INFECTION AND IMMUNITY, Apr. 1993, p. 1432–1441
0019-9567/93/041432-10$02.00/0

Vol. 61, No. 4

Eicosanoid Production by Mouse Peritoneal Macrophages during *Toxoplasma gondii* Penetration: Role of Parasite and Host Cell Phospholipases

J. F. THARDIN,[1] C. M'RINI,[1] M. BERAUD,[1] J. VANDAELE,[1] M. F. FRISACH,[1] M. H. BESSIERES,[1,2] J. P. SEGUELA,[1,2] AND B. PIPY[1]*

Laboratoire de la Signalisation et de la Différenciation des Macrophages, INSERM CJF 9107, Institut Louis Bugnard, Université Paul Sabatier,[1] and Service de Parasitologie Mycologie-Chu-Rangueil,[2] 1, avenue Jean Poulhes, 31054 Toulouse Cedex, France

Received 11 August 1992/Accepted 12 January 1993

The metabolism of endogenous arachidonic acid by mouse resident peritoneal macrophages infected in vitro with *Toxoplasma gondii* was studied. Prelabeling of macrophages with [5,6,8,9,11,12,14,15-^{3}H]arachidonic acid and challenge with tachyzoites for 15 min resulted in a high mobilization of free labeled arachidonic acid (178%) in the culture medium. The parasites also triggered the synthesis of 6-keto-prostaglandin F1α (47%), prostaglandin E2 (44%), leukotrienes C4 and D4 (33%) and 5-, 12-hydroxyeicosatetraenoic acids (155%). The study indicated that during the intracellular development phase of the parasites, 6-keto-prostaglandin F1α (38%), prostaglandin E2 (31%), leukotrienes C4 and D4 (15%), hydroxyeicosatetraenoic acids (43%), and free arachidonic acid (110%) were secreted into the culture medium. Pretreatment of tachyzoites with phospholipase A_2 inhibitors (4-*p*-bromophenacyl bromide and quinacrine) and no calcium in the culture medium resulted in inhibition of tachyzoite penetration into the macrophages and a decrease of the arachidonic acid metabolism. The triggering of the arachidonic acid cascade by *T. gondii* was dependent on the active penetration of the parasites into the macrophages, whereas preincubation of the macrophages with phospholipase A_2 inhibitors did not affect penetration or free arachidonic acid release, thereby supporting a role for parasite phospholipase in the penetration process and in arachidonic acid mobilization from macrophage membrane phospholipids. Moreover, treatment of macrophages with phospholipase A_2 inhibitors decreased the activities of the cyclooxygenase and lipoxygenase pathways, also suggesting an activation of host cell phospholipase A_2 by the parasite.

Toxoplasma gondii is the etiologic agent of toxoplasmosis. A high percentage of the world's human population is chronically infected with *T. gondii*, but many of those infected do not suffer apparent untoward effects. Toxoplasmosis poses a great hazard to the fetus when the disease is contracted during pregnancy (26). Interest in this opportunistic parasite has recently increased considerably because of its role in the clinical complications of AIDS (15, 39) and in patients given immunosuppressive therapy for organ transplantation (14).

T. gondii actively invades a wide range of phagocytic and nonphagocytic cells by an energy-requiring process which is not simply a phagocytic event (20, 40). During host cell penetration, it is believed that *T. gondii* secretes rhoptry products which could facilitate its entry into host cells (18, 19, 33, 40). It has been previously reported that a soluble phospholipase increases host cell penetration by *T. gondii*, while phospholipase A_2 (PLA_2) inhibitors and antisera to PLA_2 decrease penetration in a dose-dependent manner (29). Saffer and Schwartzman (30) have recently shown that calcium-dependent PLA activity is found in the supernatant of fractions of sonically disrupted *T. gondii*; this soluble PLA activity may be involved in the release of rhoptry proteins. Phospholipase-associated penetration is probably the cell entry mechanism used by numerous obligate intracellular pathogens. Inoculation of L-cell monolayers with *Rickettsia prowazekii*, a parasitic bacterium, induces the cytotoxicity associated with phospholipase activity and hydrolysis of fatty acids from host cell phospholipids (41). Moreover, phospholipase activity could play an important role in the penetration mechanism of *R. prowazekii* (38).

PLAs represent a family of enzymes involved in the specific hydrolysis of membrane phospholipids yielding equimolar amounts of lysophospholipids and free fatty acids including arachidonic acid (AA). Free AA is a substrate for the synthesis of eicosanoids (cyclooxygenase [CO] and lipoxygenase [LO] products), biologically potent agents involved in inflammatory processes (3). Several reports support the role of eicosanoids in parasite-host cell interactions. Reiner and Malemud (23) have shown that splenic mononuclear cells from mice infected with *Leishmania donovani* synthesized two- to fivefold more prostaglandin E_2 (PGE_2) than did spleen cells from normal mice. Moreover, murine peritoneal macrophages infected with *L. donovani* synthesized increased amounts of both CO and LO AA metabolites (25). Locksley et al. (13) reported alteration of leukotriene release by mouse peritoneal macrophages ingesting *T. gondii* and formation of 11-, 12-, and 15-hydroxyeicosatetraenoic acids (HETEs).

The mechanism of triggering of the AA cascade by parasitic cells is still very unclear. Moreover, it is not sure whether the triggering of the AA cascade by *T. gondii* is linked to the activation of macrophage PLA_2 and/or to the phospholipase of the parasite. Therefore, the objective of this present study was to elucidate the origin of the PLA_2

* Corresponding author.

parasite and/or macrophage in the production of eicosanoids by the host cells. Moreover, we attempted (i) to identify the AA metabolites produced during the penetration of parasites into macrophages and (ii) to elucidate the role of PLA_2 in the interaction of *T. gondii* and macrophages.

MATERIALS AND METHODS

Reagents. Labeled [5,6,8,9,11,12,14,15-^{3}H]AA (specific activity, 166 Ci/mmol) and [5,6-^{3}H]uracil (specific activity, 44 Ci/mmol) were obtained from Amersham France. Phorbol 12-myristate 13-acetate, uracil, quinacrine dihydrochloride, 4-*p*-bromophenacyl bromide (4-*p*-BPB), indomethacin, 1-(5-isoquinolinyl)-2-methylpiperazine (H-7), and sodium dodecyl sulfate (SDS) were purchased from Sigma Chimie (La Verpillière, France); trichloroacetic acid was obtained from Prolabo (Paris, France). The culture media, Hanks' balanced salt solution (HBSS), Dulbecco's modified Eagle medium (DMEM), 199 medium with Hanks' salts, and RPMI 1640 medium, were prepared with GIBCO products (Cergy-Pontoise, France). All reagents and solvents used were of analytical grade.

Animals. OF1 (IOPS Caw) female Swiss mice (6 to 10 weeks of age) were purchased from Iffa-Credo (l'Arbresle, France) and maintained in a sterile environment to reduce infection by environmental pathogens.

Parasite culture. (i) The virulent RH strain of *T. gondii* was used in all experiments. *T. gondii* was maintained by continuous passage in female Swiss OF1 mice as follows. The mice were injected intraperitoneally with about 10^4 tachyzoites in 0.5 ml of HBSS. The parasites, which were collected by washing with 1 ml of HBSS from the peritoneal cavities of mice on day 3 of infection with *T. gondii*, were filtered free of host cells through 3.0-μm-pore-size polycarbonate membrane filters (Nuclepore Corp., Pleasanton, Calif.), centrifuged at 500 × *g* for 15 min at 4°C (Beckman GS-6R centrifuge), suspended in appropriate culture medium, and then counted. The number of tachyzoites was adjusted to 5×10^6/ml.

(ii) The RH strain of *T. gondii* was also maintained by serial passages in human MRC 5 fibroblast (BioMérieux, Lyon, France) monolayers (6, 28), virulence in mice being unaffected. Briefly, MRC 5 fibroblasts were cultivated in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum (FCS), 10 IU of penicillin per ml, and 10 μg of streptomycin per ml in 80-cm^2 flasks (Falcon; Becton Dickinson, Grenoble, France) in a humidified atmosphere at 37°C containing 5% CO_2. The confluent monolayers of MRC 5 were infected with approximately one tachyzoite of the RH strain per 100 fibroblasts. After a 48-h incubation, the tachyzoites lysed the MRC 5 fibroblasts and were collected by centrifugation. *T. gondii* prelabeled with [^{3}H]uracil was obtained after infection of MRC 5 fibroblasts for 48 h in RPMI 1640 medium with 10% FCS containing labeled uracil (20 μCi/ml) at 37°C in a humidified atmosphere containing 5% CO_2. After this time, the culture medium was collected and centrifuged at 500 × *g* for 15 min at 4°C. The labeled parasite pellet was resuspended in DMEM containing 2% lactalbumin hydrolysate and 100 μg of unlabeled uracil per ml and centrifuged twice. The number of labeled tachyzoites was then adjusted to 10^6/ml, and the radioactivity they incorporated was evaluated in a liquid scintillation counter (Pharmacia LKB 1217 Rackbeta).

