Role of adipocyte differentiation-related protein in surfactant phospholipid synthesis by type II cells

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Schultz, C. J., E. Torres, C. Londos, and J. S. Torday. Role of adipocyte differentiation-related protein in surfactant phospholipid synthesis by type II cells. Am J Physiol Lung Cell Mol Physiol 283: L288–L296, 2002. First published March 22, 2002; 10.1152/ajplung.00204.2001.—Adipocyte differentiation-related protein (ADrP) is an intrinsic lipid storage droplet protein that is highly expressed in lung. ADrP localizes to lipid storage droplets within lipofibroblasts, pulmonary cells characterized by high triacylglycerol, which is a precursor for surfactant phospholipid synthesis by alveolar type II epithelial (EPII) cells. The developmental pattern of ADrP mRNA and protein expression in lung tissue parallels triacylglycerol accumulation in rat lung. ADrP mRNA levels are relatively high in isolated lipofibroblasts, accounting for the high ADrP expression in lung. Isolated EPII cells, which do not store neutral lipids but derive them from lipofibroblasts, have low levels of ADrP mRNA expression. ADrP is found around lipid droplets in cultured lipofibroblasts, but not in EPII cells isolated from developing rat lung. After coculture with lipofibroblasts, EPII cells acquired ADrP, which associates with lipid droplets. Furthermore, 3H-labeled triolein in isolated ADrP-coated lipid droplets is a tenfold better substrate for surfactant phospholipid synthesis by cultured EPII cells than 3H-labeled synthetic triolein alone. Antibodies to ADrP block transfer of neutral lipid. These data suggest a role for ADrP in this novel mechanism for the transfer of lipid between lipofibroblasts and EPII cells.

lipid storage droplets; neutral lipids; fetal lung development

The pulmonary lipofibroblast is located in the alveolar interstitium and is distinguished by the presence of large, cytoplasmic lipid droplets (13, 36). These cells were first described by O’Hare and Sheridan in 1970 (25), and their biochemical and structural characteristics were determined during the late 1970s and early 1980s by Brody’s group (13, 19, 20, 36), which named them lipid interstitial cells. McGowan and Torday (22) have recently critically reviewed the literature on the contributions of these cells to alveolar development and have termed them lipofibroblasts to highlight their fibroblast-like phenotype.

Torday and coworkers (24, 27) have investigated the prenatal ontogeny of the fetal rat lung lipofibroblast, showing a four- to fivefold increase of triacylglycerol in isolated lipofibroblasts, paralleling that in whole lung (34), over the last 4 days of gestation. The triacylglycerol content of fetal rat lung lipofibroblasts is maximal just before the appearance of surfactant phospholipid-containing lamellar bodies in neighboring alveolar type II epithelial (EPII) cells, the site of pulmonary surfactant synthesis (30). Torday and coworkers have demonstrated in a coculture system that the triacylglycerols of fibroblast origin are used for surfactant phospholipid synthesis by EPII cells (31) and that the metabolism of these lipids in the culture system is regulated by hormones important for lung maturation (24, 27).

In most mammalian cells, neutral lipids, including those found in pulmonary lipofibroblasts, are stored in discrete lipid storage droplets, which are composed of a core of triacylglycerol and cholesterol esters surrounded by a limiting osmophilic boundary (3). Little is known about the proteins that are present at the surface of these lipid storage droplets. The first-described intrinsic lipid droplet-associated proteins were the perilipins, which localize to the periphery of the intracellular neutral lipid storage droplets in adipocytes (1, 8, 17, 28) and steroidogenic cells of the adrenal cortex, testes, and ovaries (28). Perilipins share sequence homology with adipocyte differentiation-related protein (ADrP), which was first identified as a gene expressed very early in adipocyte differentiation (12). ADrP, transfected into COS cells, has been shown to play a role in facilitated fatty acid uptake (7) and to bind fluorescent cholesterol analogs (6). ADrP mRNA has subsequently been found to be expressed in a wide variety of somatic tissues: heart, brain, spleen, liver, skeletal muscle, kidney, testes, and most pronouncedly in the lung (3, 17). The expression level of ADrP mRNA in adult mouse lung was found to be second only to that in adipose tissue, the tissue that stores the greatest amount of neutral lipid and has the highest expression.

