Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 7200–7204, December 1983
Cell Biology

Arachidonate metabolism by human polymorphonuclear leukocytes stimulated by *N*-formyl-Met-Leu-Phe or complement component C5a is independent of phospholipase activation

(chemotactic factors/arachidonic acid/lipoxygenase/chemotaxis/human neutrophils)

ROBERT M. CLANCY, CLEMENS A. DAHINDEN, AND TONY E. HUGLI

Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, CA 92037

Communicated by Hans J. Müller-Eberhard, July 18, 1983

ABSTRACT **Release of arachidonic acid by the membrane phospholipase and metabolism by the 5-lipoxygenase pathway was examined in human polymorphonuclear leukocytes (PMNs). The 5-lipoxygenase pathway is activated when PMNs are given arachidonic acid in ethanol and there is extensive metabolism to 5-hydroxyicosatetraenoic acid (5-HETE) and leukotriene B_4 (LTB_4). This activation event was shown to be altered by the ethanol because resting PMNs given arachidonic acid with bovine serum albumin fail to metabolize arachidonic acid. However, cells activated by the inflammatory agents *N*-formyl-Met-Leu-Phe (fMLF) or complement component C5a recruit the 5-lipoxygenase to metabolize exogenous arachidonic acid to 5-HETE and LTB_4. When PMNs were incubated with arachidonic acid–bovine serum albumin and challenged with fMLF or C5a (des-Arg-C5a) they produced 49–75 pmol of LTB_4 and 310–440 pmol of 5-HETE per 10^7 cells. PMNs stimulated by fMLF or C5a (des-Arg-C5a) do not induce membrane phospholipases to mobilize endogenous arachidonic acid and neither 5-HETE nor LTB_4 is formed. In contrast, PMN stimulation by the ionophore A23187 activates both the membrane phospholipase and the 5-lipoxygenase to produce 5-HETE and LTB_4 from endogenous arachidonic acid. Our results indicate that the lipoxygenase pathway is inoperative in resting PMNs but can be recruited by chemotactic factors to act on arachidonate from extracellular sources. It was previously believed that formation of 5-HETE and LTB_4 by the PMN depends solely on phospholipase to mobilize endogenous arachidonic acid. The results reported here refute this concept and indicate that the role of phospholipase activation in PMN may be overestimated. Therefore, subsequent involvement of lipoxygenase products in mediating stimulation of PMN by inflammatory factors (e.g., as in aggregation and chemotaxis) remains in question unless an exogenous source of arachidonate can be identified.**

Polymorphonuclear cells (PMNs) are a central component of the cellular response in inflammatory reactions and of paramount importance to the immune surveillance system. PMNs are attracted to inflammatory sites in a highly specific manner by stimulation from chemotactic factors. Many chemotactic factors have been described, but only those derived from the fifth component of complement (e.g., C5a and des-Arg-C5a) and synthetic analogues of the putative bacterial chemotactic factor (e.g., *N*-formyl peptides) have been thoroughly characterized (1, 2). These agents, in the nanomolar concentration range, bind and activate the PMNs by interacting with distinct receptor sites to induce directional migration of the PMN (3, 4). They also stimulate other cellular functions such as aggregation, adherence, enzyme release, and the production of toxic oxygen metabolites (3, 5, 6). Thus, in addition to attracting PMNs, chemotactic factors appear capable of modulating a number of other aspects of the inflammatory reaction.

Recently, another class of chemotactic factor was described. The biologically active principle, leukotriene B_4 (LTB_4), is a complex lipid found to be as potent on a molar basis as the formyl peptides or C5a (7–9). LTB_4 is a metabolite of arachidonic acid formed by the 5-lipoxygenase pathway. Arachidonic acid does not exist in free form but is normally esterified in triglycerides and phospholipids in the cell membrane. It has been postulated that granulocyte stimulation activates membrane phospholipase(s) to release arachidonic acid, which is then enzymatically metabolized by the 5-lipoxygenase pathway to form LTB_4 as well as a host of other bioactive lipid metabolites (10, 11).

PMNs not only respond when exposed to LTB_4 but also are a major source of this factor when stimulated by the calcium ionophore A23187 (12). Thus LTB_4 may serve as an intracellular second messenger in the receptor–response coupling of PMN stimulated by other chemotactic factors (13). Indeed, several reports support a direct role for the lipoxygenase pathway of arachidonic acid metabolism in PMN activation (9, 14, 15). In order to establish the role of LTB_4 in inflammatory reactions, the physiological conditions leading to release of this lipid mediator must first be examined.

