SECRETION OF LEUKOTRIENE C AND OTHER ARACHIDONIC ACID METABOLITES BY MACROPHAGES CHALLENGED WITH IMMUNOGLOBULIN E IMMUNE COMPLEXES*

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Recent studies in our laboratory (1) have shown that resident, mouse peritoneal macrophages respond to particulate immune complexes of immunolgobulin E (IgE) (IgE-IC)[1] with the release of arachidonic acid (20:4). We have now defined the optimum in vitro conditions for IgE-IC-mediated 20:4 metabolism and have examined the nature of the products synthesized. In this report, we demonstrate that macrophages challenged with particle-bound IgE-IC release large quantities of prostacyclin, prostaglandin (PG)E_2, and the slow-reacting substance, leukotriene C [5(S)-hydroxy-6(R)-glutathionyl-7,9,11,14-eicosatetraenoic acid] (LTC). These observations suggest that the macrophage might be a source of slow-reacting substance and other 20:4 metabolites generated during IgE-mediated allergic reactions.

Materials and Methods

Preparation of Fibronectin-coated surfaces. Fibronectin-coated plastic tissue culture dishes for the isolation of peritoneal macrophages were prepared by the method of Bevilacqua et al. (2). Briefly, 35-mm dishes containing 1 ml of a 30 mg/ml sterile solution of gelatin (type II; Sigma Chemical Co., St. Louis, MO) in water were incubated at 37°C for 2 h. The gelatin solution was then removed, and the plates were allowed to dry for at least 2 h at 37°C. To coat the dishes with fibronectin, 1 ml of human, heparinized, platelet-free plasma was added to each dish. After incubation at room temperature for 1 h, the plates were washed three times with calcium- and magnesium-free phosphate-buffered saline (Pi/NaCl).

Macrophage Cultures. Primary cultures of resident peritoneal macrophages were established from specific pathogen-free female ICR (CD-2) mice, weighing 25–30 g (The Trudeau Institute, Saranac Lake, NY). The peritoneal cavities of the mice were lavaged twice with 1 ml of minimum essential medium/alpha modified (α-MEM; K. C. Biological Inc., Lenexa, KS) containing 5 U/ml of heparin (lipo-hepin; Riker Laboratories, Inc., Northridge, CA). The cells were collected by centrifugation at 200 *g* for 10 min and resuspended at a final concentration

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[1] *Abbreviations used in this paper:* α-MEM, minimum essential medium/alpha modified; DNP, dinitrophenol; DNP-albumin, bovine serum albumin covalently modified with dinitrophenol; GSH, glutathione; HPLC, high pressure liquid chromatography; IC, immune complex; KRPG, Kreb's ringer phosphate buffer supplemented with 5.4 mM glucose; LTC, leukotriene C; PG, prostaglandin; Pi/NaCl, calcium- and magnesium-free phosphate-buffered saline; 20:4, arachidonic acid.

of 7×10^6 cells/ml in the supernatant fluid recovered from the peritoneal lavage (referred to as peritoneal lavage medium). Maintaining cultures in peritoneal lavage medium retarded the loss of the macrophages' capacity to respond to IgE-IC, which occurs with increasing incubating time in vitro (4–16 h) (1). The resulting cell suspension was added to fibronectin-coated dishes (1 ml/dish), and the cultures were incubated at 37°C in an atmosphere of 5% CO_2/95% air for 1 h. The nonadherent cells were then removed by gently washing four times with Krebs ringer phosphate buffer supplemented with 5.4 mM glucose (KRPG), and the macrophage monolayers were overlaid with fresh medium, as indicated for each experiment. Cultures prepared in this way contained $1.0 \pm 0.4 \times 10^6$ adherent cells per dish, or 60 ± 14 μg protein per dish. The adherent cells were >95% typical macrophages, as assessed by morphologic and functional criteria (1). The major contaminating cells were lymphocytes, and <0.01% of the population were mast cells.

Labeling Macrophage Cultures with [^{3}H]20:4. [5,6,8,9,11,12,14,15-^{3}H]20:4 (60–90 Ci/nmol; New England Nuclear, Boston, MA) was suspended in peritoneal lavage medium at a concentration of 1.0 μCi/ml, and 1 ml of the medium was added to the washed macrophage monolayers. After incubation for 45 min at 37°C, the cells were washed three times with KRPG and overlaid with the appropriate culture medium for each experiment. The release of ^{3}H by macrophages labeled under these conditions overestimates actual 20:4 release (in molar quantities) by 50–60%, however, the specific activities of the [^{3}H]20:4 metabolites produced are similar to one another (1).

