DYNAMICS OF LEUKOTRIENE C PRODUCTION BY MACROPHAGES*

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Slow reacting substance (SRS)[1] was first discovered by Feldberg and Kellaway (1) in the perfusate of a guinea pig lung treated with cobra venom. This material, which caused a characteristic contraction of selected smooth muscle preparations, was later found in the perfusates of sensitized guinea pig or human lungs after treatment with antigen (2, 3). Because of its physiological effects and immunologic origin, it was thought that SRS could play an important role as a mediator of immediate hypersensitivity reactions, and immunologically generated SRS was named slow reacting substance of anaphylaxis (SRS-A).

For many years, progress on the study of SRS-A was hampered by the very small quantities available. Recently, however, the use of calcium ionophore A 23187-stimulated mouse mastocytoma cells (4, 5) or rat basophilic leukemia cells (6, 7) has led to the generation of SRS in sufficient quantity to allow for their chemical characterization. These SRS were identified as metabolites of arachidonic acid (20:4) and named leukotrienes C and D (LTC and LTD). LTC, originally obtained from mouse mastocytoma cells is 5-hydroxy-6-S-γ-glutamylcysteinylglycyl-7,9,11,14-eicosatetraenoic acid, an adduct of arachidonic acid and glutathione (5). LTD, the SRS produced by rat basophilic leukemia cells, is 5-hydroxy-6-S-cysteinylglycyl-7,9,11,14-eicosatetraenoic acid (6, 7). It is believed that LTD has the properties of the SRS-A obtained from antigenically challenged guinea pig or human lung (6, 8).

In a recent paper (9), we reported that mouse peritoneal macrophages release SRS in response to a phagocytic challenge with unopsonized zymosan. This compound, known to be a metabolite of 20:4, was purified by solvent extraction, Sephadex G-25 column chromatography, and reverse-phase high pressure liquid chromatography (HPLC). Chemical characterization of the purified SRS indicated structural features, all of which were consistent with the identity of the compound as a 20:4 metabolite that contained glutathione, although characterization of the 20:4 portion of the molecule has not yet been completed. In this paper, we present a simple radiochemical method for the assay of the release of this SRS (which we designate LTC) by macrophages in vitro. Using this assay, we provide data that establish the relationship

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[1] *Abbreviations used in this paper:* α-MEM, Minimum essential alpha medium; 20:4, arachidonic acid; [³H]-20:4, [5,6,8,9,11,12,14,15-³H]20:4; FCS, fetal calf serum; HPLC, reverse-phase high pressure liquid chromatography; LTC, leukotriene C; LTD, leukotriene D; PD, calcium- and magnesium-free phosphate-buffered saline; PG, prostaglandin; PGE, prostaglandin E; SRS, slow reacting substances; SRS-A, SRS of anaphylaxis.

of LTC production to the process of phagocytosis and the release of other 20:4 metabolites by the cells.

Materials and Methods

Macrophage Cultures. Primary cultures of peritoneal macrophages were established from resident cells of female Swiss Webster mice (Taconic Farms, Germantown, N. Y.) weighing 25–30 g as previously described (10). Peritoneal cells (6×10^6) in minimum essential alpha medium (α-MEM; Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.) that contained 10% fetal calf serum (FCS) were added to 35-mm diameter plastic culture dishes. After 2 h at 37°C in 5% CO_2, cultures were washed three times in α-MEM to remove nonadherent cells. Fresh medium plus 10% FCS (1 ml) that contained 0.5 μCi/ml of [5,6,8,9,11,12,14,15-^{3}H]20:4 ([^{3}H]-20:4; New England Nuclear, Boston, Mass.) was then added to each dish, and the cells were incubated overnight (16 h).

Corynebacterium parvum-elicited Macrophages. *C. parvum*-elicited macrophages were obtained by injecting 1.4 mg formalin-killed *C. parvum* Coparvax; a gift of Dr. Tuttle, Burroughs Wellcome Co., Research Triangle Park, N. C.) intraperitoneally into each mouse. Macrophages were harvested 11–14 d later and cultured as described for resident cells.

Preparation of Unopsonized Zymosan. Zymosan was purchased from ICN, K & K Laboratories Inc., (Plainview, N. Y.), and stock solutions in α-MEM were prepared as described (11).