In this study, we examined 5-lipoxygenase metabolites of arachidonate that are formed by PMNs exposed to the chemotactic factors *N*-formyl-Met-Leu-Phe (fMLF) and C5a/des-Arg-C5a. Our results show that, unlike the calcium ionophore A23187, neither C5a/des-Arg-C5a nor fMLF stimulates PMNs to metabolize endogenous arachidonic acid into 5-hydroxyicosatetraenoic acid (5-HETE) or LTB_4. However, 5-HETE, LTB_4, and other metabolites of the lipoxygenase pathway are formed from exogenous arachidonic acid by PMNs stimulated with chemotactic factors. Conversely, exogenous arachidonic acid is not metabolized by PMNs in the resting state. These results indicate the phospholipase(s) that releases intracellular arachidonic acid is either poorly activated or unactivated when PMNs are stimulated by fMLF or C5a. Conversely, the 5-lipoxygenase that does not metabolize arachidonic acid in resting PMNs is activated by fMLF and C5a.

METHODS

Cell Preparation and Reaction Conditions. Venous blood from human adult donors was collected into sodium heparin (20

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: monoHETE, monohydroxyicosatetraenoic acid; diHETE, dihydroxyicosatetraenoic acid; 5-HETE, 5-hydroxyicosatetraenoic acid; LTB_4, (5*S*,12*R*)-*cis-trans-trans*-dihydroxyicosatetraenoic acid (leukotriene B_4); PMN, polymorphonuclear cell; fMLF, *N*-formyl-Met-Leu-Phe; C5a, a fragment of the fifth component of complement.

units/ml of blood). PMNs were purified according to the method of Dahinden and co-workers (16), which involved separating erythrocytes by layering the blood on methylcellulose/metrizoate followed by purifying the leukocyte-rich supernatant on Ficoll/Hypaque. The residual erythrocytes were removed by hypotonic lysis and the PMNs were washed twice in Gey's solution. The PMN preparation contained less than 0.5% mononuclear cells and less than 0.01% platelets. This purification method has a low platelet contamination compared to cell preparations that use dextran in the first purification step. The PMNs were resuspended at 2.5×10^7 cells per ml in a modified Tyrode buffer. The bicarbonate concentration of the Tyrode buffer was reduced to 1.6 mM, and 10 mM Hepes, 2 mM glucose, and 1% fatty acid-free bovine serum albumin (Miles; hereafter referred to as albumin) were added.

Arachidonic acid (99% pure, Calbiochem or Nu Chek Prep) was stored under N_2 gas in methanol at −20°C. When cells were challenged with arachidonic acid in the presence of albumin, an aliquot of arachidonic acid was dried under N_2 gas and the arachidonic acid was suspended in the modified Tyrode buffer by vigorously swirling on a Vortex mixer. For the activation of cells by chemotactic factors, 1 ml of the cell suspension was incubated for 5 min at 37°C and then another 5 min after addition of arachidonic acid. After a total of 10 min at 37°C, the cells were challenged with fMLF, C5a, or des-Arg-C5a.

Analysis of Arachidonate Products. For the analysis of leukotrienes, the cells were lysed by addition of 1 vol of methanol per sample volume. The cell debris was spun down and the supernatant was recovered. The pellet was washed with 1 vol of methanol, and after centrifugation, the wash was combined with the reaction supernatant. After acidification to pH 3, fractions enriched by mono- and dihydroxyicosatetraenoic acid (monoHETE and diHETE) were isolated by the procedure of Borgeat and Samuelsson (12). This procedure involved extraction with diethyl ether followed by silicic acid chromatography of the material recovered in the ether layer.

The hydroxyarachidonates were separated on a Nucleosil C18 HPLC column employing a Waters 6000A pump and U6K injector using a carrier solvent composed of methanol/water/acetic acid (75:25:0.01, vol/vol). A variable wavelength detector (Waters model 450) was used to monitor leukotriene elution. Separation of the different isomers of LTB_4 was performed by chromatography on a Nucleosil C18 HPLC column. The flow rate was 1 ml/min, and absorbance was monitored at 270 or 237 nm. UV spectra of separated components were recorded in methanol with a Cary 219 spectrophotometer.