(iii) Killed tachyzoites were prepared by exposure to UV irradiation for 2 h (fluence, 200 J m^{-2}). They were then washed with HBSS (pH 7.2) before being used for experiments. The viability of *T. gondii* tachyzoites after UV irradiation was tested by two methods, (i) the trypan blue exclusion test (>98 ± 5% of the tachyzoites were killed as determined by six separate irradiation experiments), and (ii) infection of mice and scoring mortality every day. Two groups of seven mice were infected intraperitoneally with about 10^4 irradiated or viable tachyzoites. Under these conditions, all the control mice, which received viable tachyzoites, succumbed after 5 days of infection with *T. gondii*, whereas mice receiving irradiated tachyzoites survived.

(iv) To investigate the involvement of *T. gondii* phospholipase in AA production and parasite penetration, tachyzoites were pretreated with inhibitors of PLA_2: 1 μM 4-*p*-BPB or 50 μM quinacrine (1, 12). In some experiments, parasites were also pretreated with 100 μM H-7, a protein kinase C (PKC) inhibitor. On cell-free cultures, tachyzoites were incubated with appropriate inhibitors for 10 min at 37°C, washed twice, and used for infecting the macrophages. These inhibitor-treated parasites appeared motile under light microscopy, and viability was higher than 95% as determined by the trypan blue exclusion test.

Preparation of peritoneal macrophages. Resident peritoneal macrophages were obtained by the technique of de Maroussem et al. (5). Briefly, the peritoneal cells were collected by washing the peritoneal cavity with 5 ml of cold (4°C) 199 medium with Hanks' salts containing 20 IU of heparin per ml. The cells collected were centrifuged at 400 × *g* for 8 min and resuspended to a final concentration of 5×10^6 cells per ml in DMEM with 44 mM $NaHCO_3$ buffer (pH 7.2) containing 1% FCS. After 2 h of culture, more than 98% of the adherent cells were nonspecific esterase positive and had the morphological appearance of macrophages when examined by May-Grünwald and Giemsa staining.

AA, CO, and LO product release by resident macrophages. Macrophage AA metabolite production was evaluated by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) after in vitro incorporation of labeled AA into the macrophages (8). Briefly, the cell suspensions (5×10^6 cells per ml) were placed in 24-well Falcon plates (10^6 cells per well) and incubated at 37°C for 4 h with labeled AA (1 μCi per well) in 300 μl of DMEM containing 1% FCS. After 4 h of culture, the labeled macrophages were washed twice with DMEM without FCS and containing 2% lactalbumin hydrolysate. The washed, labeled cells were then incubated for 15 min in 500 μl of DMEM with 2% lactalbumin hydrolysate, in the presence of *T. gondii* (ratio of tachyzoites/macrophages, 1:2).

To investigate the effect of extracellular calcium on AA metabolite production by macrophages infected with *T. gondii*, we washed the above-mentioned labeled macrophages twice in HBSS without Ca^{2+} or Mg^{2+} ($[Ca^{2+}] < 2$ μM), and infection was done in the same medium.

To investigate host cell PLA_2 involvement in the AA cascade, we pretreated prelabeled macrophages for 10 min with PLA_2 inhibitors (quinacrine dihydrochloride [50 μM] or 4-*p*-BPB [1 μM]) and with a CO inhibitor (1 μM indomethacin) or a PKC inhibitor (100 μM H-7). Metabolic inhibitors were added to the culture medium in sterile dimethyl sulfoxide, the final concentration of which in the assay was 0.05% (controls included). The cell monolayer was washed twice to remove metabolic inhibitors and then was infected with *T. gondii*. After metabolic inhibitor removal, macrophage viability as judged by trypan blue exclusion was more than 95%.

After the 15-min challenge in the above experiments, the culture medium was collected and the various labeled AA oxygenation products (LO and CO metabolites) released by

macrophages were extracted and evaluated by TLC and HPLC. The labeled infected macrophages were washed twice with DMEM containing 2% lactalbumin hydrolysate (or with Ca^{2+}- and Mg^{2+}-free HBSS) and were cultured for an additional 60 min at 37°C in the absence of extracellular parasites. After this period, the culture medium was collected and centrifuged and the various AA metabolites were extracted and separated by TLC and HPLC.

Assay of LO and CO metabolites and free labeled AA. The culture medium supernatants were acidified to pH 5.4 with 1 N HCl and extracted by chromatography on Varian Bond Elut C18 columns (Analytichem). AA metabolites were eluted with methanol (5 ml). This procedure was very efficient for extracting both CO and LO metabolites (extraction efficiency was greater than 95%). The methanol samples were evaporated to dryness under nitrogen. The residues were dissolved in 80 μl of methanol, and prostaglandins (PGs) and leukotrienes (LTs) dissolved in methanol were quantitatively applied to thin-layer silica-gel plates (LK-6-DF Whatman), which had been previously activated (1 h at 100°C). The solvent system used for the separation of PGs and LTs was the organic phase of ethyl acetate-water-isooctane-acetic acid (110:100:50:20, vol/vol/vol/vol) (32). Samples were monitored by rapid scanning with a Berthold detector for AA metabolite identification and quantification. The results are expressed as femtomoles of labeled AA equivalents in each peak identified as described previously (7).

PGs and LTs resuspended in chromatographic solvent were subjected to reverse-phase HPLC. A Hewlett-Packard 1090 liquid chromatograph was used with a Hewlett-Packard 5-μm C18 column (200 by 4 mm). The solvent acetonitrile-water-acetic acid (35:65:0.6, vol/vol/vol) buffered to pH 5.0 with triethylamine was used at a 1.1-ml/min flow rate. AA metabolites were eluted by an isocratic phase for 7 min; then, from 7 to 37 min, a linear acetonitrile gradient increased the final acetonitrile percentage to 91%. During the final 10 min, an isocratic phase permitted HETE and AA elution. The radioactivity in the effluent was monitored by a Berthold LB506D model HPLC radioactivity detector. The retention time of each metabolite was determined by comigration with authentic radiolabeled standards whose retention times were as follows: 6-keto-PGF1α, 7 min, 5 s; TXB2, 9 min, 15 s, PGF2α, 11 min, 15 s; PGE_2, 13 min, 50 s; PGD2, 15 min, 5 s; LTC4, 16 min, 20 s; LTD4, 18 min, 30 s; LTB4, 23 min, 40 s; 12-HETE, 34 min; 5-HETE, 35 min; and AA, 46 min, 30 s.

Kinetics of *T. gondii* penetration into macrophages. (i) To investigate the mechanism of *T. gondii* penetration into mouse resident peritoneal macrophages, we used [^{3}H]uracil-prelabeled *T. gondii* and evaluated the kinetics of radioactivity incorporated into host cells against time. For each assay, 10^6 macrophages (monolayers in Falcon multiwell plates) cultured in 0.5 ml of DMEM containing 2% lactalbumin hydrolysate at 37°C and 5% CO_2 were infected with either viable labeled *T. gondii* or irradiated labeled *T. gondii* for 15, 30, and 60 min (ratio of tachyzoites/macrophages, 1:2).

(ii) To investigate the influence of temperature on parasite penetration, we investigated *T. gondii* penetration into macrophages at 4°C with DMEM containing 2% HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.2). Under these culture conditions, resident macrophages were maintained for 1 h at 4°C before *T. gondii* challenge.

(iii) To investigate the role of calcium ions, macrophages and tachyzoites were washed twice with HBSS medium without Ca^{2+} or Mg^{2+} and then infected with prelabeled *T. gondii* at 37°C.

