Macrophages primed by overnight culture demonstrate a marked stimulation of surfactant protein A degradation

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Bates, Sandra R., J in Xu, Chandra Dodia, and Aron B. Fisher. Macrophages primed by overnight culture demonstrate a marked stimulation of surfactant protein A degradation. Am. J. Physiol. 273 (Lung Cell. Mol. Physiol. 17): L831–L839, 1997.—The current study examined whether long-term culture of macrophages affects their metabolism of surfactant components. Compared with freshly isolated resting macrophages in culture for 1 h, macrophages attached to plastic dishes for 24 h showed evidence of conversion to a “primed” state with 1) an altered morphology characterized by a larger size, ruffled membranes, lamellipodia, and a “foamy” appearance after attachment to glass and 2) a fivefold greater respiratory burst in response to phorbol 12-myristate 13-acetate stimulation. On incubation with iodinated surfactant protein A (SP-A), the 24-h alveolar or tissue macrophages showed a 5- or a 23-fold greater increase in SP-A degradation, respectively, than macrophages cultured for 1 h. Conditioned media experiments demonstrated that the elevated rate of SP-A degradation after prolonged culture was not a result of proteases secreted by the macrophages. Incubation of cells with NH4Cl reduced the degradation of SP-A to a similar extent (to 33% of control values) in resting and primed tissue macrophages. On the other hand, length of time of cell culture did not affect macrophage uptake and degradation of [3H]dipalmitoylphosphatidylcholine in mixed unilamellar liposomes. Thus freshly isolated resting tissue and alveolar macrophages can be primed to specifically increase their rate of SP-A degradation. Activation of macrophages associated with lung disease may be important for SP-A metabolism and surfactant function.

THE ALVEOLAR MACROPHAGE is important in maintaining the ability of the lung to preserve sterility and to respond to injury. These macrophages are derived ultimately from peripheral blood monocytes that have been recruited into the lung tissue by chemotactants. Once in the lung, cytokines promote their maturation into interstitial macrophages, which can then move into the alveolar space in response to appropriate signals (for review see Ref. 25). Alveolar macrophages release a variety of products, including cytokines, enzymes, biologically active lipids, and oxygen metabolites, and have >50 ligand-specific membrane receptors on the cell surface (for review see Ref. 41).

In addition to host defense, evidence is accumulating that alveolar macrophages also play an important role in the turnover of surfactant through uptake and degradation of surfactant components. In vivo studies have documented the presence of tubular myelin figures and surfactant protein A (SP-A) within macrophages lining the alveolar space (36, 45). In vitro studies have demonstrated the ability of alveolar macrophages isolated by lung lavage to degrade phospholipid (32, 48), surfactant protein B (5), and SP-A (6, 48). Macrophage surface receptors specific for SP-A have been described (14, 37, 38) and recently isolated (13). Because SP-A has been shown to modulate phospholipid uptake (4, 43, 47), secretion (18, 40), and catalysis (21) by type II cells, macrophage degradation of SP-A may contribute to the regulation of surfactant levels in the alveoli.

On injury to the lung, the inflammatory cell population of the lung increases (3) and surfactant components are altered (16). Although alveolar macrophages provide the first line of defense in the lower airways, when the system is overwhelmed, materials may pass through a damaged epithelial barrier into the interstitium, where they may be engulfed by interstitial macrophages (for review see Ref. 10). Pulmonary macrophage appearance can be dramatically changed from small, round cells to large “foamy” cells, depending on the etiology of lung damage (44). Such morphological changes may reflect alterations in cell biochemistry that would affect the metabolism of surfactant by these primed or “activated” macrophages and provide a possible mechanism whereby changes in surfactant levels occur. To convert a nonresponsive macrophage to a responsive or “primed” state in vivo, macrophages are elicited by injection of thymocytes or endotoxin (26). In vitro, adherence of human macrophages to protein-coated surfaces primed the macrophages for a massive respiratory burst on exposure to cytokines (35), whereas macrophages adhered to plastic produced less reactive oxygen species (ROS). Thus resting and primed macrophages can be activated on stimulation, but the degree of responses differs quantitatively. We noted that alveolar and tissue macrophages markedly altered their morphological appearance after 24 h of attachment to a culture dish and centrifugation onto glass slides. The cells became large and vacuolated with many pseudopodia, physical characteristics of active macrophages. We compared freshly isolated resting macrophages with 24-h macrophages to determine whether priming by this mechanism alters the uptake and degradation of surfactant components.

