Migration of Human Inflammatory Cells into the Lung Results in the Remodeling of Arachidonic Acid into a Triglyceride Pool

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Summary

Increasing evidence suggests that the metabolism of arachidonic acid (AA) may be different in inflammatory cells isolated from blood or migrating into tissues. To explore the possibility that changes in AA metabolism between blood and tissue inflammatory cells could be due in part to a different content or distribution of AA in glycerolipid classes, we studied these parameters in six human inflammatory cells isolated from blood (eosinophils, monocytes, neutrophils, and platelets) or from the lung tissue (mast cells and macrophages). Lung cells generally had a higher total cellular content of AA than that found in the blood cells. In addition, both mast cells and macrophages had a large endogenous pool of AA associated with triglycerides (TG), containing 45 and 22% of their total cellular AA, respectively. To address the hypothesis that cells migrating into the lung had a higher cellular level of AA and a larger AA pool in TG, we studied neutrophils isolated from the bronchoalveolar lavage (BAL) of patients with adult respiratory distress syndrome. BAL neutrophils had a fourfold increase in cellular AA as compared with blood neutrophils and contained 25% of their AA in TG versus 3% in blood neutrophils. BAL neutrophils also had a higher number of cytoplasmic lipid bodies (8 ± 3/cell) relative to blood neutrophils (2 ± 1/cell). High concentrations of free AA were also found in the cell-free BAL fluid of adult respiratory distress syndrome patients. To explore whether changes in BAL neutrophils may be due to the exposure of the cells to high concentrations of exogenous AA found in BAL, we incubated blood neutrophils in culture with AA (10–100 μM) for 24 h. Neutrophils supplemented with AA had a 10-fold increase in the amount of AA associated with TG and a sixfold increase in the number of lipid bodies. In addition, supplementation with AA induced a dose-dependent formation of hypodense cells. Taken together, these data indicate that human inflammatory cells undergo a fundamental and consistent remodeling of AA pools as they mature or enter the lung from the blood. These biochemical and morphological changes can be mimicked in vitro by exposing the cells to high levels of AA. This mechanism may be responsible for the changes in AA mobilization and eicosanoid metabolism observed in tissue inflammatory cells.

Eicosanoids are oxygenase-derived metabolites of arachidonic acid (AA)[1] involved in the pathogenesis of inflammatory and allergic reactions (1–3). The amount and the profile of eicosanoids produced by inflammatory cells is dictated by a variety of biochemical factors, including the activity of biosynthetic enzymes (phospholipases A_2, prostaglandin synthases, and lipoxygenases) and the availability of AA in cellular glycerolipid pools (4–7). A number of studies have shown that AA metabolism can be both quantitatively and qualitatively different in inflammatory cells obtained from blood and those resident in or migrating into tissues (8, 9). For example, blood monocytes produce different quantities and a distinct profile of eicosanoids from those generated by tissue macrophages (10, 11). In

[1] *Abbreviations used in this paper:* AA, arachidonic acid; ARDS, adult respiratory distress syndrome; BAL, bronchoalveolar lavage; $^2H_8$-AA, octadeuterio AA; $^2H_3$-SA, trideuterio stearic acid; LA, linoleic acid; NICI-GC/MS, negative ion chemical ionization–gas chromatography/mass spectrometry; NL, neutral lipids; OA, oleic acid; PI, phosphatidylinositol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; TG, triglyceride.

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addition, eosinophils migrating into the lung metabolize AA differently than those isolated from blood, and these differences are associated with phenotypic and density changes of the cells (12–14). Part of these differences may be related to microenvironmental factors in the lung, such as cytokines and growth factors, to which tissue cells are exposed (15, 16). For example, several cytokines have been shown to substantially influence enzymes involved in AA metabolism (17, 18). However, there is increasing evidence that remodeling of AA into certain glycerolipid pools or in distinct subcellular locations may be an important factor in regulating AA metabolism in these inflammatory cells. Defining these factors may help elucidate the pathophysiological role of inflammatory cells that move into or reside in the lung.

Recent studies have emphasized the importance of the cellular compartmentalization of AA metabolism and, in particular, of the subcellular distribution of glycerolipid pools of AA as well as of the enzymes that act on these pools. For example, both high molecular weight phospholipase A_2 and 5-lipoxygenase appear to translocate from a cytosolic to a nuclear location during cell activation (19–21). It is thought that this translocation is necessary to move these enzymes to their AA-containing substrates. 5-lipoxygenase–activating protein has also been localized to the nuclear envelope (21). Studies by S. T. Reddy and H. R. Herschman suggest that one isotype of PG H synthase (PG/H synthase II) uses exogenous AA while another isotype (PG/H synthase I) uses glycerolipid pools of AA within the cell (7). Our studies in mast cells indicate that there are distinct glycerolipid pools at separate cellular locations that supply AA that forms leukotrienes or AA that is released unmetabolized from the cell (22, 23).

