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Research Article

Recent studies have shown that alveolar macrophages (AM) are able to release leukotrienes (LTs). Since cigarette smoking inhibits the cyclooxygenase pathway of arachidonic acid metabolism in the AM, we evaluated the LT production by AM from smokers and nonsmokers. AM were obtained from 35 volunteers, 16 nonsmokers, and 19 smokers. The cells were incubated under various conditions including stimulation with 30 microM arachidonic acid, 2 microM ionophore A23187, or both. Each experiment was performed in parallel using cells from a smoker and a nonsmoker. Lipoxygenase products were analyzed by reverse-phase high performance liquid chromatography. After stimulation, nonsmokers' AM produced LTB4 and 5-hydroxy-eicosatetraenoic acid (5-HETE). In incubations of AM with arachidonic acid and ionophore, the amounts of products formed were: LTB4, 317 +/- 56 pmol/10(6) cells and 5-HETE, 1,079 +/- 254, mean +/- SEM. No metabolites were generated under control conditions (no stimulation). In all incubations performed, the peptido-LTs (LTC4, LTD4, and LTE4) were undetectable. In comparison with AM from nonsmokers, those from smokers showed a 80-90% reduction of 5-HETE and LTB4 synthesis (P less than 0.05 to P less than 0.001 according to stimulatory conditions). This defective lipoxygenase metabolite production in AM from smokers was observed over a wide range of stimuli concentrations and incubation times; AM from smokers also had lower levels of intracellular (esterified) 5-HETE than nonsmokers' AM. […]

Decreased Leukotriene B_4 Synthesis in Smokers' Alveolar Macrophages In Vitro

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Abstract

Recent studies have shown that alveolar macrophages (AM) are able to release leukotrienes (LTs). Since cigarette smoking inhibits the cyclooxygenase pathway of arachidonic acid metabolism in the AM, we evaluated the LT production by AM from smokers and nonsmokers. AM were obtained from 35 volunteers, 16 nonsmokers, and 19 smokers. The cells were incubated under various conditions including stimulation with 30 μM arachidonic acid, 2 μM ionophore A23187, or both. Each experiment was performed in parallel using cells from a smoker and a nonsmoker. Lipoxygenase products were analyzed by reverse-phase high performance liquid chromatography. After stimulation, nonsmokers' AM produced LTB_4 and 5-hydroxy-eicosatetraenoic acid (5-HETE). In incubations of AM with arachidonic acid and ionophore, the amounts of products formed were: LTB_4, 317±56 pmol/10^6 cells and 5-HETE, 1,079±254, mean±SEM. No metabolites were generated under control conditions (no stimulation). In all incubations performed, the peptido-LTs (LTC_4, LTD_4, and LTE_4) were undetectable. In comparison with AM from nonsmokers, those from smokers showed a 80–90% reduction of 5-HETE and LTB_4 synthesis ($P < 0.05$ to $P < 0.001$ according to stimulatory conditions). This defective lipoxygenase metabolite production in AM from smokers was observed over a wide range of stimuli concentrations and incubation times; AM from smokers also had lower levels of intracellular (esterified) 5-HETE than nonsmokers' AM. We also studied blood polymorphonuclear leukocytes (PMNL) and no difference in the synthesis of 5-lipoxygenase products in these cells was noticed between smokers and nonsmokers. These data show that cigarette smoking causes a profound inhibition of the 5-lipoxygenase pathway in AM but not in blood PMNL.

Introduction

In the study of lung diseases related to tobacco smoking, special attention has been given to smoking-induced alterations in the morphology and metabolism of alveolar macrophages (AMs)[1] (1–4). The AM, as a phagocytic cell, plays a major role in lung defense mechanisms (5). Because of its ability to release an array of biologically active molecules, including chemotactic factors, complement components, platelet activating factor (6, 7), and the recently described leukotriene (LT) B_4 (8, 9), the AM is considered to be an important regulator of inflammatory reactions in the lung (7).

Leukotrienes (LTs) constitute a newly recognized family of metabolites of arachidonic acid (10), with very potent biological properties: LTC_4, LTD_4, and LTE_4 cause both vasoconstriction and bronchoconstriction (11), and LTB_4 is an effective chemotactic factor for polymorphonuclear leukocytes (PMNL) in vitro (12). In addition, LTB_4 causes PMNL to adhere to vascular endothelium and migrate into extravascular tissues in vivo (13). LTB_4 is generated by neutrophils (14–16), monocytes (17, 18), and AM (8, 9). Its synthesis is initiated by a variety of inflammatory stimuli including phagocytosis (19) and chemotactic substances (20). These observations suggest a role for LTB_4 in inflammation. The capacity of AM to synthesize this potent chemotactic substance and consequently, to recruit PMNL to a site of inflammation, may be an important defense mechanism in the lung.