(iv) The role of phospholipase in *T. gondii* penetration was studied with macrophages or labeled parasites pretreated for 10 min with 1 μM 4-*p*-BPB or 50 μM quinacrine before challenge at 37°C. Treated macrophages and/or treated tachyzoites were washed twice before macrophage infection with labeled *T. gondii* to remove metabolic inhibitors. After the challenge with prelabeled *T. gondii*, the cell monolayers were washed twice with 2 ml of DMEM, and the macrophages were disrupted in 1% SDS containing 100 μg of unlabeled uracil per ml and precipitated with 0.3 N trichloroacetic acid overnight at 4°C. The contents of the wells were collected by suction on glass fiber filters with an automated cell harvester (Titertek; Flow Laboratories), washed extensively with cold 0.3 N trichloroacetic acid and 95% alcohol, and dried, and the radioactivity was incorporated into RNA counted with BCS scintillation fluid (Amersham) in a liquid scintillation counter.

Assay of *T. gondii* proliferation in macrophages. The intracellular growth of *T. gondii* in monolayer cultures was measured by monitoring [^{3}H]uracil incorporation in RNA into *T. gondii* as described previously (34). Different control experiments were performed to evaluate [^{3}H]uracil incorporation into uninfected macrophages and into free tachyzoites. For each assay, 0.2×10^6 macrophages were cultivated for 2 h in 96-well Falcon plates in DMEM with 1% FCS at 37°C and 5% CO_2 and used for infection as follows. (i) Macrophage monolayers were infected with 2×10^4 tachyzoites (ratio of tachyzoites/macrophages, 1:10) for 1, 2, or 3 h in DMEM containing 2% lactalbumin hydrolysate at 37°C and 5% CO_2. (ii) In some experiments, the macrophages were infected with *T. gondii* without calcium in the culture medium. (iii) Macrophages were infected with irradiated *T. gondii* tachyzoites. (iv) In selected experiments, macrophages or parasites were pretreated as described above with PLA_2 inhibitors, PKC inhibitor, or CO inhibitor.

After challenge of macrophages with tachyzoites (1, 2, and 3 h), the monolayers were washed to eliminate extracellular parasites and the macrophages parasitized by tachyzoites were cultured for another 24 h in the presence of labeled uracil (2 μCi per well). The monolayers were washed and disrupted, and the radioactivity incorporated into RNA was counted with BCS scintillation fluid (Amersham) as indicated.

Statistical analysis. Data are expressed as mean ± standard error. For each experiment, the data were analyzed by one-way analysis of variance and the multiple comparison method of Tukey (11).

RESULTS

AA metabolite production by resident mouse peritoneal macrophages during infection with *T. gondii*. After 15 min, challenge of AA prelabeled mouse peritoneal macrophages with viable *T. gondii* caused an increased mobilization of free labeled AA (Fig. 1A) in the culture medium (178% compared with control macrophages; $P < 0.01$). During this phase of tachyzoite penetration into the macrophages, TLC analysis showed (Fig. 1A) that macrophages synthesized both CO metabolites (PGI2, measured as 6-keto-PGF1α, and PGE_2) and LO metabolites (LTC4 and LTD4 and HETEs [unseparated 5- and 12-HETE]). 6-Keto-PGF1α and PGE_2 increased by 47 and 44% respectively ($P < 0.05$); LTC4 and LTD4 increased by 33% and HETEs increased by 155% ($P < 0.01$) in response to *T. gondii* infection compared with

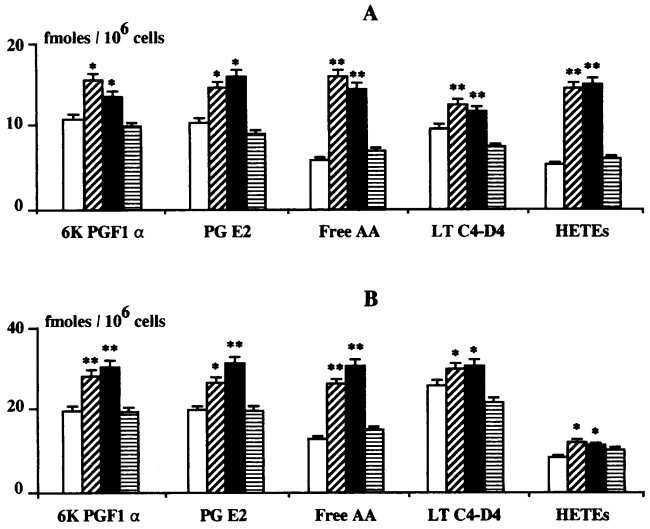

FIG. 1. PG and LT production by mouse peritoneal resident macrophages during *T. gondii* infection. (A) [^{3}H]AA-prelabeled macrophages were infected by *T. gondii* at a ratio of 1:2 tachyzoites/macrophages for 15 min; PGs and LTs secreted in the culture medium were analyzed. (B) Infected host cells were washed and incubated in the absence of extracellular tachyzoites for an additional 60 min, and the eicosanoids were evaluated in the culture medium. □, uninfected macrophages. Macrophages infected with viable tachyzoites from ascitic fluid (▨), with viable tachyzoites from human fibroblast monolayer (■), or with irradiated tachyzoites (▤). The results are the means of three experiments. The bar represents the standard error. The asterisks (*) indicate significant modifications compared with cultured uninfected macrophages: *, $P < 0.05$; **, $P < 0.01$.

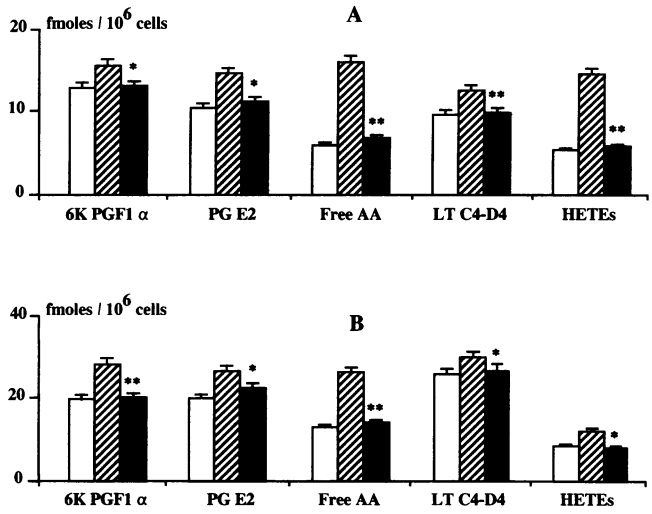

FIG. 2. Effect of culture medium without calcium on PG and LT production by mouse peritoneal resident macrophages during *T. gondii* infection. [^{3}H]AA-prelabeled macrophages incubated in medium without calcium were infected with *T. gondii* at a ratio of 1:2 tachyzoites/macrophages for 15 min and an additional 60 min as indicated in the legend to Fig. 1. Eicosanoids were evaluated in the culture medium after 15 min (A) and 60 min (B) of infection. □, uninfected macrophages, without extracellular calcium. Infected macrophages with extracellular calcium (▨) or without extracellular calcium (■) in the culture medium. The results are the means of three experiments. The bar represents the standard error. The asterisks (*) indicate significant modifications compared with the infected macrophages in the presence of calcium: *, $P < 0.05$; **, $P < 0.01$.

control macrophages (Fig. 1A). By contrast, insignificant increases in labeled material comigrating with standard metabolites of LTB4, TXB2, and PGD2 were noted (data not shown). HPLC analysis demonstrated that the HETEs unseparated by TLC were composed of 20% 5-HETE and 80% 12-HETE. Infection with the *T. gondii* from mouse ascitic fluid and that maintained in human MRC 5 fibroblast monolayers both caused release of labeled AA metabolites of the CO and LO pathways in the extracellular medium. The quantity and profiles of the metabolic products were similar in both cases.

No significant increase in AA production was observed in macrophages infected with irradiated *T. gondii* tachyzoites. The results are shown in Fig. 1A.

After 15 min of challenge, infected prelabeled macrophages were cultured for a further 60 min in the absence of extracellular tachyzoites. As shown in Fig. 1B, during this period, supernatants derived from infected macrophages contained increased amounts of free AA (plus 110%, $P < 0.01$), 6-keto-PGF1α (plus 38%, $P < 0.01$), PGE_2 (plus 31%, $P < 0.05$), LTC4 and LTD4 (plus 15%, $P < 0.05$), and HETEs (20% 5-HETE, 80% 12-HETE) (plus 43%, $P < 0.05$), as determined by TLC and HPLC. The increase in HETEs observed after 15 min was reduced to 57% after a 60-min incubation. Insignificant increases of the macrophage CO and LO profile were detected after 60 min of challenge with irradiated tachyzoites (Fig. 1B).

