

REGULATION OF ARACHIDONIC ACID METABOLISM BY MACROPHAGE ACTIVATION*

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Macrophages represent a major source of cyclo-oxygenase and lipoxygenase products (1, 2). In recent reports we established a set of defined conditions for the rapid and quantitative determination of these arachidonic acid (20:4)¹ metabolites using macrophages prelabeled with tritiated 20:4 (1,2). With these procedures, it has been possible to define the relationship between phagocytosis and prostaglandin synthesis (1) or leukotriene C production (2) and the role of specific phases of the phagocytic process in the initiation of 20:4 metabolism (3).

It is well established that the secretory activities of the macrophage are dependent upon the in vivo environment (4–6) and that both elicited and activated macrophages secrete products not released by resident cells (7). In this paper, we examine the production of 20:4 oxygenated products by murine peritoneal macrophages stimulated in vivo by both inflammatory and immunologic agents and stimulated in vitro by lymphokines. Our results emphasize that diminished macrophage 20:4 metabolism is a consequence of the in vivo activated state.

Materials and Methods

Macrophages. Normal peritoneal macrophages were established from cells of 25–30 g female Swiss-Webster mice (Taconic Farms, Germantown, NY) or from ICR mice (Trudeau Institute, Saranac Lake, NY). Proteose peptone (PP), heart infusion broth (HIB), and thioglycollate (THIO)-elicited macrophages were obtained from mice injected intraperitoneally (i.p.) 4 d earlier with 1 ml of phosphate-buffered saline containing a 1% (wt/vol) solution of PP or HIB and a 4% (wt/vol) solution of THIO, all from Difco Laboratories, Detroit, MI. *Cornibacterium parvum* (CP)-activated cells were obtained 11–14 d after either an i.p. or an intravenous (i.v.) injection with 1.4 mg of formalin-killed CP (Coparvax, Burroughs Wellcome, Co., Research

* Supported by a grant-in-aid from the Squibb Institute for Medical Research, and by grants 79-1009 from the American Heart Association, AI-07012 and AI-16963 from the National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD, and RF-78021 from the Rockefeller Foundation.

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¹ Abbreviations used in this paper: 20:4, arachidonic acid; BCG, Bacille Calmette-Guérin; CP, *Cornibacterium parvum*; FCS, fetal calf serum; HETE, hydroxy-eicosatetraenoic acids; HIB, heart infusion broth; HPLC, high-performance liquid chromatography; i.p., intraperitoneal; i.v., intravenous; 6-ketoPGF_{1α}, 6-keto prostaglandin F_{1α}; α-MEM, minimum essential alpha medium; PD, calcium and magnesium-free phosphate buffer; PGE₂, prostaglandin E₂; PGF_{2α}, prostaglandin F_{2α}; PP, proteose peptone; THIO, thioglycollate; TXB₂, thromboxane B₂.

Triangle Park, NC). Where appropriate, animals injected i.p. or i.v. with CP were boosted i.p. with 1.4 mg of CP or with 1×10^7 to 2×10^7 heat-killed Pasteur type Bacille Calmette-Guérin (BCG) 3 d before harvesting the macrophages. Macrophages were also taken 3–4 wk after mice were injected either i.p. or i.v. with 2×10^7 viable BCG. Boosted animals were injected i.p. with 2×10^7 autoclaved BCG 3 d before harvest.

Macrophage Cultivation. Primary cultures were established from peritoneal exudates as described by Cohn and Benson (8). Approximately 6×10^6 resident peritoneal cells or 10×10^6 cells from treated mice were suspended in 1 ml of minimum essential alpha medium (α -MEM, Gibco, Grand Island Biological Co., Grand Island, NY) containing 10% fetal calf serum (FCS) and added to 35-mm diam plastic culture dishes. After 2 h at 37°C in 5% CO₂/95% air, the cultures were washed three times in calcium- and magnesium-free phosphate-buffered saline (PD) to remove nonadherent cells and incubated overnight in fresh α -MEM plus 10% FCS or α -MEM plus 10% FCS and lymphokine. Fresh media were added daily.

Lymphokine-activated Macrophages. BCG-stimulated spleen cell supernates were prepared as described (9) and stored at -70°C after sterilization by filtration. Control supernatants were obtained from cultures of spleen cells from normal mice incubated with autoclaved BCG or alone.

Synthesis of 20:4 Oxygenated Metabolites. Macrophage cultures maintained in α -MEM and 10% FCS were labeled for 16 h with 0.5 μ Ci of [5,6,8,9,11,12,14,15-³H]20:4 ([³H]20:4, 62.2 Ci/mmol, sp act; New England Nuclear, Boston, MA). At the end of the labeling period, the cultures were washed three times with PD, overlaid with α -MEM (no serum), and the phagocytic stimulus added to initiate 20:4 release and metabolism. Media were removed after incubation for the appropriate periods under 5% CO₂/95% air at 37°C. Cell monolayers were scraped into 1 ml of 0.05% Triton X-100 (Rohm and Haas, Co., Philadelphia, PA), and protein was determined by the method of Lowry et al. (10), with bovine serum albumin as the standard. Aliquots of media and Triton X-100 cell lysates were removed for radioactivity determinations.