Because cigarette smoking inhibits the cyclooxygenase-related metabolism of arachidonic acid in the AM (21, 22) and is associated with an increased incidence of infectious diseases in the lung (23, 24), we undertook a comparative study of LTB_4 production by AM in smokers and nonsmokers. For comparison, we also studied the 5-lipoxygenase products synthesis in blood PMNL from smokers and nonsmokers.

Methods

Subjects. 35 subjects volunteered for this study: 16 nonsmokers, 7 males/9 females, with a mean age of 27.4±1.8 yr (mean±SEM); and 19 smokers, 9 males/10 females, 28.4±1.5 yr. In the nonsmokers' group, one subject was an exsmoker who had quit 3 yr before the study. Smokers consumed 20 cigarettes or more a day and had smoked for at least 3 yr (13.3±2.1 pack-year [1 pack-year = one pack of cigarettes each day for 1 yr, or the equivalent]). None of the subjects smoked marijuana, and all were free of respiratory symptoms suggestive of asthma. None had symptoms of cold, nor had taken any medication for one month before this study. After having given informed consent, the subjects underwent pulmonary function tests (volumes, flows, and diffusion capacity), blood sampling, and bronchoalveolar lavage (BAL). Blood sampling and BAL were performed in the morning, and smokers were asked to refrain from smoking after midnight before testing.

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1. *Abbreviations used in this paper:* AM, alveolar macrophage; BAL, bronchoalveolar lavage; 5-HETE, 5S-hydroxy-6,8,11,14-(E,Z,Z,Z)-eicosatetraenoic acid; 15-HETE, 15S-hydroxy-5,8,11,13-(Z,Z,Z,E)-eicosatetraenoic acid; HPLC, high performance liquid chromatography; LT, leukotriene; LTB_4, 5S,12R-dihydroxy-6,8,10,14-(Z,E,E,Z)-eicosatetraenoic acid; LTC_4, 5S-hydroxy-6R-S-glutathionyl-7,9,11,14-(E,E,Z,Z)-eicosatetraenoic acid; LTD_4, 5S-hydroxy-6R-S-cysteinylglycyl-7,9,11,14-(E,E,Z,Z)-eicosatetraenoic acid; LTE_4, 5S-hydroxy-6R-S-cysteinyl-7,9,11,14-(E,E,Z,Z)-eicosatetraenoic acid; PGB_2, prostaglandin B_2; PMNL, polymorphonuclear leukocyte. UV, ultraviolet.

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Lidocaine (Astra Scientific International, Inc., Santa Clara, CA) and BAL was carried out by instilling aliquots of 20–60 ml of sterile 0.9% saline solution into a segmental or subsegmental bronchus of the right middle lobe through a fiberoptic bronchoscope and by aspirating the fluid gently with a syringe (25). The fluid recovered was kept on ice until it was centrifuged (250 *g*, 10 min, 4°C). Cells were resuspended at concentrations of 0.8 to 4 × 10^6 cells/ml in Dulbecco's phosphate-buffered saline solution (PBS) without Ca^{++} and Mg^{++}. Cell viability, measured by Trypan Blue exclusion, was always higher than 90%. Differential cell counts were performed both on Wright-Giemsa and nonspecific esterase-stained cytocentrifuged preparations.

Blood samples (30 ml) were collected in EDTA and centrifuged (200 *g*, 15 min, room temperature). PMNL were obtained as described previously by successive dextran sedimentation, NH_4Cl lysis, and centrifugation on Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, NJ) cushions (14). The PMNL were finally resuspended at concentrations ranging from 7 to 12 × 10^6 cells/ml in PBS (Ca^{++} and Mg^{++} free). PMNL suspensions were 98% pure and the platelet contamination determined on contrast phase microscope was <1 platelet/leukocyte.