Effect of extracellular calcium on AA cascade triggered by *T. gondii*. Eicosanoid production in response to *T. gondii* was examined in prelabeled macrophages incubated with or without extracellular calcium in order to examine the effect of extracellular calcium on triggering the AA cascade during infection with *T. gondii*. In these culture conditions, without extracellular calcium ($[Ca^{2+}] < 2$ μM), uninfected resident macrophages spontaneously secreted PGs and LTs. The quantity and profile of these metabolic products were comparable to those of macrophage secretions in the presence of extracellular calcium in the medium.

Figure 2 clearly shows that calcium depletion brings the level of AA and various derivatives back to that observed in the absence of infection by *T. gondii*. Seen in another way, as indicated in Fig. 2A, when prelabeled macrophages were cultivated with tachyzoites for 15 min in the absence of extracellular calcium, significant decreases of free AA ($P < 0.01$, by 56%), of 6-keto-PGF1α and PGE_2 ($P < 0.05$, by 20 and 22%, respectively), and of LTC4 and LTD4 and of HETEs ($P < 0.01$, by 20 and 61%, respectively) were observed in comparison with infected macrophages in the presence of extracellular calcium.

The infected macrophage monolayer cultured for 60 min in the absence of extracellular tachyzoites and without calcium in the culture medium also produced less CO and LO metabolites than infected macrophages cultured for 60 min in the presence of extracellular calcium (Fig. 2B). The decreases of free AA (48%) and 6-keto-PGF1α (27%) production were significant ($P < 0.01$). PGE_2, LTC4 and LTD4, and HETE production decreased significantly ($P < 0.05$), by 14, 9, and 40%, respectively, compared with that of cultures in the presence of extracellular calcium.

To examine whether the absence of extracellular calcium altered macrophage eicosanoid production by a mechanism independent of the level of extracellular calcium, we exam-

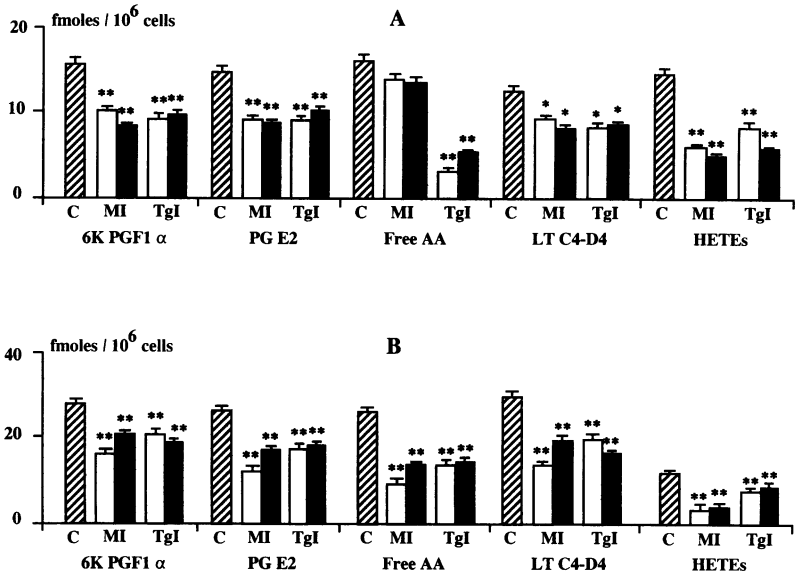

FIG. 3. Effect of the PLA_2 inhibitors 4-*p*-BPB and quinacrine on PG and LT production by mouse peritoneal resident macrophages during *T. gondii* infection. [^{3}H]AA-prelabeled macrophages that were not treated or were with the PLA_2 inhibitors (1 μM 4-*p*-BPB or 50 μM quinacrine) were infected with *T. gondii* tachyzoites themselves not treated or pretreated with the inhibitors (ratio of tachyzoites/macrophages, 1:2). The challenges were performed for 15 min and for an additional 60 min as indicated in the legend to Fig. 1. Eicosanoids were evaluated in the culture medium after 15 min (A) and after 60 min (B) of infection. C, ▨ (control group), macrophages not treated with PLA_2 inhibitors and infected with untreated *T. gondii*. MI, macrophages pretreated with PLA_2 inhibitors and infected with untreated *T. gondii*. TgI, macrophages not treated with PLA_2 inhibitors and infected by *T. gondii* pretreated with PLA_2 inhibitors; □, macrophage or tachyzoite pretreatments by 4-*p*-BPB; ■, macrophage or tachyzoite pretreatments with quinacrine. The results are the means of three experiments. The bar represents the standard error. The asterisks (*) indicate significant modifications compared with macrophages infected with *T. gondii* tachyzoites without PLA_2 inhibitor: *, $P < 0.05$; **, $P < 0.01$.

ined the eicosanoid production in response to 100 nM of phorbol 12-myristate 13-acetate, a PKC activator, in cells cultured for 60 min with or without calcium. The increase in eicosanoid production observed with phorbol 12-myristate 13-acetate was similar in the medium without calcium (data not shown).

Effects of the PLA_2 inhibitors 4-*p*-BPB and quinacrine on AA cascade triggered by *T. gondii*. To investigate PLA_2 involvement in triggering the AA cascade and to examine whether the PLA of *T. gondii* was responsible for eicosanoid production, the effects of 4-*p*-BPB and quinacrine (PLA_2 inhibitors) on the *T. gondii*-induced increase in eicosanoid production were determined. In our experimental conditions, 4-*p*-BPB (1 μM) and quinacrine (50 μM) had no toxic effects on tachyzoites or macrophages as evaluated by the trypan blue exclusion test.

As indicated in Fig. 3A, the increase in free AA observed with *T. gondii* for 15 min was unaffected by PLA_2 inhibitors, while other metabolites (6-keto-PGF1α, PGE_2, HETEs) decreased significantly ($P < 0.01$), by 33, 39, and 57%, respectively; LTC4 and LTD4 decreased ($P < 0.05$) by 25% in comparison with infected macrophages without PLA_2 inhibitors.

During the incubation of cultures for a further 60 min, the mobilization of free labeled AA and the production of 6-keto-PGF1α, PGE_2, LTC4 and LTD4 and HETEs by infected macrophages preincubated with PLA_2 inhibitors were decreased ($P < 0.01$) by 67, 41, 52, 52, and 70%, respectively, in comparison to parasitized macrophages without PLA_2 inhibitors (Fig. 3B).

To examine whether the PLA of *T. gondii* was responsible for eicosanoid production, we preincubated tachyzoites with the PLA_2 inhibitors as indicated above. When AA-prelabeled macrophages were incubated for 15 min at 37°C with *T. gondii* pretreated with PLA_2 inhibitors, mobilization of free labeled AA decreased (80%, $P < 0.01$) in comparison with that of macrophages infected by *T. gondii* without PLA_2 inhibitors (Fig. 3A). In parallel, the production of 6-keto-PGF1α, PGE_2, and HETEs decreased ($P < 0.01$) by 44, 39, and 43%, respectively; the production of LTC4 and LTD4 decreased ($P < 0.05$) by 35% (Fig. 3A). Macrophages infected for 15 min with *T. gondii* treated or not by PLA_2 inhibitors were incubated for an additional 60 min at 37°C without extracellular parasites. During this second phase of parasitized-macrophage culture, the mobilization of free labeled AA (48%) and the production of CO (6-keto-PGF1α, 23%; PGE_2, 33%) and LO (LTC4 and LTD4, 30%; HETEs, 40%) metabolites by macrophages parasitized with *T. gondii* pretreated with PLA_2 inhibitors were decreased in comparison with those of macrophages infected with *T. gondii* not treated with PLA_2 inhibitors (Fig. 3B).

Effects of CO and PKC inhibitors indomethacin and H-7, respectively, on AA cascade triggered by *T. gondii*. As shown in Fig. 4, 1 μM indomethacin markedly inhibited the production of 6-keto-PGF1α and PGE_2 ($P < 0.01$, by 32 and 29%, respectively) by infected macrophages in comparison with infected macrophages without indomethacin. In the same experimental conditions, indomethacin did not significantly enhance the level of labeled AA or of LO metabolites.

