Iron increases expression of iron-export protein MTP1 in lung cells

FUNMÉI YANG,1 XINCHAO WANG,2 DAVID J. HAILE,3 CLAUDE A. PIANTADOSI,4 AND ANDREW J. GHIO5

1Departments of Cellular and Structural Biology and 3Medicine, University of Texas Health Science Center, San Antonio, Texas 78284; 2Center for Environmental Medicine and Lung Biology, University of North Carolina, Chapel Hill, 27599; 4Department of Internal Medicine, Duke University Medical Center, Durham, 27710; and 5National Health and Environmental Effects Research Laboratory, United States Environmental Protection Agency, Research Triangle Park, North Carolina 27711

Received 16 April 2002; accepted in final form 16 May 2002

Yang, Funmei, Xinchao Wang, David J. Haile, Claude A. Piantadosi, and Andrew J. Ghio. Iron increases expression of iron-export protein MTP1 in lung cells. Am J Physiol Lung Cell Mol Physiol 283: L932–L939, 2002.—Accumulation of reactive iron in acute and chronic lung disease suggests that iron-driven free radical formation could contribute to tissue injury. Safe transport and sequestration of this metal is likely to be of importance in lung defense. We provide evidence for the expression and iron-induced upregulation of the metal transporter protein-1 (MTP1) genes in human and rodent lung cells at both the protein and mRNA levels. In human bronchial epithelial cells, a 3.8-fold increase in mRNA level and a 2.4-fold increase in protein level of MTP1 were observed after iron exposure. In freshly isolated human macrophages, as much as an 18-fold increase in the MTP1 protein level was detected after incubation with an iron compound. The elevation in expression of MTP1 gene was also demonstrated in iron-instilled rat lungs and in hypotransferrinemic mouse lungs. This is similar to our previous findings with divalent metal transporter-1 (DMT1), an iron transporter that is required for iron uptake and intracellular iron trafficking. These studies suggest the presence of iron mobilization and/or detoxification pathways in the lung that are crucial for iron homeostasis and lung defense.

OXIDATIVE STRESS PLAYS A CENTRAL role in the pathogenesis of both acute and chronic pulmonary disease. Generation of reactive oxygen intermediates (ROIs) can be dependent on the presence of catalytic transition metals which can trigger the Fenton chain reaction. Incompletely or loosely coordinated iron can be released during tissue injury. This catalytically active metal can contribute heavily to subsequent tissue injury. In fact, accumulation of reactive iron has been demonstrated in a number of lung diseases including acute respiratory distress syndrome (ARDS) and cystic fibrosis (5, 16, 23). Consequently, sequestration of iron into a less-reactive form is likely to be important in lung defense. Animal studies have shown that iron chelators can prevent lung injury that is secondary to iron-driven free radical formation (19). However, effective iron-chelating drugs that do not inflict damage on healthy tissues have not been developed. A lack of knowledge about iron metabolism in the lung hampers a complete understanding of its role in both normal and diseased states, thus precluding the development of specific therapeutic interventions.

Recently there have been several major findings regarding iron absorption and export at the cellular level, including the discovery of two transmembrane iron transporters: divalent metal transporter-1 (DMT1, also known as DCT1 or SLC11A2) and metal transporter protein-1 (MTP1, also known as ferroportin 1, Ireg1, or SLC11A3). These two proteins present most abundantly in enterocytes but are also synthesized in other cells including macrophages in the liver (Kupffer cells) and spleen. In the duodenal epithelium, DMT1 is present mainly in the apical membrane (15), whereas MTP1 is localized to the basolateral membrane (1, 7, 20). Several lines of evidence indicate that DMT1 and MTP1 are required for iron uptake from the intestinal lumen to the duodenal epithelium and for iron export to the circulation, respectively. The ability of these two proteins to import and export iron across the plasma membrane has been demonstrated in Xenopus oocytes and cultured mammalian cells (1, 7, 15, 20, 22, 24). Interestingly, although mutations in DMT1 result in a defect in intestinal iron absorption and erythroid iron use in rodents (8, 9), a mutation in the MTP1 gene leads to hemochromatosis and iron toxicity in affected humans (21). Mice with sex-linked anemia (SLA) that were defective in hephaestin, which is a protein that is required for the function of MTP1 in the intestine, were...
unable to export iron from the intestinal epithelial cells despite total-body iron depletion (25). In addition, zebrabfish that carried MTP1 mutations were impaired in the transport of iron from maternally derived yolk stores to the circulation (7). Conversely, overexpression of MTP1 in tissue-culture cells resulted in intracellular iron depletion (1). The exact roles of DMT1 and MTP1 in other tissues have not been established.