METHODS

Lung cell preparation and staining. Alveolar macrophages were isolated from pathogen-free male Sprague-Dawley rats (500 g) by lung lavage, as described previously (5). The alveolar macrophages (4 × 106) were plated in 35-mm plastic dishes (Costar, Cambridge, MA) for 1 h at 37°C in minimal essential medium (MEM) with 10% fetal calf serum (FCS). After 1 h, the cells were washed three or more times to remove...
red blood cells and other nonadherent cells. Ninety-nine percent of the adherent cells were alveolar macrophages (5). The cells were then used for study or refeed MEM containing 10% FCS and were examined 24 h later. Interstitial macrophages were isolated from minced rat lungs. The lungs were perfused, lavaged eight times with 7 ml of calcium-free phosphate-buffered saline containing 10 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, 0.2 mM ethylenediaminetetraacetic acid, and 6 mM glucose, minced with a tissue chopper, shaken in a 37°C water bath for 3 min, filtered through nylon mesh, and centrifuged (7). The tissue macrophages (4 × 10⁵) were transferred to 35-mm plastic dishes (Costar) after 1 h of incubation at 37°C in MEM with 10% FCS. The plates then were washed at least three times to remove nonadherent cells, including red blood cells. The resultant preparation contained <15% nonmacrophage cell types, with lymphocytes as the principal contaminating cell. The cells were studied immediately or refeed MEM containing 10% FCS and were studied 24 h later. After 24 h, ~75% of the alveolar and tissue macrophages had detached from the plastic, and these cells then were washed off the dishes. Only macrophages that remained attached to the dishes were used in experiments, and the viability of these cells was >98% by vital dye exclusion. For light-microscopic examination, the macrophages were scraped from the dish, attached to a glass slide using a cyto spun (Shandon, Pittsburgh, PA), stained using a Kwik-Diff stain kit and visualized by microscopy under oil.

Type II cells were isolated from the lungs of male Sprague-Dawley rats by methods previously described (12). Briefly, after clearance of blood by perfusion, the lavaged lungs were digested with elastase and minced. The crude cell preparation was panned on immunoglobulin G-coated bacteriological plates and plated overnight on 35-mm plastic tissue culture dishes (Costar) at 3 × 10⁴ cells/dish in MEM with 10% FCS at 37°C. Purity of the final preparation was >90%, with macrophages being the primary contaminating cell type.

Surfactant and SP-A isolation. Bronchoalveolar lavage fluid was obtained from normal (slaughterhouse) bovine lungs or patients with alveolar proteinosis. Surfactant was isolated from NaCl-NaBr gradient after centrifugation (20). The bovine and human SP-A were purified from surfactant according to the butanol extraction method described by Hawgood et al. (24). SP-A was iodinated using Iodo-gen (Pierce, Rockford, IL), as described previously (6), and was dialyzed against tris(hydroxymethyl)aminomethane-buffered saline to remove free NaI⁻⁻. An aliquot of ¹²⁵I-SP-A was analyzed for protein concentration and trichloroacetic acid (TCA) precipitability. The specific activity of the radiiodinated SP-A preparations was 200–2,000 cpm/µg, and 97% of the labeled SP-A was precipitable with TCA. The ligand was stored and used within 2 wk of iodination.

