Pulmonary-specific expression of tumor necrosis factor-α alters surfactant lipid metabolism

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Carroll, James L. Jr., Diann M. McCoy, Stephen E. McGowan, Ronald G. Salome, Alan J. Ryan, and Rama K. Mallampalli. Pulmonary-specific expression of tumor necrosis factor-α alters surfactant lipid metabolism. Am J Physiol Lung Cell Mol Physiol 282: L735–L742, 2002. First published September 28, 2001; 10.1152/ajplung.00120.2001.—Tumor necrosis factor (TNF-α) is a major cytokine implicated in inducing acute and chronic lung injury, conditions associated with surfactant phosphatidylcholine (PtdCho) deficiency. Acutely, TNF-α decreases PtdCho synthesis but stimulates surfactant secretion. To investigate chronic effects of TNF-α, we investigated PtdCho metabolism in a murine transgenic model exhibiting lung-specific TNF-α overexpression. Compared with controls, TNF-α transgenic mice exhibited a discordant pattern of PtdCho metabolism, with a decrease in PtdCho and disaturated PtdCho (DSPtdCho) content in the lung, but increased levels in alveolar lavage. Transgenics had lower activities and increased immunoreactive levels of cytidylyltransferase (CCT), a key PtdCho biosynthetic enzyme. Ceramide, a CCT inhibitor, was elevated, and linoleic acid, a CCT activator, was decreased in transgenics. Radiolabeling studies revealed that alveolar reuptake of DSPtdCho was significantly decreased in transgenic mice. These observations suggest that chronic expression of TNF-α results in a complex pattern of PtdCho metabolism where elevated lavage PtdCho may originate from alveolar inflammatory cells, decreased surfactant reuptake, or altered surfactant secretion. Reduced parenchymal PtdCho synthesis appears to be attributed to CCT enzyme that is physiologically inactivated by ceramide or by diminished availability of activating lipids. Disaturated phosphatidylcholine; choline kinase; choline phosphotransferase

PULMONARY SURFACTANT is a heterogeneous material that is essential for life, consisting of key hydrophobic proteins and lipids that maintain alveolar patency (35). Disaturated phosphatidylcholine (DSPtdCho) is the major surface-active lipid component of surfactant that is produced by the alveolar type II epithelial cell via the CDP-choline pathway. The initial step in this pathway involves phosphorylation of choline to cholinephosphate by the enzyme choline kinase (CK, EC 2.7.1.32). The second step involves conversion of cholinephosphate to CDP-choline. This latter step is a slow, energy-requiring reaction catalyzed by the regulatory enzyme CTP:phosphocholine cytidylyltransferase (CCT, EC 2.7.7.15) (17). The terminal reaction coupling diacylglycerol to CDP-choline is catalyzed by cholinephosphotransferase (CPT, EC 2.7.8.2). To date, most of the interest in PtdCho synthesis has focused on the regulatory mechanisms for CCT because of its important role in controlling intracellular PtdCho content (19, 32).

Tumor necrosis factor-α (TNF-α) is a small polypeptide cytokine released in the lung primarily by alveolar macrophages (6). In addition to diverse effects promoting the acute and chronic inflammatory response, TNF-α also regulates surfactant metabolism. For example, TNF-α inhibits the synthesis of the surfactant apoproteins, induces PtdCho degradation, and decreases the biosynthesis of PtdCho (3, 37, 40). Recently, we demonstrated that TNF-α inhibits PtdCho synthesis both in vitro and in vivo by inhibiting the activity of CCT (21, 36). In contrast to these negative effects of the cytokine on phospholipid synthesis, TNF-α also stimulates PtdCho secretion in primary alveolar epithelial cells (5). Thus TNF-α could simultaneously trigger several events directly related to PtdCho metabolism and processing. Presumably, the net effect of the cytokine on effector pathways such as synthesis, secretion, and intraalveolar processing of PtdCho will likely dictate the availability of surfactant lipid necessary to maintain the integrity of a stable surface-active film.

Although much of the prior work has improved our understanding of how TNF-α affects surfactant lipid metabolism, essentially all studies to date have investigated short-term exposures to the cytokine; long-term effects have not been addressed. Sustained or intermittent TNF-α release is a plausible mechanism in the setting of chronic alveolitis where high TNF-α exists in tissue as a receptor-bound or membrane-associated form (4). This process appears to occur in late-phase acute lung injury (4, 15). Long-term TNF-α exposure

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may also be a feature of chronic lung disorders such as bronchopulmonary dysplasia and idiopathic pulmonary fibrosis, which are also associated with surfactant abnormalities (29, 31).