Preparation of Unopsonized Zymosan. Zymosan was purchased from ICN K & K Laboratories Inc., Plainview, NY, and prepared by the method of Bonney et al. (3). Stock suspensions (20 mg/ml) in α-MEM were stored at −80°C until use.

Antibodies. Rabbit anti-dinitrophenol (DNP) IgG (a generous gift of Dr. Nicholas A. Pawlowski, The Rockefeller University, New York) was obtained from New Zealand white rabbits immunized by multiple injections of DNP-modified keyhole limpet hemocyanin in complete Freund's adjuvant. The antibodies were purified by affinity chromatography on trinitrophenol-modified albumin-Sepharose 4-B columns, as described (4). Stock solutions of purified IgG contained 1.2 mg/ml of antibody protein in Pi/NaCl plus 0.02% (wt/vol) sodium azide. Mouse monoclonal anti-DNP IgE (H1 DNP-ε-26.82) was prepared as described (5). Stock solutions (2 mg/ml) in Pi/NaCl were stored at −20°C.

Preparation of IC-coated particles. Dinitrophenol-conjugated bovine serum albumin (DNP-albumin) containing 11 mol of DNP/mol of albumin was prepared by incubating albumin (Armour Pharmaceutical, Phoenix, AZ) at a concentration of 50 mg/ml with 15 mM dinitrobenzene sulfonic acid (Eastman Kodak Co., Rochester, NY) in 0.3 M sodium carbonate for 10 h at 25°C, followed by extensive dialysis (4). DNP-albumin-coated latex particles were prepared by a modification of a previously described method (6). Briefly, 100 μl of a 10% (wt/vol) suspension of carboxylate-modified latex beads (0.86-μm Diam; The Dow Chemical Company, Indianapolis, IN) was washed three times and suspended in 1 ml of 0.2 M sodium acetate buffer, pH 5.6. After the addition of 0.012 g of *N*-hydroxysuccinimide and 0.019 g of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (both from Pierce Chemical Co., Rockford, IL), the beads were incubated for 10 h at 25°C with gentle agitation. The latex was then washed once with fresh acetate buffer and suspended in 1 ml of a 1 mg/ml solution of DNP-albumin in 0.1 M sodium carbonate buffer, pH 9.4. After incubation for 45 min at 25°C, 0.075 g of glycine was added, and the beads were incubated for an additional 15 min. The latex was then washed four times, suspended in 1 ml of Pi/NaCl plus 0.02% sodium azide, and stored at 4°C.

IC-coated latex beads were prepared by adding 25 μg of IgE or 30 μg of IgG as undiluted stock solutions to 25 μl of the DNP-albumin latex suspension. After incubation at 37°C for 30 min, the beads were washed three times in Pi/NaCl and suspended in 1 ml of α-MEM. For challenge of macrophage cultures, each 35-mm dish was overlaid with 1 ml of the final beads suspension. At this dose, macrophages received 4.1 μg of particle-bound IgE or 5.5 μg of particle-bound IgG per dish, as determined from the radiolabel content of beads opsonized with antibodies labeled with ^{125}I. Latex particles prepared in this way promoted maximum 20:4 release by the cultures.

Analysis of Total [^{3}H]20:4 Metabolites Released by Macrophages. [^{3}H]20:4-labeled macrophages

were washed and overlaid with α-MEM containing zymosan (160 μg/dish) or latex particles. The cultures were subjected to centrifugation for 5 min at 250 *g* and incubated for 2 h at 37°C to allow 20:4 release and metabolism to occur. The medium was then removed and placed on ice, and aliquots (50 μl/ml of α-MEM) were taken for radioactivity measurements. The cell monolayers were washed twice with KRPG and scraped into 500 μl of a 0.05% (wt/vol) solution of Triton X-100 (Rohm and Haas Co., Philadelphia, PA). Duplicate aliquots of the cell lysates were removed for assay of radioactivity (50 μl) and cell protein (100 μl).

The 20:4 metabolites in the culture medium were extracted by the method of Unger et al. (7). Briefly, to 1 ml of medium was added 1 ml of ethanol and 10 μl of 88% (wt/wt) formic acid, and the resultant solution was extracted twice with 1 ml of chloroform containing 0.005% (wt/vol) butylated hydroxytoluene (Sigma Chemical Co.). The lower (chloroform) phases were combined, evaporated to dryness under nitrogen, and dissolved in the appropriate starting solvent for further purification by reverse-phase high pressure liquid chromatography (HPLC).