The purity of the SP-A preparation was monitored using sodium dodecyl sulfate-polyacrylamide gel electrophoresis according to the method of Laemmli (27), as described previously (6). Autoradiographic analysis of radiolabeled ¹²⁵I-SP-A showed no evidence of degradation products or contaminating proteins. Iodinated SP-A was added to surfactant with gentle mixing and placed on ice for 30 min before use. The ¹²⁵I-labeled bovine SP-A was reconstituted with surfactant at a labeled protein-to-surfactant phospholipid ratio of 1:100 and a labeled SP-A protein-to-unlabeled SP-A protein in surfactant ratio of 1:17, assuming that the amount of SP-A in bovine surfactant was 40% of total protein (6).

Interaction of SP-A with cells. Cells were incubated with radiolabeled SP-A for the indicated time period at 37°C. To terminate the experiment, the medium was removed and processed as outlined below. The cells were washed three times with MEM and twice with phosphate-buffered saline to remove unbound ligand. The cells were then dissolved in 0.2 N NaOH (total cell-associated SP-A). Aliquots were taken to measure radioactivity and protein (30).

The medium from the experiment was centrifuged to remove any cells and precipitated with cold 10% TCA. Insulin was added to serve as a carrier protein. The intact precipitated protein was removed by centrifugation, and the supernatant was treated with KI and H₂O₂ and was extracted with chloroform to remove any free labeled iodide, as described previously (6). An aliquot of the aqueous fraction of the ¹²⁵I-labeled TCA-soluble, chloroform-extracted supernatant containing ¹²⁵I-labeled amino acids and/or small protein fragments was counted (5, 23). This material represents SP-A degradation products released from the cell into the culture media (23), as outlined in detail previously (5). SP-A protein association or degradation by the cells was calculated from the radioactivity and protein in the cell extract and the specific activity of the SP-A. Medium with ¹²⁵I-SP-A was incubated on empty dishes in each experiment, and any ¹²⁵I counts were subtracted from the radioactivity found in the presence of cells. Radioactive iodine counts from empty dishes found in the NaOH were <10%, and those in the media as degradation products were <20%. Separate dishes of cells were used to determine the amount of cellular protein per dish present at the initiation of each experiment and served as the denominator in the quantitation of the amount of SP-A degraded per milligram of protein, since experimental manipulations caused detachment of a fraction of the cells. Because of the variation in cellular metabolism between experiments, the extent of cell association and degradation (as ng SP-A/mg cell protein) at a specified concentration of SP-A or time of experiment was set equal to 100% (control) and the remaining data were expressed as a percentage of control. This allowed comparison of data from several experiments.

For conditioned media experiments, macrophages were incubated with medium for 2 h, the medium was centrifuged to remove detached cells, and the conditioned medium was transferred to empty plastic dishes (35 mm; Costar). Fresh medium was added to the macrophages and to other empty dishes. ¹²⁵I-labeled bovine SP-A (8.6 µg/ml in surfactant) was added to all dishes, and the cells were incubated for 2 h. Then the medium was removed and assayed for SP-A degradation products. Degradation that occurred with fresh medium on empty dishes was subtracted from the data.
Measurement of ROS production. ROS generation was followed using the fluorescent detection of dichlorofluorescein (DCF), formed by the oxidation of the nonfluorescent precursor DCFH (DCFH-DA) according to the method described by Cathcart et al. (11). 2',7'-Dichlorofluorescin diacetate (1 mM; Eastman Kodak, Rochester, NY) in ethanol was hydrolyzed to DCFH with 0.01 N NaOH at room temperature for 30 min and neutralized by a 1:40 dilution in Krebs-Ringer buffer. Macrophages were incubated for 20 min in 2 ml of Krebs-Ringer buffer containing 5 µM DCFH, 12.5 µg/ml horseradish peroxidase (Sigma Chemical, St. Louis, MO), and 1 mM glucose with or without PMA at 50 ng/ml. DCF fluorescence was measured on a Hitachi fluorescence spectrophotometer using the excitation and emission wavelengths of 490 and 530 nm, respectively. By use of H₂O₂ as a standard, the assay was linear from 100 nM to 1 µM H₂O₂. The rate of ROS formation was continuously measured over a 20-min period by analysis of 0.3 ml of media taken at several time points. The rate of ROS production was calculated from the linear portion of the curves following the initial lag phase and was expressed in arbitrary fluorescence units.

