Deletion of the Transmembrane Transporter ABCG1 Results in Progressive Pulmonary Lipidosis*

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We show that mice lacking the ATP-binding cassette transmembrane transporter ABCG1 show progressive and age-dependent severe pulmonary lipidosis that recapitulates the phenotypes of different respiratory syndromes in both humans and mice. The lungs of chow-fed Abcg1−/− mice, >6-months old, exhibit extensive subpleural cellular accumulation, macrophage, and pneumocyte type 2 hypertrophy, massive lipid deposition in both macrophages and pneumocytes and increased levels of surfactant. No such abnormalities are observed at 3 months of age. However, gene expression profiling reveals significant changes in the levels of mRNAs encoding key genes involved in lipid metabolism in both 3- and 8-month-old Abcg1−/− mice. These data suggest that the lungs of young Abcg1−/− mice maintain normal lipid levels by repressing lipid biosynthetic pathways and that such compensation is inadequate as the mice mature. Studies with A-549 cells, a model for pneumocytes type 2, demonstrate that overexpression of ABCG1 specifically stimulates the efflux of cellular cholesterol by a process that is dependent upon phospholipid secretion. In addition, we demonstrate that Abcg1−/−, but not wild-type macrophages, accumulate cholesterol ester droplets when incubated with surfactant. Together, these data provide a mechanism to explain the lipid accumulation in the lungs of Abcg1−/− mice. In summary, our results demonstrate that ABCG1 plays essential roles in pulmonary lipid homeostasis.

Surfactant is a complex mixture of lipids and proteins that coats the alveolar sacs thus reducing surface tension and preventing the collapse of the lungs (1–3). It is composed of 85% phospholipids, of which 33% is dipalmitoyl-phosphatidylcholine, 10% neutral lipids (predominantly cholesterol) and 5% proteins that include surfactant proteins SP-A, SP-B, SP-C, and SP-D (1–3).

Surfactant is synthesized and secreted by pneumocytes type 2; the lipid, together with SP-A, -B, and -C, is transferred into characteristic intracellular inclusions called lamellar bodies, which subsequently fuse with the plasma membrane to secrete the contents into the air space (1–5). Once secreted, the lamellar bodies unravel to form highly organized lipid-protein structures termed “tubular myelin,” phospholipid-rich sheets and vesicles (1, 3). Subsequently, the vesicles are taken up by the type 2 cells for recycling and degradation, by resident alveolar macrophages for degradation, or are cleared to the airways (1–3). Interestingly, surfactant is not only critical for the proper ventilatory function of the lung, but it also plays a role, together with alveolar macrophages, in the innate defense system that provides the first barrier against exogenous agents (6–8).

Several human diseases, which include pulmonary alveolar proteinosis (PAP), respiratory distress syndrome of the newborn and cholesterol ester storage disease, have been described which are associated with altered surfactant and/or the presence of lipid-loaded macrophages and/or pneumocytes in the lung (9–14). Similar phenotypes have been noted in several knock-out mice (15–18). Studies with both humans and mice have identified a number of proteins that are necessary for normal pulmonary lipid metabolism; they include granulocyte/macrophage-colony stimulatory factor (GM-CSF) (10, 11), GM-CSF receptor (16), ABCA1 (17), ABCA3 (12, 13), lysosomal acid lipase (LAL) (9, 15), SP-B, -C, and -D (14, 18). These data suggest that pneumocytes type 2 and alveolar macrophages play essential roles in regulating surfactant levels and lung function.