Even though the cellular compartmentalization of different glycerolipid pools of AA appears to be important in AA mobilization and eicosanoid generation, little is known about the location and function of individual glycerolipid pools. In general, the major glycerolipid pools of AA within inflammatory cells that supply AA have been assumed to be located within membrane phospholipids. However, even this basic assumption has been challenged by recent studies that indicate a large pool of AA associated with triglycerides (TG) in human macrophages (24).

The goal of the current study was to begin to test the hypothesis that human inflammatory cells, reaching their final maturation in the lung or migrating in the lung during an inflammatory reaction, metabolize AA differently because they remodel their AA into unique glycerolipid pools at distinct subcellular locations. This study indicates that most human blood inflammatory cells (neutrophils, monocytes, and platelets) contain a small pool of AA in TG, but this pool is substantially larger in lung resident cells (mast cells and macrophages). In addition, the quantity of AA associated with the TG pool is directly correlated with the total content of AA in each cell. Finally, our data show that the increase in AA within the TG pool is associated both in vitro and in vivo with an increased number of cytoplasmic lipid bodies and with the formation of hypodense cells.

Materials and Methods

Materials. Radiolabeled [3,6,8,9,11,12,14,15-^{3}H]-AA (80 Ci/mmole) was purchased from New England Nuclear Products (Boston, MA). AA, octadeutero AA ($^2H_8$-AA), and trideutero stearic acid ($^2H_3$-SA) were purchased from BIOMOL Research Laboratories Inc. (Plymouth Meeting, PA). α-Naphthyl acetate esterase kit, Ficoll, Percoll, and insulin/transferrin/sodium selenite supplement were purchased from Sigma Chemical Co. (St. Louis, MO). Essentially fatty acid–free human serum albumin, pyridine, and pentafluorobenzoyl chloride were purchased from Aldrich Chemical Co. (Milwaukee, WI). Nile red was purchased from Polysciences Inc. (Warrington, PA). RPMI 1640 and FCS were obtained from GIBCO BRL (Gaithersburg, MD). All solvents were HPLC grade and were purchased from Fisher Scientific Co. (Norcross, GA).

Cell Preparation. Neutrophils, eosinophils, monocytes, and platelets were isolated from the peripheral blood of healthy donors after overnight fasting. Neutrophils were prepared by dextran sedimentation followed by centrifugation over Ficoll (density 1.077), as previously described (25). Eosinophils were purified by a negative selection technique reported by Hansel et al. using immunomagnetic beads (Perseptive Diagnostics, Cambridge, MA) coated with a mAb anti-CD16 (3G8 monoclonal, a generous gift from Dr. Jay Unkeless, Mount Sinai School of Medicine, NY) (26). Monocytes were isolated from the mononuclear cell fraction floating over the Ficoll gradient (11). Mononuclear cells were suspended (10^6/ml) in RPMI 1640 containing 2 mM glutamine, 100 μg/ml penicillin, and 100 μg/ml streptomycin and were incubated for 4 h in plastic dishes at 37°C in 5% CO_2. After the incubation, nonadherent cells were removed by washing the plates twice with RPMI 1640. Adherent cells were identified as >90% monocytes by α-naphthyl acetate esterase staining. Contaminating platelets adhering to the surface of monocytes at the end of the incubation were <2% as visualized by phase-contrast microscopy. Platelets were isolated from platelet-rich plasma, as previously described (27). Lung macrophages and mast cells were obtained from the lung parenchyma of patients undergoing thoracic surgery according to previously described techniques (28). Briefly, macrophages were isolated by flotation over discontinuous Percoll gradients followed by adherence to plastic dishes. Macrophage purity was determined by α-naphthyl acetate es-

Table 1. *Total Arachidonate Content of Human Inflammatory Cells*

Cells	*n*	Purity range	AA
		%	*nmoles/10^6 cells*
Blood cells			
Monocyte	3	90–95	5.4 ± 1.1
Platelet	5	98–100	0.01 ± 0.004
Neutrophil	4	97–99	1.3 ± 0.1
Eosinophil	4	91–96	3.7 ± 0.3
Tissue lung cells			
Mast cell	4	85–92	8.6 ± 2.4
Macrophage	5	93–97	5.3 ± 0.5

Inflammatory cells were isolated as described. Lipids were then extracted by the method of E. G. Bligh and W. J. Dyer (30), and the total AA content in all lipids was measured by NICI-GC/MS.

terase staining. Mast cells were obtained by enzymatic digestion of the lung tissue followed by countercurrent centrifugation elutriation and flotation over Percoll density gradients. Mast cell purity was determined by Alcian blue staining. The number of preparations and the range of purity of cells used in this study are reported in Table 1.

Isolation of Neutrophils from the Bronchoalveolar Lavage (BAL) of Patients with Adult Respiratory Distress Syndrome (ARDS). BAL fluid was obtained from three patients with ARDS. In all three patients, ARDS developed as a sequela of pneumonia. BAL was performed by injecting 3 60-ml aliquots of prewarmed saline. Recovery of the fluid ranged from 50 to 60%. The average number of neutrophils in the BAL fluids was 6.5×10^6 cells/ml with a purity ranging from 48 to 67%. After a brief (15-s) hypotonic lysis to eliminate contaminating erythrocytes, cells were suspended (10^6 cells/ml) in RPMI 1640 without FCS and incubated for 60 min in plastic dishes. This procedure allowed the adherence of macrophages, which were the major contaminating cells of our preparations. At the end of the incubations, nonadherent cells were harvested and counted. The purity of neutrophils after this procedure was 82 ± 2%.