To investigate long-term expression of TNF-α, Miyazaki et al. (28) generated a transgenic mouse expressing the murine TNF-α gene driven by the human surfactant protein C promoter. In this model, TNF-α mRNA was selectively expressed within the alveolar epithelium. Morphologically, transgenic mice exhibit lymphocytic alveolitis, and by 6 mo of age the mice had features typical of chronic interstitial fibrosis (28). In the present study, we used these mice to investigate long-term effects of TNF-α on PtdCho metabolism. We hypothesized that chronic overexpression of TNF-α will result in a decrease in surfactant synthesis. To test our hypothesis, we measured the sequential enzymatic steps involved in surfactant synthesis and key regulatory lipids in TNF-α transgenics and wild-type littermate controls.

MATERIALS AND METHODS

MATERIALS. Silica LK5D (0.25 mm × 20 cm × 20 cm) TLC plates were purchased from Whatman International ( Maidstone, England). All radiochemicals were purchased from DuPont New England Nuclear Chemicals (Boston, MA). Immunoblotting membranes were obtained from Millipore (Bedford, MA). The enhanced chemiluminescence (ECL) demonstration mixture (0.1 ml volume) contained 100 mM Tris-HCl buffer (pH 8.0), 10 mM magnesium acetate, 0.106 mM [14C]choline [specific activity ~7,000 disintegrations/min (dpm)/nmol], 10 mM ATP, and 50–100 μg of cell sample. After a 1-h incubation at 37°C, the reaction was terminated with 0.02 ml of cold 50% trichloroacetic acid. Twenty-microliter aliquots of the mixture were spotted on Whatman 3MM paper, and choline metabolites were resolved by using paper chromatography as described (21). The spots that comigrated with the radiolabeled standard choline phosphate were cut and used for scintillation counting.

The activity of CT was determined by measuring the rate of incorporation of [methyl-14C]phosphocholine into CDP-choline by a charcoal extraction method (21). All assays were performed without the inclusion of a lipid activator in the reaction mixture. Enzyme-specific activity is expressed as picomoles per minute per milligram of protein or picomoles per minute per unit of enzyme mass.

The activity of CPT was assayed as described (21). Each reaction mixture contained 50 mM Tris-HCl buffer (pH 8.2), 0.1 mg/ml Tween 20, 1 mM 1,2-dioleoylglycerol, 0.8 mM phosphatidylglycerol, 0.5 mM [14C]CDP-choline (specific activity 1,110 dpm/nmol), 5 mM dithiothreitol, 5 mM EDTA, 10 mM MgCl2, and 30–40 μg of sample. The lipid substrate was prepared by combining appropriate amounts of 1,2-dioleoylglycerol (1 mM) and phosphatidylglycerol (0.8 mM) in a test tube, drying under nitrogen gas, and sonicating on ice for 10 min before addition to the assay mixture. The reaction proceeds for 1 h at 37°C and is terminated with 4 ml of methanol-chloroform-water (2:1.7 vol/vol/vol). The remainder of the assay was performed exactly as described (27).

Immunoblot analysis. For immunoblot analysis, equal amounts of microsomal protein were used. Each sample was adjusted to give a final concentration of 60 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, 0.1% bromphenol blue, and 5% β-mercaptoethanol and heated at 100°C for 5 min. Samples were then electrophoresed through a 10% SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride.
membrane. CCT and β-actin were detected by using the ECL Western blotting detection system as instructed by the manufacturer. The dilution factor for anti-CT and anti-β-actin was 1:1,000.

Fatty acid gas chromatography. Lipids were extracted from cell homogenate (1 mg protein) according to the method of Bligh and Dyer (7). Fatty acid methyl esters (FAME) were prepared by transmethylation in the presence of 10% boron trifluoride. The FAME derivatives were separated by gas liquid chromatography and detected using flame ionization detection. The GC column packing was 10% SP-2330 on 100/120 Chromosorb W AW (Supelco).