Preparation of liposomes. Lipids were obtained from Avanti (Birmingham, AL), and tracer radiolabeled [3H]-methylcholinedipalmitylphosphatidylcholine (DPPC) was from New England Nuclear. The liposomes were composed of a mixture of lipids combined in the ratio of 0.5 mol of DPPC, 0.25 mol of egg phosphatidylcholine (PC), 0.15 mol of cholesterol, and 0.1 mol of egg phosphatidylglycerol and tracer [3H]DPPC (specific activity = 4,400 degradations·min⁻¹·nmol⁻¹). The lipids were dried under nitrogen and were resuspended in buffer. Liposomes were prepared by extrusion through polycarbonate membranes, as previously described (19), and were stored overnight at 4°C.

Uptake and degradation of DPPC by macrophages. Macrophages were incubated with [3H]DPPC-labeled liposomes at 80 µM DPPC (120 µM total PC) for 2 h at 37°C. Cells then were washed three times with MEM, harvested with trypsin (0.05% trypsin in 0.02% EDTA), and pelleted twice by centrifugation. Aliquots were taken for protein, total degradations per minute by scintillation counting, and lipid extraction using the method of Bligh and Dyer (8). The aqueous fraction was analyzed for degradations per minute. One aliquot of the lipid fraction was subjected to thin-layer chromatography on silica gel G plates run in the solvent system CHCl₃-CH₃OH-H₂O-NH₄OH-H₂O (65:35:2.5:2.5 by volume) to separate lysophosphatidylcholine from PC (19, 33). The bands were identified by exposure to I₂ vapor, scraped, and counted for degradations per minute. Another aliquot of the lipid fraction was osmicated and chromatographed on neutral alumina columns to isolate disaturated PC (31). Incorporation into the different fractions was calculated on the basis of the specific activity of DPPC. Unsaturation of PC was determined by subtraction of DPPC from total PC (19).

Statistical analysis. Values are means of at least two samples in each experiment and are presented as means ± SE for the number of experiments indicated (n) or means ± range when the number of experiments was two. Each experiment used a different preparation of macrophages. Statistical analysis was performed using the t-test with SigmaStat for Windows (Jandel, San Rafael, CA), where significance is set at P < 0.05. When SE bars are not visible on Figs. 2 and 3, they are within the symbols.

RESULTS

Light microscopy. Alveolar and tissue macrophages cultured for 1 or 24 h were cytospun onto glass slides, and stained preparations were visualized by light microscopy (Fig. 1). Most of the 1-h alveolar and tissue macrophages (Fig. 1, A and B) were circular, compact cells with darkly staining nuclei, distinct smooth plasma membranes, and few pseudopodia. On the other hand, most of the 24-h macrophages (Fig. 1, C and D) showed morphological indications of activation, such as an enlargement of the cytoplasmic area with many filopodia or lamellipodia, or a vacuolated appearance with a large, irregular, flattened shape and pale nuclei.

ROS release. One measure of the responsive state of macrophages is an increased rate of release of superoxide and H₂O₂ in primed cells compared with quiescent cells when challenged with PMA (26). To examine whether the 24-h macrophages were "primed," the freshly isolated alveolar macrophages (1-h cells) or the macrophages cultured overnight (24-h cells) were incubated without or with PMA (50 ng/ml) in the presence of 5 µM DCFH. The 1-h macrophages showed a low rate of ROS production that was stimulated over threefold by the addition of PMA (Table 1). Incubation of the 24-h macrophages with PMA resulted in a sevenfold increase in ROS production, which was significantly (5-fold) higher than that found after PMA exposure of 1-h macrophages. Similar results were seen with tissue macrophages, although the production of ROS was attenuated compared with the alveolar macrophages. PMA stimulation of the tissue macrophages resulted in a significant enhancement of ROS levels by the 24-h macrophages (2-fold) over that produced by 1-h macrophages.

