Ceruloplasmin is known to inhibit lipid peroxidation, however, in our system (Fig 1), the azide-sensitive inhibitory activity could not be detected when amounts of plasma below 15-20 μl were used. When extracts from leukocyte lysosomes were combined with 10 μl of plasma prior to initiation of peroxidation, inhibition was readily observed. This activity requires factors from both plasma and lysosomal extract since neither fraction alone could inhibit peroxidation. The levels of this activity were measured in 10 patients with a clinical diagnosis of emphysema and in 12 normal subjects. A single lysosomal extract was utilized to determine levels of the plasma factor (Fig 1A) and a single plasma to determine the levels of the lysosomal factor (Fig 1B). Finally, levels of inhibition were determined using the autologous plasma and lysosomal extract (Fig 1C). Figure 1A shows that the plasma factor activity from 2 patients with emphysema was reduced by 50% of the mean value of the normal subjects (40.2 ± 6.9% inhibition). Excluding the 2 deficient patients (21.3 ± 1.4%), the mean inhibition of the remaining 8 (43.5 ± 9.2%) was identical to that of the normal subjects. Autologous lysosomal extract was available on only one of the deficient patients. The lysosomal factor from this patient also was reduced (12.2 ± 0.7% compared to the mean value of the normal population of 40.1 ± 3.6% Fig 1B). The net effect in the autologous system (Fig 1C) was an almost undetectable level of inhibition. The lysosomal factor from the remaining patients was significantly elevated (46.8 ± 4.8%, p <0.01, t-test, t = 3.66) when compared to the normal subjects (Fig 1B).

The peroxidation inhibitor from the plasma of 1 normal subject and from 1 patient with COPD was analyzed by Sephadex G-50 column chromatography (Fig 2). This figure provides confirmatory evidence that the plasma factor is not ceruloplasmin. Antioxidant activity eluted with an M, of approximately 700. Preliminary studies using PMN lysosomal extracts indicate that the white cell factor also is of low M, (c<1,000). Citrate, used as an anticoagulant, and triton X-100 and sucrose, used during extraction of lysosomes, have no inhibitory effect in the assay. At present we do not know the identity of the plasma or lysosomal extract peroxidation inhibitors.

All normal subjects tested in this study had normal α1 Pi levels and phenotypes. The patient population included 6 PiMM, 2 PiMZ, and 2 PiZZ individuals. Two of the patients with emphysema had reduced plasma factor activity (approximately 50% of normal). The α1 Pi of these patients was identified as PiMM by isoelectric focusing. Their α1 Pi levels (functional measurement) were in the normal range. It is possible that an abnormality or deficiency in antioxidant systems in the body may contribute to the pathogenesis of emphysema in some patients, particularly in those individuals with normal levels of serum and lung protease inhibitors.

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Fibroblasts: Important Producers and Targets of Inflammatory Prostaglandins in the Lungs*


Prostaglandins are thought to be important mediators of immune and inflammatory responses within the lungs. Since fibroblasts comprise nearly one-third of lung parenchymal cells, the ability of lung fibroblasts to produce and/or respond to prostaglandins could be an important aspect of lung inflammatory mechanisms. To test this hypothesis, cultured human fetal lung fibroblasts (HFL-1) were tested for their ability to produce prostaglandins, both in unstimulated culture and in response to bradykinin, an inflammatory mediator known to affect prostaglandin production. The production of PGE1 and 6-keto-F1α, a metabolite of PGI2 by HFL-1 cells was monitored using a radioimmunoassay. Bradykinin (0.2μM) induced a greater than 5-fold increase in the production of these prostaglandins (p <0.01, all comparisons

*From the National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda.
to control). To evaluate whether such prostaglandins can alter lung connective tissue secretion by lung fibroblasts, the ability of prostaglandins to modulate the secretion of collagen and fibronectin by HFL-1 cells was determined using an enzyme immunoassay for human type I collagen and for human fibronectin. PGE, and PGE, caused a 29 ± 6% reduction in type I collagen secretion but, in contrast, a 39 ± 7% increase in fibronectin secretion (p < 0.01, all comparisons to control). In contrast, PCF, (a prostaglandin which frequently has effects opposite those of PGE, and PGE,) had no effect on type I collagen secretion (99 ± 7% of control, p > 0.2) but caused a 20 ± 3% reduction in fibronectin secretion (p < 0.05). Thus, fibroblasts are capable of specific modulation of connective macromolecule secretion in response to prostaglandins. Both through the production of prostaglandins and as a target for these mediators, lung fibroblasts play an important role in the cellular response to lung inflammation.