ABCG1 is a member of the ATP-binding cassette (ABC) family of transmembrane transporters. Incubation of human and murine macrophages with either oxidized or acetylated LDL or with agonists for the liver-X-receptor (LXR) identified

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4 The abbreviations used are: ABC, ATP-binding cassette; ACC, acetyl-coenzyme A carboxylase; BAL, bronchioalveolar lavage; CCT, CDP-choline phosphotransferase; CE, cholesterol ester; DG, diglycerides; FAS, fatty acid synthase; FFA, free fatty acids; GPAT, glycerol-3-phosphate acyltransferase; HDL, high-density lipoprotein; HMGR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; LDLR, low-density lipoprotein receptor; LXR, liver-X-receptor; MG, monoglycerides; ORO, oil red O; PA, phosphatidyl acid; PC, phosphatidyl choline; PCT, phosphatidate cytidylyltransferase; PE, phosphatidyl ethanolamine; PG, phosphatidyl glycerol; PS, phosphatidyl serine; SM, sphingomyelin; SREBP, sterol responsive element-binding protein; TG, triglycerides; UC, unesterified cholesterol.
**ABCG1 and Lung Function**

ABCG1 as a highly inducible LXR target gene (19). LXR functions as a “cholesterol sensor” and, in response to elevated intracellular levels of oxysterols, activates genes that control cholesterol and lipid metabolism (20–22). LXR target genes include four members of the ABC family, ABCA1, ABCG5, ABCG8, and ABCG1 (20–24), all of which are involved in transmembrane transport of sterols (20, 25–27). Mutations in ABCA1 or ABCG5 and ABCG8 result in Tangier disease (28) or sitosterolemia (29), respectively.

In contrast to ABCA1 or ABCG5/ABCG8, the physiological importance and function of ABCG1 is not well understood. Recent studies showed that ABCG1 facilitates cholesterol efflux from cells to mature HDL particles, phospholipid vesicles or phospholipid/apoprotein complexes, but not to lipid-poor apoA1 (30–35). In addition, studies using both Abcg1−/− and ABCG1 transgenic mice demonstrated that ABCG1 plays a critical role in controlling pulmonary and hepatic lipid homeostasis in response to a high fat, high cholesterol diet challenge (31). In the current report we extend those initial observations, and demonstrate that the lungs of chow-fed Abcg1−/− mice exhibit age-dependent abnormalities that have been previously linked with respiratory syndromes. These changes include the accumulation of lipid-filled pneumocytes type 2 and macrophages, altered surfactant composition and changes in the expression of genes involved in lipid metabolism. Thus, the present study identifies new pivotal roles for ABCG1 in controlling pulmonary homeostasis.

**EXPERIMENTAL PROCEDURES**

**Animals**—Abcg1−/−/lacZ knock-in mice on a C57Bl/6 background were generated and maintained as described (31). Where indicated, mice were fed a high fat (21%), high cholesterol (1.25%) diet for 9 weeks (Research Diets D12108). Male and female mice were used for BAL studies. All other studies utilized male mice.

**Histological Analysis**—Preparation and staining of frozen lung sections and cells were as described (31). Lungs were fixed in 10% formalin for 24 h, post-fixed in 15% ethanol, and embedded in paraffin. Sections (4 μm) were stained with hematoxylin–eosin. Electron microscopy was carried out as described (36).

**Lipid Analysis**—A-549 cells (ATCC, CCL-185) were seeded in 48-well plates (5 × 10^5 cells/well) and cultured in F-12 media + 10% fetal bovine serum. Cells were infected with Me2SO or with a secretagogue mixture (100 μmol/liter ATP, 0.1 μmol/liter phorbol-12-myristate-13-acetate, 20 μmol/liter terbutaline) (39), as indicated and efflux of radiolabeled cellular lipids to F-12 media containing 0.2% bovine serum albumin was conducted as described (31).

**RESULTS**

**Progressive Cellular and Lipid Accumulation in the Lungs of Chow-fed Abcg1−/− Mice**—The lungs of young (3-month-old) chow-fed Abcg1−/− mice and their wild-type littermates are visually indistinguishable (Fig. 1A). However, at 15 weeks, pale foci can be seen on the surface of the Abcg1−/− lungs, consistent with lipid accumulation (data not shown). By the age of 8 months, the lungs of Abcg1−/− mice are white, and clearly distinguishable from the pink lungs of wild-type mice (Fig. 1B). In contrast, other tissues, such as the liver and kidney, showed no gross visual evidence of lipid accumulation (data not shown). Blood lipid levels and whole body fat content did not differ significantly between genotypes (Table 1).