SP-A metabolism. The cell association and degradation of SP-A by alveolar macrophages either freshly isolated (1 h) or in culture for 24 h are shown in Fig. 2. Cells were incubated for 3 h at 37°C with increasing concentrations of [125I]-SP-A. The media then were removed and assayed for SP-A degradation products. The cells were harvested by dissolution in 0.2 N NaOH to determine the total SP-A associated with the cell, which includes the SP-A bound to the surface of the cell and that internalized into the cell during the 3-h incubation period. The extent of SP-A cell association and degradation for the 1- and 24-h macrophages varied with the SP-A concentration in the medium, as has been reported previously for binding of SP-A to freshly isolated alveolar macrophages (38). Cell association of SP-A, binding plus uptake, was similar for 1- and 24-h alveolar macrophages, whereas degradation of SP-A was significantly enhanced approximately fivefold with overnight culture for all concentrations of SP-A tested. For tissue macrophages (Fig. 3), the cell association of SP-A by the 24-h macrophages was similar to that of freshly isolated cells. However, there was a striking 20-fold increase in the amount of SP-A degradation by tissue macrophages cultured for 24 h compared with macrophages in culture for 1 h.

To directly compare the metabolism of SP-A by alveolar and tissue macrophages with time in culture, the cell association, degradation, and total metabolism of SP-A at one concentration in the media (0.5 µg SP-A/ml) were analyzed with the two types of macrophages present in the same experiment. Incubating the
macrophages on culture dishes for 24 h resulted in a 40% decrease (P < 0.05) or a 70% increase (P = 0.063) in the cell association of SP-A to alveolar or tissue macrophages, respectively, but had a marked stimulatory effect on the rate of SP-A degradation by these cells. There was a 5-fold (alveolar macrophages) or a 23-fold (tissue macrophages) increase in the catabolism of SP-A, resulting in a 3- to 11-fold increase in the total SP-A metabolism (binding, uptake, and degradation) of these cells. Exposure of the 1-h tissue and alveolar macrophages to SP-A reconstituted with surfactant slightly enhanced the degradation of SP-A, as indicated by the increase in the ratio of SP-A degradation to total SP-A metabolism (cf. Tables 2 and 3). However, even in the presence of surfactant, the macrophages in culture for 24 h continued to demonstrate a 6- to 10-fold higher rate of SP-A degradation than the freshly isolated cells. The metabolism of SP-A by tissue and alveolar macrophages was similar, although the 1-h tissue macrophages showed a statistically significantly lower cell association and degradation of SP-A than the alveolar macrophages (Table 2), which was not seen in the presence of surfactant (Table 3). Transition of macrophages from a quiescent to a responsive state with regard to enhancement of SP-A degradation required more than activation of the macrophages. Freshly isolated (1 h) alveolar macrophages were incubated with PMA (50 ng/ml) and 125I-SP-A (0.5 µg/ml) for 3 h. PMA-exposed macrophages showed a slightly lower cell association of SP-A (81 ± 6%) than control cells, whereas degradation of SP-A was reduced to 41 ± 5% (SE) of control values (n = 3). SP-A degradation by alveolar macrophages in culture for 1 h was previously determined to be intracellular.

Table 1. Reactive oxygen species production by macrophages

<table>
<thead>
<tr>
<th>Time in Culture</th>
<th>Alveolar Macrophages</th>
<th>Tissue Macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No additions (A)</td>
<td>PMA (B)</td>
</tr>
<tr>
<td></td>
<td>B/A</td>
<td>No additions (A)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B/A</td>
</tr>
<tr>
<td>1 h</td>
<td>0.82 ± 0.36 (5)</td>
<td>3.08 ± 0.86* (5)</td>
</tr>
<tr>
<td>24 h</td>
<td>2.24 ± 1.10 (5)</td>
<td>16.33 ± 5.38** (5)</td>
</tr>
<tr>
<td>24 h/1 h</td>
<td>2.73</td>
<td>5.30</td>
</tr>
</tbody>
</table>