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of ADrP mRNA (3). ADrP was also identified in human tissues and named adipophilin (11).

On the basis of the relatively high abundance of ADrP in lung tissue and on the evidence for a precursor-product relationship between lipofibroblast triacylglycerol and EPII cell surfactant, we have investigated the expression and role of ADrP in developing fetal rat lung lipofibroblasts and EPII cells.

MATERIALS AND METHODS

Immunohistochemical Staining of ADrP in Rodent Tissue

Three- to five-millimeter tissue sections of adult mouse lung were deparaffinized, hydrated through graded ethanol, and treated with 0.3% hydrogen peroxide in methanol. After PBS rinsing, a heat treatment was performed in 0.01 M sodium phosphate buffer, pH 7.4, with 0.5% Triton X-100 for 20 min, the cells were pelleted at 500 g at 4°C until immunostained. The cells were permeabilized with 1% saponin, which was present in all incubations after fixation. Fixed cells were washed, incubated for 60 min in quenching/blocking solution (PBS containing 0.2 M glycine and 1.25 mg of goat IgG/ml), incubated for 18 h at 4°C with antibody against ADrP, washed three times with PBS (10 min each), incubated for 60 min with labeled secondary antibody, and washed again three times with PBS (10 min each). Coverslips containing cells were inverted and mounted on coverslips and viewed on either a Leitz Diaplan microscope or Zeiss confocal microscope with appropriate filters for fluorescein or rhodamine.

Northern Blot Analysis

Total RNA was isolated from lung tissue or cultures of lipofibroblasts or EPII cells with TRIzol reagent (GIBCO-BRL) as per the manufacturer’s protocol. Whole lung tissue was excised, snap-frozen in liquid N2, and minced in ice-cold TRIzol with a Polytron; cultured cells were scraped into TRIzol and lysed by pipetting. Ten or twenty micrograms of total RNA were separated by denaturing gel electrophoresis, blotted onto filters, and hybridized under high stringency with 32P-labeled ADrP probes corresponding to the full coding sequence of ADrP or 0.8 kb of the 3′ end of ADrP as previously described (3).

Harvesting Lung Fibroblasts by Differential Adherence

Five to ten time-mated dams were used per preparation, depending on the number of experimental variables to be tested. The fetal lungs were removed into Hanks’ balanced salt solution (HBSS). The HBSS was decanted, and five volumes of 0.05% trypsin (Worthington) were added to the lung preparation. We dissociated the lungs in a 37°C water bath using a Teflon stirring bar to disrupt the tissue mechanically. Once the tissue was dispersed into a unicellular suspension (~20 min), the cells were pelleted at 500 g for 10 min at room temperature in a 50-ml polystyrene centrifuge tube. The supernatant was decanted, and the pellet was resuspended in Dulbecco's modified Eagle’s medium (DMEM; GIBCO, Grand Island, NY) containing 20% fetal bovine serum (FBS) to yield a mixed cell suspension of ~60 × 106 cells (determined by a Coulter particle counter). The cell suspension was then added to culture flasks (25 or 80 cm2) for 30–60 min to allow for differential adherence of lung fibroblasts. These cells were >95% pure fibroblasts according to vimentin staining. In some experiments, intracellular neutral lipid storage was increased by the addition of 400 μM oleic acid [coupled to fatty-free bovine serum albumin (BSA) at a ratio of 6:1 mol/mol] to the culture medium for up to 48 h.
Isolation of Fetal EPII Cells by Nycodenz Gradient