Preparation of Immune Complexes. Horseradish peroxidase and rabbit antiserum against horseradish peroxidase were the generous gift of Dr. Ralph M. Steinman of The Rockefeller University. Immune complexes were prepared at equivalence by incubating equal volumes of a solution of horseradish peroxidase (200 μg/ml in α-MEM) and antibody for 30 min at 37°C. The complexes were washed twice in calcium- and magnesium-free phosphate-buffered saline (PD) and suspended at a concentration equivalent to 20 μg/ml horseradish peroxidase (135 μg of total immune complex protein/dish) for addition to macrophage cultures.

Production of LTC by Macrophage Cultures. After the 16-h labeling period, cells were washed twice in α-MEM, and 1 ml of serumless α-MEM that contained zymosan or immune complexes was added. After incubation for specified time periods at 37°C in 5% CO_2, the medium was removed and extracted for LTC.

The cell monolayers were washed twice with PD, overlaid with 0.05% Triton X-100 (Rohm and Hass Co., Philadelphia, Pa.), and the dishes were scraped. Cell protein was determined by the method of Lowry et al. (12). Macrophages isolated from 6×10^6 peritoneal cells contained ~150 μg protein.

Extraction of LTC. Quantities given refer to 1 ml of medium. The medium was acidified to pH 3 with 10 μl 85% formic acid. Then 3.75 ml of chloroform:methanol 1:2 (vol:vol), 1.25 ml of chloroform, and 1.25 ml of water were added sequentially, with mixing after each addition. After centrifugation at 1,000 g for 15 min, the upper phase was removed and extracted with 6 ml of chloroform:ethanol 1:1 (vol:vol) and 2 ml of chloroform:ethanol 2:1 (vol:vol). The chloroform-ethanol extracts were combined and concentrated under reduced pressure. The extraction was performed at 4°C, and solvents contained 0.005% of the antioxidant butylated hydroxytoluene (Sigma Chemical Co., St. Louis, Mo.).

Silicic Acid Column Chromatography of LTC. The concentrated chloroform-ethanol extracts were dried under nitrogen, and the residues were dissolved in 0.1 ml of ethanol:water 80:20 (vol:vol). Samples were applied to columns (6 mm $\times$ 10 cm) of activated silicic acid (Silicar CC-7; Mallinckrodt Inc., St. Louis, Mo.) packed in chloroform. Columns were eluted sequentially with 5 ml of chloroform, 15 ml of 5% methanol in chloroform, and 20 ml of methanol. All solvents contained 0.005% butylated hydroxytoluene. Routinely, the solvent fractions were collected in glass scintillation vials, the first two solvents comprising fraction 1, and the third solvent comprising fraction 2. LTC eluted in fraction 2. Fractions of 2 ml were collected when elution profiles of standards and chloroform-ethanol extracts were monitored. Fractions were dried under a stream of air, water (0.5 ml) was added, and radioactivity was measured by liquid scintillation counting in Hydrofluor (National Diagnostics, Inc., Advanced Applications Institute Inc., Sommerville, N. J.).

Preparation of Radiolabeled LTC Standard. LTC was obtained from macrophage cultures prelabeled with [^{3}H]-20:4 and stimulated with zymosan. The released LTC was purified by

solvent extraction, Sephadex G-25 column chromatography, and HPLC as described previously (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 (13). 1 U SRS activity was arbitrarily defined as that amount that produced a contraction equal in amplitude to 5 ng histamine. The second fractions from silicic acid columns were dried under reduced pressure and dissolved in Tyrode's solution for testing. FPL 55712 (Fisons Pharmaceuticals, Loughborough, England) was used to test inhibition of SRS induced contractions (14).

Assay of Prostaglandin E (PGE) Release. Macrophages were isolated, prelabeled with [^{3}H]-20:4, and challenged with zymosan as described above. After the desired incubation periods, the media were removed and the prostaglandin (PG) extracted and purified by silicic acid column chromatography as described previously (15). PGE release was quantitated by radioactivity recovered in the 2.5% methanol in chloroform fraction from the columns, and is expressed as pmol of PGE/μg of cell protein.