Concentrated medium extracts were applied to a column of 5-μm Ultrasphere ODS, 4.6 mm × 25 cm (Altex Scientific Inc., subsid. of Beckman Instruments, Inc., Berkeley, CA; and Rainin Instruments Co. Inc., Woburn, MA) (HPLC system 1). The column was eluted at a rate of 1 ml/min with 60 ml of solvent 1 (methanol/water/acetic acid, 65:34.9:0.1, vol/vol/vol, adjusted to pH 5.4 with ammonium hydroxide), followed by 40 ml of solvent 2 (methanol/acetic acid, 100:0.01, vol/vol). For the characterization of cyclooxygenase metabolites, material eluting at 4–16 min from HPLC system 1 was collected, evaporated to dryness under reduced pressure, and rechromatographed on HPLC system 2 using a Waters Fatty Acid Analysis Column (Waters Associates, Inc., Milford, MA). PG were eluted at a flow rate of 2 ml/min with 100 ml of solvent 3 (water/acetonitrile/benzene/acetic acid, 76.7:23:0.2:0.1, vol/vol/vol/vol), followed by 40 ml of solvent 2 (8). Fractions from the HPLC were collected at 1-min intervals, and the radiolabel content of whole fractions or aliquots was measured by liquid scintillation counting in Hydrofluor (National Diagnostics, Inc., Advanced Applications Institute Inc., Somerville, NJ) using an LKB 1210 Ultrobeta scintillation counter (LKB Instruments, Inc., Rockville, MD). Corrections were made for counting efficiency (~40%). The recovery of 20:4 metabolites through extraction and purification procedures and their elution times on HPLC were monitored with the aid of radiolabeled standards, as described (9).

Quantitation of LTC. For the assay of LTC release, the column effluent from HPLC system 1 was monitored for absorbance at 280 nm using a Kratos Spectroflow Monitor SF770 (Kratos, Inc., Schoeffel Instrument Division, Westwood, NJ) with full-scale deflection set at 0.02 absorbance units. The monitor was calibrated using an LTC standard applied to the column in known quantities of from 60–750 pmol. At these amounts, a linear relationship was found between the quantity of LTC injected and the area under the peak corresponding to LTC in the ultraviolet absorbance elution profile. This standard curve was then used to determine molar quantities of LTC present in extracts of macrophage culture medium from the ultraviolet absorbance elution profile generated during purification by HPLC system 1.

Purification of LTC. For the characterization of LTC, [^{3}H]20:4 metabolites were extracted from macrophage culture medium as described above, and LTC was purified by silicic acid column chromatography and HPLC (9).

Bioassay of LTC. The bioassay for SRS activity was performed using an isolated guinea pig ileum in Tyrode's solution in the presence of atropine (1 μM) and pyrilamine maleate (1 μM) by the method of Chakravarty (10). 1 U of SRS activity was defined as the amount producing a contraction equal to that of 5 ng of histamine.

Assay of Intracellular Glutathione (GSH) and Cell Protein. Macrophage monolayers were washed twice with KRPG, and the cells from two 35-mm dishes were scraped together into 500 μl of 0.05% (wt/vol) Triton X-100. Samples of the lysates (190 μl) were acidified with 10 μl of 0.1 M HCl, and protein was precipitated by the addition of 10 μl of 50% (wt/vol) sulfosalicylic acid. The protein was removed by centrifugation, and 100-μl portions of the supernatants were assayed for total GSH (GSH and GSSG) by a modified 5, 5′-dithiobis (2-nitrobenzoic acid)-GSH reductase procedure (11). GSH standards contained Triton X-100, sulfosalicylic acid, and HCl in quantities identical to the samples. The protein content of Triton X-100 macrophage cell lysates was determined by the method of Lowry et al. (12) using bovine serum albumin as a standard.