RESULTS

Phospholipid and DSPtdCho analysis. Transgenic mice exhibiting chronic expression of TNF-α displayed several abnormalities in phospholipid composition compared with control littersmates. Furthermore, a distinctly different pattern of expression of phospholipids was identified in TNF-α transgenics and wild-type mice between the lung parenchyma and alveolar compartment. In whole lung homogenates, total PtdCho levels decreased from 122 ± 5 nmol/mg protein phospholipid phosphorus in controls to 58 ± 2 nmol/mg phospholipid phosphorus in TNF-α transgenics (P < 0.05). There were no other significant differences in other major phospholipids. However, in lung microsomes, TNF-α transgenics almost uniformly contained lower levels of several major phospholipids in the lung. Significantly lower levels of PtdCho, phosphatidylylycerol, and phosphatidylinositol were detected (Fig. 1A).

Because PtdCho is the major lipid of eukaryotic membranes and surfactant, we assayed DSPtdCho content, a marker of surfactant lipid. This was also reduced by 36% (control 105 ± 8 nmol phospholipid phosphorus/mg protein, TNF-α transgenics 67 ± 3 nmol phos-

Sphingolipid analysis. Sphingosine was extracted from microsomal samples (0.25–1 mg protein per sample) plus 200 pmol C20-sphingosine (an internal standard) according to the method of Bligh and Dyer. The chloroform layer was isolated and dried under nitrogen gas. The dried extracts were resuspended in 0.33 ml chloroform and 0.66 ml 0.1 M KOH in methanol and incubated at 37°C for 1 h. The samples were then mixed with 1 ml chloroform and 1 ml 1.0 M NaCl. The chloroform phase was washed with NaCl and dried under nitrogen gas. Ortho-phthalaldehyde derivatives were prepared by dissolving the dried samples in 50 μl methanol, followed by the addition of 50 μl OPA reagent (5 mg o-phthalaldehyde in 100 μl ethanol, 9.9 ml 3% boric acid, and 5 μl 2-mercaptoethanol), incubated at room temperature for 5 min, diluted with methanol-water (94:6 vol/vol), and quantitated by HPLC. Ortho-phthalaldehyde derivatives were separated on a Beckman Ultrasphere C-18 column, with methanol-water (94.6 vol/vol) mobile phase at a rate of 1 ml/min. The derivatives were detected using a Thermoseparation Products Spectra System FL3000 fluorescence detector at 340 nm excitation and 454-nm emission wavelengths. Ceramide (an N-acetylated sphingosine) was extracted from cells and resolved from sphingosine using TLC before derivatization and HPLC as described above (26).

Alveolar uptake of DSPtdCho. A mixture of [choline-methyl-14C]DSPtdCho (1.7 μCi, 7.8 μg) and cold DSPtdCho (7.8 μg) was dried under nitrogen gas. Normal saline (90 μl) and 2% brilliant blue (10 μl) were added, and the mixture was sonicated on ice for 10 min. Mice were sedated with ketamine (2–3 mg ip). The trachea was exposed, and 20 μl of the [14C]DSPtdCho mixture was instilled under direct observation. After 10 min of spontaneous breathing and careful positional rotation, the mice were killed with pentobarbital sodium (10 mg ip). Microsomal fractions were obtained as described above. Lipids were extracted, DSPtdCho was isolated, and radioactivity within the DSPtdCho spots was analyzed by scintillation counting (43).

Statistical analysis. The data are expressed as means ± SE. Statistical analysis was performed by Student’s t-test.

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phosphorus/mg protein, $P < 0.01$, Fig. 1A insert). In contrast to these parenchymal lipids, analysis of bronchoalveolar lavage lipids revealed that TNF-α transgenics exhibited a 55% increase in PtdCho (Fig. 1B, $P < 0.05$). The level of alveolar DSPtdCho was 46% greater in the TNF-α transgenics compared with control (control 1,510 ± 420 nmol phosphorus/mg protein, TNF-α transgenics 2,210 ± 660 nmol phosphorus/mg protein, Fig. 1B insert), but this finding did not reach statistical significance. There were no significant differences in other alveolar phospholipids. Collectively, these studies suggest that chronic expression of TNF-α in this murine model results in an overall decrease in the synthesis of several major phospholipids. Moreover, a divergent pattern for PtdCho between the alveolar and lung parenchymal compartment was observed in TNF-α transgenics compared with control, which may reflect differences in surfactant synthesis, secretion, or intraalveolar processing.