By 8 months, Abcg1−/− mice, but not wild-type controls, exhibit extensive subpleural cell proliferation (Fig. 1, D versus C), accumulation of oil red O-positive macrophages throughout their lungs, but especially in the subpleural regions (Fig. 1, G versus F) and multiple lymphytic foci (Fig. 1E). A similar lung phenotype was noted when Abcg1−/− mice were analyzed at 3 months of age after administration of a high fat, high cholesterol (HF/HC) diet for 9 weeks (31).
The lungs of chow-fed 8 month old Abcg1<sup>−/−</sup> mice also contained 3–6-fold increased levels of unesterified and esterified cholesterol, and total phospholipid (Table 1). These results are consistent with the increase in oil red O staining (that identifies neutral lipid) (Fig. 1) and accumulation of phospholipid-rich lamellar bodies in both pneumocytes type 2 and in extracellular spaces (see below). Phospholipid levels, determined by choline and lipid phosphorus assays (Table 1 and data not shown) were increased 5-fold in the lungs of Abcg1<sup>−/−</sup> mice. In contrast, pulmonary triglyceride levels increased <50% (Table 1). Thus, there was a specific increase in cholesterol and phospholipids in the lungs of chow-fed Abcg1<sup>−/−</sup> mice that occurred in the absence of changes in plasma lipids.

Because no evidence of subpleural cellular proliferation, lymphocytic foci, or lipid accumulation was observed in the lungs of 3-month-old Abcg1<sup>−/−</sup> mice or wild-type mice, age 3–15 months (Fig. 1, C and F and data not shown), these results identify a progressive, severe and age-related disorder/lipidosis in the lungs of Abcg1<sup>−/−</sup> mice.

**Electron Microscopy Studies Identify Abnormal Pneumocytes Type 2 and Macrophages in the Abcg1<sup>−/−</sup> Lungs—Pneumocyte type 2 cells are the sole source of surfactant.** These cells express LacZ and thus normally express ABCG1 (Fig. 1E, black arrows). The elevated phospholipid levels in the Abcg1<sup>−/−</sup> lungs (Table 1) suggested that type 2 cells might also be abnormal in the null mice. Analysis of electron micrographs demonstrated that the lamellar bodies in Abcg1<sup>−/−</sup>/type 2 cells were both larger and, although difficult to quantify, more electron dense (compare Fig. 2, A versus B), a characteristic that has been correlated with increased phospholipid levels (13). Morphometric analysis of multiple micrographs (n = 32) prepared from the lungs of 8-month-old chow-fed Abcg1<sup>−/−</sup> (Fig. 2A) and wild-type (Fig. 2B) mice demonstrated that both the number of lamellar bodies per type 2 cell, and the total number of pneumocyte type 2 cells were each increased 5-fold in the Abcg1<sup>−/−</sup> lungs (Fig. 2, D and E). Interestingly, the hypophase of Abcg1<sup>−/−</sup> lungs contained typical tubular myelin structures (Fig. 2C), consistent with secretion of surfactant from type 2 cells.

Many type 2 cells in the Abcg1<sup>−/−</sup> lungs also contained numerous intense staining highly enlarged lamellar-like structures that are presumed to be composed of phospholipids (LB*...
Altered Lipid Metabolism in Abcg1