To understand the roles of DMT1 and MTP1 in iron homeostasis in the lung, we studied the expression and regulation of these two genes in lung cells. We found that DMT1 is expressed in airway epithelial cells in humans (26). Interestingly, the expression of DMT1 without the iron-response element (IRE) was increased after exposure to iron, whereas expression of DMT1 with IRE was not increased (26). In this paper, we report the detection of MTP1 in specific lung cells. We also demonstrate an upregulation of the MTP1 gene by iron that parallels the regulation of the DMT1 gene in lung cells and discuss the possible roles of DMT1 and MTP1 in iron detoxification in the lung.

MATERIALS AND METHODS

Collection of lung tissues. Surgical specimens of human lung tissues were obtained, quick-frozen in Tissue-Tek optimum cutting temperature (OCT) compound, and stored at −80°C until cryosections were prepared. Alternatively, lung tissues were obtained, quick-frozen in Tissue-Tek optimum cutting temperature (OCT) compound, and stored at −80°C until cryosections were prepared. Alternatively, lung tissues were obtained, quick-frozen in Tissue-Tek optimum cutting temperature (OCT) compound, and stored at −80°C until cryosections were prepared. Alternatively, lung tissues were obtained, quick-frozen in Tissue-Tek optimum cutting temperature (OCT) compound, and stored at −80°C until cryosections were prepared.

Culture of BEAS-2B cells. BEAS-2B is an immortalized line of normal human bronchial epithelium derived by transfection of primary cells with simian virus-40 (SV-40) early-region genes. This particular subclone undergoes squamous differentiation in response to serum (17). Cells were grown to confluence on uncoated, plastic, 12-well plates in ketone growth medium (KGM, Clonetics), which is essentially MCDB 153 medium supplemented with 5 ng/ml human epidermal growth factor, 5 mg/ml insulin, 0.5 mg/ml hydrocortisone, 0.15 mM calcium, 30 μg/ml bovine pituitary extract, 0.1 mM ethanolamine, and 0.1 mM phosphoethanolamine. Previous investigation (13) demonstrated no difference in the response to iron of BEAS-2B cells relative to normal human bronchial epithelial cells. These cells were exposed to ferric ammonium citrate (FAC) in varying concentrations for 24 h.

Macrophage suspension. Alveolar macrophages were collected from healthy volunteers during bronchoalveolar lavage (11). These cells were exposed to FAC in varying concentrations for 24 h.

Hypotransferrinemic mice. Homozygous hypotransferrine mic mice (hpx) were obtained from matings between either hpx/hpx and hpx+/+ or hpx+/+ and hpx+/+ animals. Homozygous hpx pups were small, anemic, and pale at birth. They were maintained by weekly intraperitoneal injections of mouse serum (up to 0.3 ml; Ref. 3). The genotypes of homzygous and wild-type animals were confirmed by measuring serum concentrations of transferrin. All animals were kept in pathogen-free facilities and were routinely monitored for pathogens and viruses. After the animals were anesthetized with halothane (Aldrich, Milwaukee, WI), they were euthanized by exsanguination. Mouse lungs were intratracheally instilled with 4% paraformaldehyde, immersed in the same fixative for 16 h, and embedded in paraffin using standard histological procedures. Alternatively, lung tissue was immediately stored in liquid nitrogen and used later for the preparation of cell lysate or RNA.