Calculations. The molar quantity of LTC obtained in radiolabeled samples was calculated using the specific activity of [^{3}H]-20:4 in the macrophage phospholipids (53.7 dpm/nmol). The determination of [^{3}H]-20:4-specific activity was based on an uptake of 90% of the label added to the cultures, and incorporation of 70% of the label into the phospholipid pool (15).

Results

Assay of LTC Synthesis. In our earlier work (9), we showed that macrophages prelabeled with [^{3}H]-20:4 and stimulated with zymosan released radiolabeled LTC into the culture medium. We further demonstrated that the amount of LTC calculated from the radiolabel content of purified preparations was in excellent agreement (80%) with that estimated from amino acid analysis. Radioactivity measurements of such samples, therefore, could be used to quantitate the amount of LTC released. To make such an assay method practical, it was necessary to develop techniques that would facilitate the processing of large numbers of samples. This problem was simplified in that only radiochemical purity of LTC was required, making extensive HPLC purifications unnecessary. We therefore developed a procedure for obtaining radiochemically pure LTC as follows.

Media from macrophage cultures labeled with [^{3}H]-20:4, and subsequently challenged with zymosan, were extracted for LTC. From our previous work, we knew that the major radiolabeled contaminant of LTC in the chloroform-ethanol extracts of culture media was PG. Separation of PG and LTC was accomplished by stepwise elution of the concentrated chloroform-ethanol extracts from silicic acid columns. Fig. 1 shows the ^{3}H-elution profile from this column. Also shown are the elution profiles for [^{3}H]-PGE$_2$ and [^{3}H]-LTC standards. Three peaks of radioactivity were obtained on chromatography of media extracts (Fig. 1 C). The first two of these peaks coeluted with PGE$_2$, which was recovered in the chloroform, and 5% methanol in chloroform fractions (Fig. 1 A). The third peak from the chloroform-ethanol extracts was recovered in the methanol fraction and coeluted with the LTC standard (Fig. 1 B). These data indicated that LTC could be separated from PG and other less polar 20:4 metabolites by this procedure.

Reverse-phase HPLC was used to verify that the LTC in the methanol eluate from the silicic acid columns (fraction 2) was radiochemically pure. Fraction 2 was concentrated and applied to a column of Lichrosorb C-8 (Altex Scientific, Inc., Berkeley, Calif.) routinely used for the purification of LTC standard. A single peak of radioactivity was obtained, which had a retention time (170 min) identical with that
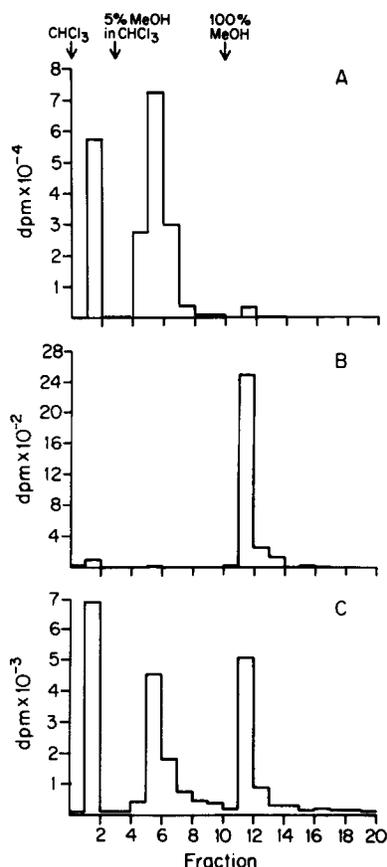
FIG. 1. ^{3}H-elution profiles of (A) [^{3}H]-PGE$_2$ and (B) [^{3}H]-LTC standards, and (C) the chloroform-ethanol extract of macrophage culture medium. Macrophages in a 35-mm diameter culture dish were prelabeled for 16 h with [^{3}H]-20:4 and then challenged with zymosan (160 μg). After 1 h, the medium was removed, extracted, and subjected to silicic acid chromatography as described in Materials and Methods. Standards were dissolved in ethanol:water 80:20 (vol:vol) and applied to identical silicic acid columns. Fractions of 2 ml were collected, dried under air, and the radioactivity determined.

of LTC standards (Fig. 2). The column effluent was also monitored for the presence of compounds with free amino groups by reaction with fluorescamine (16, 17). The ^{3}H peak corresponded to a fluorescamine-positive peak. Other, nonradioactive fluorescamine-positive peaks were also present, but these were well-resolved from the LTC. From these data, we concluded that LTC was recovered from fraction 2 of silicic acid columns, and that this LTC was free of radiolabeled contaminants.