Culture of Neutrophils Supplemented with AA. Immediately after isolation, neutrophils were suspended (2×10^6 cells/ml) in RPMI 1640 containing 1% FCS, 5 μg/ml insulin, 5 μg/ml transferrin, and 5 ng/ml sodium selenite. The cells were supplemented with 10, 50, or 100 μM AA bound to HSA (1 mg/ml). These relatively high concentrations were chosen because much of the AA provided to the cells is rapidly bound by serum components in the culture media. After a 24-h incubation period, cells were harvested and counted, and viability was determined by trypan blue staining. The percentages of cell recovery after culture were as follows (mean ± SE, $n = 4$): 82 ± 5, 79 ± 3, 71 ± 4, and 69 ± 4 for unsupplemented cells and cells supplemented with 10, 50, and 100 μM AA, respectively. The percentage of viable cells among those recovered was similar in each group and ranged from 91 to 97%.

Lipid Bodies Count and Evaluation of Hypodense Cells. Cytoplasmic lipid bodies in neutrophils from normal peripheral blood, neutrophils exposed to exogenous AA, and BAL neutrophils from ARDS patients were stained with Nile red, as previously described (29). Briefly, cells were incubated for 5 min at 22°C in the dark with Nile red (1 μg/ml). The lipid bodies were then visualized under a fluorescent microscope (IM 35; Carl Zeiss Inc., New York) using 488 nm excitation with >510 nm emission. The number of lipid bodies was counted in at least 25 cells of each preparation.

The number of hypodense cells was determined by using a cut-off density of 1.077. Freshly isolated neutrophils and neutrophils supplemented with AA were layered over 3 ml of Ficoll-Paque and centrifuged at 1,200 *g* for 20 min at 4°C. The percentage of hypodense cells was calculated by counting the number of cells remaining on the top of the gradient.

Lipid Extraction and Chromatography. Lipids were extracted from the cell pellets by a modified procedure of E. G. Bligh and W. J. Dyer in which sufficient formic acid was added to lower the pH of the aqueous phase to 3.0 (30). Cells adherent to plastic dishes (monocytes and macrophages) were directly scraped off the plate with a rubber policeman in a mixture of methanol/water (2:1, vol/vol). After the extraction, solvents were removed under a stream of nitrogen, and the lipids were resuspended in chloroform/methanol (1:1, vol/vol). Lipid classes were separated by normal-phase HPLC on silica columns using a mobile phase composed of hexane/2-propanol/ethanol/phosphate buffer (490:367:100:30, vol/vol). After 10 min of the run, the volume of phosphate buffer was increased from 30 to 50 over 5 min. The flow was maintained at 1 ml/min, and 1-ml fractions were collected throughout the run. Absorbance was monitored at 206 nm. Authentic glycerolipid standards were run before each biological sample to determine the retention times of glycerolipid classes.

Neutral lipid classes (mono-, di-, and triglycerides and free AA) were isolated by TLC over layers of silica gel G developed in hexane/diethyl ether/formic acid (90:60:6, vol/vol). Each class was located on the silica gel by parallel runs of standards and was eluted with chloroform/methanol (1:1, vol/vol). The recovery was similar for all neutral lipid classes and was always >80%.

Negative Ion Chemical Ionization–Gas Chromatography/Mass Spectrometry (NICI-GC/MS) of AA and Other Fatty Acids. The mole quantities of AA, oleic acid (OA), and linoleic acid (LA) in each lipid class isolated by normal-phase HPLC or by TLC were determined by NICI-GC/MS. $^2H_8$-AA and $^2H_3$-SA (100 ng each) were added as internal standards to each lipid fraction. Fatty acids were liberated from glycerolipids by base hydrolysis by using 2 M KOH in 75% ethanol (30 min, 60°C). The reaction was stopped by the addition of an equal amount of water, and the reaction mixture was adjusted to pH 3.0 with 6-N hydrochloric acid. Free fatty acids were extracted with diethyl ether. Recovery of internal standards was >80%. Fatty acids were then converted to pentafluorobenzylesters, and the mole quantities of fatty acids were determined by combined NICI-GC/MS by using a mass spectrometer (model 5989; Hewlett-Packard Co., Palo Alto, CA), as previously described (24). Carboxylate anions (m/z) at 279, 281, 286, 303, and 311 for LA, OA, $^2H_3$-SA, AA, and $^2H_8$-AA, respectively, were measured in the selected ion monitoring mode. The typical area beneath the peak was 2.0×10^5 to 5.0×10^5 for $^2H_8$-AA and 9.0×10^5 to 1.2×10^6 for $^2H_3$-SA.