Incubation procedures. 1-ml aliquots of AM or PMNL suspensions were added to polystyrene tubes and preincubated 5 min at 37°C. $MgCl_2$ and $CaCl_2$ were then added to the cell suspensions (0.5 and 2 mM final concentrations, respectively), and the cells were incubated with either 30 μM arachidonic acid, 2 μM ionophore A23187, 30 μM arachidonic acid plus 2 μM ionophore A23187, 10 μM [1-^{14}C]arachidonic acid (~50 mCi/mmol), plus 2 μM ionophore A23187 or with ethanol (control). Stock solutions of the ionophore A23187 and arachidonic acid were prepared in ethanol. The final concentration of ethanol in any incubation medium was 0.2%. After 5 min of incubation at 37°C, the reactions were stopped by adding 1 ml of methanol/acetonitrile (1:1, vol/vol) containing 200 ng of prostaglandin B_2 (PGB_2) as internal standard for high performance liquid chromatography (HPLC) analysis. In some experiments, time and concentration response studies were also performed. Incubation duration ranged from 2 to 60 min with 2 μM ionophore A23187 and 30 μM arachidonic acid. The different concentrations used were 2, 6, and 20 μM ionophore A23187, 9, 30, and 90 μM arachidonic acid and 2 μM ionophore A23187 with 9, 30, or 90 μM arachidonic acid in 5-min incubations.

Analysis of lipoxygenase products. Lipoxygenase metabolites of arachidonic acid were measured by reverse-phase HPLC as previously described (16) with minor modifications. The denatured cell suspensions were centrifuged to remove the precipitated material, the supernatants were acidified to pH 3 with H_3PO_4, and injected (injection volume, 1.7 ml) into a cartridge (Radial Pak C18; 100 × 8 mm, 10 μm particule size, Waters Associates, Millipore Corp., Milford, MA) without further treatment. A Guard-Pak C18 (Waters Associates) was used to protect the Radial Pak cartridge. The various metabolites were eluted at a solvent flow of 3 ml/min using three solvent mixtures (A, B, and C) as follows: step 1, time 0 to 1 min, 100% B to 75% B/25% A; step 2, time 1 to 6 min, 75% B/25% A to 66% B/34% A; step 3, time 6 to 8.5 min, 66% B/34% A to 30% B/70% A; step 4, time 8.5 to 11.8 min, 30% B/70% A (isocratic); step 5, time 11.8 to 12.2 min, 30% B/70% A to 0% B/100% A; step 6, time 12.2 to 18.5 min, 100% A (isocratic); step 7, time 18.5 to 18.6 min, 100% A to 0% A/100% C; step 8, time 18.6 to 31 min, 100% C (isocratic). Solvent compositions were: solvent A, methanol-acetonitrile-water, 30/60/10 (vol/vol/vol), containing 0.01% of H_3PO_4; solvent B, methanol-acetonitrile-water, 23/23/54 (vol/vol/vol), containing 0.01% H_3PO_4, 0.15% of tetrahydrofuran, and 0.004% of dimethylsulfoxide; solvent C, methanol-acetonitrile-water, 30/50/20 (vol/vol/vol), containing 0.06% of H_3PO_4, adjusted to pH 4.6 (apparent pH) with NH_4OH.

The elution was monitored with ultraviolet (UV) photometers (229 nm and 280 nm) and a radioactivity detector (Berthold LB 503 with a glass scintillator cell). The metabolites were identified on the basis of: (*a*) co-migration with authentic standards, (*b*) specificity of UV absorption, and (*c*) incorporation of [1-^{14}C]arachidonic acid. Their quantitation was done by measurement of peak areas and comparison with an internal standard (PGB_2) after correction for differences in absorption coefficients and attenuation settings. The lower limit of detection for the different lipoxygenase products was 2–5 ng.

Analysis of esterified lipoxygenase metabolites. In some experiments both extracellular and intracellular lipoxygenase metabolites were analyzed. Incubations of AM were stopped by centrifugation of the cell suspensions at 250 *g* for 15 min (2°C). The supernatants were collected and denatured with an equal volume of methanol/acetonitrile (1:1, vol/vol) containing 200 ng of PGB_2 and saved for HPLC analysis of the lipoxygenase products released by AM. The cells were resuspended in 1 ml of PBS (2°C) and centrifuged at 250 *g* for 15 min; the supernatant was discarded and the pellet was treated with 100 μl of NaOH 2 N and 400 μl of methanol. After 15 min at room temperature the reaction mixture was neutralized by addition of 100 μl acetic acid 2 N and diluted with 1 ml of PBS containing 200 ng of PGB_2. The samples were centrifuged to remove any particulate material and analyzed by HPLC as described above. Using this procedure for hydrolysis of 5-hydroxy-eicosatetraenoic acid (5-HETE), the release of 5-HETE from cellular lipids in human PMNL is complete within 15 min.

Statistical analysis. Blood sampling, BAL, cell incubations, and lipoxygenase product analysis were performed in pairs (nonsmoker and smoker) for all experiments but two. Results are expressed as mean±SEM. Data were analyzed using the unpaired *t* test, except for concentration and time response data, which were compared with a two-way variance analysis.