Results

The Effect of Culture Time on Macrophage 20:4 Metabolism. Macrophages explanted from the peritoneal cavity rapidly lose their responsiveness to IgE-IC when cultured for >4 h (1). Therefore, assays of 20:4 metabolism by IgE-IC-challenged macrophages required that cultures be subjected to minimum in vitro incubation periods. Because 20:4 metabolism by freshly isolated macrophages had not been examined previously, we first determined the effects of culture time on macrophages challenged with unopsonized zymosan, a stimulus frequently used in our laboratory (13, 14). Fig. 1 A shows the HPLC elution profile of the total [^{3}H]20:4 metabolites of macrophages exposed to zymosan after 17 h of culture. Two major peaks, corresponding to cyclooxygenase products (4–16 min) and LTC (22–23 min) were obtained, in addition to multiple, minor peaks having the elution characteristics of mono- and dihydroxyeicosatetraenoic acids. Separation of the cyclooxygenase metabolites revealed two

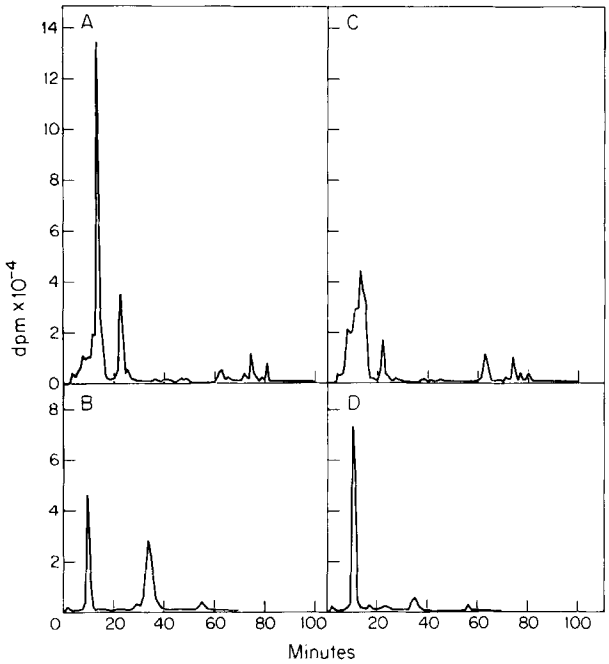

FIG. 1. Effect of culture time on macrophage 20:4 metabolism in response to zymosan. (A) Macrophages isolated by adherence for 1 h to fibronectin-coated dishes were overlaid with α-MEM containing 10% fetal bovine serum and incubated for 16 h. The cultures were then labeled for 45 min with [^{3}H]20:4 and challenged with zymosan. After 2 h of incubation, the 20:4 metabolites released by the cells were extracted from the medium and separated on HPLC system 1. (B) Aliquots (100 μl) of the fractions eluting at 4–16 min from HPLC system 1 (Fig. 1 A) were removed for radiolabel measurements. The remaining material was combined, evaporated under reduced pressure, and rechromatographed on HPLC system 2. (C and D) Conditions are identical to those for Fig. 1 A and B, respectively, except the cultures were labeled for 45 min with [^{3}H]20:4 and then challenged with zymosan immediately after isolation. The data shown are the ^{3}H elution profiles obtained from the combined medium of duplicate cultures. Relevant elution times for HPLC system 1 are: cyclooxygenase products, 4–16 min; LTC, 22–23 min; leukotriene D, 34–35 min; leukotriene E, 46–47 min; mono- and dihydroxyeicosatetraenoic acids and unreacted 20:4, 25–85 min. Elution times for HPLC system 2 are: 6-ketoPGF$_{1\alpha}$, 9–10 min, thromboxane B$_2$, 20–24 min; PGF$_{2\alpha}$, 27–29 min; and PGE$_2$, 32–37 min. The recovery of LTC through the extraction and HPLC system 1 was 60%. The recovery of 6-ketoPGF$_{1\alpha}$ and PGE$_2$ through extraction and both HPLC systems was 55%.

products (Fig. 1 B) with the elution characteristics of 6-ketoPGF$_{1\alpha}$ (the stable hydrolysis product of prostacyclin) (8–10 min) and PGE$_2$ (33–38 min). In contrast, the 20:4 metabolites of macrophages that were challenged with zymosan immediately after isolation (1 h) showed a marked reduction in the synthesis of LTC (Fig. 1 C) and PGE$_2$ (Fig. 1 D). 6-ketoPGF$_{1\alpha}$ was the single major 20:4 metabolite produced by 1-h cultures, although the percent of incorporated radiolabel released (40.3 ± 1.8%) was similar to that released by 17-h cultures (31.9 ± 0.8%).