The minced lungs from four to six litters were pooled, washed with 50 ml 140 mM NaCl, 5 mM KCl, 2.5 mM Na₃HPO₄, 10 mM HEPES, 6 mM glucose, and 0.2 mM EGTA, pH 7.4 (solution I) and filtered through 100-μm nylon mesh. The minced lungs were transferred to a trypsinizing flask containing 40 ml 140 mM NaCl, 5 mM KCl, 2.5 mM Na₃HPO₄, 10 mM HEPES, 2.0 mM CaCl₂, and 1.3 mM MgSO₄, pH 7.4 (solution II) with elastase (30 orceinelastase units/ml) and DNase (250 fg/ml) and stirred for 20 min at 37°C. Digestion was terminated by adding 5 ml of charcoal-stripped (hemorene-depleted) FBS. The cell suspension was filtered sequentially through two- and four-ply gauze, then 37- and 15-μm nylon mesh, and washed with additional solution II to a final volume of 40 ml. The cells were centrifuged at 130 g for 10 min at 20°C and resuspended in DMEM with 2% FBS for addition to Nycodenz gradients. We documented identity of fetal EPII cells by staining for presence of glycogen with specific EPII cell markers, Maclura pomifera lectin and antibody to cytokeratins 8 and 18. EPII cell preparations were found to be >90% pure by these criteria.

Organotypic Culture of Fetal Rat Lung Fibroblasts and EPII Cells

Fibroblasts and EPII cells were recombined in organotypic culture as follows: fibroblasts were incubated with 20% rat serum in MEM for 24 h to preload them with serum triacylglycerols (31). The fibroblasts were harvested from the 75-cm² tissue culture flasks using 0.25% trypsin. The cells were spun down at 500 g for 10 min and resuspended in MEM-10% FBS. The number of cells in each preparation was determined with a Coulter particle counter. Monolayer-cultured EPII cells were harvested and mixed with the fibroblasts in a 1:1 ratio, spun at 500 g for 10 min and allowed to incubate as a pellet at 37°C for 1 h in a CO₂ incubator (31). At the end of the incubation period, the cell pellet was resuspended in MEM to yield a cell suspension equivalent to 2 × 10⁶ EPIII cells/ml. One-twentieth of this cell suspension (i.e., 1 × 10⁸ fibroblasts, 1 × 10⁶ EPII cells) was then injected into Gelfoam collagen sponges (Upjohn, Kalamazoo, MI), which were hydrated with MEM. The cell-impregnated sponges were maintained in MEM at 37°C in 5% CO₂ and 95% air. At the end of the 24 h incubation period, we reisolated the EPII cells from the organotypic cultures by incubating them with 0.1% collagenase (Worthington, Freehold, NJ) in serum-free MEM to dissolve the collagen sponges; the EPII cells contained within the alveolar-like structures were collected on ice at unit gravity (5). The EPII cells were plated on glass coverslips for immunofluorescent staining. To confirm the identity of the reisolated EPII cells, they were immunostained for surfactant protein A (gift from Dr. Michael Beers, University of Pennsylvania) and found to be >97% positive, confirming that they were authentic EPII cells.

Incubation of EPII Cells with Lipid Droplets

Monolayer cultures of day 21 fetal rat EPII cells were incubated with the [3H]-labeled lipid droplets (10,000 disintegrations·min⁻¹·dpm·20 μg triacylglycerol⁻¹) isolated from fibroblasts for 24 h at 37°C in an atmosphere of 5% CO₂-air. At the end of the incubation the cells were processed for phospholipid as previously described (31).