The recovery of the biological activity of LTC after silicic acid chromatography was also examined. For this purpose, HPLC-purified [^{3}H]-LTC standard was dissolved in α-MEM, extracted, and subjected to silicic acid chromatography. Recovery of radioactivity was 50% after the extraction step and 40% after chromatography. Fig. 3 shows the results of bioassay of this LTC before and after treatment. Dose-response curves are presented for histamine and untreated LTC standard dissolved in α-MEM. The LTC caused a half-maximal contraction of the ileum at a concentration of 5 ×
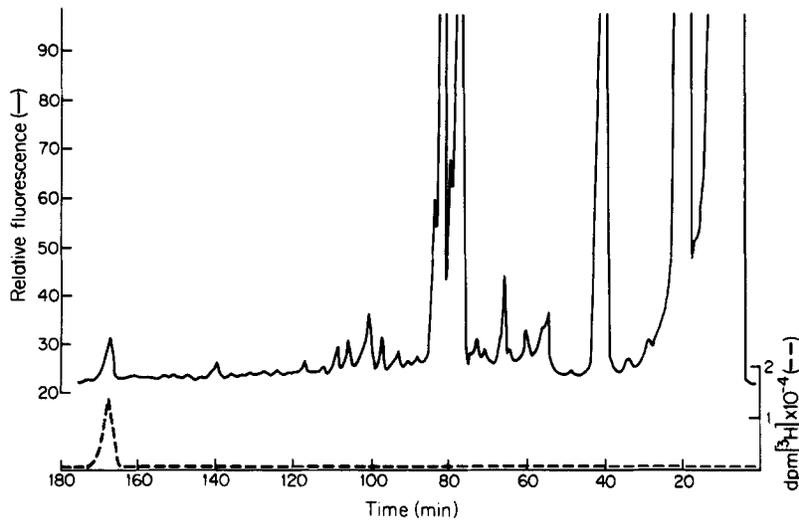
FIG. 2. HPLC elution profile of fraction 2 from silicic acid columns. Macrophages in a 35-mm dish were labeled and stimulated with zymosan as in Fig. 1. The medium was extracted and chromatographed on silicic acid. The methanol eluate (fraction 2) was dried under reduced pressure, redissolved in HPLC starting buffer, and subjected to chromatography on a column of Lichrosorb C-8 as described previously (9). Elution profiles for ^{3}H (---) and relative fluorescence after reaction with fluorescamine (—) are shown.
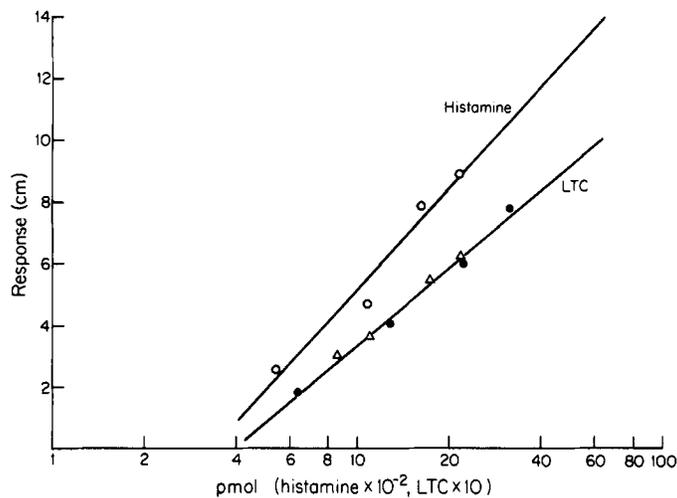
FIG. 3. Bioassay of LTC standard before and after extraction and silicic acid chromatography. [^{3}H]-LTC standard was obtained from macrophages prelabeled with [^{3}H]-20:4 and challenged with zymosan as described previously (9). Purified standard was dissolved in α-MEM (64,000 dpm/ml) and two samples (1 ml each) were extracted and chromatographed on silicic acid. Bioassay was performed on untreated standard (●), and on fraction 2 from the silicic acid columns after drying and redissolving in Tyrode's solution (△). The dose-response curve for histamine is also shown for comparison (○).