Effect of Culture Time on Intracellular GSH. The spectrum of 20:4 metabolites secreted by zymosan-challenged macrophages after 1 h in culture was similar to that of cells deficient in intracellular GSH (15). Because GSH is required for the synthesis of both LTC and PGE$_2$, we examined the GSH content of macrophages as function of culture time (Table I). Freshly isolated macrophages contained 12–16 pmol of GSH/μg of cell protein. From 3–9 h of incubation, intracellular GSH rose to a steady state level of 32–35 pmol/μg of cell protein, which was maintained thereafter. Table I also shows that, coincident with the increase in intracellular GSH, macrophages acquired an increased capacity to synthesize LTC and PGE$_2$. Thus, total radiolabel release by cells challenged with zymosan was similar in cultures incubated from 1–17 h. However, with increasing culture time and GSH content, macrophages used relatively more of the released 20:4 for LTC and PGE$_2$ synthesis and less for the production of

TABLE I

*20:4 Metabolism as a Function of Time in Culture**

Time in culture‡	GSH (pmol/μg protein)‡§	Total ^{3}H release‖	20:4 metabolite¶		
			LTC	PGE$_2$	6-ketoPGF$_{1\alpha}$
h	*pmol/μg protein*				
0	16.0 ± 4.1				
1	12.4 ± 0.4	47.5 ± 1.8	7.6	5.3	45.5
3	13.4 ± 1.6	51.0 ± 1.1	10.3	9.6	39.1
5	21.3 ± 1.7	51.3 ± 0.2	16.5	13.2	29.5
9	32.6 ± 4.7	39.9 ± 1.6	17.9	17.9	22.1
17	34.7 ± 4.8	37.7 ± 0.7	18.6	26.6	19.1

* Macrophages isolated by adherence for 1 h to fibronectin-coated surfaces were washed and overlaid with α-MEM containing 10% fetal bovine serum. After varying periods of time in culture the cells were washed, labeled with [^{3}H]20:4, and then challenged for 2 with zymosan (160 μg/dish). The 20:4 metabolites released by the cells were extracted from the culture medium and separated by HPLC systems 1 and 2. Alternatively, after the indicated incubation periods, duplicate pairs of cultures were washed and scraped into 0.05% Triton X-100 (500 λ per two dishes). The resulting cell lysates were analyzed for GSH and cell protein.

‡ Time in culture designates the total incubation period between the end of the adherence step and the measurement of GSH or the challenge with particles.

§ Mean ± range of two separate experiments.

‖ Mean ± range of duplicate cultures for the percent of total incorporated radiolabel released into the culture medium.

¶ The quantity of radiolabeled metabolite obtained from the combined medium of duplicate cultures was corrected for recovery (see legend to Fig. 1) and is expressed as the percent of total ^{3}H released. In the absence of LTC synthesis, ~2% of the radiolabeled released by macrophages is recovered in the fractions corresponding to LTC on HPLC system 1. All values for LTC were corrected for this background.

6-ketoPGF$_{1\alpha}$.

Synthesis of 20:4 Metabolites in Response to IgE. The above results indicated that incubation of macrophages for ~4 h before challenge with a stimulus would provide sufficient time for the cells to acquire the capacity for LTC and PGE_2 synthesis, with a minimum loss of their responsiveness to IgE-IC. Macrophages were therefore isolated, incubated for 3.5 h, labeled with [^{3}H]20:4 (45 min), and exposed to IgE-IC-coated latex beads. These cultures released 27.1 ± 4.9% (mean ± SD; seven experiments) of their incorporated radiolabel, in contrast to cells challenged with DNP-albumin-coated latex beads, which did not release ^{3}H above control values (5–9%).

Fig. 2 A shows the HPLC elution profile of the total [^{3}H]20:4 metabolites extracted from the culture medium of IgE-IC-challenged macrophages. Radiolabeled peaks, corresponding to cyclooxygenase metabolites (4–16 min) and LTC (22–23 min) were observed in addition to multiple minor peaks having the elution characteristics of hydroxy fatty acids. Separation of cyclooxygenase products indicated the presence of 6-ketoPGF$_{1\alpha}$ and PGE_2 (Fig. 2 B).