Measurement of Oxygen Consumption. Oxygen consumption of PMNs was determined by using a Clark type oxygen electrode (YSI Model 45, Yellow Springs Instruments). The reaction mixture contained 2 ml of modified Tyrode buffer at 37°C. The oxygen electrode was calibrated by using catalase and a known quantity of H_2O_2.

Chemotaxis. PMN chemotaxis was measured in a modified Boyden chamber (16). Cells that migrated through a micropore filter [8-μm pore size cellulose filter (Sartorius)] after incubation for 60 min at 37°C were counted.

RESULTS

In this study we attempt to define the physiologic conditions under which arachidonic acid is metabolized to LTB_4. Several published reports have stated that PMNs rapidly consume oxygen and produce superoxide anion (O_2^-) when provided an exogenous source of arachidonic acid (17). This is a particularly interesting observation because superoxide anion was shown to convert arachidonic acid to monoHETE and diHETE (18). When we challenged 10^8 PMNs with 250 nmol of arachidonic acid and measured oxygen consumption with an oxygen electrode, we observed no burst in oxygen consumption (Fig. 1). However, cells subsequently challenged with 1 μM fMLF underwent a burst of oxygen consumption reflecting stimulation of the hexose monophosphate shunt and production of O_2^- (6, 19). The respiratory burst initiated by fMLF was identical with or without arachidonic acid pretreatment of the PMNs (data not shown).

Supernatants from suspensions of stimulated cells were screened for production of monoHETE or diHETE metabolites of arachidonic acid. The lipid components in these supernatants were isolated and analyzed on HPLC. PMNs produce numerous lipoxygenase metabolites when challenged with the calcium ionophore A23187, as is shown in Fig. 2*A*. A major metabolite is (5*S*,12*R*)-*cis*-*trans*-*trans*-diHETE (LTB_4), which is designated here as compound III and has a retention time of 14.0 min. Another major metabolite is 5-HETE, which has a retention time of 45.0 min. Note that even with ionophore-stimulated cells no platelet-derived lipoxygenase products such as 12-hydroxyicosatetraenoic acid or 12-hydroxyheptadecatrienoic acid were detected, further indicating the low platelet content of our cell preparation. The supernatant from cells that were exposed only to arachidonic acid bound to albumin contain no detectable monoHETE or diHETE metabolites (Fig. 2*B*). An identical chromatographic pattern was observed when the PMNs were stimulated with fMLF in the absence of arachidonic acid. However, supernatants from cells first given arachidonic acid and then challenged by fMLF contain large quantities of 5-HETE and the isomers of LTB_4 (Fig. 2*C*).

Conversion of extracellular arachidonic acid to lipoxygenase metabolites in PMNs depends on the method in which the arachidonic acid is presented to the cells. Arachidonic acid complexed with albumin is not metabolized unless the PMNs are stimulated with 1 μM fMLF for 5 min, then LTB_4 and 5-HETE are produced. Under our reaction conditions LTB_4 is produced only by stimulated cells; these data contrast sharply with results reported by others (12, 20), who commonly present arachidonic acid to PMNs in the presence of 0.7% ethanol. Arachidonic acid/ethanol is extensively metabolized by nonstimulated PMNs. For example, when PMNs were exposed to 250 nmol of arachidonic acid per ml in 0.7% ethanol for 5 min at 37°C, the supernatant contained 31 pmol of LTB_4 and 500 pmol of 5-HETE per 10^7 cells. There also was 150 pmol per 10^7 cells of 15-HETE, a metabolite that is not detected in the arachidonate–albumin reaction mixture. When these cells were exposed to 1 μM fMLF for 5 min, there was no increase in the

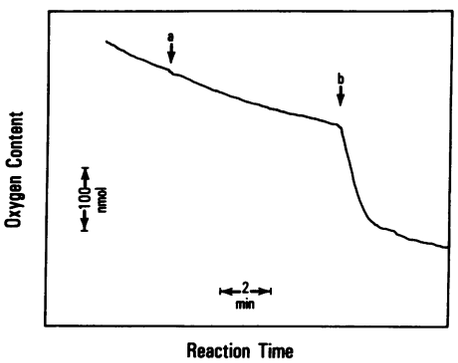

FIG. 1. Oxygen consumption by 10^8 PMNs was determined in 2 ml of modified Tyrode buffer at 37°C. Basal oxygen metabolism was recorded for the cells and then 250 μM arachidonic acid complexed to albumin was added at time point a. No increase in oxygen consumption was observed. However, when 1 μM fMLF was added to this reaction mixture (arrow b) a burst of oxygen consumption was observed.