**Choline incorporation into PtdCho.** To investigate whether TNF-α mice had lower PtdCho biosynthetic capacity compared with wild-type controls, we prepared whole lung slices and measured radiolabeled incorporation of $[^3]$Hcholine into the phospholipid (Fig. 2). Indeed, we observed that lung slices from wild-type controls exhibited a 3.8-, 4.8-, and 3.9-fold greater incorporation of $[^3]$Hcholine into PtdCho at 2, 4, and 8 h of analysis, respectively.

**Enzymes of PtdCho synthesis.** We next determined if the decrease in surfactant content in the lungs of murine TNF-α transgenics was due to diminished synthesis by assaying activities of enzymes in the CDP-choline pathway, the primary pathway for PtdCho synthesis. Compared with control littermates, TNF-α transgenic mice exhibited no differences in the activity of CK, the first committed enzyme of the CDP-choline pathway (Table 1). In contrast, the activity of CCT, the rate-limiting enzyme within this pathway, was reduced in TNF-α transgenics by 42% compared with control mice ($P < 0.05$). Interestingly, this reduced activity for CCT was associated with nearly a threefold induction of cholinephosphotransferase activity, the final enzyme in the pathway. These results suggest that chronic expression of TNF-α inhibits a key regulatory enzyme, CCT, in murine lungs, resulting in an overall decrease in PtdCho content.

**Immunoblot analysis.** We performed immunoblot analysis to determine if reduced activity of CCT in TNF-α transgenics was due to a decrease in enzyme mass. Unexpectedly, in three separate studies, we observed that transgenic mice harboring the TNF-α gene exhibited a twofold increase in CCT protein compared with wild-type littermate controls (Fig. 3A). In contrast to expression of immunoreactive CCT, levels of β-actin were unchanged between control and TNF-α transgenic mice (Fig. 3B). Thus when we expressed our results as specific activity of the enzyme using densitometric measurements of enzyme mass, the TNF-α transgenic mice exhibited an even more substantial reduction in catalytic activity relative to wild-type controls (Fig. 3C). Collectively, these results suggest that TNF-α transgenic mice have reduced CCT activity associated with an increase in enzyme mass. The enzyme protein, however, is largely physiologically inactive.

**Sphingolipids.** The data above indicate that adequate amounts of CCT protein are detected in TNF-α transgenic mice, but the enzyme is not present in an activated form. CCT is an enzyme that is primarily regulated by lipids (17). One class of lipids that have been shown to be inhibitory for PtdCho synthesis and CCT function is sphingolipids, such as sphingosine and ceramide (1, 38, 41). In addition, TNF-α has been shown to acutely increase the levels of these bioactive mediators in the lung (20). Although we observed that TNF-α transgenic mice expressed no significant differences in sphingosine content, ceramide levels were significantly increased in TNF-α transgenic mice (control 1,540 ± 430 pmol/mg protein, TNF-α transgenic 3,540 ± 220 pmol/mg protein, $P < 0.01$) (Table 2). These results suggest that induction of ceramide may be a mechanism by which CCT activity is decreased in the TNF-α transgenic mice.

**Fatty acids.** In addition to its effects on sphingolipid metabolism, TNF-α regulates the biosynthesis, uptake,
and release of fatty acids from triglycerides (13, 24, 25, 30). Unsaturated fatty acids activate CCT (22, 39). Thus we hypothesized that TNF-α might decrease the availability of key fatty acids required for enzyme 
activation. As expected, palmitic acid was the major saturated fatty acid and oleic acid the major unsaturated species in both wild-type and TNF-α transgenics (Table 3). There were no substantial differences between the groups with regard to levels of saturated fatty acids. TNF-α transgenics, however, contained a significantly lower proportion of linoleic acid but greater amounts of arachidonic acid compared with control littermates. These results suggest that the relative abundance of selective unsaturated fatty acid species might also limit CCT activation in the setting of chronic TNF-α expression.