To verify the identity of the material eluting at 22–23 min (Fig. 2 A), macrophage cultures containing a total of 5.9 mg of protein (100–35-mm dishes) were challenged with IgE-IC-coated latex after 4.5 h in culture. The 20:4 metabolites were extracted from the culture medium and subjected to silicic acid column chromatography and HPLC. Fig. 3 shows the ^{3}H elution profile from the final HPLC purification of the leukotriene-containing (methanol) eluate from the silicic acid column. The major radiolabeled peak eluted at 22–23 min. The ultraviolet spectrum of this material (Fig. 3, upper inset) showed an absorbance maximum at 280 nm, with shoulders at 270 and 292 nm, which is characteristic for LTC (16). In addition, this material produced a slow, sustained contraction of the longitudinal muscle of a standard guinea pig ileum preparation, demonstrating typical slow-reacting substance bioactivity (Fig. 3, lower inset). These data confirm the identity of this metabolite as LTC.

Based on the absorbance at 280 nm and a molar extinction coefficient of 40,000 (17), ~2.3 nmol of purified LTC was obtained. Correcting for a recovery of 30% (9) through the extraction and purification procedures, this value corresponded to a synthesis of 1.3 pmol of LTC/μg of cell protein (78 pmol/10^6 cells) by IgE immune complex-challenged macrophages. LTC comprised ~4% of the total radiolabel released by the cells.

Comparison of 20:4 Metabolites Secreted in Response to IgE and IgG-IC and Zymosan. [^{3}H]20:4-labeled macrophages were exposed for 2 h to IgE-IC- or IgG-IC-coated latex particles or zymosan after 4.5 h of culture. Under these conditions, the cells released 27.1 ± 4.9% (mean ± sd; n = 7), 40.5 ± 11.6% (mean ± range; n = 2), and 43.2 ± 15.8% (mean ± range; n = 2) of their total incorporated ^{3}H in response to IgE-IC, IgG-IC, and zymosan, respectively. The [^{3}H]20:4 metabolites released by the cells were extracted from the culture medium and separated by HPLC. Table II shows the percent of the total released radiolabel that was recovered as LTC, 6-ketoPGF$_{1\alpha}$, and PGE_2. The absolute quantity of LTC produced by the cells was determined from the ultraviolet absorbance chromatogram (280 nm) generated during the separation of total 20:4 metabolites by HPLC system 1. Quantities of the other 20:4 metabolites were then calculated from the specific activity of the LTC. Although these values (Table II) are subject to error, the data obtained for zymosan-treated cells are consistent with previously reported estimates (15) after consideration is given to the

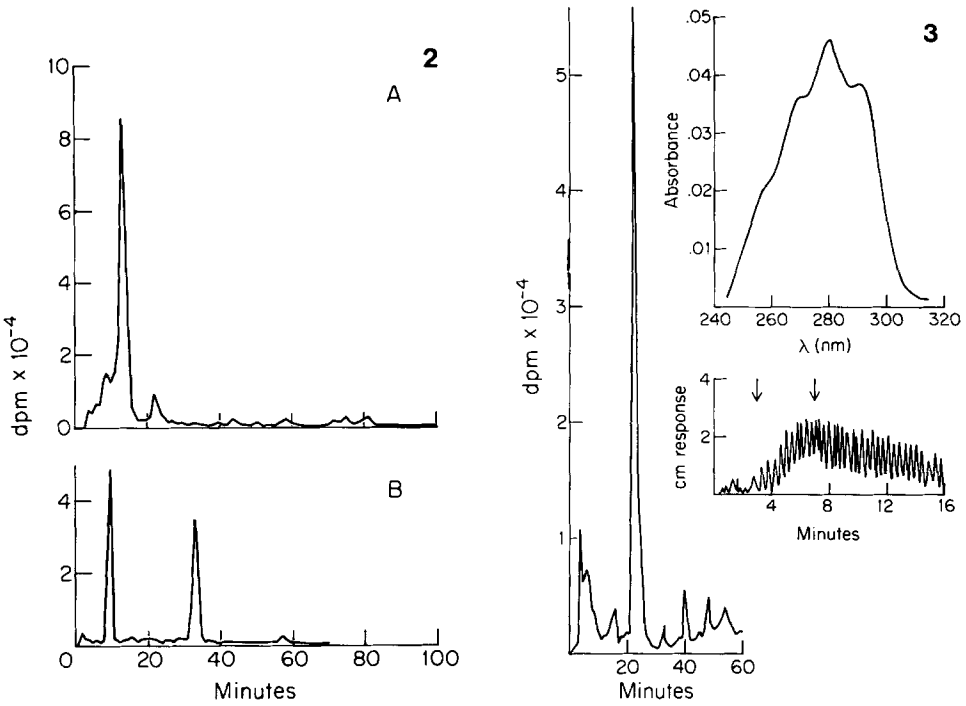

FIG. 2. 20:4 metabolites released by IgE-IC-challenged macrophages. Macrophages isolated by adherence for 1 h to fibronectin-coated surfaces were incubated for 3.5 h in peritoneal lavage medium, labeled for 45 min with [^{3}H]20:4, and challenged with IgE-IC-coated latex beads. The [^{3}H]20:4 metabolites released into the culture medium after 2 h were extracted and separated by (A) HPLC system 1 and (B) HPLC system 2, as described in Fig. 1. The data shown are the ^{3}H elution profiles obtained from the combined medium of duplicate cultures. Relevant elution times and recoveries are presented in the legend to Fig. 1.