Results

Pulmonary function tests and BAL. Statistically, smokers and nonsmokers had similar lung volumes and forced expiratory volume in 1 s, but the diffusing capacity for carbon monoxide was lower in the smoker group, $P < 0.05$. Lavage from smokers differed from those of nonsmokers in having: (*a*) a greater total cell count, (*b*) a higher percentage of AM, and (*c*) a lower percentage of lymphocytes ($P < 0.001$ for all three parameters) (Table I). Moreover, smokers' AM were larger, brown in color, and filled with dark inclusions. No significant blood contamination to the BAL was found; only a few erythrocytes were seen in some cell preparations, and platelets were not detectable.

Synthesis of 5-lipoxygenase products in AM and PMNL. Fig. 1 shows a typical profile of lipoxygenase-derived arachidonic acid metabolites after incubation of 3.2 × 10^6 AM with 10 μM [1-^{14}C]arachidonic acid and 2 μM ionophore A23187. Peaks for LTB_4 and 5-HETE were clearly evident as the two major metabolites. As expected in incubations with [1-^{14}C]arachidonic acid, both 5-HETE and LTB_4 were radiolabeled. Under all incubation conditions tested, the peptido-LTs, LTC_4, LTD_4, and LTE_4, the ω-hydroxy-LTB_4, the ω-carboxy-LTB_4, and the 15-lipoxygenase product, 15-HETE, were undetectable. The products of the nonenzymatic hydrolysis of LTA_4, i.e., the Δ6-trans-LTB_4, Δ6-trans-12-epi-LTB_4, and the 5,6-dihydroxy-eicosatetraenoic acids (5,6-DiHETEs) were formed in small quantities (each <5% of the amount of LTB_4) and for this reason were seldom measurable. When AM suspensions were incubated for 5 min at 37°C with 0.2% ethanol in the absence of stimulatory substances, 5-HETE and LTB_4 were undetectable in the incubation media.

Fig. 2 shows a typical profile of arachidonic acid metabolites released by 6.8 × 10^6 PMNL stimulated with 2 μM ionophore A23187. Peaks for LTB_4 and 5-HETE were again evident, but in contrast to the AM, the PMNL produced measurable quantities of LTC_4, ω-hydroxy-LTB_4, ω-carboxy-LTB_4, and, in incubation with arachidonic acid, 15-HETE. The PMNL also formed the Δ6-trans-LTB_4, Δ6-trans-12-epi-LTB_4, and the 5,6-DiHETEs.

*Table I. Physiologic Parameters and Bronchoalveolar Lavage Characteristics of Subjects**

Group	Physiologic parameters: Vital capacity	Total lung capacity	FEV$_1$	DLCO	Bronchoalveolar lavage characteristics: Fluid recovered (percent of infused)	Cells per ml ($\times 10^{-4}$)	Macrophages	Lymphocytes	Neutrophils
							%	%	%
Nonsmokers (n = 16)	103.4±2.8	104.5±3.0	99.4±2.9	93.9±3.0	66.7±1.9	6.4±0.6	87.8±1.2	10.8±1.2	1.4±0.3
Smokers (n = 19)	101.9±2.7	101.9±3.0	96.5±2.4	83.3±3.7	66.2±1.5	23.6±1.8	94.9±0.6	3.2±0.4	1.9±0.3
P value	NS	NS	NS	<0.05	NS	<0.001	<0.001	<0.001	NS

* Results are given as mean±SEM. Physiologic data are presented as percentage of predicted values; FEV$_1$, forced expiratory volume in 1 second; DLCO, diffusing capacity for carbon monoxide.

Effects of cigarette smoking on the synthesis of LTB$_4$ and 5-HETE in AM and PMNL. Fig. 3 shows the relative quantities of LTB$_4$ and 5-HETE synthetized by nonsmokers' and smokers' AM. In incubations with the ionophore A23187, smokers' AM produced about ten times less LTB$_4$ and 5-HETE than nonsmokers' AM: LTB$_4$, 5.6±1.5 and 57±15 pmol/10^6 cells, respectively, $P < 0.01$; 5-HETE, 12±6 and 125±44 pmol/10^6 cells, $P < 0.05$. Such a decrease in LTB$_4$ and 5-HETE production in smokers' AM was also observed with arachidonic acid: LTB$_4$, 5.4±1.3 and 61±16 pmol/10^6 cells in smokers and nonsmokers, respectively, $P < 0.005$; 5-HETE, 15±4 and 250±81 pmol/10^6 cells, $P < 0.01$. In the presence of both arachidonic acid and ionophore A23187, smokers' AM released ~15% of the amount of metabolites produced by nonsmokers' AM: LTB$_4$, 61±19 and 317±56 pmol/10^6 cells, respectively, $P < 0.001$; 5-HETE, 123±32 and 1,079±254 pmol/10^6 cells, $P < 0.001$. The profiles of arachidonic acid metabolites formed by AM were similar in all conditions studied; only the amount of products formed varied.