10^{-10} M; at this concentration it was 500 times more potent than histamine on a molar basis. Fig. 3 also shows a dose-response curve for the LTC standard recovered in silicic acid column fraction 2 from two separate extractions. These samples gave

responses identical to those of the untreated LTC, indicating that the recovered LTC had retained its full biological activity.

From the above data we could draw the following conclusions. LTC can be partially purified from macrophage culture medium by solvent extraction and silicic acid chromatography. LTC treated in this way is recovered in 40% yield with full retention of biological activity. Because the LTC is radiochemically pure, the amount of radioactivity recovered in the methanol eluates from the silicic acid columns can be used as a direct measurement of the quantity of LTC in the sample. Furthermore, because the specific activity of [^{3}H]-20:4 in the macrophage phospholipids is known (15), the radioactivity recovered can be related to the molar quantity of LTC. Therefore, the use of these techniques forms the basis of an assay for LTC release by macrophages in vitro when the cells are prelabeled with [^{3}H]-20:4 under the specified conditions.

Kinetics of Release of LTC and Other 20:4 Metabolites after Zymosan Ingestion.   Fig. 4 shows the time-course of total 20:4, PGE, and LTC release by macrophages prelabeled with [^{3}H]-20:4 and challenged with zymosan. The release of PGE and LTC followed similar kinetics and both were comparable with that of 20:4 (total ^{3}H). In each case, the levels of the metabolite increased linearly for 60 min after the addition of zymosan, and remained constant thereafter. Compared with control cultures (no zymosan), exposure to zymosan promoted a 40-fold increase in LTC synthesis and a comparable increase in PGE (15). Bioactivity measurements of the amount of released LTC
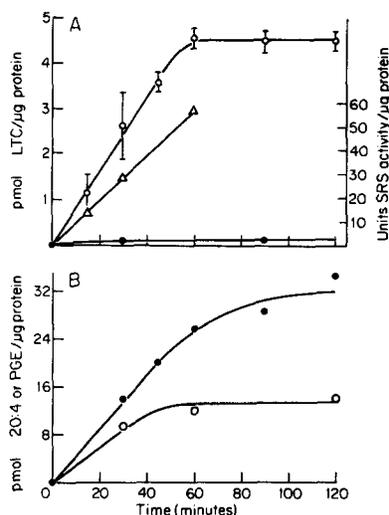

Fig. 4.   Time-course of LTC release in response to zymosan. Macrophages were isolated and prelabeled with [^{3}H]-20:4. After the 16-h labeling period, the cells were washed and challenged with 160 μg zymosan. At the specified times, the medium was removed and an aliquot counted. The PGE or LTC was extracted and purified by silicic acid chromatography. A. LTC release was calculated from fraction 2, corrected for a 40% recovery, and is the mean ± SD of triplicate cultures (O). LTC release is also shown for cultures not given zymosan (●). At selected time points, LTC in the medium from additional cultures was bioassayed for SRS activity (Δ). B. Release of total [^{3}H]-20:4 was calculated directly from the disintegrations per minute recovered in the medium and is expressed as the mean of triplicate cultures (●). PGE release was calculated from the radiolabel recovered in the 2.5% methanol in chloroform fraction from the columns, corrected for a 70% recovery, and is the mean of duplicate cultures (O). All release values are given in terms of pmol/ μg of cell protein.

(fraction 2 of the silicic acid columns) were in good agreement with those measured by radioactivity.

LTC Production and Zymosan Concentration. Macrophage cultures were challenged with different doses of zymosan, and the amount of LTC released was assessed after 90 min of incubation. The results of these experiments are presented in Fig. 5. LTC release was linear with zymosan dose up to 100 μg (coefficient of correlation = 0.98). Above this dose, progressively smaller increases in LTC release were obtained with increasing amounts of zymosan. Also shown in Fig. 5 are results from the bioassay of samples from fraction 2. These were in good agreement with the radioassay. Because previous studies indicated that cell viability was best maintained at doses of zymosan not exceeding 160 μg (W. A. Scott. Unpublished observations.), this dose was used for all subsequent experiments.