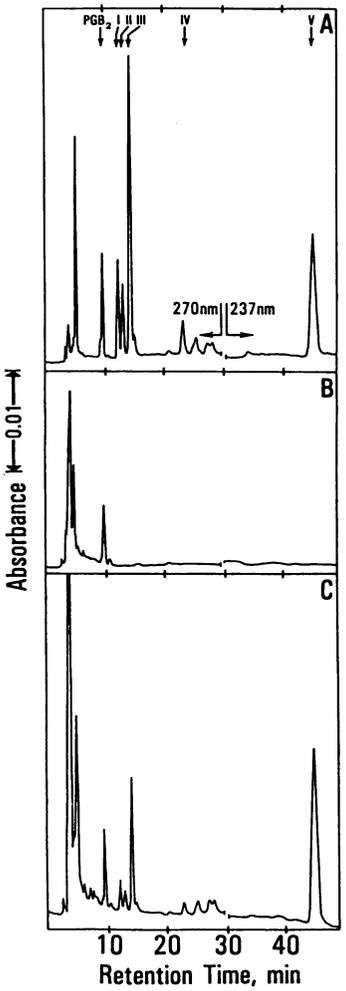

FIG. 2. HPLC analysis of the monoHETE and diHETE derivatives in supernatant fluids isolated from 2.5×10^7 PMNs. The internal standard prostaglandin B_2 (PGB_2) was added to the PMNs prior to ether extraction. (*A*) PMNs were treated with 10 μM calcium ionophore A23187 for 5 min at 37°C. Compounds I–V were detected. (*B*) PMNs were treated with 250 μM arachidonic acid–albumin. No lipoxygenase products were identified. An identical chromatographic pattern was observed when the PMNs were stimulated with fMLF in the absence of arachidonic acid. (*C*) PMNs were exposed to both 250 μM arachidonic acid–albumin and 1 μM fMLF. Compounds I–V are identified as (5*S*,12*R*)-*all-trans*-diHETE (I), (5*S*,12*S*)-*all-trans*-diHETE (II), (5*S*,12*R*)-*cis-trans-trans*-diHETE or LTB_4 (III), 5,6-diHETE (IV), and 5-HETE (V).

quantity of LTB_4 or 5-HETE produced relative to nonstimulated cells exposed to only arachidonate/ethanol.

PMNs were incubated with various concentrations of arachidonic acid complexed with albumin and then stimulated with 1 μM fMLF for 5 min. An arachidonic acid concentration of 250 μM was determined to be optimal for production of lipoxygenase metabolites (data not shown). In a second series of experiments PMNs were incubated with optimal levels of arachidonic acid–albumin and then stimulated with 1 μM fMLF as shown in Fig. 3. Conversion of arachidonic acid to LTB_4 appeared to be complete in 2–3 min. Therefore, arachidonate metabolism by cells activated in suspension follows the same kinetic pattern as O_2^- release and PMN aggregation (6, 19). Cells stimulated in the absence of arachidonic acid, as well as nonstimulated cells incubated with arachidonic acid, fail to produce 5-HETE or LTB_4.

Inflammatory agents other than the *N*-formyl peptides are capable of activating PMNs incubated with arachidonate–albumin to produce lipoxygenase metabolites, as is shown in Table 1. Cells that are stimulated with fMLF, C5a, or des-Arg-C5a in the absence of arachidonic acid do not produce 5-HETE or LTB_4. However, cells stimulated in the presence of arachidonic acid-albumin produce approximately equal quantities of LTB_4 and 5-HETE. In a dose–response study, the ED_{50} for conversion of exogenous arachidonic acid to lipoxygenase products by des-Arg-C5a and C5a was found to be 10 nM and 2 nM, respectively.

A number of donors were screened with respect to the ability of their PMNs to metabolize endogenous and exogenous arachidonic acid when activated by chemotactic factors. In all cases, no lipoxygenase metabolites were observed when cells were activated with fMLF. However, all cells incubated with arachidonic acid produced lipoxygenase metabolites when activated with chemotactic factor. The metabolism of exogenous arachidonic acid by the four donors resulted in the production (mean ± SD) of 450 ± 190 and 74 ± 30 pmol per 10^7 cells of 5-HETE and LTB_4, respectively.