Table 3. Lung fatty acid distribution

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>WT</th>
<th>TNF-α</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristate</td>
<td>1.0 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Palmitate</td>
<td>40.2 ± 2.5</td>
<td>43.0 ± 3.7</td>
<td>NS</td>
</tr>
<tr>
<td>Palmitoleate</td>
<td>5.6 ± 0.2</td>
<td>5.3 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Stearate</td>
<td>9.5 ± 0.4</td>
<td>10.7 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>Oleate</td>
<td>18.4 ± 2.0</td>
<td>13.2 ± 1.5</td>
<td>NS</td>
</tr>
<tr>
<td>Linoleate</td>
<td>15.2 ± 1.2</td>
<td>9.2 ± 0.7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Arachidonate</td>
<td>6.7 ± 0.4</td>
<td>11.2 ± 1.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Docosahexanoic</td>
<td>3.4 ± 1.0</td>
<td>5.9 ± 0.7</td>
<td>NS</td>
</tr>
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Lipids were extracted, transmethylated in the presence of 10% boron trifluoride, and resolved by gas liquid chromatography. The areas under individual peaks and retention times were compared to known fatty acid standards. The areas of individual peaks were integrated and used for quantitation using an internal standard. Three control and three TNF-α transgenic mice were used.

DSPtdCho alveolar uptake. We determined if elevated alveolar PtdCho was secondary to altered recycling of DSPtdCho. Compared with control littermates, TNF-α transgenics had a 90% reduction in radiolabeled DSPtdCho uptake (control 86,800 ± 21,100 dpm/mg protein, TNF-α transgenic 8,900 ± 2,800 dpm/mg protein, P < 0.01, Fig. 4). These data suggest that alveolar surfactant recycling is decreased in TNF-α transgenic mice, consistent with the findings of increased alveolar DSPtdCho and decreased microsomal DSPtdCho.

DISCUSSION

The goal of the present study was to investigate whether chronic expression of TNF-α downregulates surfactant PtdCho synthesis. Because of the relatively short half-life of TNF-α and limitations with repetitive in vivo administration, we felt that chronic exogenous administration of the cytokine would not be a suitable

![Fig. 3. Effect of TNF-α overexpression on cytidylyltransferase (CCT) expression. A: the amount of CCT was assayed using immunoblotting. Lanes were loaded with 100 μg microsomal protein from WT and TNF-α transgenic mice. CCT standard was derived from purified rat liver enzyme. An anti-CCT-specific rabbit polyclonal antiserum was used. B: β-actin immunoblot with similar experimental conditions. The specific activity is expressed as enzyme activity/enzyme mass [nmol-min⁻¹-mg protein⁻¹] = mmol-min⁻¹-mass unit⁻¹. Mass units were arbitrary values determined by densitometric analysis of 3 WT and 3 TNF-α transgenic lanes from the preceding immunoblot. Data are expressed as means ± SE, P < 0.05.](http://example.com/fig3.jpg)

![Fig. 4. Uptake of alveolar DSPtdCho. Animals were exposed to [14C]DSPtdCho for 10 min after intratracheal instillation. After death, lipids were extracted from equal amounts of microsomal protein and separated by TLC. Radioactivity of the DSPtdCho spots was analyzed by scintillation counting, from 3 control and 4 TNF-α transgenic animals, expressed as dpm/mg protein. Results are means ± SE, *P < 0.01.](http://example.com/fig4.jpg)
model system to test our hypothesis. Rather, we opted to use a well-characterized murine model in which TNF-α is overexpressed in alveolar epithelium to pursue our studies (28). We observed that surfactant lipid synthesis was decreased in the lungs of TNF-α transgenics as evidenced by reduced PtdCho (and DSPtdCho) content and decreased radiolabeled incorporation of choline into PtdCho, coupled with a decrease in the activity of the rate-regulatory enzyme CCT. A somewhat unanticipated finding in our studies, however, was that these changes in lung tissue were not reflected in alveolar lavage, as PtdCho and DSPtdCho were elevated in these transgenic mice. Our radiolabeling studies suggest that elevated alveolar lipids may be due to reduced reuptake of surfactant in TNF-α transgenic mice. Although TNF-α might affect other aspects of surfactant processing, the inhibitory mechanisms on PtdCho synthesis may be important in understanding cytokine signaling in the setting of chronic exposure.

Consistent with observations in other systems after acute exposure to TNF-α, the levels of DSPtdCho and phosphatidylglycerol were decreased significantly in lungs of TNF-α transgenics compared with wild-type littermate controls (2, 3, 21, 36). Phosphatidylglycerol, a minor component of surfactant lipid, may in fact originate from several different populations of pulmonary cells. However, although the decrease in lung DSPtdCho content is not specific to type II cells, DSPtdCho is the major surface-active lipid of surfactant, and it is reasonable to attribute changes in this lipid to regulation by TNF-α at the level of either lipid synthesis or degradation within the cells. We did not exclude the possibility that TNF-α might alter turnover of DSPtdCho in the transgenics. This may also be relevant because the cytokine has been shown to activate both phospholipase A2 and PtdCho-specific phospholipase C (11, 37). However, the kinetics of phospholipase activation by TNF-α usually occurs more rapidly than regulation of enzymes involved in PtdCho synthesis.