Table 1. Incorporation of [3H]oleic acid from lipofibroblast lipid droplets cells into EPII cell phospholipid

<table>
<thead>
<tr>
<th>Triacylglycerol As</th>
<th>[3H]Oleic Acid Incorporated into EPII Cell Phospholipid</th>
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<tbody>
<tr>
<td>Synthetic [3H]triolein emulsion</td>
<td>125 ± 9</td>
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<tr>
<td>Lipofibroblast [3H]lipid droplet fraction</td>
<td>1,976 ± 65</td>
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<tr>
<td>Lipofibroblast [3H]lipid droplet fraction</td>
<td>168 ± 19 (P &lt; 0.001)</td>
</tr>
<tr>
<td>Anti-ADrP IgG (2 μg/ml)</td>
<td>582 ± 48 (P &lt; 0.01)</td>
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<tr>
<td>Lipofibroblast [3H]lipid droplet fraction</td>
<td>1,790 ± 260 (NS)</td>
</tr>
<tr>
<td>Anti-ADrP IgG (0.4 μg/ml)</td>
<td>2,054 ± 113 (NS)</td>
</tr>
<tr>
<td>Lipofibroblast [3H]lipid droplet fraction</td>
<td>1,906 ± 82 (NS)</td>
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</tbody>
</table>

Values for each incubation are mean ± SD of 5 replicates. Monolayer cultures of EPII cells isolated from day 21 fetal rat lungs were incubated with 10,000 disintegrations·min⁻¹·dpm·20 μg triacylglycerol⁻¹ from 1) a synthetic [3H]triolein emulsion or 2) an adipocyte differentiation-related protein (ADrP)-coated [3H]lipid droplet fraction isolated from lipofibroblasts. Incubations with the endogenous lipid droplet fraction were with and without preimmune serum or affinity-purified anti-ADrP IgG and with an irrelevant interleukin (IL)-6 antibody. After 24 h of incubation, the amount of [3H]incorporated into type II phospholipid was measured. P values in parentheses indicate significant differences from incubations with lipofibroblast lipid droplet fraction alone (row 2). EPII, alveolar type II epithelial; NS, not significant. Anti-ADrP IgG had no effect on uptake into EPIII cells from the synthetic [3H]triolein emulsion (data not shown).
RESULTS

Histochemical Localization of ADrP in Tissue Sections

Peroxidase staining with an antibody prepared against ADrP was used to determine whether ADrP protein was expressed in lung tissue in vivo. ADrP staining was found in sections of adult mouse lung around lipid droplets (Fig. 1). These lipid droplets were in cells identified as lipofibroblasts. In addition to its presence in lipofibroblasts, ADrP staining was also found around lipid droplets at the tip of septa (Fig. 1). This pattern of lipofibroblast distribution in the interstitium and septa has previously been observed (18). The data demonstrate that ADRP protein is present in rodent lung in vivo.

Developmental Expression of ADrP mRNA and Protein in Rat Lung Tissue

Triacylglycerol content of the rat lung starts to increase 6 days before birth and plateaus during the second postnatal week of life (34). To characterize the expression of ADRP mRNA during this time of increasing neutral lipid storage, we probed total RNA isolated from fetal and postnatal rat lung with a radiolabeled ADrP cDNA probe. Expression of ADrP mRNA (1.9 kb) was low at day 14 of gestation, rose to a plateau between day −5 and day −4 before birth, peaked at day 5 after birth, and declined to a minimum at the time of weaning, day 21 postbirth (Fig. 2). Portions of the lung tissue were used also to detect ADrP protein expression by immunoblotting techniques. Expression of ADrP protein (52,000 Da) in whole lung homogenates was detected at all time points with minima at days −5 before birth and day 21 after birth, with a peak at day 5 after birth, reflecting the time course of ADrP mRNA expression (Fig. 3). These data show that the expression of ADrP closely parallels the rise and fall in neutral lipid stores in the developing rat lung.

Developmental Expression of ADrP mRNA in Lipofibroblasts and EPII Cells Isolated from Fetal Rat Lung

Lipofibroblasts containing lipid storage droplets are evident in the rat lung at gestational day 16 and increase in number in parallel with the increase in triacylglycerol content of the developing lung (31, 34). The triacylglycerol content of fetal rat lung lipofibroblasts increases fivefold from day 16 to 20 of gestation, with little or no increase in EPII cell triacylglycerol

Fig. 1. Immunostaining of mouse lung tissue with anti-adipocyte differentiation-related protein (ADrP) antibody. ADrP, identified by the brown 3,3-diaminobenzidine staining, is seen in a circular pattern around lipid droplets within lipofibroblasts (LF), as indicated by arrows. Blue staining shows nuclei identified by haematoxylin.