To further characterize the structure of lipoxygenase metabolites generated in this study, PMNs were prepared on a large scale. These cells were incubated with arachidonate–albumin and then exposed to 1 μM fMLF. Samuelsson and coworkers have rigorously characterized the structures of LTB_4, diHETE isomers, and 5-HETE (compounds I–V in Fig. 2*A*), and each of these compounds has a characteristic ultraviolet spectrum (20). We isolated compounds I–V from the reaction mixture described in the legend of Fig. 2*C* and have identified these compounds on the basis of their UV spectra. For example, the absorption spectra for compound III and compound V (Fig. 2*C*), which are shown in Fig. 4, allowed identification of these compounds as (5*S*,12*R*)-*cis-trans-trans*-diHETE (LTB_4) and 5-HETE, respectively. The spectra of compounds I, II, and IV allowed identification of these compounds as diHETE isomers; these are the same isomers as are generated from PMNs by ionophore stimulations (data not shown). Compounds I, II, and IV were identified spectrally as (5*S*,12*R*)-*all-trans*-diHETE, (5*S*,12*S*)-*all-trans*-diHETE, and the 5,6-diHETE, respectively. The lipoxygenase metabolites were also identified with respect to their ability to attract PMNs in a chemotaxis assay. LTB_4 is reportedly a potent chemoattractant (7–9), and we found LTB_4 (compound III) to be a significant chemoattractant at 1 nM and optimally active for attracting PMNs at 100

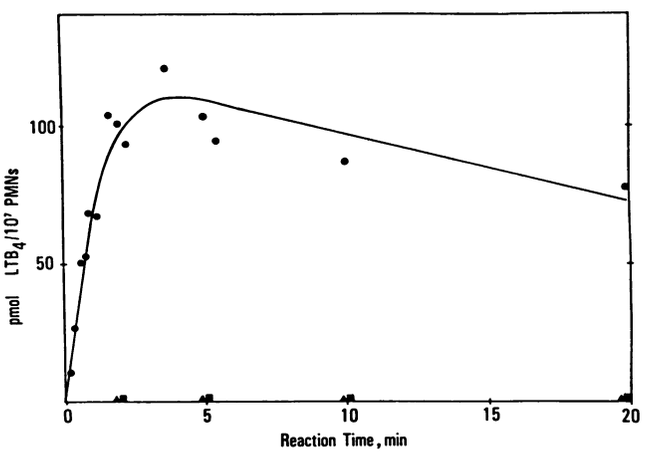

FIG. 3. Appearance of LTB_4 in the supernatant fluid of PMNs as a function of reaction time. PMNs were incubated in the presence of 1 μM fMLF (▲), 250 μM arachidonic acid (■), or 250 μM arachidonic acid and 1 μM fMLF (●). The monoHETE- and diHETE-enriched fractions were isolated as described in *Methods* and analyzed by HPLC as described in the legend of Fig. 2.

Table 1. Conversion by PMNs of exogenous arachidonic acid to lipoxygenase metabolites

Stimulant	Arachidonic acid–albumin	Lipoxygenase metabolite, pmol/10^7 cells	
		LTB_4	5-HETE
No addition	–	<2.0	<7.0
	+	<2.0	<7.0
fMLF, 1 μM	–	<0.5	<1.5
	+	75	400
C5a, 0.1 μM	–	<2.0	<7.0
	+	66	440
des-Arg-C5a, 0.1 μM	–	<2.0	<7.0
	+	49	310

LTB_4 and 5-HETE were recovered from the supernatant fluid of 2.5 × 10^7 PMNs per ml. The cells were challenged as described and the monoHETE and diHETE fractions were isolated and analyzed.

nM (data not shown). The 5-HETE (compound V) is not a chemoattractant in the 10 to 1,000 nM range.

DISCUSSION

Metabolites of arachidonic acid are hypothesized to be involved in signal transduction of responses in leukocytes stimulated by exogenous factors (13, 21). One of the better-characterized bioactive metabolites of arachidonic acid is LTB_4, a potent effector molecule for inducing chemotaxis, aggregation, and degranulation of PMNs (9, 22, 23). LTB_4 is also a potent calcium ionophore (24) and has been considered a second messenger in the receptor-mediated activation of PMNs by chemotactic factors.