20:4 oxygenated products were extracted from culture media following a modification of the procedure described by Unger et al. (11). In brief, 1 vol of absolute ethanol was added. After acidification with formic acid (85% wt/wt; 10 μ l/ml of medium, final pH ~3), media were extracted twice with 1 vol each of chloroform. The chloroform phases were combined and taken to dryness under a stream of nitrogen. This procedure was repeated twice before the 20:4 metabolites were dissolved in 0.5 ml of the appropriate starting buffer for silicic acid chromatography or reverse-phase high performance liquid chromatography (HPLC).

Prostaglandins were separated from lipoxygenase products and unreacted 20:4 by chromatography of concentrated chloroform extracts on 0.3-g columns of silicic acid (Unisil; 100–200 mesh, Clarkson Chemical Co., Williamsport, PA). Hydroxy-eicosatetraenoic acids (HETE) and unreacted 20:4 were eluted with 15 ml of chloroform. Subsequently, prostaglandins were recovered by elution with 10 ml of 5.0% methanol in chloroform. The solvent fractions were routinely collected into scintillation vials. After removal of solvents under a stream of air, radioactivity was measured by liquid scintillation counting in Hydrofluor (National Diagnostics Inc., Advanced Applications Institute, Inc., Sommerville, NJ).

For estimates of the total 20:4 oxygenated metabolites formed by macrophages, concentrated media extracts were subjected to HPLC. Columns (4.6-mm \times 25-cm) of ultrasphere C-18 (Altex Scientific Inc., Subsid. of Beckman Instruments, Inc., Berkeley, CA) were eluted isocratically with 80 ml of solvent 1 (methanol/water/acetic acid, 75/25/0.01, vol/vol/vol) followed by 40 ml of solvent 2 (methanol/acetic acid, 100/0.01, vol/vol) at a flow rate of 1 ml/min. For the identification of prostaglandins, fractions 4–10 were pooled and evaporated under nitrogen. The residues were redissolved in 0.5 ml of solvent 3 (water/acetonitrile/benzene/acetic acid, 76.7/23.0/0.2/0.1, vol/vol/vol/vol) and subjected to HPLC on ultrasphere C-18 columns eluted isocratically at a flow rate of 1 ml/min with solvent 3 (12).

Quantitation of 20:4 Metabolites. The molar quantities of 20:4 and 20:4 metabolites released by [³H]20:4-labeled resident macrophages challenged with a phagocytic stimulus can be accurately calculated from their radiolabel content, provided the specific activity of 20:4 in cell phospholipids is accurately known (1). The latter value is readily obtained from the phospholipid content (172 pmol/ μ g of cell protein) (13) and the 20:4 content (25 mol percent of phospholipid fatty acids) (1), together with the quantity of incorporated 20:4. Independent

criteria, including fatty acid analysis, radioimmunoassay, and amino acid analysis, indicated radiolabel measurements provide an accurate (80% agreement) estimate of 20:4 release, PGE₂ synthesis, and LTC production, respectively (1, 2). The radiolabel content of 20:4 metabolites recovered from silicic acid columns or HPLC was converted to pmol 20:4 metabolite/ μ g cell protein by the following formula (14):

$$\frac{\text{pmol of 20:4 metabolite}}{\mu\text{g cell protein}} = \frac{\text{dpm}_1}{\text{dpm}_2} \times 122.85,$$

where dpm₁ is the radiolabel content of the 20:4 metabolite, dpm₂ is the total radioactivity incorporated by cells, and 122.85 is a constant that includes the specific activity of 20:4 in cell phospholipid. Values for 20:4 metabolites were corrected for overall recoveries obtained after extraction and chromatography, as described previously (14). Total 20:4 release was the total quantity of radiolabel released by cells after a phagocytic stimulus. Molar quantities were calculated from the above formula using the total radiolabel in the medium as dpm₁.