Results

AA Content and Distribution in Phospholipid and Neutral Lipid Pools of Blood and Lung Tissue Inflammatory Cells. Initial experiments were performed to determine the total AA content and the distribution of endogenous AA in the glycerolipid classes of human inflammatory cells isolated either from blood (neutrophils, eosinophils, platelets, and monocytes) or from the lung tissue (mast cells and macrophages). Table 1 shows the number of preparations, the cell purity, and the total AA content in the six cells examined. The cellular content of AA within the cells was highly variable, with amounts ranging from 8.6 ± 2.4 nmoles/10^6 mast cells to 0.01 ± 0.004 nmoles/10^6 platelets. The distribution of AA in the major glycerolipid classes of the six cells is shown in Fig. 1. In all cells, phosphatidylethanolamine (PE) and phosphatidylcholine (PC) were the major AA-containing phospholipid pools, and PE was the predominant pool in neutrophils, monocytes, and platelets. As previously reported, the macrophage was the only cell in which AA could be measured in bis-monoacyl-glyceryl phosphate, an unusual phospholipid derived from phosphatidylglycerol (24). In all cells, other classes of phospholipids, including lysophospholipids, sphingomyelin, cardiolipin, and phosphatidic acid, contained a negligible amount of AA (data not shown). In contrast with the relative homogeneity of AA distribution within the phospholipid classes among the

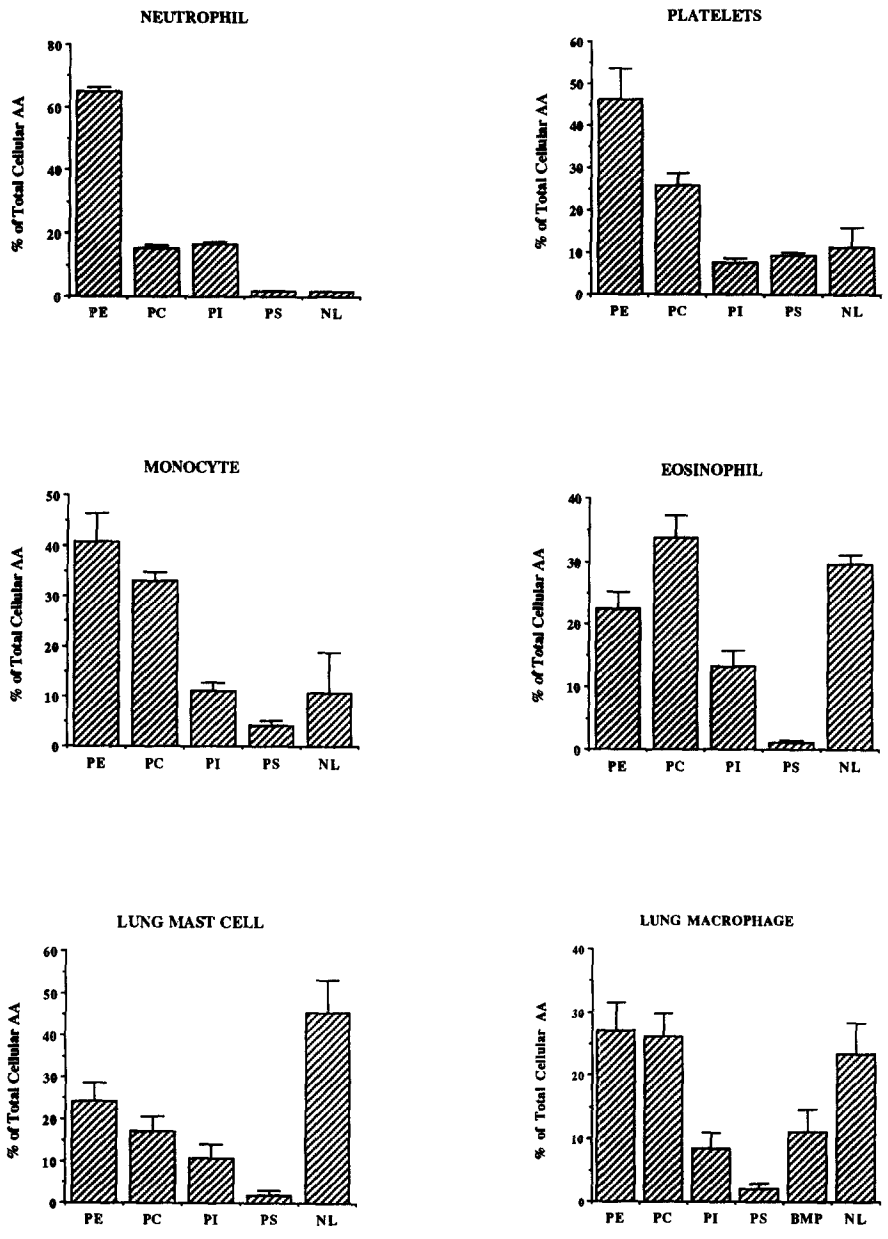

Figure 1. Distribution of AA in the glycerolipid classes of blood inflammatory cells (monocytes, platelets, neutrophils, and eosinophils) and of lung tissue inflammatory cells (mast cells and macrophages). Cells were isolated and purified as described in Materials and Methods. Lipids were extracted and separated by normal-phase HPLC. The quantity of AA in each glycerolipid class was measured by NICI-GC/MS. The results are expressed as the percentage of total cellular AA and are the mean ± SE of the number of preparations indicated in Table 1. *BMP*, bis-monoacylglyceryl phosphate.

different cell types, there was a wide variation in the amount of AA associated with neutral lipids. In particular, 45 ± 9 and 23 ± 6% of the total cellular AA was esterified in the neutral lipids of mast cells and macrophages, respectively, whereas only 3.0 ± 0.5% of cellular AA was found in this class in neutrophils.

