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Deficiency in the divalent metal transporter 1 increases bleomycin-induced lung injury

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Abstract Exposure to bleomycin can result in an inflammatory lung injury. The biological effect of this anti-neoplastic agent is dependent on its coordination of iron with subsequent oxidant generation. In lung cells, divalent metal transporter 1 (DMT1) can participate in metal transport resulting in control of an oxidative stress and tissue damage. We tested the postulate that metal import by DMT1 would participate in preventing lung injury after exposure to bleomycin. Microcytic anemia (*mk/mk*) mice defective in DMT1 and wild-type mice were exposed to either bleomycin or saline via intratracheal instillation and the resultant lung injury was compared. Twenty-four h after instillation, the number of neutrophils and protein concentrations after bleomycin exposure were significantly elevated in the *mk/mk* mice relative to the wild-type mice. Similarly, levels of a pro-inflammatory mediator were significantly increased in the *mk/mk* mice relative to wild-type mice following bleomycin instillation. Relative to wild-type mice, *mk/mk* mice demonstrated lower non-heme iron concentrations in the lung, liver, spleen, and splenic, peritoneal, and liver macrophages. In contrast, levels of this metal were elevated in alveolar macrophages from *mk/mk* mice. We conclude that DMT1 participates in the inflammatory lung injury after bleomycin with *mk/mk* mice having increased inflammation and damage following exposure. This finding supports the hypothesis that DMT1 takes part in iron detoxification and homeostasis in the lung.

Keywords Iron · Lung diseases · Ferritin · Mouse

Abbreviations

DMT1	Divalent metal transporter 1
FBS	Fetal bovine serum
FPN1	Ferroportin 1
ICPOES	Inductively coupled plasma optical emission spectroscopy
IRE	Iron responsive element
IRP	Iron regulatory proteins
MIP2	Macrophage inflammatory protein-2
Nramp	Natural resistance-associated macrophage proteins

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Introduction

Since the lung is continuously exposed to both oxygen and iron (Schroeder et al. 1987), protective mechanisms against iron-induced oxidative injury are necessary for lung health and defense. Several proteins which directly participate in iron transport and storage are synthesized by lung cells and present at relatively high levels. Among these is the transmembrane iron transporter divalent metal transporter 1 (DMT1, aka SLC11A2). DMT1 is a member of the natural resistance-associated macrophage proteins (Nramp), a small family of structurally and functionally related polypeptides which represent a group of transporters in vertebrates. These proteins have been conserved across numerous species with homologues identified in yeasts, bacteria, worms, flies, and plants. DMT1 functions to transport divalent metal cations including Fe^{2+} (Gunshin et al. 1997). In animals, it was first identified in the enterocyte of the proximal duodenum where it imports Fe^{2+} into the epithelial cell (Wood and Han 1998). However, this protein is now recognized to be expressed in most cell types as an integral membrane protein (molecular weight of 90–100 kD) modified by glycosylation (Vidal et al. 1995).

DMT1 is produced in airway epithelial cells and alveolar macrophages (Wang et al. 2002). Rather than an import of Fe^{2+} to meet the nutritional needs of a living system, this transporter appears to take part in iron transport in the lung for the purpose of its detoxification (Ghio et al. 2005). Consequently, DMT1 expression increases in lung cells after exposure to iron and pro-inflammatory stimuli (Wang et al. 2002, 2005; Ghio et al. 2005). A cycle of iron exists in the lower respiratory tract with import of catalytically-active iron up by lung cells via a DMT1-dependent pathway followed by release of the metal (in a less reactive state) via a ferroportin 1 (FPN1) dependent pathway (Turi et al. 2004).

The bleomycins are a family of glycopeptide antibiotics widely used in the treatment of selected neoplasms (Lovstad 1991). This chemotherapeutic agent forms a 1:1 complex with ferric and ferrous ions. In the presence of molecular oxygen, the bleomycin–iron complex catalyzes the generation of reactive oxygen species which not only can cause breakage of the DNA double helix (Sam et al. 1998) but can also damage other tissue components. The clinical utility of bleomycin is often limited by a dose-dependent pulmonary toxicity that leads to pneumonitis and interstitial pulmonary fibrosis (Kawai and Akaza 2003). In fact, bleomycin is commonly used to induce experimental pulmonary fibrosis in rodents as a model of a fibro-inflammatory interstitial lung disease (Snider et al. 1978; Adamson and Bowden 1974). The biological effect of bleomycin is dependent on its coordination of iron, with chelating agents such as dexrazoxane, reducing its toxic effects (Wu et al. 2004).