Kinetics of LTC Release by C. parvum-Elicited Macrophages. Macrophages were elicited by a single intraperitoneal dose of *C. parvum* and harvested after 11 d. These cells were cultured and prelabeled with [^{3}H]-20:4 by the same methods used for resident cells. Uptake of [^{3}H]-20:4 was identical in the two macrophage populations, and *C. parvum*-elicited macrophages ingested zymosan to the same extent as did resident cells. Fig. 6 shows the kinetics of LTC release by *C. parvum*-elicited macrophages after a challenge dose of 160 μg of zymosan. Also shown is LTC release by resident macrophages at 60 and 120 min. It is evident that, although the kinetics of release by *C. parvum*-elicited cells were similar to those of resident cells (compare with Fig. 4A), lower maximal levels of LTC synthesis were reached by the elicited cells (68 ± 16 dpm of LTC/μg of cell protein) as compared with resident macrophages (156 ± 30 dpm of LTC/μg of cell protein).

Production of LTC by Macrophages Exposed to Immune Complexes. Fig. 7 illustrates the kinetics of production of LTC by resident macrophages challenged with insoluble
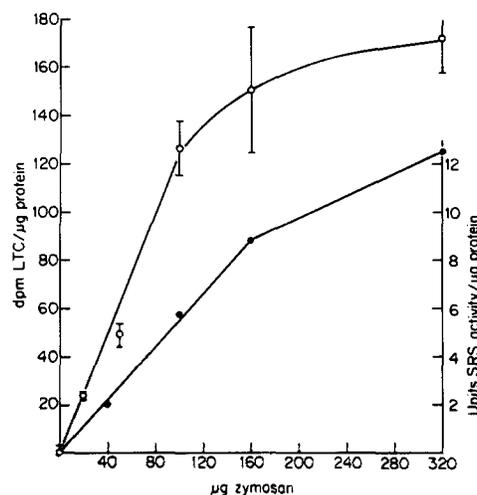

Fig. 5. Effect of zymosan concentration on LTC release. Experimental details are the same as those in Fig. 4, except that zymosan was added in the specified doses, and all cultures were incubated for 90 min. Values of radioactivity are the mean ± SD for triplicate cultures (O), and are expressed as dpm of LTC/μg of cell protein recovered in fraction 2. Values of bioactivity were obtained from dose-response determinations of individual samples in the ileum assay (●).
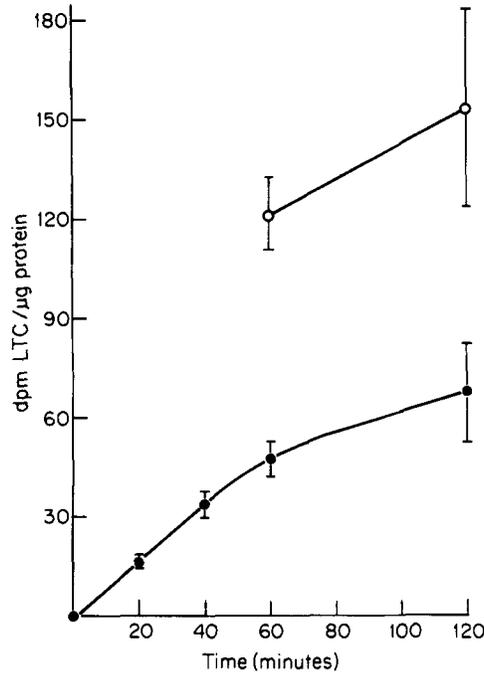
FIG. 6.   Time-course of LTC synthesis by *C. parvum*-elicited macrophages in response to zymosan. Mice were injected intraperitoneally with *C. parvum*, and macrophages were harvested after 11–14 d. Details of culture, labeling, and zymosan challenge of these cells are the same as in Fig. 4. Results given are the mean ± SD from triplicate cultures of *C. parvum*-elicited (●) and resident (○) macrophages.
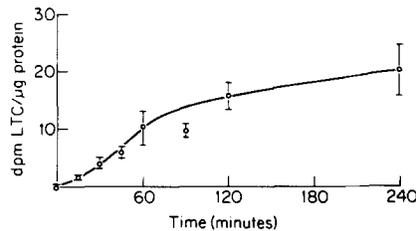

FIG. 7.   Time-course of LTC release by macrophages exposed to immune complexes. Experimental details are the same as in Fig. 4, except that macrophages were challenged with immune complexes (135 μg of immune complex protein/dish). Values are the mean ± SD from triplicate determinations.

immune complexes (135 μg of immune complex protein/dish). The kinetics of LTC production were similar to those obtained with zymosan; however, the maximal levels of LTC attained were lower (compare with Fig. 6). Dose-response studies in which doses of up to 1,300 μg of immune complex protein/dish were added indicated that no further stimulation of LTC release could be achieved with these complexes.