Radiolabeled 20:4 Metabolite Standards. HPLC of ³H-labeled standards was used to identify macrophage-derived 20:4 metabolites. [5,6,8,11,12,14,15-³H]PGE₂, [5,8,9,11,12,14,15-³H]6-keto PGF_{1 α} , [5,6,8,9,11,12,14,15-³H]PGF_{2 α} , and [5,6,8,9,11,12,14,15-³H]thromboxane B₂ (TXB₂) were purchased from New England Nuclear. ³H-labeled 5-HETE, 12-HETE, and 15-HETE were generated by published procedures using [³H]20:4 as the substrate. 5-HETE was isolated from human neutrophils exposed to calcium ionophore A 23187 (15), 12-HETE from human platelets similarly stimulated (16), and 15-HETE after incubation of [³H]20:4 with soybean lipoxygenase (17). 12-HETE and 15-HETE were extracted as described above. 5-HETE was extracted by the procedure described by Borgeat and Samuelsson (15). All three HETE were purified by HPLC in solvent 1. The elution times were 45–57, 38–39, and 34–35 min for 5-HETE, 12-HETE, and 15-HETE, respectively.

Fatty Acid Analysis. Macrophage monolayers were rinsed into isotonic saline, and the lipids were extracted as described (1). Fatty acid methyl esters were prepared by transesterification in methanolic HCl (13). The methyl esters were then analyzed by gas-liquid chromatography on 1/8-in \times 10-ft columns of 10% SP-2330 on Chromosorb WA/W (Supelco, Inc., Bellefonte, PA) at 180° with a carrier gas-flow rate of 30 ml/min.

Phagocytic Stimuli. Zymosan was purchased from ICN K and K Laboratories, Inc., Plainview, NY. Stock solutions of zymosan were prepared in an α -MEM (18). Formalin-treated CP (Coparvax, Burroughs Wellcome Co.) was centrifuged at 3,000 *g* for 20 min and suspended at a final concentration of 7 mg/ml. Stock solutions (7 mg/ml) of lyophilized BCG were prepared in α -MEM plus 0.05% Tween 80 and sonicated.

Results

In Vivo Stimulation of Macrophages: Nonspecific Inflammatory Agents. Macrophages elicited with either PP or HIB released comparable amounts (100–110%) of 20:4 and its oxygenated products as did resident cells. However, THIO cells yielded greatly reduced amounts of 20:4 metabolites (10% of resident cells), as noted by others (19). This effect could be reproduced by exposing resident macrophages *in vitro* to THIO (5 mg/ml) for 16 h with a >50% inhibition of 20:4 metabolism. Other eliciting agents used in this study (PP or HIB) were without effect on macrophage 20:4 metabolism.

In vivo Activation of Macrophages: Agents Evoking Cell-mediated Immunity. Macrophage populations obtained from animals after an *i.p.* delivery of CP demonstrated reduced levels of 20:4 metabolism. This is illustrated in Fig. 1, which shows maximum 20:4 release and prostaglandin synthesis by these cells and resident macrophages triggered with a maximum phagocytic stimulus of 160 μ g zymosan. Resident cells released 23.8–26.6 pmol 20:4/ μ g cell protein, compared with 6.3–12.6 pmol 20:4/ μ g cell protein for *i.p.* CP macrophages. Our impression from many experiments is that

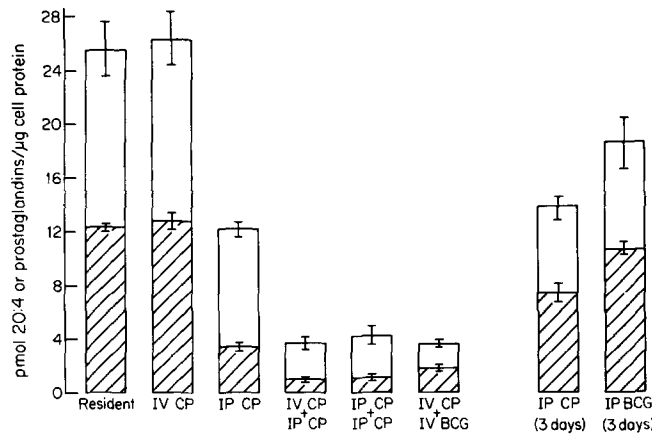


FIG. 1. 20:4 release and prostaglandin synthesis by resident and in vivo activated BCG, CP macrophages challenged with a maximum challenge of 160 μ g unopsonized zymosan. Cells were purified by adherence, labeled with [3 H]20:4 for 16 h in α -MEM plus 10% FCS, and exposed to zymosan for 90 min in α -MEM. 20:4 release was determined from the radiolabel content of medium, and prostaglandin synthesis was determined from the radiolabel content of silicic acid column eluates (5% methanol in chloroform). Injection procedures were those described in Materials and Methods, except for the two right panels in which nonimmune animals were injected 3 d before harvest of the peritoneal cells. 20:4 release is represented by the total height of each bar, and prostaglandin synthesis is represented as the height of the shaded area.

specific pathogen-free mice exhibited the greatest inhibition of 20:4 metabolism to bacterial vaccines.