Fig. 2. Northern blot of ADrP RNA from fetal and postnatal rat lung tissue. Blot of total RNA isolated from fetal rat lung tissue between −7 and +21 days of age. The RNA aliquots were normalized to the amount of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expressed per sample. ODU, optical density units. Each bar represents the mean ± SD of 5 independent observations from 5 different animals. Data were analyzed by ANOVA. *P < 0.05 vs. day −7; **P < 0.01 vs. day −7; ***P < 0.001 vs. day −7; △P < 0.001 vs. day +1; △△P < 0.0001 vs. day +1.

Fig. 3. Western blot of ADrP from fetal and postnatal rat lung tissue. Samples of fetal rat lung tissue were taken between −5 and +21 days of age, and each lane contained 100 μg of total lung protein. Each bar represents the mean ± SD of 5 independent observations from 5 different animals. Blots were scanned by densitometry and are expressed in arbitrary units. Data were analyzed by ANOVA. *P < 0.01 vs. day −5; **P < 0.001 vs. day −5; △P < 0.001 vs. day −5; △△P < 0.0001 vs. day +1; △△△P < 0.001 vs. day +15.
content (31). We examined the expression of ADrP mRNA in lipofibroblasts and EPII cells isolated from lungs of developing rat fetuses. Expression of ADrP mRNA in fetal lipofibroblasts increased with time of gestation (Fig. 4). The apparent increase in ADrP signal at day 18 of gestation is due to a gel loading inconsistency as seen in the ethidium bromide staining of the RNA. In contrast to lipofibroblasts, EPII cells, which do not store neutral lipid but are provided with lipid substrate from lipofibroblasts (31), had low levels of ADrP mRNA throughout gestation (Fig. 4). These observations indicate that lipofibroblasts, not EPII cells, are the site of high ADrP expression in lung.

Immunolocalization of ADrP in Cultures of Lipofibroblasts and EPII Cells Isolated from Fetal Rat Lung

Immunofluorescent microscopy plus an affinity-purified antibody prepared against ADrP was used to determine the cellular localization of ADrP in lipofibroblasts and EPII cells isolated from fetal rat lung at day 20 of gestation. In cultures of lipofibroblasts, ADrP staining was found associated with lipid droplets in all cells, both in a punctate pattern around smaller droplets and as bright rings around larger droplets (Fig. 5). In lipofibroblasts cultured with fatty acid-supplemented media, the amount of ADrP staining was increased in parallel with an increase in number and size of lipid droplets (Fig. 5). This increase in ADrP protein expression in lipofibroblasts cultured under conditions to increase the amount of stored neutral lipid was also detected by Western blotting (data not shown). Furthermore, the increase in ADrP protein expression was seen without a change in mRNA levels (data not shown), suggesting that a posttranslational process accounts for increased ADrP protein expression when cells increase their lipid content, as is the case for perilipin, another major lipid storage droplet protein (2).

In contrast to the observations in cultures of lipofibroblasts, ADrP staining in EPII cultures was absent from most cells. Less than 20% of these cultured cells contained ADrP staining in a punctate pattern around small lipid droplets or as rings around larger droplets (Fig. 5). Cultures of isolated EPII cells prepared by the methods described above are ≥90% pure (31). It is assumed that the majority of cells in EPII cultures that stain for ADrP are contaminating lipofibroblasts.