We tested the postulate that metal import by DMT1 would participate in preventing lung injury after exposure to bleomycin. To test this postulate, microcytic anemia mice, defective in DMT1 (Fleming et al. 1997), and wild-type mice were exposed to bleomycin via intratracheal instillation and lung injury compared.

Materials and methods

Animals

All animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio. Microcytic anemic (*mk/mk*) mice (specifically heterozygous founders; MK/ReJ Slc1a2<mk>/+) were purchased from the Jackson Laboratory (Bar Harbor, Maine). Homozygous microcytic anemia mice were derived by breeding either homozygous or heterozygous male to heterozygous female. Mice were maintained as a closed stock in the animal facility at the University of Texas Health Science Center at San Antonio in a pathogen-free condition. All animals were fed identical standard rodent diet (Harlan Teklad LM-485) ad libitum.

Treatments and collection of specimens

Mice were anesthetized using isofluorane (Webster Veterinary Supply Inc, Sterling, MA) inhalation and then intratracheally instilled with either 4 units/ml bleomycin (Sigma) in 50 µl saline or 50 µl saline alone. Twenty-four h after treatment, animals were anesthetized using isofluorane inhalation, euthanized by transecting the abdominal aorta, and tracheally lavaged with 1.0 ml normal saline. The lavage was

repeated twice and the fluids were combined. An aliquot of 200 µl lavage was used to prepare cytospin slides. These slides were stained with modified Wright's (Diff-Quick stain; American Scientific Products, McGaw Park, IL) and a total of 200 cells were counted. Neutrophils were expressed as the absolute number/ml lavage. The rest of the lavage fluid was centrifuged at 600 × *g* for 10 min to remove cells and the supernatant was collected, stored at −80°C, and used later for biochemical assays. The lungs from a second set of treated animals were fixed and used for immunohistochemical analysis. A third set of mice had lung, liver, and splenic tissue resected. These were used for quantitative analysis of non-heme iron and Perl's stain (resected liver only).

Isolation of alveolar, splenic, and peritoneal macrophages for cell non-heme iron measurement

To procure alveolar macrophages, the animal was anesthetized and euthanized. The trachea was then canulated and lavage performed five times using 0.7–1.0 ml sterile saline solution each time. The pooled fluid was centrifuged at 600 × *g* for 5 min and the cell pellet was resuspended in RPMI (Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (FBS) and 0.02 mg/ml gentamicin.

To isolate splenic macrophages, the spleen was removed from the mouse and placed in a Petri dish containing 10 ml RPMI. Forceps were used to mechanically disperse the tissue which was then placed in a conical tube. Clumps were allowed to settle. The remaining cell suspension was aspirated, transferred to a new tube, washed twice by centrifugation at 600 × *g* for 5 min, and resuspensed in RPMI with 10% FBS and 0.02 mg/ml gentamicin.

Finally, peritoneal macrophages were extracted by injecting 5 ml sterile RPMI 1640 into the peritoneal cavity. The area was massaged and the injected medium extracted. A second aliquot of 10 ml medium was injected and the process repeated. The extracted medium was centrifuged for 5 min at 600 × *g* and the pellet was suspended in RPMI with 10% FBS and 0.02 mg/ml gentamicin.

Biochemical analyses

Lavage protein levels were determined using the Pierce Coomassie Plus Protein Assay Reagent (Pierce, Rockford, IL, USA). Bovine serum albumin served as the standard. Lavage albumin concentrations were determined using an immunoprecipitin assay (Diasorin, Stillwater, MN). Concentrations of macrophage inflammatory protein-2 (MIP-2) in the lavage fluid were measured by the method of ELISA using Quantikine kits purchased from R&D Systems (Minneapolis, MN, USA). Lavage iron and ferritin concentrations were determined using a colorimetric, enzymic method (Sigma Diagnostics, St. Louis, MO) and an enzyme-linked immunoassay (Microgenics Corporation, Concord, CA), respectively.