Values are means ± SE and are expressed in arbitrary fluorescence units·min⁻¹·mg cell protein⁻¹; number of separate experiments performed in duplicate is shown in parentheses. PMA, phorbol 12-myristate 13-acetate. *Significantly different from no additions at P < 0.05. †Significantly different from 1-h macrophages at P < 0.05.
The degradation of SP-A by macrophages was significantly different (P < 0.05) from that for 1-h macrophages at 3, 7, and 9 µg SP-A/ml and for degradation of SP-A at all media concentrations of SP-A.

DISCUSSION

Alveolar macrophages freshly isolated from the lung by lavage have demonstrated the ability to bind, incorporate, and degrade SP-A (6, 37, 38, 48). The present study demonstrates that alveolar and tissue macrophages that have been in culture for 24 h have characteristics of primed macrophages with altered morphology and enhanced respiratory burst on stimulation.
The metabolism of SP-A in surfactant by macrophages after 1 or 24 h in culture is summarized in Table 3.

<table>
<thead>
<tr>
<th>Type</th>
<th>1 h in Culture</th>
<th>24 h in Culture</th>
<th>24 h/1 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cell Deg Total</td>
<td>Deg/Total, %</td>
<td>Cell Deg Total</td>
</tr>
<tr>
<td>Alveolar</td>
<td>23.9 ± 2.3</td>
<td>38.7 ± 4.0</td>
<td>62 ± 3.2</td>
</tr>
<tr>
<td>Tissue</td>
<td>15.5 ± 1.3</td>
<td>12.9 ± 3.0</td>
<td>28.4 ± 3.2</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in ng surfactant protein A (SP-A)/mg cell protein, unless otherwise indicated; n, number of experiments performed in duplicate. Alveolar or tissue macrophages were placed in culture for 1 or 24 h. After they were washed, cells were incubated with 125I-SP-A (0.5 µg/ml) for 3 h and harvested with 0.2 N NaOH. Cell, binding + incorporation; Deg, degradation; Total, total cell metabolism of SP-A. *Significantly different from 1 h in culture at P < 0.05. †Significantly different from alveolar macrophages at P < 0.05.

Furthermore, the 24-h macrophages show a substantially elevated rate of SP-A degradation compared with the fresh macrophages because of intracellular degradation of SP-A. On the other hand, macrophage uptake and degradation of phospholipid liposomes were not altered by prolonged culture time, showing differential regulation of catabolic function.

Pulmonary macrophages are distinguished primarily by their anatomic location in the lung. The alveolar macrophage is found on the surface of the alveoli in contact with the epithelial cells lining the airway and can be isolated by lavage of the alveolar compartment. The interstitial macrophage resides within the lung parenchyma, where it undergoes maturation and differentiation before migrating into the alveoli and becoming the alveolar macrophage. The interstitial macrophage is isolated by mechanical disruption of lavaged lung with or without enzymatic digestion of lung slices. It remains controversial as to whether the postlavage tissue macrophages represent subpopulations of alveolar macrophages or are, in fact, interstitial macrophages analogous to those of other organs because of the lack of distinguishing characteristics between the two cell types. The alveolar and interstitial macrophages share most of the same functional properties of phagocytic activity (28) and fibroblast growth factor production (1), although they exhibit quantitative differences in some functions, such as cytokine and oxygen radical production (29). In the present study, macrophages isolated from lung minced tissue without enzymatic digestion after extensive lung lavage are referred to as tissue macrophages. Quantitative but not qualitative differences in SP-A metabolism and ROS production were noted between the tissue and alveolar macrophages that would indicate that they were different cell types or were at a different stage of maturation.