When infected macrophages were preincubated with 100 μM H-7, free AA and CO metabolite production was not affected in comparison to untreated macrophages infected with *T. gondii* (Fig. 4). Under these conditions, LTC4 and

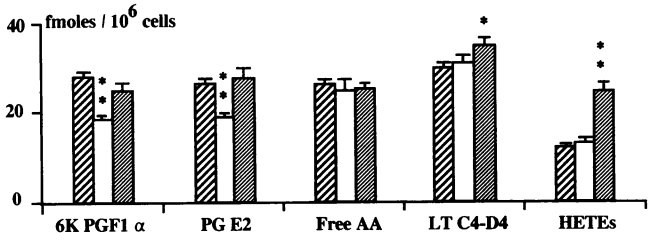

FIG. 4. Effect of indomethacin (CO inhibitor) or H-7 (PKC inhibitor) on PG and LT production by mouse peritoneal resident macrophages during *T. gondii* infection. [^{3}H]AA-prelabeled macrophages (not treated or pretreated with 1 μM indomethacin or 100 μM H-7) were infected with *T. gondii* tachyzoites at a ratio of 1:2 tachyzoites/macrophages for 15 min. Infected host cells were washed and incubated in the absence of extracellular tachyzoites for an additional 60 min. Eicosanoids were evaluated in the culture medium after 60 min of infection. ▨, macrophages infected with tachyzoites; □, macrophages pretreated with indomethacin and infected by tachyzoites; ▨, macrophages pretreated with H-7 and infected by tachyzoites. The results are the means of three experiments. The bar represents the standard error. The asterisks (*) indicate significant modifications compared with macrophages infected with *T. gondii* tachyzoites without metabolic inhibitor: *, $P < 0.05$; **, $P < 0.01$.

LTD4 metabolite production and HETE metabolite production were increased by 13 and 111%, respectively.

Effects of 4-*p*-BPB and calcium on kinetics of *T. gondii* penetration into macrophages. As shown in Fig. 5, parasites previously labeled with uracil very rapidly invaded host cells at 37°C. The penetration of labeled *T. gondii* into monolayer

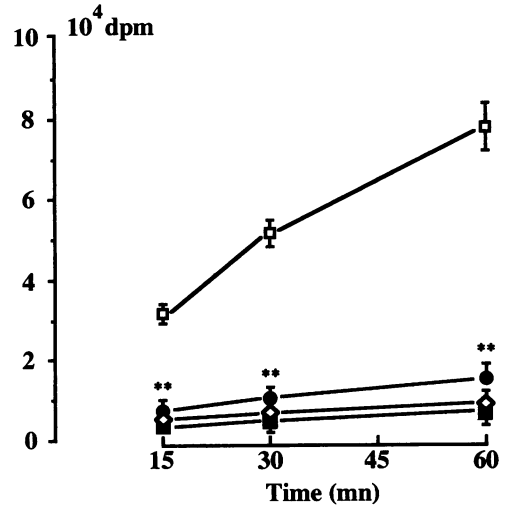

FIG. 5. Kinetics of *T. gondii* penetration in mouse peritoneal resident macrophages. Macrophages were infected with [5,6-^{3}H] uracil-labeled tachyzoites for 15, 30, or 60 min at a ratio of 1:2 tachyzoites/macrophages at 37 or 4°C. After this challenge, the cells were washed and the radioactivity incorporated into the macrophages was counted as indicated in Materials and Methods. Macrophages infected with viable prelabeled tachyzoites at 37°C (□) or 4°C (◇); macrophages infected with irradiated prelabeled tachyzoites at 37°C (●) or 4°C (■). Each point is the mean of 12 experiments. The bar represents the standard error. The asterisks (*) indicate significant modifications of radioactivity (disintegrations per minute) incorporated into infected host cells compared with viable *T. gondii* tachyzoites at 37°C ($P < 0.01$).

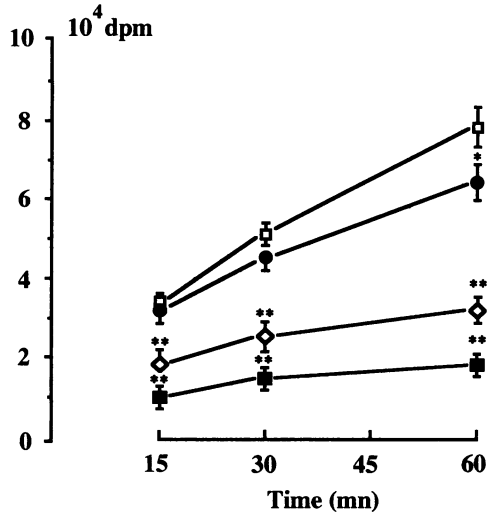

FIG. 6. Effect of the PLA_2 inhibitor 4-*p*-BPB and of calcium-free culture medium on the kinetics of *T. gondii* penetration in mouse peritoneal resident macrophages. Macrophages incubated in culture medium with calcium (not treated or pretreated with 1 μM 4-*p*-BPB) were infected with [5,6-^{3}H]uracil-labeled viable tachyzoites (not treated or pretreated with 1 μM 4-*p*-BPB) for 15, 30, or 60 min at a ratio of 1:2 tachyzoites/macrophages at 37°C. Macrophages were also infected with prelabeled viable tachyzoites in culture medium without calcium for 15, 30, or 60 min at 37°C. After this challenge, the cells were washed and the radioactivity incorporated into the macrophages was counted as indicated in Materials and Methods. □, macrophages infected with tachyzoites in the presence of extracellular calcium; ●, 4-*p*-BPB-pretreated macrophages infected with tachyzoites; ■, macrophages infected with 4-*p*-BPB-pretreated tachyzoites; ◇, macrophages infected with tachyzoites in the absence of extracellular calcium. Each point is the mean of 12 experiments. The bar represents the standard error. The asterisks (*) indicate significant modifications of radioactivity (disintegrations per minute) incorporated into infected host cells compared with viable *T. gondii* tachyzoites in culture medium with calcium: *, $P < 0.05$; **, $P < 0.01$.

cultures increased in a linear fashion for 60 min (regression coefficient highly significant, $P < 0.01$). When tachyzoites were previously irradiated, an inhibition of labeled uracil incorporation in macrophages was observed ($P < 0.01$) in culture conditions at 37°C. This inhibition ($P < 0.01$) of labeled uracil incorporation into macrophages was observed at 4°C (Fig. 5) for both irradiated and nonirradiated parasites. To evaluate the involvement of PLA_2 in *T. gondii* penetration into macrophages, the PLA_2 inhibitor 4-*p*-BPB (1 μM) was added to either the host cells or the tachyzoites as mentioned above. When the 4-*p*-BPB was added to the macrophages, there was no effect on labeled uracil incorporation into the macrophages at either 15 or 30 min of challenge in comparison to the infected control group without PLA_2 inhibitor (Fig. 6); after 60 min, however, an inhibition began to be observed ($P < 0.05$). In contrast, when tachyzoites were pretreated with 4-*p*-BPB, the kinetics of labeled uracil incorporation into macrophages was markedly inhibited after 15, 30, and 60 min of challenge ($P < 0.01$). Similar results were obtained with 50 μM quinacrine (data not shown). Figure 6 also shows that tachyzoites did not invade macrophages at 37°C in the absence of extracellular calcium ($P < 0.01$).