As we noted previously (1), resident macrophages converted >80% of the released 20:4 to oxygenated products, one-half of which were prostaglandins (Fig. 1). IP CP macrophages, in addition to diminished 20:4 release, converted a smaller percentage (25%) of the released 20:4 to prostaglandins than did resident cells.

Fig. 1 further illustrates that the route by which animals are immunized is an important determinant of macrophage 20:4 metabolism. 20:4 release and prostaglandin production by i.v. CP macrophages, in contrast to i.p. CP cells, was comparable to those of resident macrophages or somewhat elevated (100–130%, $n = 3$). Intraperitoneal boosting of animals previously injected with CP (i.v. or i.p.) effectively reduced peritoneal macrophage 20:4 metabolism to the same low level, which was 30% of i.p. CP cultures. The effect of the boost was more striking on i.v. CP than on i.p. CP cells because of the higher capacity of the former cultures to form 20:4 oxygenated products when challenged with zymosan. Immunologic specificity was not required because both i.p. BCG and i.p. CP boosts were equally effective (Fig. 1). Macrophages harvested from nonimmune mice injected i.p. with the boosting dose of CP or BCG 3 d before harvest displayed less severe reductions in 20:4 metabolism. It appeared that both sensitization of the host with the vaccine as well as a local inflammatory response, perhaps a phagocytic event, were required for maximum reduction of 20:4 metabolism.

Kinetics of 20:4 Release and Prostaglandin Synthesis. Time-course experiments supported the observation (Fig. 1) that i.p. CP cells were less effective in producing 20:4 metabolites. Although the kinetics of 20:4 release and prostaglandin synthesis by i.p. CP and resident macrophages were similar in that both were linear for 60–90 min,

the initial rates of release by i.p. CP cells were reduced (Fig. 2), which accounted for the diminished production of 20:4 metabolites. Nevertheless, the experiment in Fig. 3 demonstrated that i.p. CP macrophages retained the capacity to respond to multiple exposures of zymosan by initiating new rounds of 20:4 release and prostaglandin synthesis, as shown previously for resident macrophages (1). Both macrophage populations remained responsive provided maximum levels of ingestion were not reached. However, for each dose of zymosan, the response of i.p. CP cells was quantitatively less than that of resident cell controls.

20:4 Metabolism as a Function of Culture Time. The quantities of zymosan-induced 20:4 release and prostaglandin synthesis by i.p. CP and resident macrophages remained essentially constant over a 4-d period in culture (Fig. 4), indicating that the low levels of 20:4 metabolism by i.p. CP cells were not reversed by prolonged in vitro cultivation.

20:4 Content of Macrophages. An i.p. injection of bacterial vaccines exposes peritoneal macrophages to particles that may promote 20:4 release in vivo and result in a decreased content of 20:4 in cell phospholipid. Clearly, this could contribute to diminished 20:4 release in response to an in vitro phagocytic challenge. The data presented in Table I indicated that, although differences in phospholipid fatty acid composition were evident between resident and i.p. CP cells, the 20:4 contents were similar and would not account for the differences in 20:4 metabolite production.

20:4 Metabolites Synthesized by i.p. CP Macrophages. Fig. 5 compares HPLC profiles of 20:4 metabolites synthesized by i.p. CP and resident cells under conditions that allow mutual separation of prostaglandins and lipoxygenase products. The profiles of resident cell products were dominated by a large peak of radiolabel in the runoff

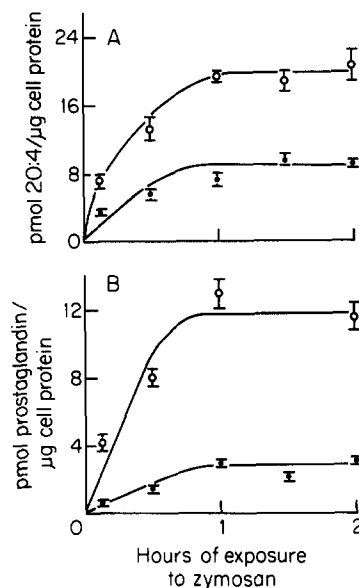


FIG. 2. Time-course of (A) total 20:4 release and (B) prostaglandin synthesis by i.p. CP macrophages. [^3H]20:4-labeled cultures were exposed to 160 μg of zymosan at $t = 0$ in α -MEM (no serum). Media were removed from duplicate plates, and the total 20:4 release was calculated from the radiolabel content of aliquots. Prostaglandins were recovered in the 5% methanol eluates of silicic acid columns. Values are the means \pm range. \circ , resident cells; \bullet , i.p. CP cells.