Intratracheal instillation of iron, asbestos, or residual oil fly ash. Adult male Sprague-Dawley rats were anesthetized with halothane and intratracheally instilled with 0.5 ml of normal (0.9%) saline or saline that contained 200 μM FAC or 500 μg of crocoddile asbes. Hpx and wild-type mice were intratracheally instilled with 50 μl of either normal saline or saline that contained 50 μg of residual oil fly ash (ROFA). Animals were killed 24 h after exposure, and lung tissues were collected for analysis of MTP1 gene expression.

RNA isolation. Total RNA was extracted from the lung tissue homogenates using TRIzol reagent (Life Technologies) according to the protocol provided by the vendor. To isolate RNA from BEAS-2B cells cultured in 12-well plates, cells were lysed with 4 M guanidine thiocyanate (Boehringer Mannheim, Indianapolis, IN), 50 mM sodium citrate, 0.5% sarcosyl, and 0.01 M dithiothreitol. After cells were dislodged from wells with scrapers (Costar), lysates were sheared with four passes through a 22-gauge needle and extracted with a phenol-chloroform mixture. After extraction, RNA was precipitated from the aqueous phase.

RT-PCR. RNA (100 ng total) was reverse transcribed using Moloney’s murine leukemia virus reverse transcriptase (Life Technologies). Quantitative PCR was performed using TaqMan polymerase, and fluorescence was detected on an ABI Prism 7700 sequence detector (PE Biosystems, Foster City, CA). MTP1 mRNA levels were normalized using the expression of GAPDH as a housekeeping gene. Relative quantifications of MTP1 and GAPDH mRNA were based on standard curves prepared from serially diluted mouse mast-cell cDNA. The following sense and antisense sequences were employed: for MTP1, 5′-AAC CGC CAG AGA GGA TGC T-3′ (sense), 5′-CCA AGG TAG AGA AAT TTG GTA-3′ (antisense), and 5′-TGG ATC CCT CCG AGA CTA CCT CAC CTG-3′ (probe); and for GAPDH, 5′-CGA GTG CCG AGT C-3′ (sense), 5′-GAA GAT GAT GAT GGG ATT TC-3′ (antisense), and 5′-CCA GCT TCC CCG TCT CAG CC-3′ (probe).

Western blot analysis. Tissue homogenates or cell lysates were used in this analysis. Cells were washed with ice-cold PBS, lysed with buffer that contained 1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS, and protease inhibitors (Cocktail Set III, Calbiochem, La Jolla, CA), and then sheared through a 22-gauge needle. Protein content was determined using the Bradford assay (Bio-Rad, Hercules, CA). Lysates that contained 70 μg of protein were mixed with an equal volume of 4X sample-loading buffer (0.5 M Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 0.7 M β-mercaptoethanol, and 0.05% bromophenol blue). Samples were separated by electrophoresis on an 8% SDS acrylamide gel and transferred to a nitrocellulose membrane (Bio-Rad). The membrane was blocked with 3% nonfat milk in PBS and incubated with an antibody directed against MTP1 (1). The membrane was stained with a horse-radish peroxidase-conjugated goat anti-rabbit IgG antibody (1:2,000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA) and developed using enhanced chemiluminescence (ECL Kit, Amersham Pharmacia Biotech).

Immunohistochemistry. Lung paraffin sections (5 μm thick) were cut, floated on a protein-free water bath, mounted on silane-treated slides (Fishers, Raleigh, NC), and air-dried overnight. The slides were heat-fixed at 60°C in a slide dryer (Shandon Lipshaw, Pittsburgh, PA) for 10 min and cooled to room temperature. Sections were then deparaffinized and hydrated to 95% alcohol. Endogenous peroxidase activity was blocked with 0.6% H2O2 in absolute methanol for 8 min. Slides were rinsed in 95% alcohol for 2 min, placed in deionized H2O, and washed in PBS. After treatment with Cyto Q

