Decreased Leukotriene B₄ 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. Lipoxigenase 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/10⁶ cells and 5-HETE, 1,079±254, mean±SEM. No metabolites were generated under control conditions (no stimulation). In all incubations performed, the peptide-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 < 0.05 to P < 0.001 according to stimulatory conditions). This defective lipoxigenase metabolite production in AM from smokers was observed over a wide range of stimulants 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 found no difference in the synthesis of 5-lipoxigenase products in these cells was noticed between smokers and nonsmokers. These data show that cigarette smoking causes a profound inhibition of the 5-lipoxigenase 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)

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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; HPLC, high performance liquid chromatography; LT, leukotriene; LTB₄; 5S,12R-dihydroxy-6,8,10,14-(Z,E,E,Z)-eicosatetraenoic acid; LTC₄, 5S-hydroxy-6R-S-glutathionyl-7,9,11,14-(E,E,Z,Z)-eicosatetraenoic acid; LTD₄, 5S-hydroxy-6R-S-cysteinylglycyl-7,9,11,14-(E,E,Z,Z)-eicosatetraenoic acid; LTE₄, 5S-hydroxy-6S-cysteinylglycyl-7,9,11,14-(E,E,Z,Z)-eicosatetraenoic acid; PGB₂, prostaglandin B₂; PMNL, polymorphonuclear leukocyte. UV, ultraviolet.
Lidocaine (Astra Scientific International, Inc., Santa Clara, CA) and BAL was carried out by instilling aliquots of 20–60 μl 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 × 10 min, 4°C). Cells were resuspended in a final concentration of 0.8 to 4 × 10⁶ cells/ml in Dulbecco’s phosphate-buffered saline solution (PBS) without Ca²⁺ and Mg²⁺. 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, NH₄Cl 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 × 10⁶ cells/ml in PBS (Ca²⁺ and Mg²⁺ 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. MgCl₂ and CaCl₂ 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 [1-¹⁴C]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 B₂ (PGB₂) 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 lipoxigenase products. Lipoxigenase 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 H₃PO₄, and injected (volume injection, 1.7 ml) into a cartridge (Radial Pak C18; 100 × 8 mm, 10 μm particle 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 H₃PO₄; solvent B, methanol-acetonitrile-water, 23/22/54 (vol/vol/vol), containing 0.01% H₃PO₄, 0.15% of tetrahydrofuran, and 0.004% of dimethylsulfoxide; solvent C, methanol-acetonitrile-water, 30/50/20 (vol/vol/vol), containing 0.06% of H₃PO₄, adjusted to pH 4.6 (apparent pH) with NH₄OH.

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 [1-¹⁴C]arachidonic acid. Their quantitation was done by measurement of peak areas and comparison with an internal standard (PGB₂) after correction for differences in absorption coefficients and attenuation settings. The lower limit of detection for the different lipoxigenase products was 2–5 ng.

Analysis of esterified lipoxigenase metabolites. In some experiments both extracellular and intracellular lipoxigenase 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 PGB₂ and saved for HPLC analysis of the lipoxigenase 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 PGB₂. 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 lipoxigenase 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 lipoxigenase-derived arachidonic acid metabolites after incubation of 3.2 × 10⁵ AM with 10 μM [1-¹⁴C]arachidonic acid and 2 μM ionophore A23187. Peaks for LTB₄ and 5-HETE were clearly evident as the two major metabolites. As expected in incubations with [1-¹⁴C]arachidonic acid, both 5-HETE and LTB₄ were radiolabeled. Under all incubation conditions tested, the peptido-LTs, LTC₄, LTD₄, and LTE₄, the ω-hydroxy-LTB₄, the ω-carboxy-LTB₄, and the 15-lipoxigenase product, 15-HETE, were undetectable. The products of the nonenzymatic hydrolysis of LTA₄, i.e., the Δ6-trans-LTB₄, Δ6-trans-12-epi-LTB₄, and the 5,6-dihydroxy-icosatetraenoic acids (5,6-DHETEs) were formed in small quantities (each <5% of the amount of LTB₄) 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 LTB₄ were undetectable in the incubation media.