Effects of 4-*p*-BPB, calcium, H-7, and indomethacin on *T. gondii* multiplication in macrophages. Uninfected macro-

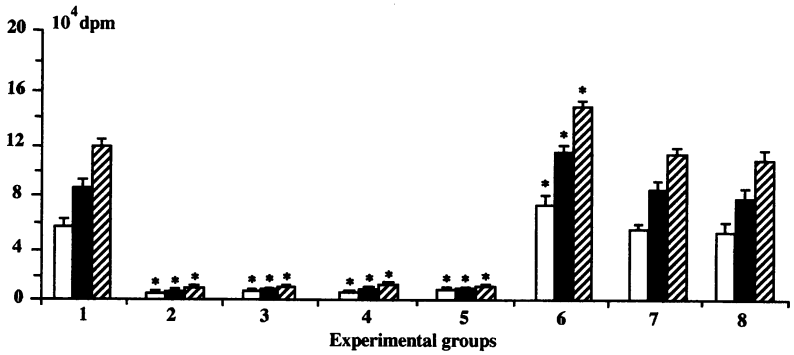

FIG. 7. Uptake of [5,6-^{3}H]uracil by *T. gondii* in mouse peritoneal resident macrophages. Macrophages pretreated with the appropriate inhibitors for 10 min (1 μM 4-*p*-BPB, 100 μM H-7, or 1 μM indomethacin) or not treated were infected with untreated *T. gondii*, or previously irradiated *T. gondii*, or *T. gondii* pretreated with the appropriate inhibitor for 10 min (1 μM 4-*p*-BPB, 100 μM H-7). The challenge (ratio of tachyzoites/macrophages, 1:10) done lasted for 1 (□), 2 (■), or 3 (▨) h. After these infection times, the cells were washed, labeled uracil was included in the culture medium for 24 h, and the radioactivity of infected host cells was counted as indicated in Materials and Methods. Columns: 1, macrophages infected with tachyzoites in the presence of extracellular calcium; 2, macrophages infected with tachyzoites in the absence of extracellular calcium; 3, macrophages infected with irradiated tachyzoites; 4, 4-*p*-BPB-pretreated macrophages infected with untreated tachyzoites; 5, macrophages infected with 4-*p*-BPB-pretreated tachyzoites; 6, H-7-pretreated macrophages infected with untreated tachyzoites; 7, macrophages infected with H-7-pretreated tachyzoites; 8, indomethacin-pretreated macrophages infected with untreated tachyzoites. The results are the means of 12 experiments. The bar represents the standard error. The asterisks (*) indicate significant modifications of the radioactivity (disintegrations per minute) incorporated into infected host cells compared with viable *T. gondii* tachyzoites in culture medium with calcium (column 1) for each infection time ($P < 0.01$).

phages and free tachyzoites did not incorporate significant amounts of labeled uracil after 24 h of incubation (data not shown). In contrast, macrophages infected with *T. gondii* showed marked uptake of labeled uracil. As shown in Fig. 7, for a ratio of 1:10 tachyzoites/macrophages, the incorporation of labeled uracil increased linearly (highly significant regression coefficient, $P < 0.01$) for 1, 2, and/or 3 h. This was also true for tachyzoites from human fibroblast monolayers under similar conditions (data not shown). Intracellular labeled uracil incorporation into monolayer cultures was decreased ($P < 0.01$) when macrophages were infected with *T. gondii* in the absence of extracellular calcium compared with that in its presence, or when the tachyzoites had been previously irradiated (Fig. 7). Macrophages or tachyzoites pretreated for 10 min with PLA_2 metabolic inhibitor (1 μM 4-*p*-BPB) also showed marked reduction of labeled uracil incorporation into monolayer cultures ($P < 0.01$) for 1, 2, and 3 h (Fig. 7). Similar results were obtained with 50 μM quinacrine (data not shown). The factor limiting incorporation into monolayer cultures of labeled uracil in the absence of extracellular calcium or when the tachyzoites had been previously irradiated or pretreated with PLA_2 inhibitor is the absence of penetration of the parasites previously observed in macrophages under the same experimental conditions.

When macrophages were pretreated with 100 μM H-7 for 10 min before infection with *T. gondii*, labeled uracil incorporation was significantly increased ($P < 0.01$) at all times in comparison with the control group (Fig. 7). In contrast, there was no significant difference in labeled uracil incorporation when the parasites were pretreated with H-7. Similar results were obtained when the macrophages were pretreated with 1 μM indomethacin which was washed away before macrophage infection with *T. gondii* (Fig. 7).

DISCUSSION

This study examined the metabolism of endogenous AA by mouse resident peritoneal macrophages infected in vitro with the highly virulent RH strain of *T. gondii*. Triggering of the AA cascade by macrophages was studied during an initial 15-min challenge with *T. gondii* (penetration process). Following this, the production of AA metabolites was studied for a further 60 min during the intracellular development phase of the parasite. The major finding of this study was that macrophages infected with *T. gondii* release arachidonate and its CO and LO metabolites during the tachyzoite penetration process. These findings are in agreement with observations describing alterations in cellular metabolism of 20:4 (AA) during infection of murine peritoneal macrophages with obligate intracellular parasites *T. gondii* (13) and *L. donovani* (23–25).

We demonstrated that resident murine peritoneal macrophages release CO products during the 15 min of challenge with *T. gondii*. This response involved selective increases of 6-keto-PGF1α and PGE_2. The synthesis of these metabolites was inhibited by indomethacin, a CO inhibitor. Specific 5-LO (LTC4 and LTD4 and 5-HETE) and 12-LO (12-HETE) metabolites of AA were also found to be elevated in the culture medium during this initial phase of penetration of the parasites into the macrophages. For this 15-min infection time, we also noted increased mobilization of free AA. The quantity and profiles of the AA metabolites obtained after challenge with *T. gondii*, maintained in human fibroblast monolayers, were very similar to those produced after challenge with *T. gondii* from mouse ascitic fluid. Locksley et al. found (13) that the major products released by resident murine peritoneal macrophages after phagocytosis of *T. gondii* were 11-, 12-, and 15-HETE. Their study showed that (i) phagocytosis was followed by production of HETEs which peaked between 90 and 120 min, (ii) *T. gondii* had to be viable, this production of HETEs being correlated with the ingestion of organisms, (iii) *T. gondii* had to replicate intracellularly after phagocytosis.

The results of our study indicated that the increase of HETEs (5-, 12-HETE) is correlated with the phase of *T. gondii* penetration into the macrophage; during the intracellular development phase in the absence of extracellular *T. gondii* tachyzoites, we found that cell culture supernatants

contained small amounts of ^{3}H-labeled material that comigrated with HETE standards in the solvent systems used for analysis by TLC and HPLC. This finding was consistent with data showing that, upon the release of HETEs from cells, the major fractions are rapidly re-esterified into phospholipid pools (2, 17, 37). On the other hand, the increase of 6-keto-PGF1α, PGE_2, LTC4 and LTD4, and free AA induced by *T. gondii* was comparable during the two phases (penetration and development).

Using uracil-labeled *T. gondii*, it was confirmed in this investigation that parasites actively invade macrophages in culture. In the experimental conditions used, phagocytosis of *T. gondii* by mouse resident peritoneal macrophages represents a small percentage of penetration (18%) compared with the active invasion of host cells by parasite. This percentage was obtained from the difference in parasite-associated macrophages when using irradiated versus nonirradiated parasites (specific activity, 0.1 μCi/10^6 irradiated or nonirradiated parasites). Phagocytosis consists of two phases: initially, an energy-independent phase of particle attachment to the phagocyte which may occur at low temperature (4°C), and later, an energy-dependent phase of ingestion. These results indicate that, in the absence of serum factors, the phase of attachment of irradiated parasites to macrophages occurring at 4°C was very minor. Also, the phase of engulfment of irradiated parasites by macrophages occurring at 37°C represents a small percentage of penetration. As a consequence, the alteration of AA metabolism in *T. gondii*-infected macrophages may not be related to membrane changes that occur during attachment and phagocytosis of parasites. It seems unlikely that the release of AA from membrane phospholipids triggered by *T. gondii* occurs as a consequence of receptor-ligand interactions. In this regard, augmentation of AA metabolism by *T. gondii* may involve mechanisms linked to active invasion by the parasite. In our study, viable *T. gondii* were required to trigger the AA cascade in mouse resident peritoneal macrophages since irradiation of tachyzoites abolished host cell penetration by *T. gondii* and subsequent prostanoid production.

Active invasion by *T. gondii* of host cells is a rapid event that requires a series of membrane changes by the host cell (35). It is thought that *T. gondii* secretes chemical factors which are active in the penetration process. Lycke and Norrby (16) isolated a "penetration-enhancing factor" from *T. gondii*; it has been established that this factor caused damage to host cell membranes and enhanced the invasion of host cells (16, 20, 21). This penetration-enhancing factor shows characteristic enzyme-like dependence on Ca^{2+}. It has also been shown that calcium is implicated in regulation of *T. gondii* surface interactions involved in host cell entry (35). Our results show that extracellular calcium is important both for the active invasion of macrophages by *T. gondii* and triggering of the AA cascade. The data of Saffer et al. (29, 30) suggest that PLA_2 could be involved in host cell invasion by *T. gondii*. The role of PLA_2 in *T. gondii* penetration was confirmed by the use of low doses of two PLA_2 inhibitors (*p*-BPB and quinacrine) to pretreat the parasite. In contrast, there was no effect on parasite penetration when macrophages were pretreated with PLA_2 inhibitors even though these inhibitors suppressed both the production of AA metabolites of the CO and LO pathways and parasite proliferation.