L933

MTP1 AFTER IRON

www.ajplung.org • VOL 283 • NOVEMBER 2002 • www.ajplung.org

Downloaded from http://ajplung.physiology.org/ by 10.220.33.5 on September 30, 2017
Background Buster (Innovex Biosciences) for 10 min, slides were incubated with rabbit anti-MTP1 antibodies (1) diluted in 1% bovine serum albumin for 45 min at 37°C in PBS. Slides were incubated with biotinylated linking antibody for 10 min at room temperature and washed with PBS before a peroxidase enzyme label was applied (Stat-Q Staining System, Innovex Biosciences). After tissue sections were incubated for 10 min at room temperature and washed with PBS, the sections were developed with 3,3′-diaminobenzidine-tetrahydrochloride for 3 min at room temperature. Sections were counterstained with hematoxylin.

In situ hybridization. Lung cryosections or paraffin sections of 5-μm thickness were prepared. For cultured cells, cytospin specimens were prepared after the cells were removed from the culture plates. Processing and hybridization of lung sections were performed as previously described by Zeller and Rogers (33). 35S-labeled single-strand RNA probes were synthesized using the Riboprobe System (Promega) according to the procedure supplied by the vendor. The two plasmid DNA templates used for synthesizing riboprobes contained a 2.7-kb human MTP1 cDNA insert and a 2.1-kb mouse MTP1 cDNA insert (1), respectively. After hybridization, sections were treated with 20 μg/ml ribonuclease A at 37°C for 30 min and washed at high stringency (50% formamide, 2× saline-sodium citrate, and 0.1% β-mercaptoethanol at 55°C). For autoradiography, slides were coated with film emulsion (Kodak NTB-2) and exposed at 4–8°C for 3–5 days. Slides were then developed with Kodak D19 developer and stained with hematoxylin and eosin.

RESULTS

Cell type-specific expression of MTP1 gene in human lung. To determine whether the MTP1 gene is expressed in the lung, we conducted in situ hybridization experiments using lung surgical specimens from patients with a variety of lung diseases including adenocarcinoma, emphysema, and fibrosis. MTP1 mRNA was predominantly detected in alveolar macrophages and airway epithelial cells in these specimens (Fig. 1). However, the levels of expression varied greatly not only among lung specimens from different individuals but also among different areas within the same lung section. This result indicates that factors that are present in different microenvironments within the lungs may affect the expression of the MTP1 gene. The distribution of MTP1 protein in lung cells was demonstrated by the procedure of immunohistochemistry. Again, MTP1 protein was present mainly in airway epithelial cells and alveolar macrophages (Fig. 2). A lower level of MTP1 was detected in endothelial cells. In some lung specimens where MTP1 gene expression was elevated, immunostain could also be seen in other lung cells including types 1 and 2 pneumocytes. A semiquantitative comparison of MTP1 gene expression in different types of lung cells is summarized in Table 1.

Dosage-dependent increase of MTP1 mRNA and protein by iron in human bronchial epithelial cells. Because reactive iron has been detected in many diseased lung tissues, we studied the effect of iron on the expression of MTP1 in lung cells. This study was conducted first using BEAS-2B cells. Cells were exposed to different concentrations of FAC for 24 h. The levels of MTP1 mRNA were compared by the procedure of real-
time RT-PCR using the level of GAPDH mRNA as an internal control. The ratios of MTP1-to-GAPDH mRNA in cells treated with 0, 125, 250, and 500 μM of FAC were 0.39, 0.56, 0.80, and 1.50, respectively. The data represent the average value of four repeats and clearly demonstrate a dosage-dependent increase of MTP1 mRNA after iron treatment. As much as a 3.8-fold increase in MTP1 mRNA level was observed in cells exposed to 500 μM of iron compared with cells incubated without iron.