Non-heme iron concentrations in resected tissues and collected cells were quantified using inductively coupled plasma optical emission spectroscopy (ICP-OES; Model Optima 4300D, Perkin Elmer, Norwalk, CT) operated at a wavelength of 238.204 nm. Employing a standard method (Torrance 1980), non-heme iron in resected tissues was measured after adding 10.0 ml 3 N HCl/10% trichloroacetic acid/gram tissue, heating to 70°C for 18 h, and centrifuging at 20,000 × *g* for 10 min. Metal concentrations were determined in the supernatant. Cells were similarly placed in 1.0 ml 3 N HCl/10% trichoroacetic acid, hydrolyzed to 70°C for 18 h, centrifuged at 20,000 × *g* for 10 min, and iron determined in the supernatant using ICPOES.

Staining for iron and immunohistochemistry for ferritin

Lungs (at inspiration) and livers were inflation-fixed with 10% formalin for 24 h and then transferred to 70% ethanol. The stain for iron was Perl's Prussian blue while immunohistochemical staining for ferritin was accomplished as previously described (Wang et al. 2005).

Statistics

Experiments were conducted in triplicate. Unless otherwise specified, data are expressed as mean value ± standard error. Differences between multiple groups were compared using analysis of variance. The post-hoc test employed was Scheffe's test. Two-tailed tests of significance were employed. Significance was assumed at $P < 0.05$.

Results

mk/mk mice have greater lung injury after bleomycin treatment

To determine whether a deficiency in DMT1 could affect lung injury after bleomycin exposure, wild-type and *mk/mk* mice were intratracheally instilled with either bleomycin or saline. Twenty-four h after instillation, biochemical assays for indices of lung injury demonstrated that bleomycin exposure caused lung injury in both wild-type and *mk/mk* mice. However, the *mk/mk* mice had significantly more injury than the wild-type mice did as reflected by elevations in both lavage total protein (Fig. 1a) and lavage albumin (Fig. 1b) concentrations in BAL; these were approximately 40 and 25% higher, respectively, in the treated *mk/mk* mice when compared to the treated wild-type mice. No significant differences in lavage total protein and lavage albumin were detected between the wild-type and *mk/mk* mice instilled with saline indicating that basal damage due to instillation itself was not affected by genotype. Bleomycin resulted in an inflammatory response in the lungs of both *mk/mk* and wild-type mice. However, the number of neutrophils in the lavage collected from the *mk/mk* mice were elevated relative to wild-type animals following bleomycin (Fig. 1c). The lavage concentration of MIP-2 was similarly higher in the *mk/mk* mice than in the wild-type mice following bleomycin (Fig. 1d). In the saline-treated group, the percentage neutrophils and lavage concentrations of MIP-2 were equivalent in the two types of mice again supporting that the biological response due to instillation itself was not affected by genotype.

Iron homeostasis in the lungs of *mk/mk* mice was altered by bleomycin

To describe changes in iron homeostasis after bleomycin treatment, non-heme iron concentrations in the lung and liver and lavage concentrations of iron and ferritin