FIG. 3. Purification of LTC from the medium of macrophages challenged with IgE-IC. Macrophage cultures containing a total of 5.9 mg of cell protein were prepared and challenged with IgE-IC-coated latex beads, as described in the legend to Fig. 2, except that the cells were labeled with 0.3 μCi/ml of [^{3}H]20:4. The [^{3}H]20:4 metabolites released by the cells were extracted from the culture medium and subjected to silicic acid column chromatography. The methanol eluates from silicic acid columns were concentrated under reduced pressure and purified on HPLC system 1. The ^{3}H elution profile from the purification step is shown. (upper inset) Ultraviolet spectrum of material eluting at 22–23 min. (lower inset) Response of a standard guinea pig ileum preparation to the material eluting at 22–23 min. Based on the absorbance at 280 nm, 6.9 pmol (1.4 nM) of LTC was added to the organ bath.

effects of the shorter culture time used in these experiments (Table I).

Discussion

The production of LTC by mouse peritoneal macrophages in response to a stimulus of IgE and antigen occurs under in vitro culture conditions. Preformed, particulate immune complexes of an appropriate size and geometric configuration are necessary, and cells must be challenged within a defined culture period. At least two additional parameters affect the capacity of macrophages to release and metabolize 20:4 in vitro, as noted previously (1). The first of these is the loss in the cells' capacity to release 20:4 in response to a challenge with IgE-IC. Consequently, culture conditions that maximize 20:4 release require the use of freshly explanted cells. The second parameter

TABLE II

*20:4 Metabolites Synthesized by Macrophages in Response to Immune Complexes and Zymosan**

Metabolite	Stimulus					
	IgE‡		IgG§		Zymosan§	
	Percent of released ^3H	pmol/μg cell protein	Percent of released ^3H	pmol/μg cell protein	Percent of released ^3H	pmol/μg cell protein
LTC	4 ± 1	1.0 ± 0.3	5 ± 1	1.9 ± 0.9	14 ± 5	5.9 ± 1.9
PGE_2	19 ± 6	4.7 ± 1.5	14 ± 2	5.3 ± 0.8	11 ± 3	4.6 ± 1.3
6-keto$PGF_{1\alpha}$	33 ± 3	8.2 ± 0.8	34 ± 2	13 ± 1	34 ± 5	14 ± 2

* Macrophages isolated by adherence for 1 h to fibronectin-coated dishes were incubated for 3.5 h in peritoneal lavage medium, labeled with [^3H]20:4 (45 min), and challenged with maximum doses of IgG- or IgE-IC-coated particles or zymosan. After 2 h of incubation, the 20:4 metabolites released by the cells were extracted from the combined medium of duplicate cultures and separated on HPLC systems 1 and 2. The radiolabel content of HPLC fractions corresponding to LTC (system 1) and to PGE_2 and 6-keto$PGF_{1\alpha}$ (system 2) was quantitated, corrected for recovery, and used to determine the percent of the total ^3H released that was converted to each metabolite. In the case of LTC, values were also corrected for radiolabel background as described in the legend to Table I. The quantity of LTC released by the cells was determined from the absorbance (280 nm) elution profile generated from HPLC system 1. This value was then used to estimate molar quantities of the other 20:4 metabolites using the formula: pmol metabolite/μg cell protein = M/L × X, where M and L are the percent of the total released 20:4 recovered as the metabolite and LTC, respectively, and X is the molar quantity of LTC produced by the cells. This calculation is based on the finding that the specific activities of the 20:4 metabolites released by the cells under these conditions are similar (1).

‡ Values are the mean ± range of separate experiments.