Freshly isolated alveolar macrophages took up slightly more SP-A than tissue macrophages, whereas alveolar macrophages degraded two to three times as much SP-A. Although both types of pulmonary macrophages increased their rates of SP-A degradation after activation by prolonged time in culture, tissue macrophages demonstrated a greater change with time in culture (23-fold) than the alveolar macrophages (5-fold). On the other hand, ROS production stimulated by PMA was higher in alveolar than in tissue macrophages. However, the phospholipid metabolism of the pulmonary macrophages was quite similar, as reflected in the rates of uptake and degradation of DPPC.

Macrophages are usually studied as freshly isolated cells in suspension or allowed to attach to a dish for a short period of time. The priming of pulmonary macrophages by prolonged culture has not been reported directly, although Prokhorova et al. (39) noted a greater sensitivity to lipopolysaccharide and interferon-γ stimulation of nitric oxide production after overnight culture of interstitial macrophages than in freshly isolated cells, data that would be consistent with an increase in reactive state. In addition, a 24-h exposure of macrophages to serum proteins causes rabbit alveolar macrophages to release reactive oxygen intermediates (22), induces maturation of human monocytes as assessed by increases in intracellular enzymatic activity (34), and stimulates peritoneal macrophage spreading (9). Primed and activated macrophages demonstrate a greater rate of cell metabolism than resting cells, as exemplified by a more rapid spreading on glass, higher glucose oxidation, more effective killing of microorganisms, and elevated content of lysosomal enzymes (42).

In this study, we have shown that alveolar and tissue macrophages show morphological and biochemical evidence of priming when placed in tissue culture over-
macrophages, it could not account for the 10- to 23-fold increase in degradation seen with the tissue macrophages. Finally, the data on the production of ROS provide additional evidence that priming of the cells has occurred during 24 h of culture. If the macrophage population at 1 h contained one-fourth primed cells (equivalent to the 24 h cells) and three-fourths resting cells, then PMA stimulation should have increased ROS production 7.3-fold for 1-h macrophages, which is equivalent to 24-h macrophages. However, the 1-h cells were stimulated only 3.8-fold by PMA exposure.

Prolonged culture of macrophages did not substantially affect the cell association of SP-A but markedly enhanced the degradation of SP-A. The fact that binding plus uptake was not altered suggests that the substrate for the degradative enzymes did not change, and thus the catabolic activity must have increased. Because the results are expressed on a “per milligram of cell protein” basis, the increase in cell size means an increase in enzymatic activity per cell. Such a rise in catabolic activity could occur through several mechanisms, including an increase in enzymatic activity, a change in kinetic properties of degradative enzymes, an augmentation in the amount of enzyme, a change in the type of enzymes, or an alteration in intracellular compartmentation. Activated macrophages have been shown to have enhanced antimicrobial activity (2) and an elevation in the content of lysosomal enzymes (for review see Ref. 41). An enrichment in the level of degradative enzymes would be consistent with the enhanced ability to degrade SP-A shown in our data. The increase in degradative ability required prolonged time in culture, since activation of alveolar macrophages with PMA for 3 h served to reduce, not enhance, SP-A degradation.

Our previous studies using freshly isolated alveolar macrophages (6) indicated that the degradation of SP-A by type II cells and macrophages during 24 h of culture could potentially result in a fourfold increase in catabolism of SP-A on a per milligram of cell protein basis, assuming that all the lost cells were nonresponsive. Although this is close to the 5-fold increase observed for alveolar macrophages during 24 h of culture, it is possible that the culture conditions select for cells primed during the 1-h incubation and that 24 h of culture are not necessary to convert the cells to a more responsive state. Another possibility is that the macrophages during 24 h of culture act as frustrated phagocytes, in that they try to “ingest” the tissue culture dish, which they recognize as foreign material. Several pieces of evidence support the conclusion that prolonged culture is priming the cells, and that, rather than selection, is responsible for their increased activity. Light microscopy indicated that the 24-h macrophages, particularly the alveolar macrophages, have a morphology entirely different from that of the 1-h cells and were typified by enlarged, foamy cells with many lamellipodia. Second, a 75% loss of macrophages from the culture dish, which they recognize as foreign material.

