of leukotrienes by human monocytes on stimulation of their phagocytic receptor for particulate activators. *J. Immunol.* 132:3034.
38. Ferreri, N.R., W.C. Howland, and H.L. Spiegelberg. 1986. Release of leukotrienes C_4 and B_4 and prostaglandin E_2 from human monocytes stimulated with aggregated IgG, IgA, and IgE. *J. Immunol.* 136:4188.
39. Tripp, C.S., A. Wyche, E.R. Unanue, and P. Needleman. 1986. The functional significance of the regulation of macrophage Ia expression by endogenous arachidonate metabolites in vitro. *J. Immunol.* 137:3915.
40. Ford-Hutchinson, A.W. 1990. Leukotriene B_4 in inflammation. *Crit. Rev. Immunol.* 10:1.
41. Martin, T.R., B.P. Pistorese, E.Y. Chi, R.B. Goodman, and M.A. Matthay. 1989. Effects of leukotriene B_4 in the human lung. Recruitment of neutrophils into the alveolar spaces without a change in protein permeability. *J. Clin. Invest.* 84:1609.
42. Stringfellow, D.A., F.A. Fitzpatrick, F.F. Sun, and J.C. McGuire. 1978. Prostacyclin biosynthesis in activated, stimulated and normal mouse peritoneal cell populations. *Prostaglandins.* 16:901.
43. Humes, J.L., S. Burger, M. Galavage, F.A. Kuehl, Jr., P.D. Wightman, M.E. Dahlgren, P. Davies, and R.J. Bonney. 1980. The diminished production of arachidonic acid oxygenation products by elicited mouse peritoneal macrophages: possible mechanisms. *J. Immunol.* 124:2110.
44. Scott, W.A., N.A. Pawlowski, H.W. Murray, M. Andreach, J. Zrike, and Z.A. Cohn. 1982. Regulation of arachidonic acid metabolism by macrophage activation. *J. Exp. Med.* 155:1148.
45. Tripp, C.S., K.M. Leahy, and P. Needleman. 1985. Thromboxane synthase is preferentially conserved in activated mouse peritoneal macrophages. *J. Clin. Invest.* 76:898.
46. Boraschi, D., S. Censini, M. Bartalini, and A. Tagliabue. 1985. Regulation of arachidonic acid metabolism in macrophages by immune and nonimmune interferons. *J. Immunol.* 135:502.
47. Boraschi, D., S. Censini, M. Bartalini, P. Ghiara, P.D. Simplicio, and A. Tagliabue. 1987. Interferons inhibit LTC_4 production in murine macrophages. *J. Immunol.* 138:4341.
48. Wahl, L.M., M.E. Corcoran, S.E. Mergenhagen, and D.S. Finbloom. 1990. Inhibition of phospholipase activity in human monocytes by IFN-γ blocks endogenous prostaglandin E_2–dependent collagenase production. *J. Immunol.* 144:3518.
49. Nichols, F.C., and S.W. Garrison. 1987. Interferon-γ potentiation of lipopolysaccharide-induced eicosanoid release from human monocytes. *J. Interferon Res.* 7:121.
50. Browning, J.L., and A. Ribolini. 1987. Interferon blocks interleukin 1–induced prostaglandin release from human peripheral monocytes. *J. Immunol.* 138:2857.