Fig. 2 shows a typical profile of arachidonic acid metabolites released by 6.8 × 10⁵ PMNL stimulated with 2 μM ionophore A23187. Peaks for LTB₄ and 5-HETE were again evident, but in contrast to the AM, the PMNL produced measurable quantities of LTC₄, ω-hydroxy-LTB₄, ω-carboxy-LTB₄, and, in incubation with arachidonic acid, 15-HETE. The PMNL also formed the Δ6-trans-LTB₄, Δ6-trans-12-epi-LTB₄, and the 5,6-DHETEs.
Table 1. Physiologic Parameters and Bronchoalveolar Lavage Characteristics of Subjects*

<table>
<thead>
<tr>
<th>Group</th>
<th>Vital capacity</th>
<th>Total lung capacity</th>
<th>FEV₁</th>
<th>DLCO</th>
<th>Fluid recovered (percent of infused)</th>
<th>Cells per ml (×10⁴)</th>
<th>Macrophages</th>
<th>Lymphocytes</th>
<th>Neutrophils</th>
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<td></td>
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<td>%</td>
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<tr>
<td>Nonsmokers</td>
<td>(n = 16)</td>
<td>103.4±2.8</td>
<td>104.5±3.0</td>
<td>99.4±2.9</td>
<td>93.9±3.0</td>
<td>66.7±1.9</td>
<td>6.4±0.6</td>
<td>87.8±1.2</td>
<td>10.8±1.2</td>
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<tr>
<td>Smokers (n = 19)</td>
<td>101.9±2.7</td>
<td>101.9±3.0</td>
<td>96.5±2.4</td>
<td>83.3±3.7</td>
<td>66.2±1.5</td>
<td>23.6±1.8</td>
<td>94.9±0.6</td>
<td>3.2±0.4</td>
<td>1.9±0.3</td>
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<tr>
<td>P value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>NS</td>
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* Results are given as mean±SEM. Physiologic data are presented as percentage of predicted values; FEV₁, forced expiratory volume in 1 second; DLCO, diffusing capacity for carbon monoxide.

Effects of cigarette smoking on the synthesis of LTB₄ and 5-HETE in AM and PMNL. Fig. 3 shows the relative quantities of LTB₄ and 5-HETE synthetized by nonsmokers' and smokers' AM. In incubations with the ionophore A23187, smokers' AM produced about ten times less LTB₄ and 5-HETE than nonsmokers' AM: LTB₄, 5.6±1.5 and 57±15 pmol/10⁶ cells, respectively, P < 0.01; 5-HETE, 12±6 and 125±44 pmol/10⁶ cells, P < 0.05. Such a decrease in LTB₄ and 5-HETE production in smokers' AM was also observed with arachidonic acid: LTB₄, 5.4±1.3 and 61±16 pmol/10⁶ cells in smokers and nonsmokers, respectively, P < 0.005; 5-HETE, 15±4 and 250±81 pmol/10⁶ 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: LTB₄, 61±19 and 317±56 pmol/10⁶ cells, respectively, P < 0.001; 5-HETE, 123±32 and 1,079±254 pmol/10⁶ 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 1.

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Figure 1. Reverse-phase HPLC analysis of arachidonic acid metabolites formed by human AM stimulated with 2 μM ionophore A23187 and 10 μM [1-14C]arachidonic acid. The cell suspension consisted of 3.2 × 10⁶ cells in 1 ml PBS, of which 94% were AM. The cell suspension was preincubated for 5 min at 37°C before addition of Mg²⁺ and Ca²⁺ 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 PGB₂, 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: LTB₄, 400 pmol; 5-HETE, 1,110 pmol. C₃₀H₄₄O₆, 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 × 10⁶ 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: α-OH-LTB₄, 310 pmol; LTB₄, 420 pmol; 5-HETE, 550 pmol; LTC₄, 65 pmol. See legend to Fig. 1 for details of incubation and analysis.
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 \( \mu M \) 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 \( \mu M \) 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 alkaline hydrolysis of the intact washed AM previously incubated 2 to 60 min in the presence of 2 \( \mu M \) ionophore A23187 and 30 \( \mu M \) 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 lipooxygenase 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*  