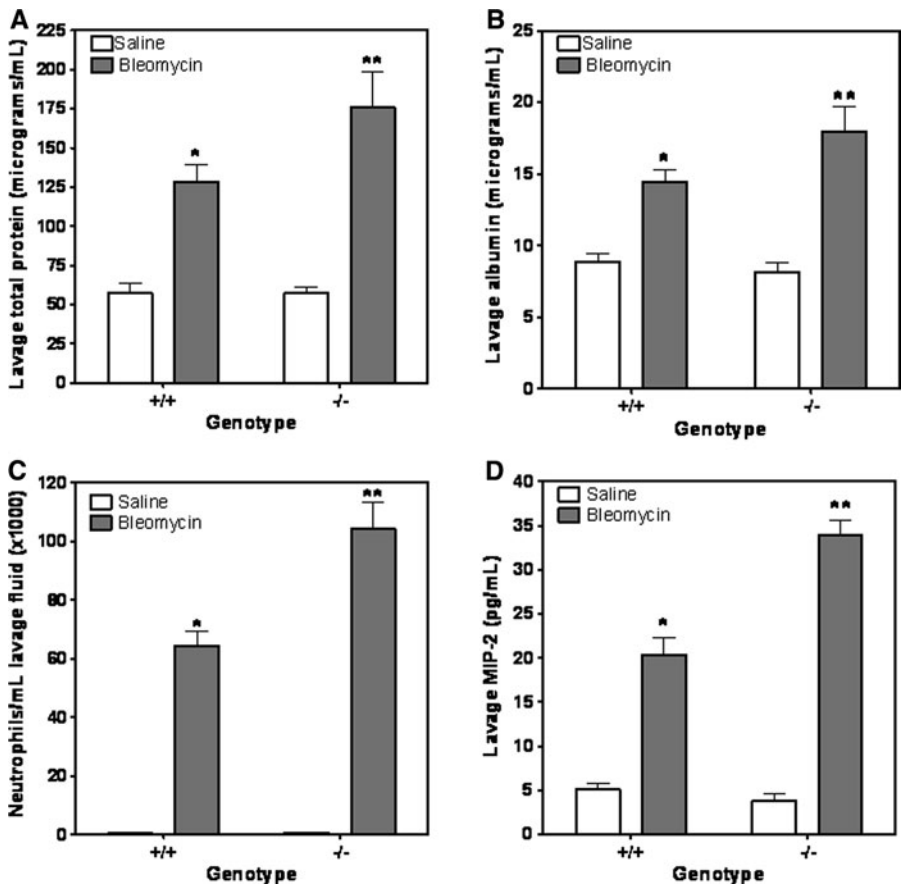

Fig. 1 *mk/mk* (−/−) mice had more severe lung injury after bleomycin exposure than wild-type (+/+) mice. Total protein (**a**), albumin (**b**), neutrophils (**c**), and macrophage inflammatory protein-2 (MIP-2) (**d**) in lavage fluids were compared among wild-type and *mk/mk* mice instilled with either saline or bleomycin 24 h after treatment. In both wild-type and *mk/mk* mice, indices of injury (**a**, **b**) and inflammation (**c**, **d**) were higher in mice treated with bleomycin. However, the increase in all indices was significantly higher in *mk/mk* than wild-type mice. * Significantly higher relative to saline treated groups. ** Significantly higher relative to bleomycin-treated wild-type animals

were measured in mice instilled with saline and bleomycin. Due to the missense mutation for DMT1 carried by the *mk/mk* mice, there is diminished intestinal iron absorption and the animals are anemic and iron-depleted in many tissues including the lung (Fig. 2a) and liver (Fig. 2b). Twenty-four h following bleomycin treatment, lung non-heme iron concentrations were increased in both *mk/mk* and wild type mice (Fig. 2a). Comparable to the lung, non-heme iron concentrations in the liver were significantly elevated in both types of mice following bleomycin exposure (Fig. 2b). The lavage concentrations of iron (Fig. 2c) and ferritin (Fig. 2d) were increased several fold in *mk/mk* and wild-type mice after bleomycin treatment when compared to their saline-treated counterparts. However, changes were greater in *mk/mk* mice relative to wild-type mice. There was no significant difference in either lavage iron or ferritin between *mk/mk* and wild-type mice in the saline-treated control group. Increased concentrations of lavage iron and ferritin after exposure supported a disruption in lung iron homeostasis and metal accumulation following bleomycin.

Despite *mk/mk* mice having significantly lower lung non-heme iron concentrations than wild-type mice, lavage concentrations of iron and ferritin were higher in *mk/mk* mice than in wild-type mice after bleomycin treatment. The diminished ability of the *mk/mk* animals to re-establish iron homeostasis after bleomycin is consistent with greater lung damage in these mice relative to the wild-type mice.