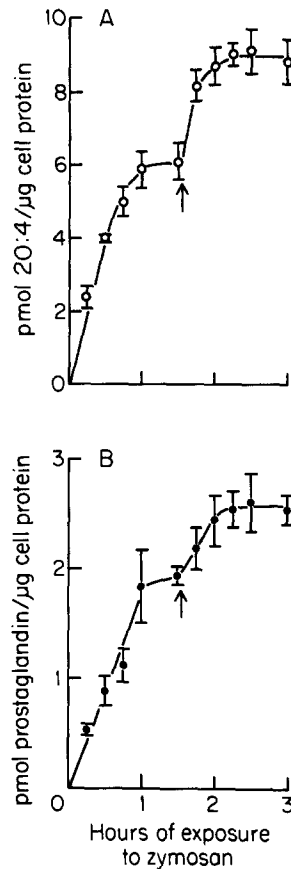


FIG. 3. Restimulation of i.p. CP macrophage 20:4 release (top) and prostaglandin synthesis (bottom). After the 16-h labeling period with $0.5 \mu\text{Ci } [^3\text{H}]20:4$, the cultures were exposed to $60 \mu\text{g}$ of zymosan at $t = 0$ in α -MEM (no serum). Media were removed from duplicate cultures at the indicated times and processed as described in Fig. 2. An additional $100 \mu\text{g}$ of zymosan was added to the remaining cultures at 90 min (arrow). The media were harvested at subsequent times and processed. Values are the means \pm range. Similar results were obtained in two separate experiments.

volume of the column that consists of prostaglandins (80%) together with smaller amounts (20%) of unidentified polar 20:4 metabolites. Also evident are mono-HETE (fractions (25–40) and components with elution characteristics (15) of di- and tri-HETE. Contrasting this situation, a major percentage (70%) of the 20:4 released by i.p. CP cells was recovered as unreacted fatty acid. As a result, prostaglandin and HETE production were reduced proportionally. This finding indicates that the small percentage of 20:4 converted by i.p. CP macrophages to prostaglandins (Fig. 1) occurs because of a lack of metabolism rather than shunting of the fatty acid into the lipoygenase pathway.

We next examined the cyclo-oxygenase products formed by resident and i.p. CP macrophages. Resident cells synthesized 6-ketoPGF $_{1\alpha}$ and PGE $_2$ in the ratio of 1:1.5 (Fig. 6). PGE $_2$ was also the major prostaglandin produced by i.p. CP populations, but the relative levels of 6-ketoPGF $_{1\alpha}$ were reduced, and substantial amounts of a product with elution characteristics of TXB $_2$ were recovered. The proportions of cyclo-oxygen-

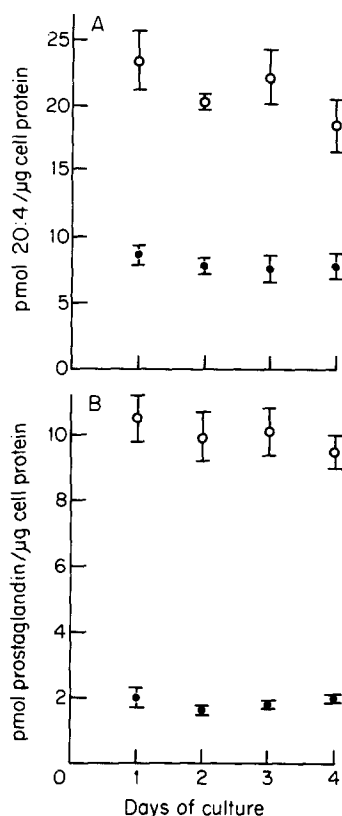


FIG. 4. Effect of cultivation time on (A) total 20:4 release and (B) prostaglandin synthesis by resident and i.p. CP macrophages. Cells purified by adherence were cultured in α -MEM plus 10% FCS for the indicated periods of time. During the final 16 h of cultivation, the cultures were labeled with [3 H]20:4. At the end of this period, the cultures were washed, overlaid with α -MEM, and exposed to 160 μ g of zymosan. Media were removed from duplicate cultures and processed as described in Fig. 2. Values are the means \pm range. \circ , resident cells; \bullet , i.p. CP cells.

ase metabolites of these CP-elicited cells was 1:4.1:16.8 for 6-ketoPGF_{1 α} , TXB₂, and PGE₂. These qualitative and quantitative differences in prostaglandins formed by the two macrophage populations were evident in three separate experiments.

In related experiments, the synthesis of leukotriene C by macrophages from specific pathogen-free mice immunized i.p. with CP was 2–3% of resident cell controls (C. A. Rouzer, personal communication).