We observed that when PMNs were stimulated by chemotactic factors they failed to produce either 5-HETE or LTB_4 (see Fig. 5). This result suggests that either the membrane phospholipase(s) is not mobilizing or the 5-lipoxygenase pathway is not converting the arachidonic acid to active products. However, PMNs incubated with exogenous arachidonic acid and then stimulated with fMLF or C5a produce sizeable quantities of both 5-HETE and LTB_4. From this result we conclude that the 5-lipoxygenase is indeed functional but that the membrane phospholipases fail to release arachidonic acid in PMNs stimulated with chemotactic factors. Therefore, it seems unlikely that lipoxygenase products, in particular LTB_4, are responsible for coupling receptor-mediated signals in PMNs to intracellular metabolic events as has previously been suggested (13–15, 21). Furthermore, we failed to detect free endogenous arachidonic acid (i.e., nonesterified) when PMNs were stimulated by chemotactic factors as measured by gas chromatography with a lower limit of detection of 8 pmol per 10^7 cells (unpublished observation). Consequently, we conclude that stimulation of PMNs by chemotactic factors is independent of phospholipase activation and fails to mobilize arachidonic acid.

We cannot exclude the possibility that LTB_4 acts intracellularly at a concentration well below our detection limit. However, even in a scaled-up experiment that lowers our detection limits to 0.5 pmol per 10^7 cells, we were not able to measure production of LTB_4 or other 5-lipoxygenase metabolites by PMNs in the absence of exogenous arachidonic acid. We observed that LTB_4 is a significant chemoattractant at 1–10 pmol/ml and, because LTB_4 is not being released in this concentration range, it seems unlikely that lipoxygenase products, in particular LTB_4, are responsible for coupling receptor-mediated signals in PMNs to intracellular metabolic events as has previously been suggested (13–15, 21).

The hydrolysis of arachidonic acid in PMN membranes is controlled at the level of the phospholipase, and in addition

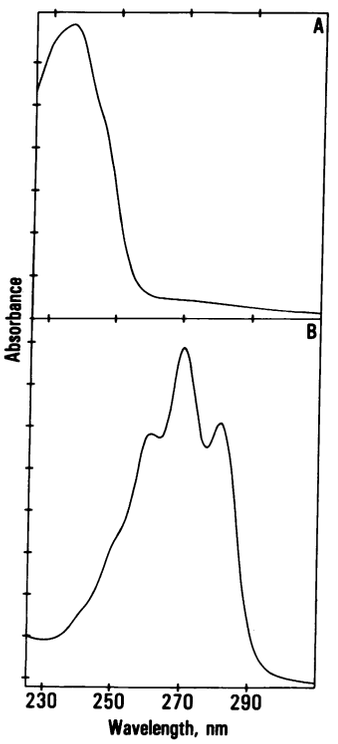

FIG. 4. UV spectra for the major monoHETE and diHETE compounds isolated from the supernatant fluid of PMNs treated with 250 μM arachidonic acid and 1 μM fMLF as described in the legend of Fig. 2C. UV spectra for compounds V and III are recorded in methanol and are given in *A* and *B*, respectively.

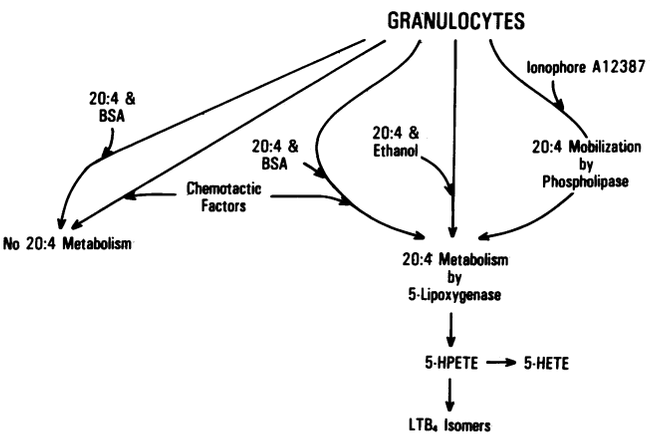

FIG. 5. Metabolism of arachidonic acid (20:4) in PMNs by the membrane phospholipase and the 5-lipoxygenase pathway. The 5-lipoxygenase pathway is activated when PMNs are exposed to arachidonic acid in ethanol. This metabolism is due to the effect of ethanol, because when PMNs are given arachidonic acid–bovine serum albumin (BSA) there is no metabolism by resting PMNs. However, cells activated by the chemotactic factors (fMLF or C5a) recruit the 5-lipoxygenase to metabolize exogenously given arachidonic acid–albumin to leukotriene metabolites. PMNs stimulated by chemotactic factors in the absence of arachidonic acid–albumin do not form leukotriene metabolites and therefore do not recruit membrane phospholipase to mobilize endogenous arachidonic acid. This result is in contrast to PMN stimulation by the ionophore A23187, which activates both the membrane phospholipase and the 5-lipoxygenase to produce leukotriene metabolites from endogenous arachidonic acid. The 5-lipoxygenase converts arachidonic acid to 5-hydroperoxyicosatetraenoic acid (HPETE), which is then further metabolized to 5-HETE and LTB_4 isomers.