Fig. 4. Northern blot analysis of RNA from lipofibroblasts (LF) and alveolar type II epithelial (EPII) cells from fetal rat lung. Blots of total RNA (20 μg/lane) isolated from LF (A) or EPII cells (C) were probed with radiolabeled ADrP cDNA probes. B and D: ethidium bromide staining (EtBr) of 28S and 18S ribosomal RNA in the gels used to prepare the blots in A and C, respectively.

Fig. 5. Immunofluorescence staining of EPII cells and LF stained with anti-ADrP antibodies. Cultured EPII cells (top, EPII) isolated from rat lung at day 20 of gestation have minimal ADrP staining. Cultured LF (middle) isolated from rat lung at day 20 of gestation show ADrP staining in all cells. This ADrP staining is increased in LF cultured in fatty acid (FA)-supplemented medium (bottom, LF+FA) due to increased number and size of lipid droplets.
Immunofluorescent ADrP in Cultures of EPII Cells After Coculture with Lipofibroblasts

As noted in the Introduction, Torday and coworkers (31) have shown that in a coculture system, lipofibroblasts can use their neutral lipid stores to supply lipid substrate to EPII cells for surfactant production. We therefore explored the possible role of ADrP in the transfer of lipid between these two types of cells. Lipofibroblasts and EPII cells were isolated separately, combined in coculture for 24 h, and then the EPII cells were reisolated and examined for the presence of ADrP by immunofluorescent microscopy. As also shown in Fig. 5, isolated EPII cells not cocultured with lipofibroblasts contain minimal ADrP (Fig. 6, left). After coculture with lipofibroblasts, nearly all reisolated EPII cells contained lipid droplet ADrP that stained positively for ADrP (Fig. 6, right). Because the coculture incubation is for 24 h, it is possible that ADrP expression reflects the uptake of lipids during the coincubation phase of the experiment. It is also possible that ADrP has a role in the transfer of lipid between lipofibroblasts and epithelial type II (TII) cells. Furthermore, preliminary results indicate that addition of an antagonist of parathyroid hormone-related protein (PTHrP) receptors [PTHrP-(7–34) amide] to the coculture medium blocked both the increased staining of ADrP and lipid accumulation in reisolated EPII cells (C. J. Schultz, J. S. Torday, and C. Londos; unpublished observations). These data suggest that the accumulation of ADrP in EPII cells may be associated with a receptor-mediated mechanism.

**Immunoinhibition of the Transfer of Lipid Between Lipofibroblasts and EPII Cells by Antibodies to ADrP**

To address the possible role of ADrP in the transfer of lipids from lipofibroblast cells to EPII cells, we asked the following question: Is triacylglycerol encased in a coating of ADrP more efficiently transferred to TII cells than triacylglycerol droplets lacking ADrP? We further asked whether anti-ADrP antibodies could interfere with such transfer.

3H-labeled, ADrP-coated endogenous lipid droplets isolated from lipofibroblast cells were incubated with unlabeled cultured EPII cells, and the incorporation of the 3H label into surfactant phospholipid produced by EPII cells was measured. As a control, EPII cells were incubated with an emulsion of BSA and synthetic triolein containing an equal amount of lipid and 3H label [10,000 disintegrations/min (dpm)]; these control cells incorporated 125 dpm of radiolabel into phospholipid (Table 1). Incubation of EPII cells with ADrP-coated lipid droplets increased the incorporation of 3H label into surfactant by 16-fold compared with incubation with synthetic 3H-labeled lipid (Table 1). Addition of preimmune serum or an irrelevant interleukin-6 antibody to this incubation had minimal effect on this incorporation (Table 1). However, the addition of antibodies against ADrP to this incubation decreased the amount of 3H label found in surfactant phospholipid in a concentration-dependent manner, with a maximal inhibition of 90% (Table 1). These data suggest that ADrP is involved in the shuttling of lipid from lipofibroblasts to EPII cells.