We observed that the decrease in PtdCho mass in TNF-α transgenics was secondary to a decrease in the activity of CCT. Although CPT activity was markedly elevated in these studies, overall it did not offset the reduction in CCT function, as PtdCho content in tissue remained decreased in the TNF-α transgenics compared with control. These results further support a rate-regulatory role for CCT within the biosynthetic pathway for PtdCho (33). CCT activity is controlled in cells by several mechanisms, including lipid regulation, reversible phosphorylation, and regulation at the level of enzyme protein and mRNA (17). A somewhat surprising and consistent finding was that TNF-α transgenics had increased CCT protein content compared with wild-type mice (Fig. 3A). Thus when our functional data are expressed as a ratio of specific activity of CCT per unit of enzyme mass, TNF-α transgenics expressed CCT activity that was markedly reduced compared with control mice (Fig. 3C). We next determined whether there might be concurrent alteration in the levels of regulatory lipids for the enzyme. CCT is inhibited by short-chain ceramides, sphingosine, and lysophosphatidylcholine, whereas activity is stimulated in the presence of phosphatidylglycerol, phosphatidylinositol, and unsaturated fatty acids (1, 8, 17). When we assayed levels of these lipids, ceramide content was increased and linoleic acid reduced in TNF-α transgenics compared with control. The results with ceramide are important because TNF-α has been shown to increase the levels of this bioactive lipid by triggering sphingomyelin hydrolysis, its generation from higher order sphingolipids including glucosylceramide, or via de novo synthesis (9). To our knowledge, decreases in linoleic acid in the setting of TNF-α exposure have not been described, and such changes together with reduced anionic lipids would be expected to decrease PtdCho synthesis and CCT activity. The induction of arachidonic acid in TNF-α transgenics, however, is well described after acute cytokine exposure in other systems and might serve as a proinflammatory mediator (2, 10, 34). Collectively, our studies strongly suggest that surfactant phospholipid synthesis in TNF-α transgenics is impaired as a consequence of decreased CCT activity. This reduction in CCT activity appears to be associated with a large pool of membrane-associated enzyme that is catalytically inhibited by ceramide and diminished availability of activating lipids.

In contrast to effects on lung PtdCho content, the alveolar levels of PtdCho were significantly increased in the TNF-α transgenic mice compared with control. There are several potential explanations for these data. First, chronic TNF-α expression might regulate surfactant secretion, uptake, or intraalveolar processing. In this regard, Benito and Bosch (5) showed that TNF-α stimulates surfactant lipid secretion in primary type II cells via a protein kinase-dependent mechanism. In these studies, chronic TNF-α expression appears to decrease pulmonary epithelial uptake of alveolar PtdCho. However, in these studies we did not specifically isolate the lamellar body fraction to determine if alveolar DSPtdCho is recovered within this intracellular surfactant storage compartment. Our preliminary studies do show parallel changes in recycling activity within the cytosol. This, coupled with the tenfold higher activity of DSPtdCho reuptake in microsomes, does suggest that recycling is a global phenomenon within all compartments of the lung. The regulatory mechanisms for this decrease require further elucidation. Second, reduced alveolar DSPtdCho levels in parallel to parenchymal levels may not have been observed because expression of TNF-α was not global but restricted to alveolar epithelium in this model. Because alveolar macrophages are not only a major source of TNF-α but also participate in DSPdCho uptake, TNF-α expression in these cells would be expected to add another level of control by stimulating intraalveolar lipid degradation (42). Finally, our alveolar lipid results might be attributed to secondary effects of TNF-α overexpression. For example, histologically the alveoli have increased numbers of lympho-
cytes, and it is possible that increased PtdCho in our
lavage might, in part, originate from membranes re-
covered from these inflammatory cells after apoptosis
or necrosis. Although each of these explanations might
be relevant, the results indicate that TNF-α transgenic
mice display a unique phospholipid profile that will
require additional characterization.

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