The amounts of MTP1 protein in cell extracts derived from iron-treated and untreated BEAS-2B cells were analyzed by Western blot. As shown in the immunoblot (Fig. 3), a protein band with molecular mass ~90–110 kDa reacted strongly with the anti-MTP1 antibody. In agreement with what was observed for MTP1 mRNA, there was a FAC dosage-dependent increase of this 90–110-kDa protein. A 2.7-fold increase in the intensity of this band was detected in cells exposed to 500 μM of FAC compared with cells incubated in the absence of iron (Fig. 3). MTP1 has a calculated molecular mass of 62 kDa, and a 60–65-kDa MTP1 band has been observed in intestines (20). However, a band with a higher molecular mass (~110 kDa) has also been consistently detected in liver and spleen lysate in mice (Yang and colleagues, unpublished results). The reactivity of this band to anti-MTP1 antiserum was specifically inhibited in the presence of MTP1 peptide that was used to produce and purify the anti-MTP1 antiserum. Other investigators also reported the detection of MTP1 bands with molecular masses 80 and 110 kDa higher than 62 kDa in rat-brain homogenates (4). These bands may reflect posttranslational modification of the protein. The nature of the difference between the MTP1 reactive bands derived from intestine and other tissues or cells is not clear at the present time. A comparison of the levels of MTP1 gene expression in BEAS-2B cells exposed to different concentrations of iron is summarized in Table 2.

Iron increases MTP1 gene expression in human alveolar macrophages. We investigated the regulation of MTP1 gene expression by iron in freshly isolated human alveolar macrophages. A significant increase in the level of MTP1 mRNA after iron treatment was demonstrated by in situ hybridization (Fig. 4) in which signals for MTP1 mRNA (silver grain) were much higher in the iron-treated than in the untreated cells. When the amounts of MTP1 protein in cell extracts were analyzed via Western blot, a FAC dosage-dependent increase in the amount of MTP1-reactive protein bands was observed. Analysis of the band of ~90–110 kDa (Fig. 5, arrow) revealed 11-, 17-, and 18-fold increases in cells exposed to 125, 250, and 500 μM FAC, respectively, compared with cells incubated in the absence of FAC. The response of the MTP1 gene to iron in alveolar macrophages appeared to be much stronger than what we found in bronchial epithelial cells. Two other bands with smaller molecular masses could also be seen in the immunoblot. The nature of these bands is not clear but could represent the proteolytic products of the high-molecular-mass band.

Table 1. Comparison of levels of MTP-1 gene expression in different lung cells

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>MTP1 mRNA Level</th>
<th>MTP1 Protein Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Airway epithelial cells</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Alveolar macrophages</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Type I and type II cells</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

*Semiquantitative analysis of metal transporter protein-1 (MTP1) mRNA levels was based on amount of silver grains observed in each cell type in in situ hybridization experiments. †Comparison of MTP1 protein levels in different cell types was based on amount of immunostain observed in immunohistochemical studies.

Table 2. Quantitative analysis of MTP1 gene expression in BEAS-2B cells exposed to iron

<table>
<thead>
<tr>
<th>Ferric Ammonium Citrate, μM</th>
<th>n</th>
<th>0</th>
<th>125</th>
<th>250</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>90–110-kDa MTP1*</td>
<td>2</td>
<td>1.00</td>
<td>1.57</td>
<td>1.71</td>
<td>2.73</td>
</tr>
<tr>
<td>MTP1 mRNA†</td>
<td>4</td>
<td>1.00</td>
<td>1.44</td>
<td>2.08</td>
<td>3.85</td>
</tr>
</tbody>
</table>

Values are means; n, no. of experiments. *Relative protein amounts were analyzed by using a densitometer to measure intensities of 90–110-kDa MTP1-reactive bands in immunoblots. †MTP1 mRNA levels were measured via real-time RT-PCR; data were calculated from MTP1 mRNA/GAPDH mRNA ratios.
Exposure of rat lung to iron or asbestos increases level of MTP1 protein. The iron-responsive upregulation of MTP1 gene expression in lung cells was investigated in vivo. Adult rats were intratracheally instilled with 0.5 ml of 200 μM FAC 24 h before the collection of lung tissues, whereas control animals received 0.5 ml of saline. In control animals, a low level of immunostaining for MTP1 was seen in epithelial cells in the upper large airways; however, no immunostain could be detected in the most distal airways and alveolar macrophages (Fig. 6A). In contrast, a high level of MTP1 proteins was detected in most airway epithelia and alveolar macrophages in animals treated with FAC (Fig. 6, B and C).