Concentration and time response data are shown in Table

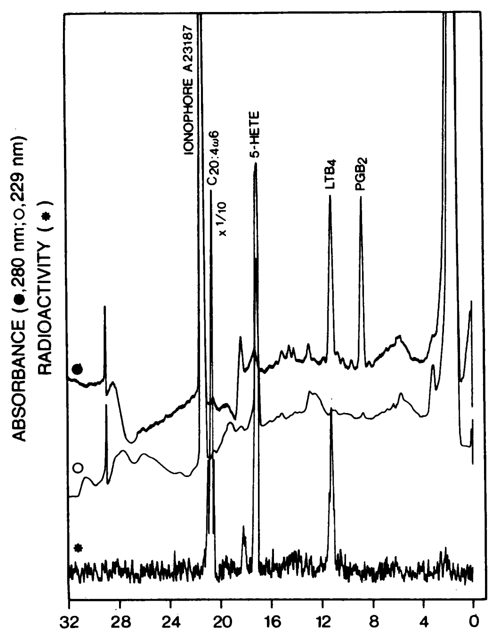

Figure 1. Reverse-phase HPLC analysis of arachidonic acid metabolites formed by human AM stimulated with 2 μM ionophore A23187 and 10 μM [1-^{14}C]arachidonic acid. The cell suspension consisted of 3.2 × 10^6 cells in 1 ml PBS, of which 94% were AM. The cell suspension was preincubated for 5 min at 37°C before addition of Mg^{++} and Ca^{++} salts and stimulatory substances. After 5 min of incubation, the reaction was stopped by adding 1 ml of methanol/acetonitrile (1:1, vol/vol) containing 200 ng PGB$_2$, and arachidonic acid metabolites were analyzed by reverse-phase HPLC. Attenuation settings of UV photometers were 0.02 and 0.05 OD unit (full scale) at 280 and 229 nm, respectively. The settings of the radioactivity monitor were 3,000 cpm and 3 s, respectively, for the range and time constant. The spike observed slightly above 28 min of elution time on both UV tracings is due to a change in the pH of the solvent. Total amounts of compounds in the sample were as follows: LTB$_4$, 400 pmol; 5-HETE, 1,110 pmol. $C^{20}:^4\omega^6$, arachidonic acid.

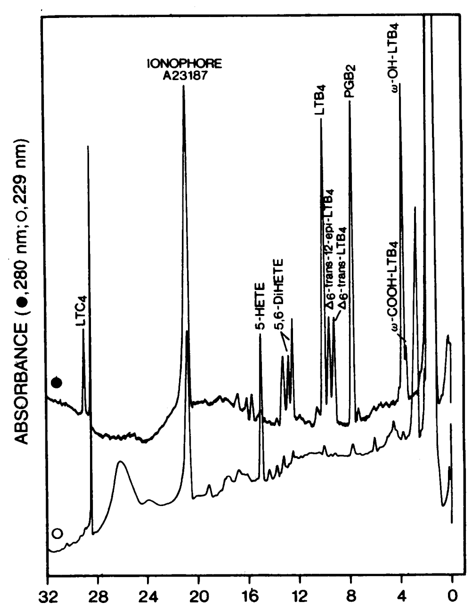

Figure 2. Reverse-phase HPLC analysis of arachidonic acid metabolites formed by human PMNL stimulated with 2 μM ionophore A23187. The cell suspension consisted of 6.8 × 10^6 cells in 1 ml PBS, of which 98% were PMNL. The platelet contamination was ~1 platelet/PMNL. Total amounts of compounds in the sample were as follows: ω-OH-LTB$_4$, 310 pmol; LTB$_4$, 420 pmol; 5-HETE, 550 pmol; LTC$_4$, 65 pmol. See legend to Fig. 1 for details of incubation and analysis.