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*Data are expressed in pmol/10\(^6\) cells, mean±SEM. AM were incubated in pairs (nonsmoker and smoker) I. with different concentrations of ionophore A23187, arachidonic acid, and arachidonic acid in presence of 2 \( \mu M \) ionophore A23187. II. with ionophore A23187 (2 \( \mu M \)) and arachidonic acid (30 \( \mu M \)) for 2 to 60 min. Lipooxygenase 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, \( P < 0.01 \).
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 LTB₄ and LTC₄ (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 octadecysilyl silica followed by an HPLC analysis using a different reverse-phase HPLC system. They found LTB₄, 5-HETE, and small amounts of Δ6-trans-LTB₄ and Δ6-trans-12-epi-LTB₄, but no peptido-LTs. Studying adherent AM, Fels et al. (8) demonstrated the release of LTB₄ by AM stimulated with A23187 using HPLC techniques, whereas Damon et al. (26) and MacDermot et al. (27) identified LTB₄ and LTB₄, 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 B₂ elute between 2 and 8 min; radiolabeled metabolites were not evident in this area of the chromatograms of both AM and PMNL [1-¹⁴C]arachidonic acid metabolites (Fig. 1; see Ref. 16 and 18 for HPLC profiles of [1-¹⁴C]arachidonic acid metabolites in human PMNL). Assuming a similar incorporation of the ¹⁴C-label into cyclooxygenase and lipoxygenase products, 5–10 ng of prostaglandins or thromboxane B₂ 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-

![Graph](image)

**Figure 4.** Measurement of intracellular (c) and extracellular (o) LTB₄ and 5-HETE in smokers' and nonsmokers' AM (1 x 10⁶ 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 LTB₄. The results are the mean of two experiments; open symbols, nonsmokers; closed symbols, smokers. Intracellular levels of LTB₄ were undetectable. See methods for detailed procedures.

**Discussion**

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**Table III. Lipoxygenase Products from Blood PMNL**

<table>
<thead>
<tr>
<th></th>
<th>Incubations with arachidonic acid (n = 6)</th>
<th>Incubations with arachidonic acid and ionophore A23187 (n = 7)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>5-HETE</td>
<td>15-HETE</td>
</tr>
<tr>
<td>Nonsmokers</td>
<td>15±9</td>
<td>29±24</td>
</tr>
<tr>
<td>Smokers</td>
<td>8±3</td>
<td>32±14</td>
</tr>
</tbody>
</table>

* Data are expressed in pmol/10⁶ 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.
lipoxynase activities (P. Poubelle and P. Borgeat, data to be published). Goetzl (29) reported the synthesis of lipoxynase 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-lipoxynase product synthesis; furthermore, any significant participation on their part should be accompanied by ω-OH-LTB4 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 LTBA4. A defective synthesis of 5-lipoxynase 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 lipoxynase metabolites indicated that differences in the metabolism of arachidonic acid through the 5-lipoxynase 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 LTBA4) 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 LTBA4 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-lipoxynase product synthesis in blood PMNL (Table III).

The present finding of altered 5-lipoxynase product formation in smokers' AM is analogous to the previous reports that cigarette smoking inhibits prostanoids 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-lipoxynase product synthesis in cigarette smokers' AM remains unknown. The decreased 5-HETE and LTBA4 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, LTBA4 hydrolase activity or product reacylation.

Since LTBA4 is a potent chemotactic agent for blood phagocytes (12) and AM are able to release LTBA4 on appropriate stimulation (27), a decreased production of LTBA4 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-lipoxynase product synthesis among smokers' AM.

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