We also studied the effects of asbestos, which is associated with elevations in tissue iron, on the expression of the MTP1 gene. Adult rats were exposed to asbestos by intratracheal instillation of 500 μg of crocidolite asbestos. The amounts of MTP1 protein were analyzed by immunohistochemistry 24 h after treatment. As shown in Fig. 6D, immunostain for MTP1 can be found in the area where asbestos fibers are present but is negligible in the region where the fiber is absent (data not shown).

MTP1 level is increased in lung in a mouse model of iron overload. In previous studies, we investigated the effects of altered iron homeostasis on the defense mechanism of the lung using the hpx mouse model. Hpx mice are defective in transferrin gene and are anemic but develop tissue-iron overload (3). Interest-
ingly, these mice are resistant to both metal-rich-particle-induced and hyperoxia-induced lung injuries (12, 30). The \textit{hp} \textsubscript{x} mice have elevated expressions of iron-storage and iron-transport proteins including ferritin and lactoferrin despite the lack of transferrin. To determine whether MTP1 gene expression is altered in the lung of this animal model of iron overload, we conducted immunohistochemical studies on lung sections derived from homozygous \textit{hp} \textsubscript{x}, heterozygous \textit{hp} \textsubscript{x}, and wild-type mice. Although only a low level of MTP1 protein was detected in the untreated wild-type mouse lungs (Fig. 7A), a high level of MTP1 was detected in the lungs of the homozygous mouse (Fig. 7B). In the \textit{hp} \textsubscript{x} mouse lungs, a heavy immunostain for MTP1 could be seen not only in airway epithelial cells and alveolar macrophages, but also in many other lung cells. In heterozygous mouse lungs, immunostain for MTP1 was higher than that in the wild-type mice but much lower than that in homozygous mice (data not shown). In this study, we also included tissue sections derived from wild-type mouse lungs that were exposed to a ROFA, which is an industrial, metal-rich particle that contains a high amount of iron. The MTP1 gene expression was strongly augmented in the lung after the animals were tracheally instilled with 50 \textmu{g} of ROFA (Fig. 7C) and resembled what was observed in homozygous \textit{hp} \textsubscript{x} mouse lungs.

\textbf{DISCUSSION}

In this study, we have shown that MTP1, as similarly observed for DMT1 (26), is expressed at a high level in lung cells under certain physiological or pathological conditions. Our findings support the importance of iron homeostasis in lung defense. The expression of the MTP1 gene varied greatly not only among different human-lung specimens but also among different regions within a given lung specimen, which suggests that microenvironments could affect the expression of the MTP1 gene. Several factors including iron (1, 20, 34), zinc (29), hypoxia (20), lipopolysaccharide (Yang and colleagues, unpublished results), and inflammatory cytokines (Liu and coworkers, unpublished results) could affect the MTP1 gene expression. Our study demonstrated that iron is a key modulator in the regulation of the MTP1 gene in the lung. In duodenal epithelial cells, both DMT1 and MTP1 genes are inversely regulated by body iron stores and dietary iron (1, 6, 14, 20, 32). In contrast, MTP1 gene expression in the Kupffer cells of the liver is upregulated in iron-replete mice and downregulated in iron-deprived mice (1). We have shown that iron augments the expression of the MTP1 gene in airway epithelial cells and alveolar macrophages in vivo and in cultured cells. This is similar to our previous findings on the regulation of the DMT1 gene by iron (26). The molecular mechanism(s) for the differential regulation of DMT1 and MTP1 by iron in different tissues is not known but may be related to tissue-specific roles of these two proteins in iron homeostasis. DMT1 and MTP1, which play key roles in iron absorption in the intestine, may be responsible for iron recycling and detoxification in the lung. Similarly, MTP1 expression in liver and spleen was related to scavenging iron from senescent erythrocytes (1). We hypothesize that upregulation of the DMT1 and MTP1 genes in response to the elevated iron level is crucial for lung health.