The neutral lipid fraction from mammalian cells is generally composed of various molecules including mono-, di-, and triglycerides and unesterified fatty acids. To determine which of these neutral lipid classes contained AA, we isolated the neutral lipids of mast cells and macrophages by TLC and measured the mole quantities of AA into each class by GC/MS (Table 2). Direct mass measurements of neutral lipids in these two cell types indicated that most (~80%) of AA was esterified in TG.

Two important observations were made from these initial experiments. First, cells that have a high content of cellular AA distribute a larger portion of their AA into a TG pool. Second, inflammatory cells found in the lung (mast cells and macrophages) contain large quantities of AA and a large pool of AA in TG.

AA Content and Distribution in Neutrophils Isolated from the BAL Fluid of Patients with ARDS. The data reported above indicated that inflammatory cells resident in the lung contain both large amounts of total cellular AA and a high amount of AA esterified into TG when compared with most cells circulating in the blood. We therefore hypothesized that cell migration into the lung is associated with changes in AA content and distribution. To test this hypothesis directly, we studied neutrophils, a cell in the blood

Table 2. *Distribution of AA in Neutral Lipid Classes of Mast Cells and Macrophages*

Neutral lipids	Percentage of AA in neutral lipids	
	Lung mast cell	Lung macrophage
Monoglycerides	2.1	3.8
Diglycerides	2.5	6.3
Triglycerides	83.3	81.3
Free AA	12.1	8.6

Lung mast cells and macrophages were purified from human lung tissue as described in Materials and Methods. Lipids were extracted and the neutral lipid classes were isolated by TLC. The amount of AA in each neutral lipid class was determined by NICI-GC/MS. The data are expressed as the percentage of the total neutral lipid–associated AA and are the mean of two preparations of mast cells and two preparations of macrophages.

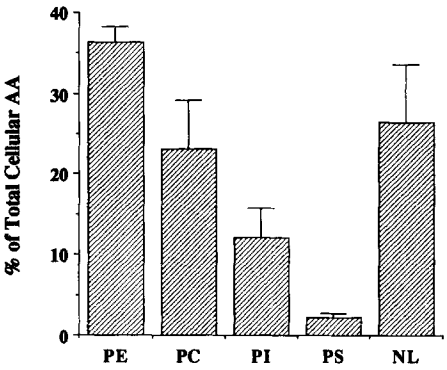

Figure 2. Distribution of AA in the glycerolipid classes of neutrophils isolated from BAL of three patients with ARDS. Cells were isolated and purified as described in Materials and Methods. Lipids were extracted and separated by normal-phase HPLC. The quantity of AA in each class was measured by NICI-GC/MS. The results are expressed as the percentage of total cellular AA and are the mean ± SE of three experiments.

that has a low amount of cellular AA and a small pool of AA in TG, in an in vivo model of migration into the lung. In this set of experiments, neutrophils were isolated from the BAL fluid of three patients with ARDS. This syndrome is characterized by severe respiratory distress, hypoxemia, and lung edema. Almost invariably, patients with ARDS have a marked neutrophil infiltration of the airways (31).

Total AA content and distribution of AA among glycerolipid classes was determined in neutrophils isolated from BAL of ARDS patients. The average content of cellular AA in these cells was 4.3 ± 1.2 nmoles/10^6 cells, a value almost fourfold higher than that found in peripheral blood neutrophils from healthy donors (1.3 ± 0.4 nmoles/10^6 cells). Fig. 2 shows the distribution of AA in glycerolipid classes of BAL neutrophils from ARDS patients. In all patients, the amount of AA in the neutral lipid pool was ~30-fold larger than it was in peripheral blood neutrophils from healthy donors (Fig. 1). In one of the three patients, the TG pool was the largest AA-containing glycerolipid class in the BAL neutrophils. Further analysis of the fatty acids in the TG pool indicated that other fatty acids, in addition to AA, were increased in this pool in the BAL neutrophils. Table 3 shows that the mole quantities of LA, OA, and AA in TG of BAL neutrophils increased 7-, 9-, and 13-fold, respectively, when compared with peripheral neutrophils from normal donors. Moreover, the ratios of LA to AA and OA to AA were significantly reduced in TG of BAL neutrophils, suggesting some selectivity for AA incorporation into TG.