Lipoprotein Profiles in Sheep Plasma and Lung Lymph

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The lung has long been recognized as an organ active in lipid metabolism. This is hardly surprising when one considers the large quantities of lipids needed for the extensive cellular plasmalemmal membranes making up the alveolar capillary interface and for lung surfactant biosynthetic processes. Although previous studies have implicated that the lung can hydrolyze circulating triglycerides, very few data are available concerning the uptake and metabolism of circulating lipoproteins by the lung.

The sheep caudal mediastinal lymph node cannulation preparation provides access to an extravascular compartment believed to be quite similar to that present in the lung interstitial fluid. In addition, this system yields sufficient lymph for studies on lung lipoprotein distribution, structure and composition (including apolipoproteins). In the present study, ultracentrifugal analysis of sheep plasma and lung lymph were carried out as a first step in the systematic characterization of lung lymph lipoproteins.

Five adult female range sheep weighing 40-50 kg. underwent cannulation and externalization of the effluent duct of the caudal mediastinal lymph node. A cannula in the superior vena cava via the left external jugular vein provided for venous blood sampling.

Lymph and plasma total protein, albumin, triglyceride and cholesterol levels were determined by standard laboratory methods. After 8-fold concentration and preliminary preparative ultracentrifugation at 40,000 rpm to obtain various lipoprotein fractions, lipoprotein concentrations and distributions were determined by computerized analysis of data from schlieren patterns of analytic ultracentrifugal runs at 52,640 rpm of the isolated low density lipoprotein (LDL) in a baseline solution of density 1.20 g/ml. Isolated plasma and lymph LDL and HDL were negatively stained and examined by electron microscopy (EM), particle sizes (200 particles per fraction) being obtained using a computerized program and digitizer which calculates particle diameters.

A summary of the plasma and lymph protein, triglyceride, cholesterol, HDL and LDL concentrations is shown in Table 1. The values for albumin and globulin concentrations and for the lung lymph/plasma ratios (0.82 for albumin and 0.56 for globulin) are in approximate agreement with those in the literature. The mean plasma triglyceride values of 9.1 ± 4.6 mg/dl is approximately 1/4 that of man and is consistent with the very low chylomicrons and very low density lipoproteins (VLDL) of sheep blood. Likewise, the mean concentration of plasma cholesterol of 43 ± 10 mg/dl is only approximately 1/4 that of man. The mean triglyceride and cholesterol concentrations for lung lymph of 6.5 ± 1.5 mg/ml and 18.3 ± 4.7 mg/ml are approximately 71% and 42% of values in the plasma compartment, respectively.

Circulating triglyceride and cholesterol are delivered to tissue and cells in the form of lipoproteins. We thus examined ultracentrifugal patterns of sheep plasma and lymph in order to determine whether lipoprotein distribution is similar in both the vascular and extravascular compartments of the lung. As shown in a representative pattern (Fig.1), plasma LDL (S0-20 consists of a broad peak between S0-18). Lung lymph LDL material is reduced in concentration as compared with plasma, and also forms a broad peak between S0-18. Plasma HDL typically consists of a single slow-moving peak with a mean flotation peak at F1,00 of 2.49 ± 0.26. In contrast, lung lymph HDL is not only reduced in total mass, but also contains a less dense, faster floating component with F1,00 of 7.35 ± 0.67 in addition to the slower component with a peak F1,00 of 2.21 ± 0.29. There was no evidence of VLDL lipoproteins in the S20-400 interval in any of the patterns.

The fast floating components of lung lymph HDL suggest that the lung interstitial HDL are heterogeneous and contain larger and less dense particles than plasma. This possibility was examined by studying the EM features of lung lymph and plasma HDL. As shown in Table 2, which represents an analysis carried out in 2 sheep (300 particle counts per sheep HDL or LDL), plasma HDL consist of relatively uniform, spherical particles with a mean diameter of 94 Å, whereas lung lymph HDL clearly possesses at least 2 distinct populations of spherical particles of approximately 96 Å and 135 Å in

Table 1—Protein, Triglyceride, Cholesterol, LDL and HDL Concentrations in Sheep Plasma and Lung Lymph

<table>
<thead>
<tr>
<th></th>
<th>Albumin (mg/dl)</th>
<th>Globulin (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>HDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>2.8 ± 0.5</td>
<td>3.2 ± 0.6</td>
<td>9.1 ± 4.6</td>
<td>43 ± 10</td>
<td>58 ± 10</td>
<td>100 ± 12</td>
</tr>
<tr>
<td>Lung Lymph</td>
<td>2.3 ± 0.4</td>
<td>1.8 ± 0.2</td>
<td>6.5 ± 1.5</td>
<td>18.3 ± 4.7</td>
<td>30 ± 2</td>
<td>47 ± 8</td>
</tr>
</tbody>
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