Other Phagocytic Stimuli. We explored the question of whether 20:4 metabolism promoted by a zymosan challenge was representative of the macrophage response to phagocytic stimuli. For this purpose, 20:4 metabolism of cultured macrophages was compared after an in vitro challenge with zymosan, BCG, or CP. The kinetics of 20:4 release and prostaglandin synthesis were similar to those shown in Fig. 4, regardless of the stimulus. Likewise, the same cyclo-oxygenase and lipoxygenase products were recovered. With resident cells, BCG promoted higher levels of 20:4 metabolism than did zymosan or CP at maximum stimulatory concentrations. However, BCG and CP at these levels (1 mg/ml and 1.2 mg/ml, respectively) led to cell loss that was not evident with zymosan. Diminished prostaglandin synthesis by i.p. CP macrophages

TABLE I
Phospholipid Fatty Acid Composition of Resident Macrophages and Activated Macrophages from Mice Injected i.p. with C. parvum

Fatty acid	Mol percent of fatty acid	
	Resident cells	Activated cells
14:0	3.6	2.4
16:0	28.0	26.2
16:1	2.0	5.3
18:0	24.5	19.7
18:1	10.0	10.5
18:2	6.3	10.7
20:4	25.7	25.0
Saturated/unsaturated	0.78	1.07

Cultures purified by adherence were incubated overnight in α -MEM plus 10% FCS. The media were removed, and lipids were extracted from cells scraped into isotonic saline. Fatty acid analyses of isolated phospholipids were determined by gas-liquid chromatography after transesterification.

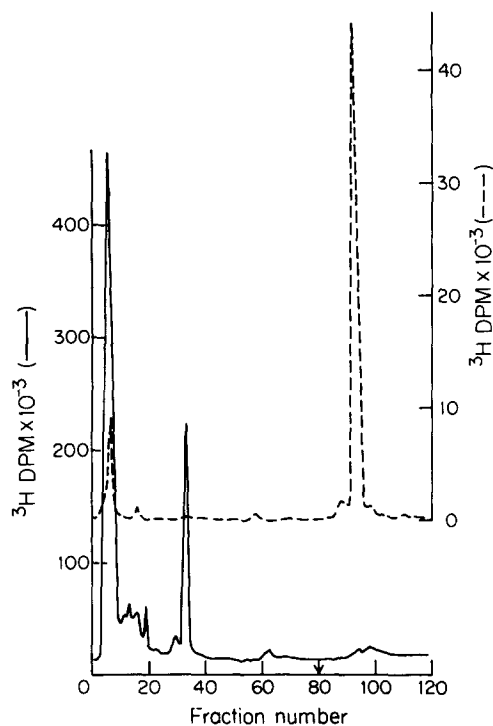


FIG. 5. HPLC profile of 20:4 oxygenated metabolites released by resident and i.p. CP macrophages after a 90-min exposure to 160 μ g of zymosan. Media from duplicate [3 H]20:4-labeled cultures were pooled and extracted. The 20:4 metabolites dissolved in solvent 1 were applied to a C18 ultrasphere column. Prostaglandins and HETE were resolved (fractions 1-80) with solvent 1, and unreacted 20:4 (fractions 81-120) was recovered by elution (arrow) with solvent 2. In parallel HPLC runs, tritiated prostaglandin standards were recovered in fractions 4-10, and tritiated mono-HETE standards were recovered in fractions 25-40. (—) resident cells; (---) i.p. CP cells.

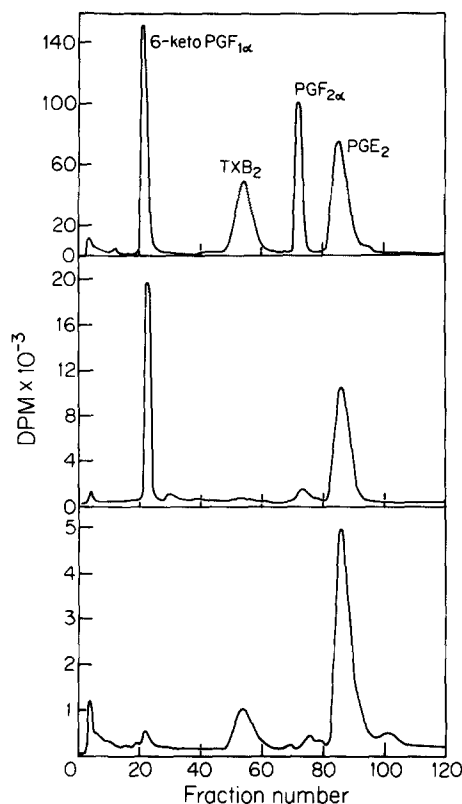


FIG. 6. Cyclo-oxygenase products synthesized by resident and i.p. CP macrophages. The prostaglandin-containing fractions (4–10) eluted from an ultrasphere C-18 column (Fig. 5) were pooled, taken to dryness under a stream of nitrogen, and redissolved in solvent 3. Cyclo-oxygenase products were resolved on the same ultrasphere C-18 column with solvent 3 at a flow rate of 1 ml/min. Top panel, commercial radiolabeled standards; middle panel, resident macrophage products; bottom panel, i.p. CP macrophage products.

occurred with all three phagocytic stimuli, and BCG and CP were clearly less effective than zymosan.