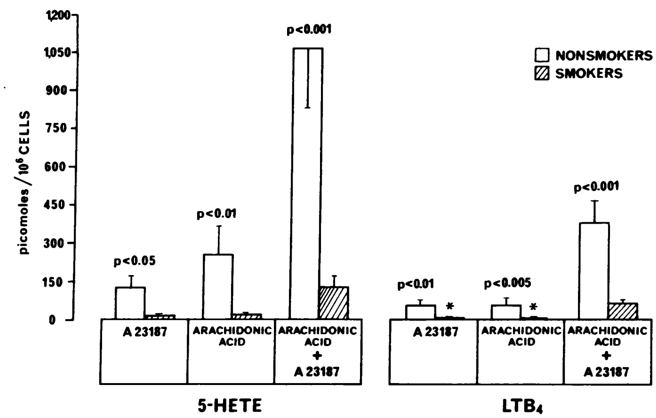

Figure 3. LTB_4 and 5-HETE synthesis by smokers' and nonsmokers' AM. AM were incubated in PBS with 2 μM ionophore A23187 (n = 6), 30 μM arachidonic acid (n = 12), or with 30 μM arachidonic acid and 2 μM ionophore A23187 (n = 13). No spontaneous release of 5-HETE and LTB_4 was found in control experiments (incubation with 0.2% ethanol, data not shown). Data are expressed as mean±SEM. *Values inferior to 10 pmol.

II. Nonsmokers' AM released more lipoxygenase products than smokers' AM at all concentrations of stimuli and incubation times studied, $P < 0.01$. Increasing the concentration of ionophore A23187 from 2 to 10 μM had no effect on 5-HETE and LTB_4 productions in the two cell populations. Increasing the concentration of arachidonic acid from 9 to 90 μM significantly stimulated the production of 5-HETE ($P < 0.005$); the increase of LTB_4 production observed was not statistically significant. Time courses of 5-HETE and LTB_4 production were parallel in smokers' and nonsmokers' AM. The formation of the two compounds was nearly maximum after 2 min of incubation with the stimuli. The levels of 5-HETE and LTB_4 were stable up to 10 min of incubation and slowly declined at longer incubation times.

The AM content in 5-HETE esters was also measured by HPLC analysis. Substantial amounts of 5-HETE were found after alcaline hydrolysis of the intact washed AM previously incubated 2 to 60 min in the presence of 2 μM ionophore A23187 and 30 μM arachidonic acid. Fig. 4 shows the amounts of 5-HETE and LTB_4 released by smokers' and nonsmokers' AM and also the amount of intracellular 5-HETE in the two cell populations. Both the released and intracellular 5-HETE were strongly depressed in smokers' AM. In the same experiments nonsmokers' AM released 5 to 10 times more LTB_4 than the smokers' AM did over the incubation times studied, but intracellular LTB_4 was not detectable in either AM populations.

Blood PMNL from nonsmokers and smokers were also compared for lipoxygenase product synthesis. Nonsmokers' and smokers' PMNL synthesized similar amounts of LTB_4, 5-HETE, and 15-HETE both in incubations with arachidonic acid alone or with arachidonic acid and ionophore (Table III). In the presence of ionophore alone, PMNL did not release 15-HETE (Fig. 2) and no significant difference was found in the production of LTB_4 and 5-HETE by smokers' and nonsmokers' cells (data not shown).

*Table II. LTB_4 and 5-HETE Synthesis by Nonsmokers' and Smokers' AM: Concentration and Time Response Data**

		5-HETE		LTB_4	
	n	Nonsmokers	Smokers	Nonsmokers	Smokers
I. Concentration (*μM*)					
Ionophore A23187					
2	3	126±53	8±5	88±12	8±1
6	3	153±61	5±5	122±10	10±2
20	3	154±50	13±7	85±14	9±1
Arachidonic acid					
9	3	36±7	5±5	16±4	9±2
30	3	167±33	15±7	40±7	8±1
90	3	312±75	24±13	74±30	14±6
Ionophore A23187 (2 μM) + arachidonic acid					
9	4	394±101	89±31	286±80	75±25
30	4	675±143	161±56	446±123	108±29
90	4	726±144	102±26	367±91	61±13
II. Time (*min*)					
2	4	808±212	211±52	508±166	142±26
5	4	859±216	196±58	584±197	131±26
10	4	886±210	211±37	598±193	128±29
20	4	733±157	159±73	482±130	103±36
60	4	331±103	75±29	274±45	43±16

* Data are expressed in pmol/10^6 cells, mean±SEM. AM were incubated in pair (nonsmoker and smoker) I. with different concentrations of ionophore A23187, arachidonic acid, and arachidonic acid in presence of 2 μM ionophore A23187. II. with ionophore A23187 (2 μM) and arachidonic acid (30 μM) for 2 to 60 min. Lipoxygenase products were measured by reverse-phase HPLC. Smokers' AM released smaller amounts of 5-HETE and LTB_4 than nonsmokers' AM at all stimulus concentrations and incubation times studied, $P < 0.01$.