It has been recognized for several years that the density of eosinophils and neutrophils changes as they migrate into the lung tissue (32–34). In addition, an increased number of cytoplasmic lipid bodies has been associated with the in vitro formation of hypodense neutrophils and eosinophils (35, 36). Therefore, we hypothesized that cytoplasmic lipid bodies could be the cellular site of the TG pool of AA. If this were the case, neutrophils from ARDS patients would be expected to contain an increased number of lipid bodies as compared with blood neutrophils. This hypothesis was addressed in the next set of experiments. Fig. 3 shows the microscopic appearance of lipid bodies stained with fluorescent Nile red in neutrophils freshly isolated from the BAL fluid or from peripheral blood of a normal donor. Cytoplasmic lipid bodies were significantly more numerous in BAL neutrophils from ARDS patients than they were in normal peripheral blood cells (8 ± 3 vs 2 ± 1/cell, respectively). We postulated that a key mechanism leading to the increase in total cellular AA, in AA associated with TG,

Table 3. *Fatty Acid Content of Neutral Lipids of Normal Blood Neutrophils and BAL Neutrophils*

Neutrophils	LA	OA	AA	Ratio LA/AA	Ratio OA/AA
		nmols/10^6 cells			
Blood neutrophils	0.12 ± 0.02	0.53 ± 0.09	0.06 ± 0.02	3.35 ± 1.83	12.26 ± 4.63
BAL neutrophils	0.84 ± 0.25	4.81 ± 1.44	0.78 ± 0.14	1.05 ± 0.18	5.84 ± 1.02

Peripheral blood neutrophils and neutrophils from BAL of ARDS patients were isolated as described in Materials and Methods. The neutral lipids were isolated by normal-phase HPLC, and fatty acids were isolated by base hydrolysis. The mole quantities of LA, OA, and AA were determined by NICI-GC/MS. The data are expressed as nmoles of fatty acid/10^6 cell and are the mean ± SE of three experiments.

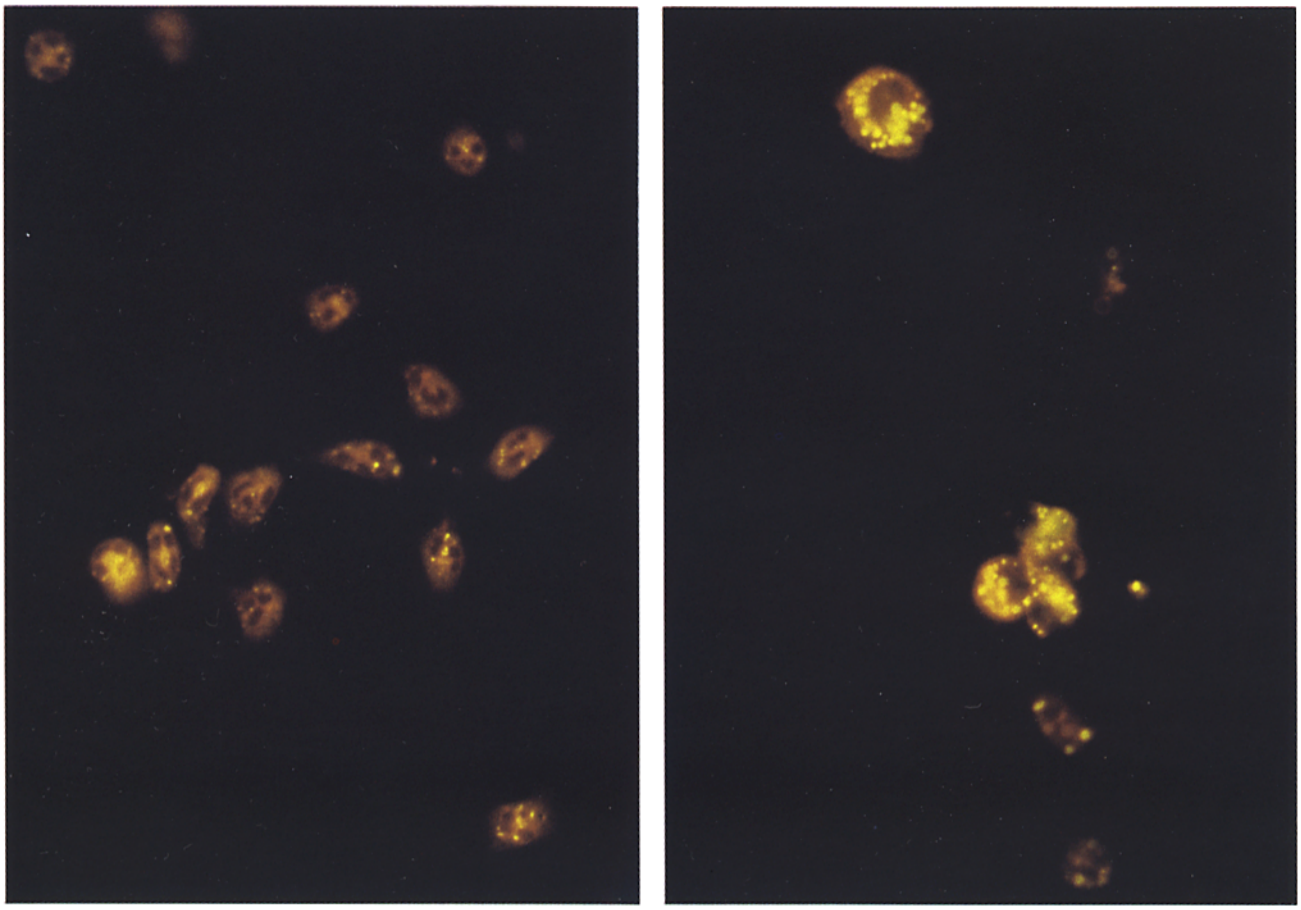

Figure 3. Fluorescent microphotograph of cytoplasmic lipid bodies in neutrophils isolated from the blood of a normal volunteer (*left*) and from the BAL of a patient with ARDS (*right*). ×20.