Moreover, the involvement of a PLA activity during the process of host cell invasion by tachyzoites is supported by their pretreatment with anti-PLA_2 serum to *Ophiophagus hannah* (king cobra) venom (Sigma Chemical Co., St. Louis, Mo.), pretreatment with various dilutions (10, 50, or 100 IU/ml) resulting in a significant reduction ($P < 0.01$) of the rate of incorporation of labeled parasites into macrophages after 15, 30, and 60 min of challenge (data not shown). This causes free AA mobilization and AA metabolite production during infection of the macrophages to fall back to the levels observed in the absence of infection with *T. gondii* (data not shown). Our results on penetration of *T. gondii* into host cells after pretreatment with anti-PLA_2 serum are in agreement with those of Silverman et al. (36), who were working on the *Rickettsia rickettsii* penetration.

Our results clearly show that *T. gondii* triggers the mobilization of the macrophage phospholipid AA, demonstrated (i) by a sharp rise in free AA and (ii) by the synthesis of eicosanoids. The eicosanoid synthesis reaction corresponds to the scheme in which the activation of PLA_2 produces a substrate (AA) for eicosanoid synthesis. The inhibition of AA mobilization and eicosanoid production after treatment of the parasite with PLA_2 inhibitors tends to support this reaction scheme. When the macrophages were pretreated with the PLA_2 inhibitors, the level of free AA coming from the PLA_2-induced mobilization reaction was not decreased, whereas the production of eicosanoids was inhibited. This result is in contradiction with the above scheme and poses the problem of finding out how eicosanoid production is inhibited while the level of AA remains unchanged.

A certain number of suggestions can be made to explain this apparent discrepancy. The PLA_2 of the parasite could bring about a rapid mobilization of the macrophage AA, giving rise mainly to an increase in the extracellular free AA pool (in the culture medium) without stimulating eicosanoid synthesis. Subsequently, penetration of the parasite into the target cell could bring about an activation of the PLA_2 of the macrophages leading to the mobilization of a second AA pool, the actual substrate pool for eicosanoid synthesis. Treatment of *T. gondii* with PLA_2 inhibitors inhibits the penetration of the parasite into macrophages and thus the formation of the AA pool which parallels tachyzoite penetration. The inhibition of *T. gondii*'s PLA_2 would then prevent the activation of the macrophage's PLA_2 owing to the absence of cell penetration. This chain of events would lead to the inhibition of eicosanoid synthesis observed in our results.

The treatment of macrophages with PLA_2 inhibitors, which does not prevent entry of the parasite into the macrophages, would not remove the first phase of AA mobilization caused by the PLA_2 of the parasite. On the other hand, the treatment would inhibit mobilization of the second AA pool which is dependent on the macrophage PLA_2 and thus the synthesis of eicosanoids from that pool. So the increase in free AA still observed after treatment of the macrophages with PLA_2 inhibitors would correspond to the first phase of AA mobilization produced by the parasite. Although the inhibition of free AA and eicosanoids does not appear during the first phase of *T. gondii* penetration, it does appear in the proliferation of the parasite.

Thus, our results suggest a role for both parasite and host cell PLA_2 in AA release from host cell membrane phospholipids. First, parasite phospholipase could trigger AA mobilization from the phospholipids, altering host membrane fluidity, which could then facilitate invasion of host cells by the parasites. Indeed, the increase of free AA during the rapid penetration phase in the presence of *T. gondii* in the culture medium was inhibited only when it was the parasites which were pretreated with PLA_2 inhibitors; the increase of free AA was not affected when the macrophages were

pretreated. Second, parasite penetration could activate the host cell PLA_2. This hypothesis is supported by the fact that after treatment of macrophages with PLA_2 inhibitors, CO and LO metabolite production was inhibited during the penetration phase of the parasites into the macrophages and during the development phase of *T. gondii*.

The release of radiolabeled AA from membrane phospholipids is the initial event in the formation of CO and LO 20:4 metabolites. Two pathways of 20:4 release have been described, a PLA_2-mediated release of 20:4 or the sequential action of a PLC and diacylglycerol lipase producing free AA, diglycerol, and monoglycerol products (4). Available evidence supports both activation of PLA_2 and inhibition of arachidonate reacylation as mechanisms contributing to increased levels of free AA (22). Activation of PKC has been demonstrated to initiate AA metabolism in a variety of cell types, in particular, in mouse peritoneal macrophages (22). In contrast, PKC inhibits acyl transferase and arachidonate reacylation (4, 10). The mechanism by which viable *T. gondii* induce an activation of host cell PLA_2 is not dependent on PKC activation since a potent inhibitor of PKC, H-7, had no inhibitory effect on mobilization of free AA or of CO 20:4 metabolites. These results have been confirmed with staurosporine (data not shown). In contrast, the increase of 5- and 12-HETE induced by PKC inhibitors suggests that PKC could be involved in LO activation and/or in HETE reincorporation into macrophage phospholipids. The reincorporation of HETEs into phospholipids could explain the decrease of HETE production during the proliferation phase of the parasite in the absence of *T. gondii* in the culture medium.

In addition, studies on AA metabolism and on *T. gondii* proliferation after pretreatment of macrophages with inhibitors acting on PLA_2, CO, and PKC suggest that AA mobilization by PLA_2 is involved in parasite proliferation. Although the inhibition of the proliferation of parasites in monolayer cultures when the tachyzoites had been previously treated with PLA_2 inhibitor depends on the absence of penetration of the parasites into macrophages, the same is not true for the absence of proliferation observed when the macrophages had been previously pretreated with PLA_2 inhibitors. This process was not modified by CO inhibitor. Inversely, *T. gondii* proliferation was increased by PKC inhibitors, which increased the total amount of LO metabolites in the culture medium. These results suggest the involvement of arachidonate and/or AA metabolites of the LO pathway in *T. gondii* proliferation.

In conclusion, a role has been shown for parasite phospholipase in AA release from host cell membrane phospholipids. The AA mobilization from phospholipids altering host membrane fluidity could facilitate the invasion of the host cells by parasites. The mobilization of free AA and the production of CO and LO 20:4 metabolites found here during infection by *T. gondii* and reported by other investigators during infection by *L. donovani* (23–25), *Schistosoma mansoni* (9, 31), and *S. japonicum* (27) suggest that the interaction of the parasites with membrane phospholipid may be a generalized mechanism. Additional studies are required to assess whether AA mobilization and eicosanoid production represent a critical event in the mechanisms by which parasites infect host cells and thus proliferate.

ACKNOWLEDGMENTS

We thank P. Rajagopalan-Levasseur for the critical reviewing of the manuscript and J. J. Pocidalo for helpful hints. We thank M. C. Souqual and C. Sabardu for their assistance in the realization of this work. The constant interest and support of H. Binz, Director of Immunology and Biotechnology, Center Pierre Fabre Medicaments, Le Puy Saint-Martin, 74164 Saint Julien-en-Genevois Cedex, France, are gratefully acknowledged.

This work was funded by INSERM and Pierre Fabre Medicaments.