Table 6. Comparison of metabolism of PC liposomes by type II cells and macrophages

<table>
<thead>
<tr>
<th>Fate of PC, % uptake during 2-h incubation</th>
<th>DPPC degradation into [3H]metabolites</th>
<th>Retained as DPPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uptake, µg DPPC/mg cell protein</td>
<td>Aquous and lysoPC</td>
<td>Unsat. PC</td>
</tr>
<tr>
<td>Type II cells</td>
<td>1.19*</td>
<td>45†</td>
</tr>
<tr>
<td>Alveolar macrophages</td>
<td>0.58</td>
<td>49</td>
</tr>
</tbody>
</table>

Pulmonary cells were incubated for 2 h with 0.08 mM [3H]choline-labeled DPPC liposomes. Uptake, amount of DPPC taken up over 2-h time period. Degradation, amount of DPPC recovered as degradation products (aqueous and lysoPC plus unsaturated PC). Reutilization, recovery of [3H]labeled unsaturated phospholipids; because all label was added as [3H]labeled saturated phospholipids, recovery of label in unsaturated phospholipids indicates degradation of saturated PC and reutilization of [3H]labeled products for unsaturated phospholipids. Retained as DPPC, phospholipids recovered as saturated phospholipids; this could be due to retention of saturated phospholipids or reassembly of degradation products into saturated phospholipids. *Data from Table 3 of Chinoy et al. (12) recalculated for DPPC uptake. †Data from Fisher and Dodia (19).
occurred within the cell. The present work determined that this was the case regardless of the state of activation. When the pH within intracellular acidic organelles was raised with a lysosomotropic weak base, breakdown of SP-A was inhibited in all the macrophage populations, supporting an important role for such sites in SP-A degradation by pulmonary macrophages. Furthermore, as the external concentration of SP-A was raised, the extent of SP-A degradation was parallel to the increase in cell association of SP-A, demonstrating the dependence of degradation on the amount of cell-associated substrate. Finally, extracellular enzymes that may have been released by primed macrophages (41) did not participate in SP-A catabolism, since medium conditioned by 24-h macrophages did not degrade SP-A.

It was of interest that, whereas SP-A degradation was markedly altered with time in culture, phospholipid degradation was not affected. Such data imply that, once internalized, degradation of surfactant phospholipid and proteins occurs in separate cellular compartments or that only one set of degradative enzymes was activated. Segregation of DPPC and SP-A after uptake has been noted in the isolated, perfused lung (20) and in morphological (48) and biochemical studies (42) of isolated type II cells. The metabolism of DPPC liposomes by macrophages shares some but not all characteristics of type II cells. Table 6 compares the uptake, reutilization, and degradation of phospholipid liposomes by the two cell types. Whereas type II cells incorporated two times as much PC as macrophages, both cell types actively degraded the phospholipid. Sixty-five percent of the PC incorporated into type II cells was recovered as aqueous degradation products, lysophosphatidylcholine, or unsaturated PC, whereas macrophages degraded 50%. Consistent with the expected low rate of macrophage PC synthesis, there was no evidence of reutilization of the liberated [3H]choline by macrophages, whereas type II cells readily resynthesized unsaturated PC from the choline initially associated with the DPPC. The 20% choline “reutilization” by the type II cells shown in Table 6 represents a lower limit, since it does not include choline reincorporated into DPPC.

In conclusion, we have shown that freshly isolated interstitial and alveolar macrophages were converted to a responsive state during prolonged culture. These macrophages produced ROS and degraded SP-A, but not phospholipid, at an elevated rate. In several physiologic states, including acute endotoxemia, pulmonary interstitial and alveolar macrophages demonstrate enhanced phagocytosis and production of reactive oxygen intermediates (45) and could be more active in their metabolism of SP-A. Although possible effects of various pulmonary diseases on the rate of surfactant lipid and protein turnover by macrophages in intact tissue remain to be determined, the present study demonstrates that the capacity of pulmonary macrophages to degrade SP-A depends on their state of activation.

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REFERENCES