and in the number of lipid bodies in the lung cells may be the exposure to high concentrations of fatty acids. To begin to test this hypothesis in ARDS patients, the levels of unsaturated fatty acids were measured in the cell-free BAL fluids by GC/MS. BAL fluids from ARDS patients contained 0.08 ± 0.03 nmol/ml of AA, a value almost threefold higher than that found in six BAL fluids from normal volunteers (0.03 ± 0.01 nmol/ml).

Influence of the Extracellular Concentration of AA on the Amount of AA Esterified into TG in Blood Neutrophils In Vitro. The data obtained in vivo in patients with ARDS strengthened the hypothesis that inflammatory cells migrating in the lung tissue had an increased content of AA and a larger pool of AA in TG as well as an increased number of lipid bodies. Furthermore, these data suggested that exposure of the cells to high concentrations of extracellular AA could contribute to these biochemical and morphological changes. To further test this hypothesis, an in vitro model was developed to determine whether raising the intracellular levels of AA would result in the accumulation of AA into TG. In these experiments, we incubated the neutrophils in culture for 24 h with increasing concentrations of exogenous AA (10–100 μM). Fig. 4 shows that exposure of neutrophils to exogenous AA induced a dose-dependent increase in the quantity of AA found in neutral lipids. Previous studies demonstrated that the bulk of AA in this neutral lipid fraction was in TG (37, 38). TG became the major AA-containing glycerolipid in neutrophils supplemented with 50 or 100 μM AA. Unsupplemented levels of AA in TG increased by as much as 10-fold in supplemented neutrophils. The only phospholipid class that significantly increased its AA content was PC. In contrast, the AA content of PE, phosphatidylinositol (PI), or phosphatidylserine (PS) was not changed by the supplementation procedure.

To confirm that the presence of AA in the TG pool was associated with cytoplasmic lipid bodies and with the hypodense phenotype, neutrophils were exposed in vitro to various concentrations of exogenous AA, and the number of lipid bodies together with the percentage of hypodense cells was measured (Table 4). These data show that AA induces a significant increase in the number of lipid bodies per cell. Furthermore, supplementation with AA also increased the percentage of hypodense neutrophils in a dose-dependent fashion so that >50% of neutrophils exposed to 100 μM AA for 24 h were hypodense.

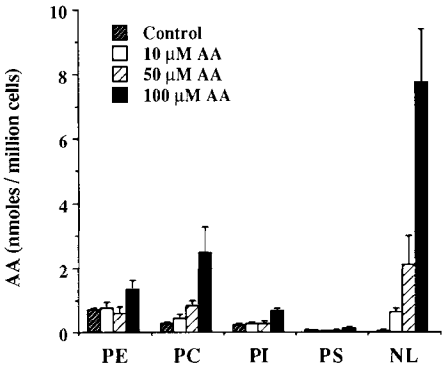

Figure 4. Distribution of AA in the glycerolipid classes of neutrophils isolated from the blood of normal donors and supplemented in vitro with increasing concentrations of AA for 24 h. At the end of the incubation, cells were harvested, and then lipids were extracted and separated by normal-phase HPLC. The quantity of AA in each class was measured by NICI-GC/MS. The results are expressed as nanomoles of AA/10^6 cells and are the mean ± SE of four experiments.

Table 4. *Effect of AA Supplement of Cellular AA Content, Number of Lipid Bodies, and Formation of Hypodense Cells*

Supplement	AA content	Lipid bodies	Hypodense cells
	nmoles/10^6 cells	*No./cell*	*%*
None	1.17 ± 0.21	3.1 ± 0.6	23.5 ± 3.9
10 μM AA	2.11 ± 0.47	6.4 ± 1.3	31.8 ± 5.7
50 μM AA	3.87 ± 1.12	12.9 ± 3.4	46.3 ± 8.5
100 μM AA	12.43 ± 3.73	17.9 ± 4.5	52.5 ± 2.4

Neutrophils from peripheral blood of healthy donors were isolated and incubated with various concentrations of AA. At the end of the incubation, the cells were harvested and lipids were extracted. Total cellular AA was measured by NICI-GC/MS. Lipid bodies were stained with Nile red and counted under a fluorescent microscope. Hypodense cells were determined after centrifugation over Ficoll-Paque density gradients (density 1.077). These data are the mean ± SE of four experiments.