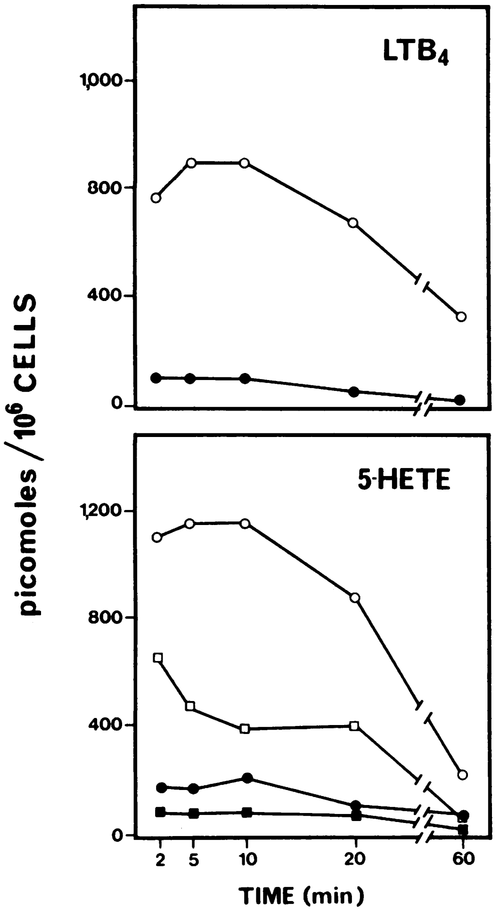

Figure 4. Measurement of intracellular (□) and extracellular (○) LTB_4 and 5-HETE in smokers' and nonsmokers' AM (1×10^6 cells) stimulated with 2 μM ionophore A23187 and 30 μM arachidonic acid for the indicated incubation times. Incubation was terminated by centrifugation of cells at 2°C. AM were washed once with cold PBS (2°C) and the cells were treated with a solution of NaOH for hydrolysis of esterified 5-lipoxygenase products. Supernatants (incubation media) and cell hydrolysates were analyzed by reverse-phase HPLC for determination of intracellular and extracellular 5-HETE and LTB_4. The results are the mean of two experiments; open symbols, nonsmokers; closed symbols, smokers. Intracellular levels of LTB_4 were undetectable. See methods for detailed procedures.

Discussion

In the present study arachidonic acid metabolites were analyzed using a reverse-phase HPLC system developed recently in our laboratory (16). The procedure used does not involve extraction, concentration, or derivatization; the sample needs only be denatured, centrifuged to remove particulate matter, and acidified before injection. Because of the simplicity of the sample preparation procedure, loss and deterioration of arachidonic acid metabolites are minimal, and recoveries exceed 90% calculated from the amounts of tritium-labeled LTB_4 and LTC_4 (carrier-free) injected (16).

Our results on the metabolism of arachidonic acid by suspensions of AM are in excellent agreement with those of Martin et al. (9) obtained under comparable experimental conditions. Their analysis of lipoxygenase products included an extraction of the incubation medium on octadecylsilyl silica followed by an HPLC analysis using a different reverse-phase HPLC system. They found LTB_4, 5-HETE, and small amounts of Δ6-trans-LTB_4 and Δ6-trans-12-epi-LTB_4, but no peptido-LTs. Studying adherent AM, Fels et al. (8) demonstrated the release of LTB_4 by AM stimulated with A23187 using HPLC techniques, whereas Damon et al. (26) and MacDermot et al. (27) identified LTD_4 and LTB_4, respectively, using mass spectrometry. The synthesis of 5-lipoxygenase and 15-lipoxygenase products by blood PMNL has been investigated in detail previously (14, 16, 28), and the profile of products reported in the present study is in excellent agreement with these reports.

Except for the 12S-hydroxy-5,8,10-heptadecatrienoic acid (HHT), cyclooxygenase products are not detectable by UV photometry at the wavelengths used in this study (229 and 280 nm) for the analysis of lipoxygenase products. In the HPLC system used, prostaglandins and thromboxane B_2 elute between 2 and 8 min; radiolabeled metabolites were not evident in this area of the chromatograms of both AM and PMNL [1-^{14}C]arachidonic acid metabolites (Fig. 1; see Ref. 16 and 18 for HPLC profiles of [1-^{14}C]arachidonic acid metabolites in human PMNL). Assuming a similar incorporation of the ^{14}C-label into cyclooxygenase and lipoxygenase products, 5–10 ng of prostaglandins or thromboxane B_2 would have been detectable.