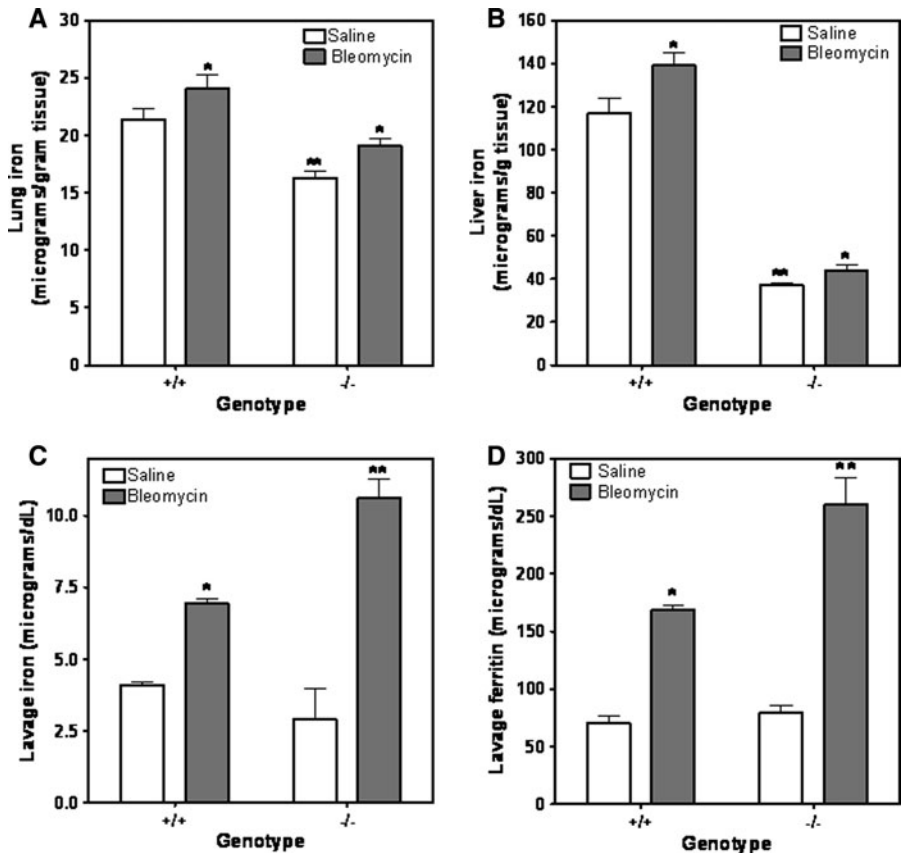

Fig. 2 *mk/mk* (−/−) mice showed greater iron disturbance in the lung after bleomycin exposure than wild-type (+/+) mice. Wild-type mice had higher non-heme iron concentrations in lung (**a**) and liver (**b**) than *mk/mk* mice but bleomycin increased non-heme iron in these two organs. Lavage iron (**c**) and ferritin (**d**) were compared among wild-type mice instilled with either saline or bleomycin 24 h after treatment. Both were increased in both wild-type and *mk/mk* mice after bleomycin treatment but the increase was much greater in *mk/mk* than in wild-type mice. In **a** and **b**: * Significantly higher relative to saline treated groups. ** Significantly lower relative to saline-treated wild-type animals. In **c** and **d**: * Significantly higher relative to saline treated groups. ** Significantly higher relative to bleomycin-treated wild-type animals

Bleomycin treatment resulted in accumulation of iron in alveolar macrophages

We investigated alterations of cell iron content in the lung after bleomycin treatment by staining for ferritin and iron. The most significant alteration in cellular iron concentrations was observed in alveolar macrophages. In wild-type animals (Fig. 3a, c), ferritin did not stain in lung instilled with saline (Fig. 3a) but was observed in alveolar macrophages after bleomycin treatment (Fig. 3c). In *mk/mk* mice (Fig. 3b, d), ferritin was detected in alveolar macrophages both before (Fig. 3b) and after (Fig. 3d) bleomycin treatment but levels of expression in these phagocytes were very high after bleomycin (Fig. 3d). Stainable iron was higher in alveolar macrophages from *mk/mk* relative to wild-type mice in both saline- (Fig. 4a, b) and bleomycin-treated (Fig. 4c, d) groups. Although the Perl's stain is not a sensitive procedure for the detection of iron, the results are consistent with that obtained with ferritin staining experiment. In wild-type mice (Fig. 4a, c), no iron staining in alveolar macrophages can be seen in mice instilled with saline (Fig. 4a) but iron staining was visible in mice instilled with bleomycin (Fig. 4c). In *mk/mk* mice, iron staining of alveolar macrophages was light in animals instilled with saline (Fig. 4b) but was obvious in mice instilled with bleomycin (Fig. 4d). In all the lung specimens studied, no other type of cell appeared to have significant staining for either ferritin or iron.