REFERENCES

1. **Blackwell, G. J., and R. J. Flower.** 1983. Inhibition of phospholipase. Br. Med. Bull. **39:**260–264.
2. **Bonser, R. W., M. I. Siegel, S. M. Chung, R. T. McConnel, and P. Cuatrecasas.** 1981. Esterification of an endogenously synthesized lipoxygenase product into granulocyte cellular lipids. Biochemistry **20:**5297–5301.
3. **Bonta, I. L., and M. J. Parnham.** 1978. Prostaglandins and chronic inflammation. Biochem. Pharmacol. **27:**1611–1623.
4. **Burgoyne, R. D., and A. Morgan.** 1990. The control of free arachidonic acid levels. Trends Biochem. Sci. **15:**365–366.
5. **de Maroussem, D., B. Pipy, M. Beraud, P. Derache, and J. R. Mathieu.** 1985. [1-^{14}C]arachidonic acid incorporation into glycerolipids and prostaglandin synthesis in peritoneal macrophages: effect of chloramphenicol. Biochim. Biophys. Acta **834:**8–22.
6. **Derouin, F., M. C. Mazeron, and Y. J. F. Garin.** 1987. Comparative study of tissue culture and mouse inoculation methods for demonstration of *Toxoplasma gondii*. J. Clin. Microbiol. **25:** 1597–1600.
7. **Forgue, M. F., B. Pipy, M. Beraud, E. Pinelli, C. Cambon, A. Didier, M. C. Souqual, and J. Vandaele.** 1991. Possible implication of arachidonic acid metabolism in the decrease of chemiluminescence production after exposure of murine peritoneal macrophages to diethylnitrosamine and tumour promoter, 12-*O*-tetradecanoylphorbol-13-acetate. Carcinogenesis **12:**449–457.
8. **Forgue, M. F., B. Pipy, M. Beraud, M. C. Souqual, and J. M. Combis.** 1990. 1-Naphthyl *N*-methyl carbamate effect on intra- and extracellular concentrations of arachidonic acid metabolites, and on the chemiluminescence generation by mouse peritoneal macrophages. Int. J. Immunopharm. **12:**155–163.
9. **Fusco, A. C., B. Salafsky, and K. Delbrook.** 1986. *Schistosoma mansoni*: production of cercarial eicosanoids as correlates of penetration and transformation. J. Parasitol. **72:**397–404.
10. **Fuse, I., T. Iwanaga, and H. H. Tai.** 1989. Phorbol ester, 1,2-diacylglycerol, and collagen induce inhibition of arachidonic acid incorporation into phospholipids in human platelets. J. Biol. Chem. **264:**3890–3895.
11. **Keppel, G.** 1973. Design and analysis: a researcher's handbook, p. 658. Prentice-Hall, Inc., Englewood Cliffs, N.J.
12. **Lanni, C., and E. L. Becker.** 1985. Inhibition of neutrophil phospholipase A_2 by *p*-bromophenylacyl bromide, nordihydroguaiaretic acid, 5,8,11,14-eicosatetraenoic acid and quercetin. Int. Arch. Allergy Appl. Immunol. **76:**214–217.
13. **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:**6922–6926.
14. **Luft, B. J., Y. Naot, F. G. Araujot, E. B. Stinson, and J. S. Remington.** 1983. Primary and reactivated *Toxoplasma* infection in patients with cardiac transplants: clinical spectrum and problems in diagnosis in a defined population. Ann. Intern. Med. **99:**27–31.
15. **Luft, B. J., and J. S. Remington.** 1988. Toxoplasmic encephalitis. J. Infect. Dis. **157:**1–6.
16. **Lycke, E., and R. Norrby.** 1966. Demonstration of a factor of *Toxoplasma gondii* enhancing the penetration of *Toxoplasma* parasites into cultured host cells. Br. J. Exp. Pathol. **47:**248–256.
17. **Mathur, S. N., E. Albright, and F. J. Field.** 1988. Incorporation of lipoxygenase products into cholesteryl esters by acyl-CoA: cholesterol acyltransferase in cholesterol-rich macrophages. Biochem. J. **256:**807–814.
18. **Nichols, B. A., and M. L. Chiappino.** 1987. Cytoskeleton of *Toxoplasma gondii*. J. Protozool. **34:**217–226.
19. **Nichols, B. A., M. L. Chiappino, and G. R. O'Connor.** 1983. Secretion from the rhoptries of *Toxoplasma gondii* during host-cell invasion. J. Ultrastruct. Res. **83:**85–98.

20. **Norrby, R.** 1970. Host cell penetration of *Toxoplasma gondii*. Infect. Immun. **2**:250–255.
21. **Norrby, R.** 1970. Immunological study of the host cell penetration factor of *Toxoplasma gondii*. Infect. Immun. **3**:278–286.
22. **Pfannkuche, H. J., V. Kaever, D. Gemsa, and K. Resch.** 1989. Regulation of prostaglandin synthesis by protein kinase C in mouse peritoneal macrophages. Biochem. J. **260**:471–478.
23. **Reiner, N. E., and C. J. Malemud.** 1984. Arachidonic acid metabolism in murine leishmaniasis (donovani): ex-vivo evidence for increased cyclooxygenase and 5-lipoxygenase activity in spleen cells. Cell. Immunol. **88**:501–510.
24. **Reiner, N. E., and C. J. Malemud.** 1985. Arachidonic acid metabolism by murine peritoneal macrophages infected with *Leishmania donovani*: in vitro evidence for parasite-induced alterations in cyclooxygenase and lipoxygenase pathways. J. Immunol. **134**:556–563.
25. **Reiner, N. E., L. A. Schultz, and C. J. Malemud.** 1988. Eicosanoid metabolism by *Leishmania donovani* infected macrophages: mouse strain responses in prostanoid synthesis. Am. J. Trop. Med. Hyg. **38**:59–64.
26. **Remington, J. S., and G. Desmonts.** 1983. Toxoplasmosis, p. 143–263. *In* J. S. Remington and J. O. Klein (ed.), Infectious diseases of the fetus and newborn infant, 2nd ed. The W. B. Saunders Co., Philadelphia.
27. **Rogers, M. V., K. J. Henkle, V. Herrmann, D. J. McLaren, and G. F. Mitchell.** 1991. Evidence that a 16-kilodalton integral membrane protein antigen from *Schistosoma japonicum* adult worms is a type A_2 phospholipase. Infect. Immun. **59**:1442–1447.
28. **Roques, C., M. H. Bessières, C. Canut, and J. P. Séguéla.** 1985. Etude du comportement en culture cellulaire MRC 5 d'une souche kystogène de *Toxoplasma gondii*. Bull. Soc. Fr. Parasitol. **1**:159–162.
29. **Saffer, L. D., S. A. Long Krug, and J. D. Schwartzman.** 1989. The role of phospholipase in host cell penetration by *Toxoplasma gondii*. Am. J. Trop. Med. Hyg. **40**:145–149.
30. **Saffer, L. D., and J. D. Schwartzman.** 1991. A soluble phospholipase of *Toxoplasma gondii* associated with host cell penetration. J. Protozool. **38**:454–460.
31. **Salafsky, B., Y. S. Wang, A. C. Fusco, and J. Antonacci.** 1984. The role of essential fatty acids and prostaglandins in cercarial penetration (*Schistosoma mansoni*). J. Parasitol. **70**:656–660.
32. **Salmon, J. A., and R. J. Flower.** 1982. Extraction and thin layer chromatography of arachidonic acid metabolites. Methods Enzymol. **86**:478–493.
33. **Schwartzman, J. D.** 1986. Inhibition of a penetration-enhancing factor of *Toxoplasma gondii* by monoclonal antibodies specific for rhoptries. Infect. Immun. **51**:760–764.
34. **Sharma, S. D., J. R. Catteral, and J. S. Remington.** 1986. Parasiticidal activity of macrophages against *Toxoplasma gondii*. Methods Enzymol. **132**:626–636.
35. **Sibley, L. D., J. L. Krahenbuhl, G. M. Adams, and E. Weidner.** 1986. Toxoplasma modifies macrophage phagosomes by secretion of a vesicular network rich in surface protein. J. Cell Biol. **103**:867–874.
36. **Silverman, D. J., L. A. Santucci, N. Meyers, and Z. Sekeyova.** 1992. Penetration of host cells by *Rickettsia rickettsii* appears to be mediated by a phospholipase of rickettsial origin. Infect. Immun. **60**:2733–2740.
37. **Stenson, W. F., M. W. Nickells, and J. P. Atkinson.** 1983. Esterification of monohydroxy fatty acids into the lipids of a macrophage cell line. Prostaglandins **26**:253–262.
38. **Walker, D. H., W. T. Firth, J. G. Ballard, and B. C. Hegarty.** 1983. Role of phospholipase associated penetration mechanism in cell injury by *Ricketsia rickettsii*. Infect. Immun. **40**:840–842.
39. **Wanke, C., C. U. Tuazon, A. Kovacs, T. Dina, D. O. Davis, N. Barton, D. Katz, M. Lunde, C. Levy, F. K. Conley, H. C. Lane, A. S. Fauci, and H. Masur.** 1987. *Toxoplasma* encephalitis in patients with acquired immune deficiency syndrome: diagnostic and response to therapy. Am. J. Trop. Med. Hyg. **36**:509–516.
40. **Werk, R.** 1985. How does *Toxoplasma gondii* enter host cells? Rev. Infect. Dis. **7**:449–457.
41. **Winkler, H. H., and E. T. Miller.** 1981. Immediate cytotoxicity and phospholipase A, p. 327–333. *In* W. Burgdorfer and R. L. Anacker (ed.), Proceedings of Rocky Mountain Laboratory Conference on Rickettsiae and Rickettsial Diseases. Academic Press, Inc., New York.