A BCG1 and Lung Function

FIGURE 2. Altered pneumocytes type 2 in 8-month-old chow-fed Abcg1−/− mice. Representative electron micrographs of Abcg1−/− (A) and wild-type (B) pneumocytes type 2 (original magnification: 9,900×). Note the increased number and density (staining) of lamellar bodies (LB) in Abcg1−/− cells. The hypophase of the Abcg1−/− lungs contains normal tubular myelin (TM) (C) (original magnification: ×38,000). Pneumocytes type 2 cell number (D) and the relative area of lamellar bodies within each type 2 cell (E) were determined in electron micrographs (n = 32) from Abcg1−/− (closed bars) and wild-type (open bars) lungs. Numerous aberrantly enlarged and intensely stained lamellar bodies as well as cholesterol clefts were often noted in sections of type 2 cells from Abcg1−/− mice (F). LB, lamellar body; LB*, aberrant lamellar body; TM, tubular myelin; Pnu2, type 2 cell; CC, cholesterol cleft. *, p ≤ 0.01.

in Fig. 2F). Such enlarged structures were not included in the quantification shown in Fig. 2, D and E. Interestingly, cholesterol crystals were also observed in Abcg1−/− type 2 cells (Fig. 2F). In contrast, enlarged lamellar-like structures or cholesterol crystals were never observed in the type 2 cells of wild-type mice (data not shown).

A BCG1 Deletion Affects Surfactant and Gene Expression of Pneumocyte Type 2 Cells—We next performed bronchoalveolar lavages (BAL) to determine whether a null mutation of Abcg1 also affected surfactant. Analysis of the surfactant from Abcg1−/− mice indicated that it contained increased levels of soluble proteins (2.3 versus 1.1 mg; Abcg1−/− versus wild-type) and a 3-fold increase in the level of choline-containing phospholipids (Table 1). The latter result is in agreement with the analysis of total phospholipids in the lung (Table 1). In addition, the number of macrophages recovered in the BALs from Abcg1−/− mice were increased >10-fold (data not shown), consistent with a significant enrichment of these cells in the alveolar space (Fig. 1).

The data of Fig. 3 demonstrate that expression of Sp-B, Sp-C, and Abca3, all known to be involved in surfactant secretion and function, were decreased ~50% in the lungs of Abcg1−/− chow-fed mice. However, because the number of type 2 cells increased 5-fold in Abcg1−/− lungs (Fig. 2D), the data suggest that the concentration of SP-B, SP-C, and ABCA3 in each Abcg1−/− type 2 cell would be reduced >80%. Together, the data of Table 1 and Figs. 2 and 3 demonstrate that deletion of Abcg1 results in major changes in surfactant metabolism by pneumocytes type 2.

Cholesterol and Phospholipid Secretion from A-549 Pneumocyte Type 2 Cells—The human carcinoma-derived A-549 cells have been used extensively as a model for pneumocytes type 2 (40). To determine whether ABCG1 expression affects surfactant secretion, we infected A-549 cells with an empty adenovirus or an adenovirus containing the mouse Abcg1 cDNA and then incubated the cells with radioactive choline or cholesterol to radiolabel endogenous lipids. Western blot analysis shows that ABCG1-FLAG protein was expressed (Fig. 4A). Consistent with a previous report (39), the efflux of [3H]choline-labeled phospholipids to the media was significantly enhanced following treatment of the A-549 cells with a secretagogue mixture (ATP, phorbol ester, and terbutaline) (Fig. 4B). Strikingly, expression of ABCG1 did not affect phospholipid efflux from the A-549 cells (Fig. 4B).

In contrast, ABCG1 overexpression resulted in increased efflux of cellular cholesterol to the media by a process that was dependent on the presence of secretagogues (Fig. 4C). The data of Fig. 4C shows that the increase in cellular efflux occurred after a delay of ~2 h. These data suggest that the enhanced efflux of cellular cholesterol that occurs when ABCG1 is overexpressed in A-549 cells is dependent on the prior exocytosis of lipid-rich lamellar bodies that presumably then function as sterol acceptors.