**DISCUSSION**

We have previously shown that fibroblasts take up and store neutral lipid (24), whereas EPII cells do not (31); paradoxically, when fibroblasts are cocultured with EPII cells, neutral lipid is transferred from fibroblasts to EPII cells and targeted to surfactant phospholipids (31), suggesting a “docking and trafficking” mechanism. We subsequently found that EPII cells produce prostaglandin E2 (33), which stimulates the secretion of neutral lipid by fibroblasts, explaining why fibroblasts release neutral lipid to EPII cells in culture but leave the mechanism of lipid uptake and targeting unexplained. The demonstration of uptake of neutral lipid coated with ADrP in the present experiments may explain why processing of neutral lipid by fibroblasts is necessary for this mechanism of neutral lipid trafficking.
The mRNA expression of ADrP, the neutral lipid droplet-associated protein, in adult rodent lung is second only to that in adipose tissue, the tissue that stores the greatest amount of neutral lipid and exhibits the highest expression of ADrP mRNA (3). In this study, we found ADrP protein expression in sections of rodent lung tissue, localized around lipid droplets (Fig. 1). We also report that ADrP is developmentally expressed in fetal and newborn rat lung, paralleling the accumulation of neutral lipid in the lung tissue (Figs. 2–4). Furthermore, the ADrP expression was localized to lipofibroblasts (Fig. 5), the pneumocyte characterized by cytoplasmic neutral lipid droplets. In contrast, we found minimal expression of ADrP in fetal rat EPII cells (Figs. 5 and 6), the pneumocytes that lie adjacent to lipofibroblasts in the alveolar interstitium and are the site of pulmonary surfactant synthesis.

Mesenchymal cells lie in close apposition to the epithelium and play a central role in the growth (14) and differentiation (9) of epithelial cells into EPII cells. Lipofibroblasts first appear during the canalicular phase of fetal rat lung development, and triacylglycerol content is maximal just before the appearance of surfactant-containing lamellar bodies in neighboring EPII cells (30). Despite such apparent evidence for a precursor-product relationship between fibroblast triacylglycerols and EPII cell surfactant phospholipids, there was no empirical evidence for the existence of such a mechanism until Torday and coworkers (31) demonstrated that triacylglycerols of fibroblast origin are used for surfactant phospholipid synthesis by EPII cells in culture.

Initially, Torday et al. (31) demonstrated the accumulation of triacylglycerol by the developing fetal rat lung fibroblast, increasing four- to fivefold between days 18 and 22 of gestation, with no increase in EPII cell triacylglycerol content. It was later revealed that isolated fetal rat lung fibroblasts, but not EPII cells, actively take up lipid and package it into triacylglycerol, providing a mechanistic explanation for the observed accumulation of triacylglycerols by fibroblasts but not by EPII cells in vivo.

In subsequent studies (31), the investigators loaded the fibroblasts with radiolabeled triacylglycerol and nonlabeled triacylglycerol and recombined them with EPII cells in organotypic coculture to evaluate transit and metabolism of fibroblast triacylglycerol by EPII cells. There was quantitative transfer of triacylglycerol from fibroblasts to EPII cells, resulting in a threefold increase in the saturated phosphatidylcholine content of the EPII cells. The rate of fibroblast [3H]triacylglycerol incorporation into EPII cell phospholipids was compared with the rate of incorporation of extracellular [14C]glucose. Both triacylglycerol and glucose were incorporated into EPII cell phospholipids, particularly disaturated phosphatidylcholine and phosphatidylglycerol, the principal surfactant phospholipids. The rate of triacylglycerol incorporation into disaturated phosphatidylcholine and phosphatidylglycerol was 10- to 24-fold higher, respectively, than that of glucose. These data suggested the existence of a specific mechanism for the shuttling of triacylglycerol from the fibroblast to the EPII cells.