In Vitro Exposure of Resident Macrophages to Lymphokines. Exposure to a 1–8 dilution of BCG lymphokine for 72 h was sufficient to morphologically activate resident macrophages. However, 20:4 release and prostaglandin (6-ketoPGF_{1α} and PGE₂) synthesis by such cells was not significantly different from those of controls (no lymphokine) or cultures exposed to control supernatants. Dose-response experiments in which BCG lymphokines were varied from 2 to 25% in the culture medium also failed to either enhance or diminish zymosan-mediated 20:4 metabolism. A similar lack of effect was noted when 20:4 metabolism was monitored at 24-h intervals during 4-d cultivation of resident cells in a 1–8 dilution of BCG lymphokine.

Modification of *in vivo* activated cells to release 20:4 products therefore reflects factors in addition to lymphokine-derived products.

Discussion

We previously concluded that resident peritoneal macrophages are a major source of 20:4 metabolites. In this report, we examined the capacity of populations of *in vivo*

stimulated inflammatory macrophages to convert 20:4 to cyclo-oxygenase and lipoxigenase products. We questioned whether a relationship exists between macrophage activation and the level of 20:4 production in response to an in vitro phagocytic stimulus. As shown in Fig. 7, there is a correlation ($r = 0.91$) between the capacity to inhibit intracellular *Toxoplasma gondii* replication, one measure of macrophage activation (9), and decreased prostaglandin synthesis. This comparison included resident cells, macrophages variously activated in vivo with BCG or CP, and macrophages elicited with nonmicrobial inflammatory stimuli. The close relationship between diminished prostaglandin release and augmented toxoplasmatatic activity illustrates that decreased 20:4 metabolism is related to the degree of macrophage activation.

Among the macrophage populations thus examined, THIO cells represented the single exception to this correlation. It is likely that components of THIO broth directly inhibited the production of 20:4 metabolites by resident cells in vitro, as previously found (20) for H_2O_2 release and tumor cell destruction by activated macrophages. The fact that anti-toxoplasma activity was spontaneously lost on prolonged cultivation (48 h) of i.p. CP cells (H. Murray, unpublished results) without a concomitant increase in zymosan-induced 20:4 metabolism (Fig. 4) suggested the inverse relationship between these two macrophage activities is not necessarily causal.²

Diminished macrophage 20:4 metabolism, as a result of activation, supported the notion that the resident tissue macrophage might have a unique role in acute inflammatory situations. This long-lived cell is undoubtedly one of the first elements of the immune system exposed to infection and tissue injury. As such, it can respond

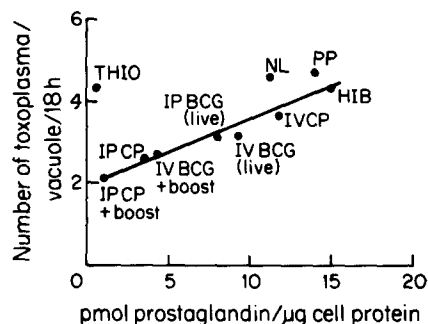


FIG. 7. Antitoxoplasma activity vs. the capacity for prostaglandin synthesis of resident, inflammatory, and activated macrophage populations. The abbreviations are those used in the text. The ordinate indicates the mean number of toxoplasms per intracellular vacuole 18 h after infection of macrophage cultures. Thus, the greater the degree of inhibition of toxoplasma replication, the greater the degree of macrophage activation. These values were taken from an overlapping study (9) in which common reagents were used to elicit cells for measurements of 20:4 oxygenated products and antitoxoplasma activity, thus providing valid comparisons for the two macrophage functions. Data are provided for the eleven macrophage populations in which both antitoxoplasma activity and PGE_2 synthesis resulting from a maximum zymosan stimulus were measured. Values for PGE_2 release are given in the text, except for BCG-activated cells. Methods for the determination of PGE_2 synthesis by these macrophage populations are given in Materials and Methods or in the legend to Fig. 1. In calculating the correlation coefficient between reduced antitoxoplasma activity and enhanced capacity for prostaglandin synthesis, data for THIO macrophages were omitted.

² This point is further emphasized from studies of in vitro activated macrophages obtained by lymphokine treatment. Although the high level of 20:4 metabolism and failure to inhibit toxoplasma replication (9) provided the expected correlation (Fig. 7), lymphokine induces resident macrophages to destroy other intracellular parasites [*L. enrietti* (21) and *T. cruzi* (22)].

to inflammatory stimuli in the absence of humoral factors by producing large quantities of 20:4 oxygenated products (Table II) that can have immediate consequences on the vasculature (23) and on the immune system (24). The considerable synthetic capacity of the macrophage is compared in Table II with that of guinea pig neutrophils for which similar quantitative data are available.