there is control of arachidonic acid metabolism at the level of the 5-lipoxygenase. When we expose PMNs to arachidonic acid–albumin there are no 5-lipoxygenase products formed in the resting state. In contrast, the results from fMLF- or C5a-stimulated cells indicate the 5-lipoxygenase is functional and products are formed. One interpretation of this result is that the 5-lipoxygenase system is activated in fMLF- or C5a-stimulated PMNs entirely independent of the phospholipase(s). We also hypothesize that modulation of 5-lipoxygenase activity provides a means whereby arachidonic acid metabolism in these cells is directed either towards the cycloxygenase pathway or lipoxygenase pathway. In a recent study monocytes were found to release prostaglandins and leukotrienes in variable ratios depending on the nature of the stimulus, and the investigators hypothesized that different arachidonic acid pools were being utilized (25). We suggest that differential activation of the lipoxygenase system provides another tenable explanation for variations in prostaglandin and leukotriene levels in monocytes and perhaps in other leukocytes.

Evidence that the lipoxygenase pathway might be involved in PMN activation comes from the studies showing that PMNs are stimulated directly by exogenous arachidonic acid to release HETEs and LTB_4 (12, 20) and to stimulate aggregation and O_2^- release (17, 26). However, our studies show that human PMNs isolated from normal individuals are stimulated when given a combination of arachidonic acid and ethanol, which apparently activates the 5-lipoxygenase pathway for PMNs. This also may be the reason why Karnovsky and co-workers (17) observed a burst of oxygen consumption when PMNs were given arachidonic acid in ethanol. When PMNs are presented with arachidonic acid complexed with albumin there is no metabolism of arachidonic acid to 5-lipoxygenase products nor is there a burst of oxygen consumption in resting PMNs (Fig. 1). Other studies used rabbit exudate cells, and the lipoxygenase in these rabbit cells may be activated due to species differences or simply due to the variations in the mode of collection. Therefore, the concept that free arachidonic acid is automatically transformed to HETEs and leukotrienes by PMNs is incorrect and requires further evaluation.

Numerous studies have provided evidence that the lipoxygenase pathway might be involved when PMNs are activated by chemotactic factors (9, 14, 15, 21). However, most of the evidence is indirect and based on inhibitor studies that are difficult to interpret. In fact, in one of these studies it was later shown that the lipoxygenase inhibitor actually interferes with binding of the chemotactic factor to the PMN receptor (27). Dahinden and Fehr (28) have recently shown that certain inhibitors of arachidonic acid metabolism, in particular pyrazolone derivatives, inhibit fMLF-induced PMN activation both *in vitro* and *in vivo*. This inhibition is unrelated to its action as an enzyme inhibitor but occurs because the compound specifically decreases binding of fMLF to its receptor. Consequently, the inhibitor studies may be invalid for demonstrating the direct involvement of lipoxygenase metabolites in PMN activation by chemotactic factors.

These studies focus on cell activation by fMLF or C5a and the regulation of enzymes involved in HETE and leukotriene production. Our data suggest that arachidonate metabolism in human PMNs stimulated by fMLF or C5a is independent of membrane phospholipase activation. However, PMNs stimulated by chemotactic factors express a functional 5-lipoxygenase pathway and perhaps *in vivo* PMNs utilize arachidonic acid that is derived from stimulated platelets (29), monocytes (25), or other sources such as damaged tissue. Fluctuations in exogenous arachidonate levels may provide a significant modulation of the inflammatory response by controlling the level of lipoxygenase products formed by circulating granulocytes.

Note Added in Proof. Compounds III and V, isolated from PMNs given arachidonate/albumin and chemotactic factors, were further purified by silicic acid straight-phase HPLC and analyzed by gas chromatography/mass spectroscopy. Separation of these compounds on a silicic acid column revealed that they were homogeneous, and no other contaminating arachidonate metabolites were detected. The gas chromatography/mass spectroscopy results for compounds III and V were identical to those previously published for LTB_4 and 5-HETE.