Quantities of LTC Released by Mouse Macrophages under Different Experimental Conditions.   Results obtained from a number of experiments indicated that macrophages stimulated with zymosan (160 μg) released ~3–6 pmol of LTC/μg of cell protein after 1–2 h incubation. This estimate was derived from the radiolabel recovered in fraction 2 from silicic acid columns, corrected for a 40% recovery, and converted to pmol as described in Materials and Methods. The release of 3–6 pmol/

μg of cell protein corresponded to a bioactivity of 35–70 U/μg of cell protein. In most studies, the radioactivity recovered in fraction 2 comprised, on the average, 6–12% of the total ^{3}H released by the cells. Correcting this value for a 40% recovery, it may be concluded that as much as 15–30% of the released 20:4 is converted to LTC by resident cells under these conditions.

C. parvum-elicited macrophages released ~3 pmol of LTC/μg of cell protein when challenged with zymosan (160 μg). This represented 50% of the amount of LTC released by resident cells in parallel cultures (6 pmol of LTC/μg of cell protein). Stimulation of resident cells with immune complexes led to a maximal release of 1.3 pmol of LTC/μg of cell protein, ~20% of that obtained with zymosan.

Discussion

Progress in the study of SRS has been slow as a result of a number of technical problems that have existed in the past. The bioassay for SRS, although highly sensitive, lacks specificity, and conveys no information concerning the nature of the substance(s) being tested. Lack of complete information concerning the structure of the molecule impeded work on its biochemistry. Finally, no purified, normal cell population was available that could be shown to release SRS in response to a physiologically relevant stimulus in vitro. This prevented the detailed study of the regulation of SRS release. The recent chemical characterization of LTC and LTD (5, 6) has provided important information necessary to overcome these problems; however, much remains to be done to elucidate the cell(s) of origin, control of biosynthesis, and metabolism of these compounds.

The finding that mouse peritoneal macrophages release LTC in response to an inflammatory stimulus was consistent with other work that implied that the mast cell may not be the cell of origin of SRS as was originally thought (18–21). The macrophage offered obvious advantages for the detailed study of the release of LTC because purified cell populations were readily available, and because methods for the labeling of macrophage phospholipid with [^{3}H]-20:4 had already been developed for the study of PG production (15). Furthermore, a simple method for the extraction of LTC from macrophage culture medium had been devised, allowing the recovery of the compound under very mild conditions (9).

We took advantage of the above available techniques to devise the radiochemical assay for the release of LTC from mouse peritoneal macrophages described in this paper. Using the combination of solvent extraction and silicic acid chromatography, radiochemically pure LTC was obtained from culture medium in a 40% yield with full retention of biological activity. The results obtained from assay of radiolabel recovered as LTC were in good agreement with those obtained from bioassay. The radioassay offered several advantages over the bioassay, however. Large numbers of samples could be processed rapidly, in large part because of the step-wise nature of separations on silicic acid columns. The inherent variability in sensitivity from one ileum preparation to another was no longer of concern, and the problems presented by substances that interfere with the bioassay (PG, histamine, serotonin) were eliminated.

We used the radioassay to study some of the parameters affecting LTC synthesis by macrophages in vitro. Results from these experiments indicated that the kinetics of LTC synthesis as well as its dose-response relationship to zymosan challenge were

very similar to results obtained for PG and total 20:4 release. In both cases, the kinetics of release of the 20:4 metabolites closely followed those of phagocytosis of zymosan obtained previously (15), and increasing amounts of the metabolites were synthesized in response to increasing quantities of zymosan ingested. These findings are consistent with the hypothesis that a phagocytic stimulus triggers 20:4 release by an inducible phospholipase, and that the 20:4 is then metabolized by cyclooxygenase (giving PG) and lipoxygenase (yielding hydroxyeicosatetraenoic acids and LTC).