Activated macrophages, as shown in this study for i.p. CP cells, responded to in vitro inflammatory stimuli by a burst of 20:4 release and the subsequent synthesis of cyclo-oxygenase and lipoxygenase products. Although their phagocytic capacity appears unimpaired (W. A. Scott, unpublished results), 20:4 metabolism was reduced. Down regulation was evident at the level of the inducible phospholipase. In addition, there is a failure to quantitatively metabolize released 20:4 together with specific inactivation (or inhibition) of the prostacyclin synthetase, as noted earlier (19), and of enzyme(s) for leukotriene C synthesis. The implication of these findings is that activated macrophages within chronic inflammatory foci release a mixture of 20:4 metabolites distinct from resident cells (Table II). The elimination of the vasoactive agents prostacyclin and leukotriene C, coupled with an overall reduction in synthetic capacity, might be a factor in limiting the immune response. Activated macrophages, however, might continue to be a considerable source of 20:4 metabolites when compared to other cell types (Table II).

The factors that regulate macrophage 20:4 metabolism remain to be elucidated. Our results suggest from a comparison of i.p. CP and i.v. CP cells that a reduced synthetic capacity is a localized response to bacterial antigens. The lessened production

TABLE II
Arachidonic Acid Metabolites

Cell type	Stimulus	Product	Amount produced (pmol/mg cell protein)	References
Resident mouse peritoneal macrophage	160 μ g zymosan	PGE ₂	12,000-15,500	Scott et al. (1)
		6-keto PGF _{1α}	5,000-7,000	
		HETE	2,500-4,000	Rouzer et al. (2)
		Leukotriene C	<u>3,000-6,000</u>	
			23,200-31,700	
<i>C. parvum</i> -elicited mouse peritoneal macrophages	160 μ g zymosan	PGE ₂	2,500-3,500	
		6-keto PGF _{1α}	100-200	
		TXB ₂	600-700	
		HETE	700-1,000	
		Leukotriene C	<u>80-110</u>	
			3,900-5,400	
Guinea pig neutrophils	10 ⁻⁶ M calcium Ionophore A23,187	PGE ₂	1.9	Bokoch and Reed (26)
		6-keto PGF _{1α}	0.9	
		TXB ₂	10.2	
		PGF _{2α}	0.95	
		5-HETE	<u>30.2</u>	
			44.15	

after an i.p. administration of BCG or CP might result because macrophages have undergone a round of 20:4 release and metabolism in vivo (25). This point of view is supported by the finding that both bacteria are potent triggers of macrophage 20:4 metabolism. Clearly, additional factors are involved in regulating macrophage 20:4 metabolism, as shown by the priming effect of a systemic (i.v.) immunization when followed by an i.p. boost. 20:4 metabolism by the resulting macrophage population was decreased significantly below that of cells from nonimmune animals given the same boosting injection. In vitro activation of macrophages with spleen cell products indicated that factors in addition to lymphokines are required to regulate the release of 20:4 products.

It is now appropriate to examine the relative contributions of several variables, such as the rates of antigen clearance and deposition, the influx of mononuclear phagocytes into the inflammatory site, and the role of the host immune system, in controlling the synthesis of inflammatory lipids by macrophages.

Summary

Levels of zymosan-induced arachidonic acid (20:4) metabolism by peritoneal macrophages elicited with inflammatory agents and resident macrophages were similar. Thioglycollate (THIO)-elicited macrophages represented the exception; however, the diminished metabolism by these cells was reproduced by exposing resident cells to 5 mg/ml THIO broth in vitro. In contrast, reduced prostaglandin synthesis by macrophages from mice variously treated with the immunologic agents, *Cornbacterium parvum* or Bacille Calmette Guérin (BCG), closely correlated with enhanced antitoxoplasma activity, one measure of macrophage activation. This relationship, although not causative, suggested that the capacity for 20:4 metabolism is a function of the macrophage activation state. Modulation of macrophage 20:4 metabolism in vivo apparently required factors in addition to lymphocyte-derived products. Treatment of resident macrophages in vitro with BCG lymphokine was without effect on 20:4 release or prostaglandin synthesis.

Activated macrophages from animals inoculated i.p. with *C. parvum* exhibited reduced 20:4 release and also failed to metabolize 70% of the 20:4 released in response to a zymosan stimulus. Consequently, the quantities of 20:4 metabolites formed were significantly less than expected from 20:4 release. These activated macrophages displayed greatly reduced synthesis of prostacyclin and leukotriene C compared with other 20:4 metabolites. It appeared that factors that regulate macrophage 20:4 metabolism influence the level of the inducible phospholipase and synthetic enzymes for specific 20:4 oxygenated products.

Received for publication 7 December 1981.

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