§ Values are the mean ± SD of four separate experiments.

concerns changes in intracellular GSH levels that occur as a function of culture time. In comparison with macrophages incubated for 10 h or more, the GSH content of freshly isolated macrophages is low, and these cells have a diminished capacity to secrete the GSH-dependent 20:4 metabolites, PGE_2 and LTC. In previous experiments, the depletion of intracellular GSH to levels observed in freshly explanted cultures (10–15 pmol/μg of cell protein) led to a qualitatively similar but less severe inhibition of LTC and PGE_2 synthesis (15). The reasons for the changes of GSH content as a function of culture time are unknown; however, an interesting possibility is that the adherence of macrophages to a substrate results in an accelerated loss of GSH similar to that observed during the phagocytosis of zymosan (18).

IgE-IC and IgG formed on the surface of latex particles stimulated the secretion of considerable quantities of LTC by macrophage cultures; however, the percentage of the total released 20:4 that was used for LTC synthesis was lower with IC than with zymosan. In contrast, the percentage of the fatty acid substrate converted to cyclooxygenase products did not vary with these particulate stimuli. In a recent report (1), we also examined the release of 20:4 by macrophages exposed to IgE-IC- and IgG-IC-coated Sephadex beads. These particles are too large to be ingested by the cells, but induce maximum levels of 20:4 release similar to the corresponding latex beads that are avidly interiorized. Studies of 20:4 metabolism in response to IC-coated Sephadex beads indicated that the quantities of PGE_2 and 6-keto$PGF_{1\alpha}$ released are comparable to those elicited by latex-bound IC; however, the synthesis of LTC induced by IC-coated Sephadex beads was highly erratic and consistently lower than with other stimuli. Although these results suggest that particle interiorization may be required for optimum LTC formation, previous studies (18) using cytochalasin D-treated cells

indicated that normal levels of zymosan-induced LTC synthesis occur in the presence of a >95% inhibition of phagocytosis. Clearly, factors in addition to the capacity to trigger 20:4 release determine the effects of a stimulus on macrophage 20:4 metabolism. The observation that different stimuli vary in their ability to promote the synthesis of cyclooxygenase and lipoxygenase products suggests distinct regulatory mechanisms for these two major metabolic pathways.

In addition to other inflammatory stimuli, IgE-IC mediate the release of large quantities of 20:4 metabolites by resident peritoneal macrophages. To date, no other normal mammalian cell has been shown to synthesize comparable quantities of 20:4 metabolites in response to a stimulus of IgE and antigen. Macrophages are ubiquitously distributed throughout the body, and many are localized in perivascular spaces. The 20:4 metabolites secreted by these cells in response to IgE-IC, including LTC, PGE_2, and prostacyclin, are all highly vasoactive compounds that effect changes in vascular tone and permeability. As a consequence, the macrophage might be a major primary source of products that mediate the vascular sequelae of IgE-mediated immediate hypersensitivity reactions.

Summary

Resident mouse peritoneal macrophages release the slow-reacting substance leukotriene C (LTC) on exposure to particulate IgE immune complexes. Because these cells lose their responsiveness to an IgE stimulus after 4 h in culture, maximum release of 20:4 metabolites is observed before this time. However, a similar diminution in 20:4 metabolism was not observed with a zymosan stimulus. Freshly explanted cells are deficient in intracellular glutathione (GSH) (12.4 ± 0.4 pmol/μg cell protein), but GSH increases to a steady state value of 30–35 pmol/μg of cell protein between 3 and 9 h of culture. Because GSH is required for the synthesis of LTC and prostaglandin (PG)E_2, cultures challenged immediately after explanation have a diminished capacity to synthesize these 20:4 metabolites and release prostacyclin as the major product. By 4–5 h in culture, macrophages form significant amounts of LTC and PGE_2. Under optimum conditions of maximum responsiveness to an IgE stimulus and GSH content (after 4 h of culture), macrophages challenged with latex beads coated with IgE immune complexes synthesize 1.0 ± 0.3 pmol of LTC/μg cell protein (60 ± 18 pmol/10^6 cells) in addition to prostacyclin (8.2 ± 0.8 pmol/μg cell protein) and PGE_2 (4.7 ± 1.5 pmol/μg cell protein). These amounts are quantitatively similar to the arachidonic acid metabolites produced by macrophages challenged with IgG immune complex-coated latex beads or zymosan. These data demonstrate that macrophages produce large quantities of LTC and other 20:4 metabolites in response to particle-bound IgE and antigen, provided that the appropriate in vitro conditions are met. The macrophage might, therefore, be a major source of slow-reacting substance and other 20:4 metabolites generated during IgE-mediated reactions in vivo.

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