Altered Lipid Metabolism in Abcg1−/− Alveolar Macrophages—To better understand the metabolic changes that specifically occur in the foamy pulmonary macrophages that accumulate in the lungs of chow-fed older Abcg1−/− mice, we recovered cells after BAL. Greater than 95% of these cells correspond to mac-
rophages. Alveolar macrophages obtained from Abcg1−/− mice were enlarged, many appeared to be multinucleated giant cells, and >90% stained positive for neutral lipid (Fig. 5A, right panels). To our surprise, freshly plated Abcg1−/− cells routinely contained giant crystals of unknown composition (Fig. 5A, upper right panel). In contrast, BAL-derived macrophages from wild-type mice were smaller, uniform in size and <5% stained with oil red O (Fig. 5A, left panels). In addition, giant cells and extracellular crystals were notably absent from control cells (Fig. 5A; data not shown).

Electron microscopy studies identified numerous Abcg1−/− macrophages that not only contained lipid droplets, but surprising numbers of cholesterol crystals (Fig. 5B). Importantly, the Abcg1−/− macrophages contained lamellar bodies (Fig. 5B), indicating that these cells retained the capacity to clear surfactant from the hypophase in vivo.

The Abcg1−/− alveolar macrophages also exhibited altered lipid synthesis, as determined by the incorporation of [14C]oleic acid into lipids (Fig. 5, C and D). Quantification of the different lipid species recovered after thin layer chromatography indicated that incorporation of [14C]oleic acid into CE, TG, and specific phospholipids (PC and PS/PA) was increased in the Abcg1−/− cells (~14-, 3-, and 3-fold, respectively) (Fig. 5, C and D). These changes are not a result of altered endogenous fatty acid pool sizes, since incorporation of the radiolabeled fatty acid into other lipids (DG, MG, SM, PG) was similar in both genotypes (Fig. 5, C and D). Importantly, the incorporation of exogenous fatty acid into cholesterol esters was much greater in Abcg1−/− macrophages, suggesting an excess accumulation of unesterified cholesterol in these cells.

As expected, based on the abnormal cell appearance and altered lipid metabolism, gene expression in the freshly isolated wild-type and Abcg1−/− alveolar macrophages differed significantly; compared with control cells, expression of numerous genes involved in lipid synthesis (Fas, Gpat, Hmgcr, and Srebp-2) were repressed, whereas LXR target genes (Srebp-1c, Lxra, and Abca1) were induced in the lipid-loaded Abcg1−/− macrophages (Fig. 5E). The increased expression of the SREBP-1c target gene Scd-1 appears anomalous and suggests that Scd-1 may also be activated by LXR. In this regard, it has been reported that hepatic Scd-1 mRNA levels are induced in response to dietary cholesterol by a mechanism independent of SREBP-1c maturation, suggesting that it may provide additional unsaturated fatty acids that are utilized to esterify excess toxic cholesterol (41, 42).

Nonetheless, the changes in gene expression in Abcg1−/− alveolar macrophages are consistent with increased cellular levels of lipids/sterols.

For comparative purposes, we also quantified the levels of selective mRNAs in the lungs of the 8-month-old chow-fed mice; the data of Fig. 5F indicate that the expression of a number of genes involved in fatty acid (Acc, Fas), cholesterol (Hmgcr, Srebp-2), and triglyceride (Gpat) synthesis were also repressed in the 8-month-old Abcg1−/− lungs as compared with the control mice. In contrast, the LXR target gene Abca1 was induced in the Abcg1−/− lungs (Fig. 5F). However, although the changes in these gene expression profiles determined in isolated alveolar macrophages and whole lung were generally similar, they were not identi-
cal; for example, Scd-1 mRNA levels in the lungs of wild-type and Abcg1^{−/-} mice were similar, whereas they differed significantly in isolated macrophages (Fig. 5, F versus E). Such differences were not unexpected because there is considerable cellular heterogeneity, and presumably cell-specific gene regulation, in the intact lung. Taken together, the results of Fig. 5 demonstrate...
strate that lipid homeostasis in Abcg1<sup>−/−</sup> alveolar macrophages and lungs as a whole is severely disrupted.