Besides AM, BAL fluid contained a small number of lymphocytes and neutrophils (Table I). It seems very unlikely that lymphocytes could contribute significantly to the synthesis of 5-lipoxygenase products in the AM suspensions studied. Their count was low and we have previously found that blood lymphocytes suspensions obtained by centrifugation on Ficoll-Paque cushions and depleted of monocytes by adherence, do not show detectable 5-lipoxygenase activity (18). Moreover, in recent studies we have been unable to show any lipoxygenase activity in human blood lymphocytes (~95% pure) obtained by centrifugal elutriation, whereas the PMNLs and monocytes isolated in the same experiments clearly show 5-lipoxygenase and 15-

*Table III. Lipoxygenase Products from Blood PMNL**

	Incubations with arachidonic acid (n = 6)			Incubations with arachidonic acid and ionophore A23187 (n = 7)		
	5-HETE	15-HETE	LTB_4	5-HETE	15-HETE	LTB_4
Nonsmokers	15±9	29±24	0.5±0.3	371±101	85±33	70±11
Smokers	8±3	32±14	0.5±0.3	284±53	80±33	64±11

* Data are expressed in pmol/10^6 cells, mean±SEM. Blood PMNL preparations were incubated in pair (nonsmoker and smoker) under various conditions: 0.2% ethanol (control), 30 μM arachidonic acid, or 30 μM arachidonic acid and 2 μM ionophore A23187. The arachidonic acid metabolites were analyzed by reverse-phase HPLC. Compounds were not detectable in control incubations. Statistical analyses showed no significant difference between smokers and nonsmokers.

lipoxygenase activities (P. Poubelle and P. Borgeat, data to be published). Goetzl (29) reported the synthesis of lipoxygenase products by human lymphocytes but it must be emphasized that their lymphocyte suspensions contained 20% of unidentified cells, probably monocytes.

The BAL neutrophil count was even lower (<4.3%) than that of lymphocytes, and this small number of cells might account for only marginal (<1% as calculated from data of Table I and Fig. 3) 5-lipoxygenase product synthesis; furthermore, any significant participation on their part should be accompanied by ω-OH-LTB_4 production (Fig. 2) (30–31), a metabolite not found in alveolar cell suspensions in this study. The findings, in BAL from smokers, of an increased number of cells, increased percent of AM, decreased percent of lymphocytes and the morphologic changes of AM, were similar to those already reported (1–4, 7).

The main goal of this study was to compare the capacity of smokers' and nonsmokers' AM to synthesize LTB_4. A defective synthesis of 5-lipoxygenase products was found among AM from smokers. This was observed in all stimulatory conditions studied, over a wide stimulus concentration range and various incubation times. Moreover, measurements of intracellular lipoxygenase metabolites indicated that differences in the metabolism of arachidonic acid through the 5-lipoxygenase pathway in smokers' and nonsmokers' AM were observable not only at the level of the products released but also at the level of the intracellular reacylated 5-HETE. The esterification of 5-HETE has been shown in PMNL and macrophages previously (32, 33). It was also observed that dihydroxy derivatives of arachidonic acid (such as LTB_4) were not subject to reacylation in cellular lipids (33) in agreement with the present study. Martin et al. (9), who also included smokers and nonsmokers in their study, did not find any difference in LTB_4 synthesis by AM between both groups. However, since the number of subjects investigated was small (four smokers, two nonsmokers) and experiments were not carried out in pairs (i.e., smoker vs. nonsmoker), their study does not provide conclusive data on this particular point. In contrast to the AM we did not find any effect of smoking on 5-lipoxygenase product synthesis in blood PMNL (Table III).

The present finding of altered 5-lipoxygenase product formation in smokers' AM is analogous to the previous reports that cigarette smoking inhibits prostaglandins and thromboxane synthesis in AM (21, 22). In one of these studies (21), it was proposed that decreased prostaglandin and thromboxane synthesis in smokers' AM was caused by a defect at the level of phospholipid hydrolysis. The mechanism that leads to the inhibition of 5-lipoxygenase product synthesis in cigarette smokers' AM remains unknown. The decreased 5-HETE and LTB_4 production observed, even in the presence of exogenous arachidonic acid, suggests a defect at the level of the dioxygenation reaction, rather than at the level of arachidonic acid release, LTA_4 hydrolase activity or product reacylation.

Since LTB_4 is a potent chemotactic agent for blood phagocytes (12) and AM are able to release LTB_4 on appropriate stimulation (27), a decreased production of LTB_4 by AM may impair their ability to regulate inflammatory responses in the lung. Smokers are known to suffer from more frequent infectious lung diseases than are nonsmokers (23, 24). Such a predisposition to infection may be related, in part, to defective AM function. Further research is needed to delineate the pathophysiologic consequences of the altered 5-lipoxygenase product synthesis among smokers' AM.

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