Iron accumulation in alveolar macrophages, but not other macrophages, is much higher in *mk/mk* than in wild-type mice

Since *mk/mk* mice are known to be iron-deficient (Edwards and Hoke 1975), our finding that the iron content of alveolar macrophages was higher in *mk/mk* than in wild-type mice, not only in bleomycin-treated but also in saline-treated control groups, was particularly interesting. To determine whether this was unique to alveolar macrophages, the iron content in various tissues as well as different types of macrophages derived from *mk/mk* and wild-type mice were

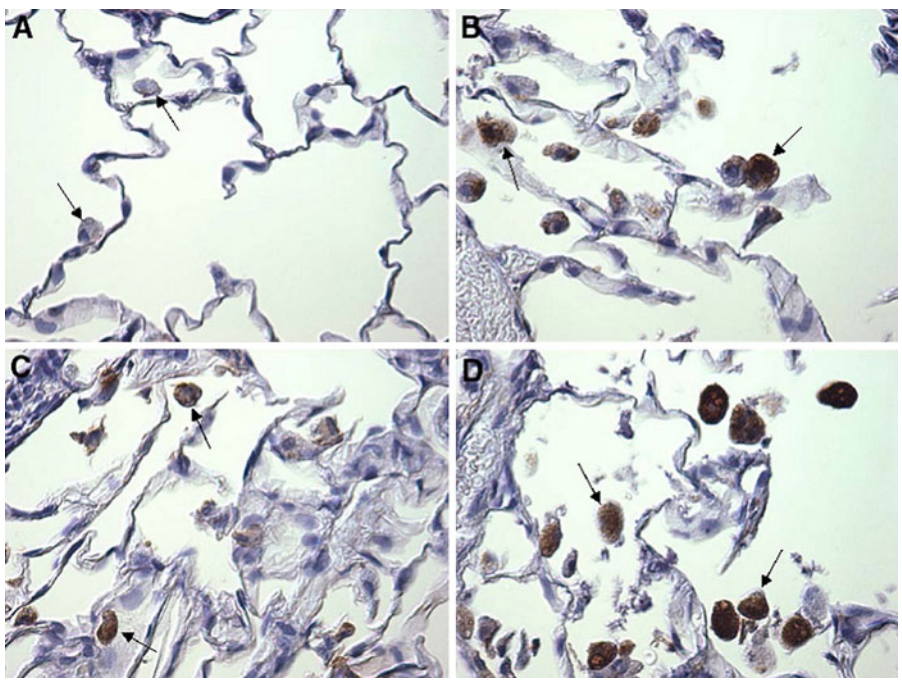

Fig. 3 Comparison of cellular ferritin in lung sections of wild-type and *mk/mk* mice after bleomycin treatment. Lung sections derived from wild-type (**a**, **c**) and *mk/mk* (**b**, **d**) mice instilled with saline (**a**, **b**) or bleomycin (**c**, **d**) for 24 h were stained with anti-ferritin antibody. While bleomycin treatment increased ferritin content in alveolar macrophages (*arrows*) in both wild-type and *mk/mk* mice, alveolar macrophages in *mk/mk* mice had significantly higher ferritin level than that in wild-type mice