We also analyzed the expression of selected genes in 3-month-old mice that had been fed either chow or a high fat, high cholesterol (HF/HC) diet for 9 weeks. Despite the fact that the lungs of the 3-month-old chow-fed Abcg1<sup>−/−</sup> mice show no evidence of lipid accumulation (Fig. 1A) (31), the expression of Acc, Fas, Scd-1 and Gpat, but surprisingly not Hmgcr or Ldlr, were significantly decreased, as compared with their wild-type littermates (Fig. 6). These data suggest that subtle changes in intracellular lipids of the 3-month-old chow-fed Abcg1<sup>−/−</sup> mice result in repression of these lipid biosynthetic genes. This proposal is consistent with the observation that these same genes were all repressed to similar levels following administration of a HF/HC diet to either young wild-type or Abcg1<sup>−/−</sup> mice (Fig. 6, HF/HC). Interestingly, Dgat expression was increased both in the lungs of young chow-fed Abcg1<sup>−/−</sup> mice and in response to the HF/HC diet (Fig. 6). We conclude that the lungs of young chow-fed Abcg1<sup>−/−</sup> mice repress lipogenic gene expression in an attempt to minimize changes in intracellular lipid levels, but that as these mice age from 3 to 8 months, this repression is inadequate and massive pulmonary lipid accumulation occurs.

**FIGURE 6.** Genes involved in lipid metabolism are disregulated in Abcg1<sup>−/−</sup> lungs. Wild-type and Abcg1<sup>−/−</sup> mice (n = 4/genotype) were fed chow or a high fat, high cholesterol (HF/HC) diet for 9 weeks prior to analysis at 3 months of age. RNA was isolated and gene expression determined using RT-qPCR. Data are expressed as mean ± S.E. for wild-type (open bars) and Abcg1<sup>−/−</sup> (closed bars) mice. *, p = 0.05 Abcg1<sup>−/−</sup> versus wild-type; §, p ≤ 0.05 HF/HC diet versus chow in wild-type mice.

**FIGURE 7.** Surfactant promotes cholesterol ester accumulation in Abcg1<sup>−/−</sup> macrophages. A, oil red O staining of wild-type and Abcg1<sup>−/−</sup> peritoneal macrophages after incubation with surfactant (100 µg phospholipid/ml) for 16 h. Alternatively, macrophages were incubated with [3H]cholesterol-labeled surfactant (1 µCi/ml; 100 µg phospholipid/ml) for 24 h, and then in fresh media for 6–48 h. Cellular lipids were extracted and analyzed by TLC. Areas corresponding to unesterified and esterified cholesterol (UC and CE, respectively) were recovered, and the 3H content determined by scintillation. No differences in cholesterol distribution (%) were observed at the different time points studied (up to 48 h) in each genotype. Consequently, the values for each genotype were pooled and represented as mean ± S.E. Radio-labeled cholesterol esters were undetectable in the original media containing [3H]cholesterol-labeled surfactant (data not shown). *, p ≤ 0.01.
contrast, these lipid droplets were absent or markedly reduced in wild-type macrophages (Fig. 7A).

In separate studies, freshly isolated peritoneal macrophages were incubated with $[^3H]$cholesterol-labeled murine surfactant (100 μg/ml of phospholipid) for 24 h and the cells then analyzed for the presence of radiolabeled unesterified and esterified cholesterol. The data show that Abcg1$^{-/-}$ macrophages accumulated far more radiolabeled cholesterol esters as compared with the wild-type macrophages (56% versus 17%) (Fig. 7B). The demonstration that surfactant-derived cholesterol is preferentially esterified in Abcg1$^{-/-}$ macrophages and that these cells then accumulate oil red O positive lipids provides a mechanism to explain the formation of foam cells and enhanced cholesterol accumulation in the lungs of these mice. The data are consistent with the proposal that Abcg1$^{-/-}$ alveolar macrophages clear cholesterol/surfactant from the hypophase but are defective in their ability to promote sterol efflux and maintain cellular sterol homeostasis.