In the current study, immunofluorescence using anti-ADrP antibodies showed that the minimal expression of ADrP in EPII cells, before coculture with lipofibroblasts (Figs. 5 and 6), was greatly increased along with the transfer of lipid from lipofibroblasts, after coculture with lipofibroblasts (Fig. 6). In addition, anti-ADrP antibodies blocked the transfer of lipid in the coculture system (Table 1). Both of these observations suggest an important role of ADrP in the mobilization of intracellular lipid stores from lipofibroblasts to EPII cells and in fetal lung maturation. In this context, ADrP has been found on the surface of fat globules secreted by mammary epithelial cells (11). However, in the current study in all cultures of lipofibroblasts and cocultures with EPII cells, we found no evidence of secreted ADrP, by either Western blotting or radiolabeled protein techniques. During the time of increased triacylglycerol accumulation in the developing lung, cytoplasmic projections are present between lipofibroblasts and EPII cells (16, 19), giving rise to the possibility that the lipid transfer may occur along these connections.

McGowan et al. (21) have recently evaluated the roles of lipoprotein receptors and apolipoprotein E (apoE) in the accumulation of circulating lipoproteins by lipofibroblasts. Because there was no correlation between developmental age of the lipofibroblasts and their lipoprotein receptors or apoE expression, they concluded that such changes must, alternatively, be due to the amounts of lipoprotein in circulation. The concentration of triglyceride in fetal rat circulation is 40-fold lower than in fetal rat lung lipofibroblasts (10, 31), and although it increases in the postnatal period, it does not correlate with the pattern of triglyceride content in developing lipofibroblasts (10, 30). Furthermore, Torday and coworkers (24, 27) have shown that steroid hormones have a direct effect on the rate of lipofibroblast triglyceride accumulation, indicating that this process is regulated at the cellular level. In contrast to the dissociation of circulating triglyceride levels during the perinatal period from the ontogeny of triglycerides in lipofibroblasts, the pattern of lipofibroblast expression of ADrP is consistent with its hypothesized role in lipofibroblast triglyceride accumulation.

Lung surfactant production is widely recognized to be under hormonal regulation (23). In the lipofibroblast-EPII cell coculture system, dexamethasone was shown to stimulate lipofibroblast triacylglycerol incorporation into EPII cell disaturated phosphatidylcholine by 40% (31), indicating that the mechanism of triacylglycerol mobilization from the fibroblast to the EPII cells is hormonally regulated. Preliminary studies indicate that the increase in ADrP expression in EPII cells after coculture with lipofibroblasts is blocked by incubation with an antagonist of parathyroid hormone-related protein (C. J. Schultz, J. S. Torday, and C. Londos; unpublished observations), a regulator of lung maturation (32). This preliminary finding suggests that endogenous PHrP promotes the lipid trans-
fer between lipofibroblasts and EPII cells and the change in ADRP expression in EPII cells that accompanies this transfer.

The possible involvement of ADRP, a protein intrinsic to intracellular lipid droplets, in the transfer of triacylglycerol from lipofibroblasts to EPII cells suggests a novel and yet undefined mechanism for the trafficking of neutral lipids between these two types of cells. No ADRP protein was found in the culture medium of lipofibroblasts alone or in cocultures with EPII cells. This observation, in combination with the close apposition and cellular projections between lipofibroblasts and EPII cells, would suggest that the lipid shuttling mechanism between these two types of cells is not similar to that of circulating lipoproteins, which involves secretion and possible uptake of whole lipid particles. In this context, TIP47, a recently described protein highly homologous to ADRP (4), has been reported to bind to the cytoplasmic domain of mannose 6-phosphate receptors and mediate receptor uptake and targeting to the lysosomal compartment. This pathway is very similar to the processing of lipids and proteins for surfactant phospholipid synthesis and storage within lamellar bodies, which are modified lysosomes (26). Interestingly, a separate study has also demonstrated that TIP47 targets to lipid storage droplets (37). Furthermore, we have previously shown that lipofibroblasts secrete lipid and that prostaglandin E2 stimulation of ADRP expression in EPII cells that accompanies this transfer.

ADRP IN LUNG LIPOFIBROBLASTS

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