This is publication number IMM 3017 from the Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, California. R.M.C. is supported by a National Research Service Award Individual Postdoctoral Fellowship (HL06692). T.E.H. is supported by Public Health Service Grants HL16411 and AI17354. C.A.D. is supported by the Swiss National Science Foundation.

1. Fernandez, H. N., Henson, P. M., Otani, A. & Hugli, T. E. (1978) *J. Immunol.* **120**, 109–115.
2. Schiffmann, E., Corcoran, B. A. & Wahl, S. M. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1059–1062.
3. Chenoweth, D. E. & Hugli, T. E. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3943–3947.
4. Niedel, J., Wilkinson, S. & Cuatrecasas, P. (1979) *J. Biol. Chem.* **254**, 10700–10706.
5. Becker, E. L., Showell, H. J., Henson, P. M. & Hsu, L. S. (1974) *J. Immunol.* **112**, 2047–2054.
6. Dahinden, C. A., Fehr, J. & Hugli, T. E. (1983) *J. Clin. Invest.* **72**, 113–121.
7. Ford-Hutchinson, A. W., Bray, M. A., Doig, M. V., Shipley, M. E. & Smith, M. J. H. (1980) *Nature (London)* **286**, 264–265.
8. Goetzl, E. J. & Pickett, W. C. (1981) *J. Exp. Med.* **153**, 482–487.
9. Palmblad, J., Malmsten, C. L., Uden, A. M., Radmark, O., Engstedt, L. & Samuelsson, B. (1981) *Blood* **58**, 658–661.
10. Borgeat, P., Hamberg, M. & Samuelsson, B. (1976) *J. Biol. Chem.* **251**, 7816–7820.
11. Murphy, R. C., Hammarström, S. & Samuelsson, B. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4275–4279.
12. Borgeat, P. & Samuelsson, B. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2148–2152.
13. Becker, E. L. & Stossel, T. P. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 2949–2952.
14. Showell, H. J., Naccache, P. H., Shaafi, R. I. & Becker, E. L. (1980) *Life Sci.* **27**, 421–426.
15. Smolen, J. E. & Weissmann, G. (1980) *Biochem. Pharmacol.* **29**, 533–538.
16. Dahinden, C., Galanos, C. & Fehr, J. (1983) *J. Immunol.* **130**, 857–862.
17. Badwey, J. A., Curnutte, J. T. & Karnovsky, M. L. (1981) *J. Biol. Chem.* **256**, 12640–12643.
18. Fridovich, S. E. & Porter, N. A. (1981) *J. Biol. Chem.* **256**, 260–265.
19. Lehmeyer, J. E., Snyderman, R. & Johnston, R. B., Jr. (1979) *Blood* **54**, 35–45.
20. Borgeat, P. & Samuelsson, B. (1979) *J. Biol. Chem.* **254**, 7865–7869.
21. Hirata, F., Corcoran, B. A., Venkatasubramanian, K., Schiffmann, E. & Axlerod, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2640–2643.
22. Naccache, P. H., Molski, T. F. P., Becker, E. L., Borgeat, P., Picard, S., Vallerand, P. & Shaafi, R. I. (1982) *J. Biol. Chem.* **257**, 8608–8611.
23. Hafstrom, I., Palmblad, J., Malmsten, C. L., Radmark, O. & Samuelsson, B. (1981) *FEBS Lett.* **130**, 146–148.
24. Serhan, C. N., Fridovich, J., Goetzl, E. J., Dunham, P. B. & Weissmann, G. (1982) *J. Biol. Chem.* **257**, 4746–4752.
25. Humes, J. L., Sadowski, S., Galavage, M., Goldenberg, M., Subers, E., Bonney, R. J. & Kuehl, F. A., Jr. (1982) *J. Biol. Chem.* **257**, 1591–1594.
26. O'Flaherty, J. T., Showell, H. J., Becker, E. L. & Ward, P. A. (1979) *Am. J. Pathol.* **95**, 433–444.
27. Atkinson, J. P., Simchowitz, L., Mehta, J. & Stenson, W. F. (1982) *Immunopharmacology* **4**, 1–9.
28. Dahinden, C. & Fehr, J. (1980) *J. Clin. Invest.* **66**, 884–891.
29. Pickett, W. C., Jesse, R. L. & Cohen, P. (1977) *Biochim. Biophys. Acta* **486**, 209–213.