**Altered Hematocrit Values in Aged Abcg1$^{-/-}$ Mice**—The results from Figs. 1–7 suggest that the pulmonary function of Abcg1$^{-/-}$ mice might be affected. As shown in Table 2, hematocrit levels were increased significantly ($p \leq 0.001$) in 8-month-old Abcg1$^{-/-}$ mice. In contrast, hematocrit levels were similar in 3-month-old wild-type and Abcg1$^{-/-}$ mice (Table 2). These data suggest that lung function is indeed compromised in Abcg1$^{-/-}$ mice in an age-dependent manner, consistent with the changes in pulmonary lipid levels, macrophages, and type 2 cells.

**DISCUSSION**

In the current report we demonstrate that mice defective in ABCG1 exhibit an age-related, progressive pulmonary disease that has many of the properties associated with respiratory distress syndromes; by the age of 8 months, the lungs of chow-fed Abcg1$^{-/-}$ mice exhibit cellular accumulation, massive lipid deposition in macrophages and pneumocytes type 2, and accumulation of excessive levels of surfactant phospholipid.

Studies with both patients and mice have linked respiratory syndromes to mutations in genes encoding proteins involved in macrophage differentiation and surfactant secretion. These include GM-CSF, SP-B, SP-C, SP-D, ABCA3, ABCA1, and LAL (11–18). Together, these reports illustrate the important roles of pulmonary macrophages and pneumocytes type 2 in regulating lipid, and especially surfactant, metabolism.

The findings that pulmonary pneumocytes type 2 and macrophages accumulate massive amounts of sterol and/or phospholipid in chow-fed Abcg1$^{-/-}$ mice, together with the observation that surfactant levels are also increased significantly, identify a critical role for ABCG1 in maintaining normal lipid metabolism in the lung. The observation that these changes in the lungs of Abcg1$^{-/-}$ mice occur in the absence of altered plasma lipid levels suggests that pulmonary sterol/lipid homeostasis is highly sensitive to minor changes in local lipid metabolism within the lung. Interestingly, deletion of Sp-D also results in the appearance of cholesterol crystals in alveolar macrophages and in alveolar proteinosis (18). However, to our knowledge, no functional role for SP-D in controlling sterol accumulation has been reported.

The accumulation of excessive numbers of dense and enlarged lamellar bodies within Abcg1$^{-/-}$ type 2 cells (Fig. 2), suggests that surfactant metabolism is abnormal. Nonetheless, we conclude that Abcg1$^{-/-}$ type 2 cells retain the capacity to secrete surfactant since surfactant levels (recovered after BAL) increased ~5-fold and tubular myelin is present in the alveolar hypophase of Abcg1$^{-/-}$ mice.

Recent studies have linked mutations in ABCA3 (13) or ABCA1 (17) to respiratory distress syndrome and altered surfactant secretion in humans and mice, respectively. Thus, the decrease in Abca3 that occurs in the lungs of Abcg1$^{-/-}$ mice (Fig. 3) may contribute to the accumulation of lamellar bodies in Abcg1$^{-/-}$ type 2 cells (Fig. 2, A, D, E). Additional studies will be needed to determine whether the increased number of type 2 cells in the Abcg1$^{-/-}$ lungs compensates for any defect in surfactant/lamellar body secretion. However, the mechanism by which ABCG1 controls surfactant metabolism is unknown at this time. Recent studies have indicated that ABCA1 promotes surfactant efflux from pneumocytes type 2 (43, 44). Thus, we conclude that lipid homeostasis in the lung is particularly sensitive to mutations in ABCG1, ABCA1, and ABCA3, all of which modulate lipid flux in macrophages and/or type 2 pneumocytes.

The current study also highlights the importance of pulmonary macrophages in metabolizing surfactant cholesterol. To our knowledge, the critical role of macrophages in this process has not been previously reported. Abnormal expression of lipid metabolic genes were noted in both 3- and 8-month-old Abcg1$^{-/-}$ mice despite the fact that the lungs of the younger mice are histologically normal (Figs. 1 and 6). These latter data suggest that subtle changes in lipid signaling molecules in the lungs of 3-month-old chow-fed Abcg1$^{-/-}$ mice result in altered expression of genes controlling lipid homeostasis.