C. parvum is well recognized as a nonspecific stimulator of the immune system. Peritoneal macrophages obtained from mice injected with C. parvum exhibit increased tumoricidal and antimicrobial activities in vitro (22, 23). Comparisons of LTC released by these cells and resident cells after an in vitro challenge with a phagocytic stimulus may provide useful information as to the capacity of macrophages to synthesize LTC in chronic and acute inflammatory sites. Our findings indicate that macrophages obtained after a single intraperitoneal injection of C. parvum release 50% less LTC than resident cells. Similarly, Humes et al. (24) reported decreased PG release by C. parvum-elicited macrophages. These findings together would seem to indicate that 20:4 metabolism by C. parvum-elicited macrophages is different from that of resident cells. Further work is being conducted in this laboratory to better define the nature of these effects.

Macrophages are known to recognize and ingest immune complexes via their Fc receptor for IgG. Because macrophages are exposed to immune complexes in their role as inflammatory cells, it is of interest to know if these particles can serve as a trigger for LTC release. We have shown that macrophages do synthesize LTC in response to a challenge with immune complexes, although a lower maximal release was obtained when the cells were stimulated with zymosan. Total 20:4 release in response to immune complexes was also lower than that obtained from zymosan challenge. It is not known why immune complexes appear to be a less potent trigger of 20:4 release than zymosan. Factors such as size and class of antibody used in their formation may influence the efficiency of immune complexes to stimulate 20:4 release.

In our previous work, we estimated that the release of LTC by resident macrophages after challenge with zymosan (160 μg) was 2.1 pmol/μg of cell protein, comprising 10–15% of the total 20:4 released and possessing a bioactivity of 24 U/μg of cell protein (9). In the present studies, higher values of LTC released were obtained (3–6 pmol/μg of cell protein, 15–30% of the 20:4 released, and 35–70 U/μg of cell protein). These differences may be attributed to different lots of zymosan and their ability to stimulate 20:4 metabolism. The values presented here better reflect the capacity of the macrophage for LTC release.

That mouse peritoneal macrophages release LTC in response to inflammatory stimuli cannot be disputed. This is a clear example of the synthesis of an SRS by a purified normal cell population in response to an immunologically relevant stimulus in which the product has been chemically characterized and quantitated. Information of this type is vital to the understanding of the physiologic and pathologic role of SRS. The findings presented here raise some interesting questions. What role, if any, does the macrophage play in the generation of SRS in an immediate hypersensitivity reaction? If the macrophage is involved, what is the trigger that stimulates its release of leukotriene? What is the physiological significance of leukotriene release in the peritoneal cavity? What are the target tissues? Does leukotriene release play a role in

chronic inflammatory reactions where the involvement of macrophages is well known? It is clear that our present understanding of the biochemistry, immunology, and physiology of SRS must be broadened extensively to accommodate the rapidly growing knowledge of the leukotrienes.

Summary

A method for the radiochemical assay of LTC production by mouse peritoneal macrophages in vitro is presented. The method involves labeling macrophages in culture with [5,6,8,9,11,12,14,15-^{3}H]20:4 followed by stimulation of arachidonic acid (20:4) release under the experimental conditions desired. Radiolabeled leukotriene C (LTC) is recovered from the culture medium by extraction and silicic acid chromatography in 40% yield with full retention of biological activity. Because this LTC is radiochemically pure, the quantity of LTC release may be estimated from the amount of radioactivity in the sample.

Use of the radioassay to study parameters affecting LTC synthesis by macrophages indicated that the time course of LTC synthesis and its relationship to the dose of a phagocytic stimulus (zymosan) were very similar to those of prostaglandin (PG) release. LTC release was also similar to that of PG in that lower levels of both metabolites were produced by *Corynebacterium parvum*-elicited macrophages than by resident cells. Finally, LTC release was stimulated in response to a challenge with antigen-antibody complexes, but lower maximal levels were attained than those with zymosan.

The data presented here are consistent with the hypothesis that challenge of macrophages with a phagocytic stimulus leads to the release of 20:4 by an inducible phospholipase. Cyclooxygenase and lipoxygenase then compete for the released 20:4, leading to the production of PG, hydroxyeicosatetraenoic acids, and LTC.

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