Discussion

Results in this study support the hypothesis that migration of inflammatory cells into the lung or other sites where there are large concentrations of AA induces a fundamental and consistent remodeling of AA pools, leading to morphologic and densitometric changes in the cells. This postulate is supported by the following five lines of evidence. First, cells reaching their maturation or moving into the lung (mast cells, macrophages, and lung neutrophils) contain larger quantities of cellular AA than most inflammatory cells circulating in the blood (eosinophils, neutrophils, and platelets). Second, resident lung cells contain a large amount of their AA esterified into TG. In fact, this pool contains the bulk of AA in all glycerolipids (including phospholipids) in the human lung mast cells. Third, blood inflammatory cells like the neutrophils, which do not normally contain a significant TG pool, acquire large quantities of AA in TG as they migrate into the lung in vivo. Fourth, the acquisition of AA into the TG pool is associated with an increased number of cytoplasmic lipid bodies and with a reduction in cell density, as determined by Ficoll-Paque density gradients. Fifth, the accumulation of AA in TG, an increase in the number of lipid bodies, and a reduction in cell density can all be seen simultaneously in vitro by exposure of the cells to high concentrations of AA.

Over the last 5 yr, several studies have focused on the cellular and functional characterization of inflammatory cells as they move into tissues. For example, in asthma, the appearance of hypodense eosinophils in the lung has been correlated to disease severity (39). However, the precise toxic role that these cells have in diseases such as asthma has not been clearly established (40). It is known that lung inflammatory cells have a different capacity to synthesize oxygenase-derived metabolites of AA (eicosanoids) when compared with their respective blood counterparts (9–11, 40). The current finding that inflammatory cells that either reside in or migrate into the lung have fundamentally different glycerolipid pools of AA raises important questions as to the role of these pools in AA metabolism.

One hypothesis is that AA-containing TG in lipid bodies are major sources of AA for eicosanoids. This postulate is supported by recent studies by A. M. Dvorak and colleagues that have demonstrated that lipid bodies contain prostaglandin G/H synthase, the key enzyme responsible for prostaglandins, and thromboxane biosynthesis (41–43). However, it is not known whether there is an enzyme(s) (TG lipase) in these cellular organelles that mobilizes AA in response to cell activation from the large reservoir of TG. This activity would be necessary to initiate eicosanoid biosynthesis.

An alternative hypothesis is that TG serves as an expandable pool of AA to protect cells and tissues from extremely high concentrations of AA. For example, BAL fluids from the ARDS patients in our study contained 30 ng/ml of unesterified AA. It has been calculated that the amount of saline injected into the bronchus to perform BAL would dilute the alveolar lining fluid ~50- to 100-fold (44). Thus, the concentrations of free AA to which the lung neutrophils were exposed may range from 5 to 10 μM. Tissue damage could be extensive if inflammatory cells used such a large amount of AA to synthesize proinflammatory eicosanoids. Tissue cells may have the capacity to remove large quantities of unesterified AA from tissues by using the TG pool. In the case of ARDS, neutrophils appear to be loaded with AA in TG and with cytoplasmic lipid bodies. In fact, they have an AA content in their TG pool that is increased ~30-fold, with little change in the AA content of phospholipids. This hypothesis is further supported by our in vitro studies, which demonstrate a direct correlation between the exposure of neutrophils to different concentrations of AA and the appearance of AA in TG as well as the number of lipid bodies.

Finally, a third hypothesis suggests that the TG pool of AA represents a biochemical marker for inflammatory cells exposed to prolonged stimulation (24). In this case, TG may act as a pool to recapture AA released intracellularly during pathophysiological conditions in which a massive activation of phospholipase A_2 or other AA-mobilizing enzymes occurs. This hypothesis is supported by the observation that AA content in TG increases in rat hearts during myocardial ischemia, a condition generally associated with an intense AA mobilization from the phospholipid pools (45, 46).

The biochemical pathway responsible for the appearance of AA-containing TG has not been elucidated. However, the current findings suggest that the size of TG may be rapidly expanded in tissue cells, since the content of fatty acids other than AA, such as LA and OA, also increased in TG of BAL neutrophils from ARDS patients. Previous studies from this and other laboratories have suggested that AA-containing TG can be rapidly synthesized in neutrophils and HL-60 cells by de novo glycerolipid biosynthesis (37, 47). This pathway of formation of TG is supported by the observation that a key intermediate, 1,2-diarachidonoyl-*sn*-glycerol, can be isolated when neutrophils are exposed to high concentrations of AA. Neutrophils supplemented with AA also produce 1,2-diarachidonoyl-*sn*-glycero-3-phosphocholine. Both 1,2-diarachidonoyl-*sn*-glycerol and 1,2-diarachidonoyl-*sn*-glycero-3-phosphocholine are likely products of the de novo glycerolipid biosynthesis and not of the deacylation–reacylation pathway.

In conclusion, this paper centers on the observation that inflammatory cells that reside in or move into the lung have different glycerolipid pools of AA than inflammatory cells circulating in the blood. This remodeling of AA pools may represent a crucial event that alters AA metabolism during inflammatory diseases of the lung such as ARDS and asthma.

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