We have found that iron upregulates MTP1 gene expression in alveolar macrophages and airway epithelial cells at both mRNA and protein levels. This suggests a transcriptional regulation of the MTP1 gene in the lung cells but does not rule out additional posttranscriptional or translational regulation of the gene. Despite the presence of the 5'-IRE sequence in the MTP1 mRNA and its affinity for iron-regulatory protein (IRP), an IRE/IRP-independent iron-regulatory path-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig7.png}
\caption{MTP1 gene expression is upregulated in an in vivo model of tissue-iron overload. Immunohistochemistry was conducted on lung sections derived from a wild-type mouse (A), a homozygous hypotransferrinemic mouse (B), and a wild-type mouse treated with residual oil fly ash (ROFA; C). MTP1 protein was elevated in homozygote mouse lung (B) and wild-type mouse lung treated with ROFA (A) compared with wild-type mouse lung.}
\end{figure}
way for the MTP1 gene appears to be preset in duodenal enterocytes (1, 20) and placental cells (10). Similarly, DMT1 is regulated by iron through an IRE-independent pathway in lung cells. Iron increased the expression of the non-IRE DMT1 isoform but had no effect on the expression of the IRE DMT1 isoform (26).

The complicity of iron-regulatory pathways involved in the DMT1 and MTP1 genes again implicates the vital roles of these two proteins in iron homeostasis. In iron-absorbing duodenal epithelial cells, DMT1 is the apical membrane iron transporter, whereas MTP1 is localized to the basolateral membrane. Our preliminary study, which used the techniques of immunohistochemistry, immunofluorescent labeling, and confocal microscopy, revealed that DMT1 is located in the apical region and MTP1 is in the plasma membrane of airway epithelial cells (Yang and colleagues, unpublished results). Interestingly, iron taken up by human bronchial epithelial cells cultured on a porous membrane was efficiently released across the apical but not the basolateral surface in a protein-bound form (Ghio and colleagues, unpublished results). It is likely that DMT1- and MTP1-mediated iron uptake and export form a novel iron-detoxification process in the airway epithelium. To protect the lung tissues, airway epithelial cells may take up reactive iron derived from pollutants or released from injured tissues and secrete it into the airway lumen in less-reactive forms. The secreted iron-protein complexes may eventually be expelled in sputum as was observed in normal volunteers and patients with chronic bronchitis and cystic fibrosis (2).

Although bronchial epithelial cells serve as filters to protect the conducting airways, alveolar macrophages are the scavengers that guard the lower respiratory tracts. These cells are capable of taking up large amounts of iron, although in so doing the cells become vulnerable to oxidative damage (28). Recent identification of the hemoglobin-haptoglobin scavenger receptor CD163 on macrophages (18) and the finding that alveolar macrophages synthesize a high amount of haptoglobin (31) indicate that alveolar macrophages also play a major role in scavenging hemoglobin in the lung via a haptoglobin-dependent pathway. Although it was shown that alveolar macrophages could release ferritin-bound iron especially during iron overload (27), the fate of the iron taken up by alveolar macrophages is not well understood. Expression and upregulation by iron of the DMT1 and MTP1 genes in alveolar macrophages may facilitate the mobilization and perhaps safe sequestration of iron in the lower respiratory tracks.

In summary, we have provided evidence for the expression and iron-stimulated upregulation of the recently identified iron-exporting MTP1 gene in human and rodent lung cells. Results from this study and our previous work on the DMT1 gene (26) suggest that an iron-detoxification pathway exists in the respiratory tract to protect against iron toxicity that results from environmental insults. Although the molecular mechanism involved in these pathways remains to be elucidated, it is also important to determine whether and how these pathways are altered in lung disorders.

The authors thank Emily A. Van Beveren for assistance during the preparation of the manuscript.

This work is supported by a Veterans Administration merit grant award (to D. J. Haile), National Institutes of Health Grant R01 DK-53079 (to D. J. Haile), and a Morrison Trust research grant (to F. Yang). This report has been reviewed by the National Health and Environmental Effects Research Laboratory, US Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

REFERENCES