Lipogenic and cholesterogenic pathways are regulated by the transcription factors LXR and SREBP, that are in turn regulated by cholesterol, oysterolys and polyunsaturated fatty acids (42, 45, 46). The gene expression data (Figs. 5 and 6) wherein we report the repression of lipogenic and cholesterogenic enzymes and the activation of LXR target genes in both Abcg1$^{-/-}$ lungs and alveolar macrophages are consistent with decreased nuclear levels of SREBP and the concomitant activation of LXR by sterols and oysterolys (Fig. 8). These results are also in agreement with previous reports from Kim et al. (41) and Repa et al. (42) in which, under certain dietary conditions, cholesterol-driven increases in Srebp-1 and Scd-1 mRNA were accompanied by repression of other SREBP-1c targets such as Fas and Acc. These authors speculate that maturation of SREBP-1c and the relative direct effects of SREBP and LXR on the promoters.

### Table 2

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<th>Wild-type</th>
<th>Abcg1$^{-/-}$</th>
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<tr>
<td>3-month-old$^a$</td>
<td>47.9 ± 0.5</td>
<td>48.8 ± 0.9</td>
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<tr>
<td>8-month-old$^a$</td>
<td>48.4 ± 0.6</td>
<td>53.2 ± 0.6</td>
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$^a$ n, 7 genotype.

$^b$ n, 10 genotype.

$^p \leq 0.001.$
of these genes might explain this apparent contradictory expression pattern (41, 42). Importantly, the Abcg1−/− alveolar macrophages accumulate cholesterol esters and cholesterol clefts (Figs. 1, 5, and 7) despite the induction of Abca1 mRNA levels (Fig. 5). Thus, increased ABCA1 expression is unable to compensate for the loss of ABCG1 in alveolar macrophages and the result is intracellular cholesterol accumulation.

In a previous study we noted severe hepatic steatosis when Abcg1−/− mice were fed a HF/HC diet (31). No such lipid accumulation occurs in chow-fed Abcg1−/− mice (Fig. 5E). Thus, increased Abca1 expression is unable to compensate for the loss of ABCG1 in alveolar macrophages and the result is intracellular cholesterol accumulation.

Although ABCG1 has been shown to promote efflux of cellular cholesterol to either HDL of phospholipid vesicles, but not to lipoprotein apoproteins (30–35), the molecular mechanism remains to be determined. Indeed the cellular localization of ABCG1 is controversial as the protein has been reported to be localized to the plasma membrane or to perinuclear membranes (33–35). Nevertheless, a primary defect in cholesterol mobilization in Abcg1−/− pneumocytes and alveolar macrophages is consistent with the accumulation of intracellular lamellar bodies and cholesterol ester droplets (Figs. 1, 2, 5, and 7). As outlined in Fig. 8, we hypothesize that the lung-specific lipidosis observed in chow-fed Abcg1−/− mice is the result of combined defects in both type 2 cells and alveolar macrophages. Thus, loss of ABCG1 likely results in partially defective secretion of surfactant (and accumulation of enlarged lamellar bodies) which is compensated by hyper trophy of the type 2 cells. In addition, we hypothesize that the continual uptake of cholesterol-containing surfactant by alveolar macrophages, coupled with impaired efflux of cholesterol, results over time in the generation of foam cells. Such lipid-loaded cells
ABCG1 and Lung Function

may have altered function and thus accelerate the deposition of lipid in the lung.

Although to date a human disease has not been linked to altered ABCG1 expression, the current studies suggest that mutations and/or polymorphisms in the human ABCG1 gene might lead to alterations in pulmonary function, in the absence of changes in plasma lipids. Based on our studies in the Abcg1−/− mouse, it is tempting to speculate that the activity of ABCG1 might play a role in human pulmonary lipidosis.

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