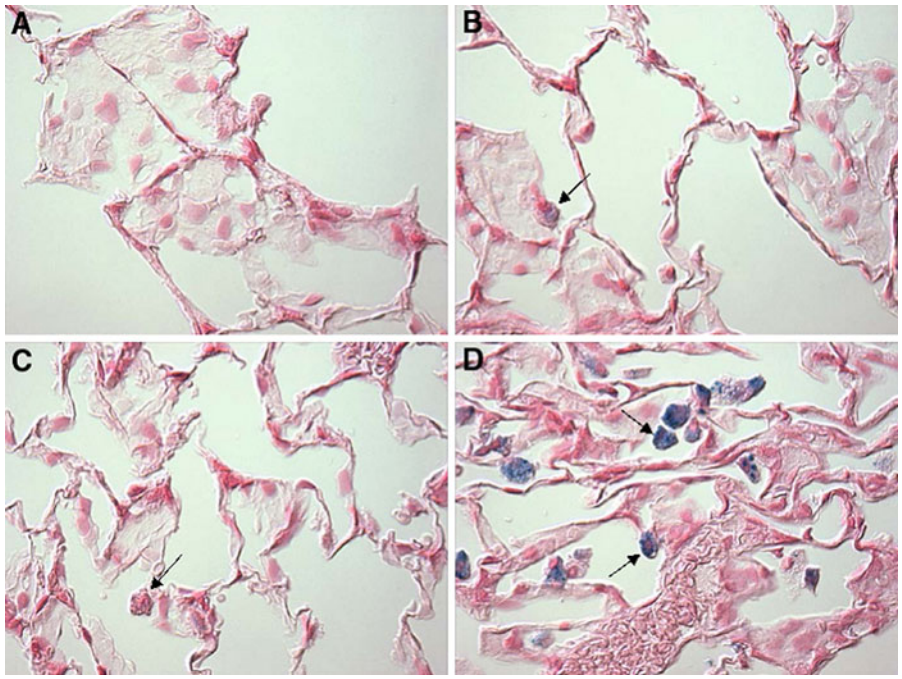

Fig. 4 Histochemical study of cellular iron in lung sections of wild-type and *mk/mk* mice after bleomycin treatment. Lung sections derived from wild-type (**a**, **c**) and *mk/mk* (**b**, **d**) mice instilled with saline (**a**, **b**) or bleomycin (**c**, **d**) for 24 h were stained with Perl's stain. No significant staining was detected in any lung cells in wild-type mice instilled with either saline (**a**) or bleomycin (**c**). In *mk/mk* mice, alveolar macrophages (*arrows*) had weak staining in animals instilled with saline (**b**) and strong staining in animals instilled with bleomycin

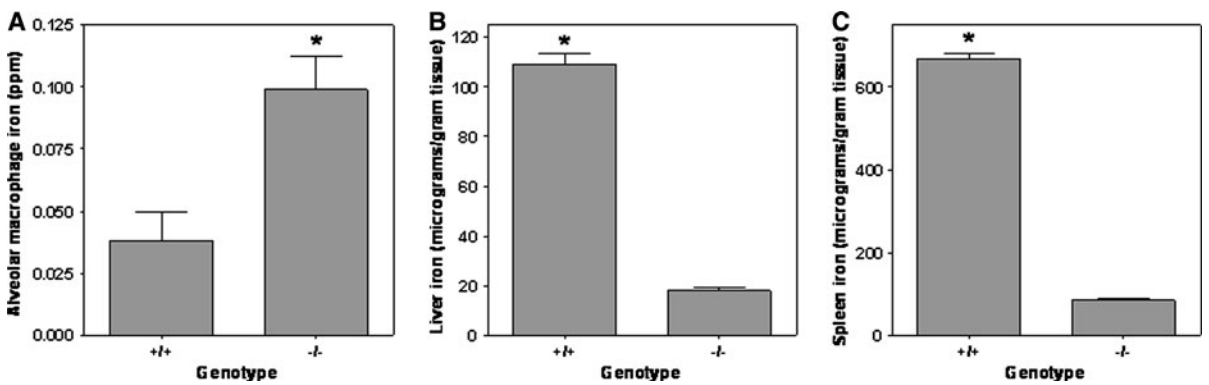

Fig. 5 Comparison of iron content in alveolar macrophages, liver, and spleen between wild-type (+/+) and *mk/mk* (−/−) mice. Iron content in alveolar macrophages (**a**), liver (**b**), and spleen (**c**) from 12 week-old wild-type and *mk/mk* mice were analyzed and compared. *mk/mk* mice have significantly higher iron in alveolar macrophages but are iron-deficient in liver and spleen when compared to wild-type mice. * Significantly higher in one genotype than the other

compared. Iron content of alveolar macrophages isolated from 12 week old *mk/mk* mice was 2.6-fold (Fig. 5a) that of age-matched wild-type mice from the same colony, while in the same group of animals, liver iron content in the wild-type mice was 5.7-fold of that from age-matched *mk/mk* mice (Fig. 5b). This is consistent with our previous finding in the bleomycin treatment experiment. Similarly, splenic iron content was higher (8.7-fold) in wild-type than in *mk/mk* mice (Fig. 5c).

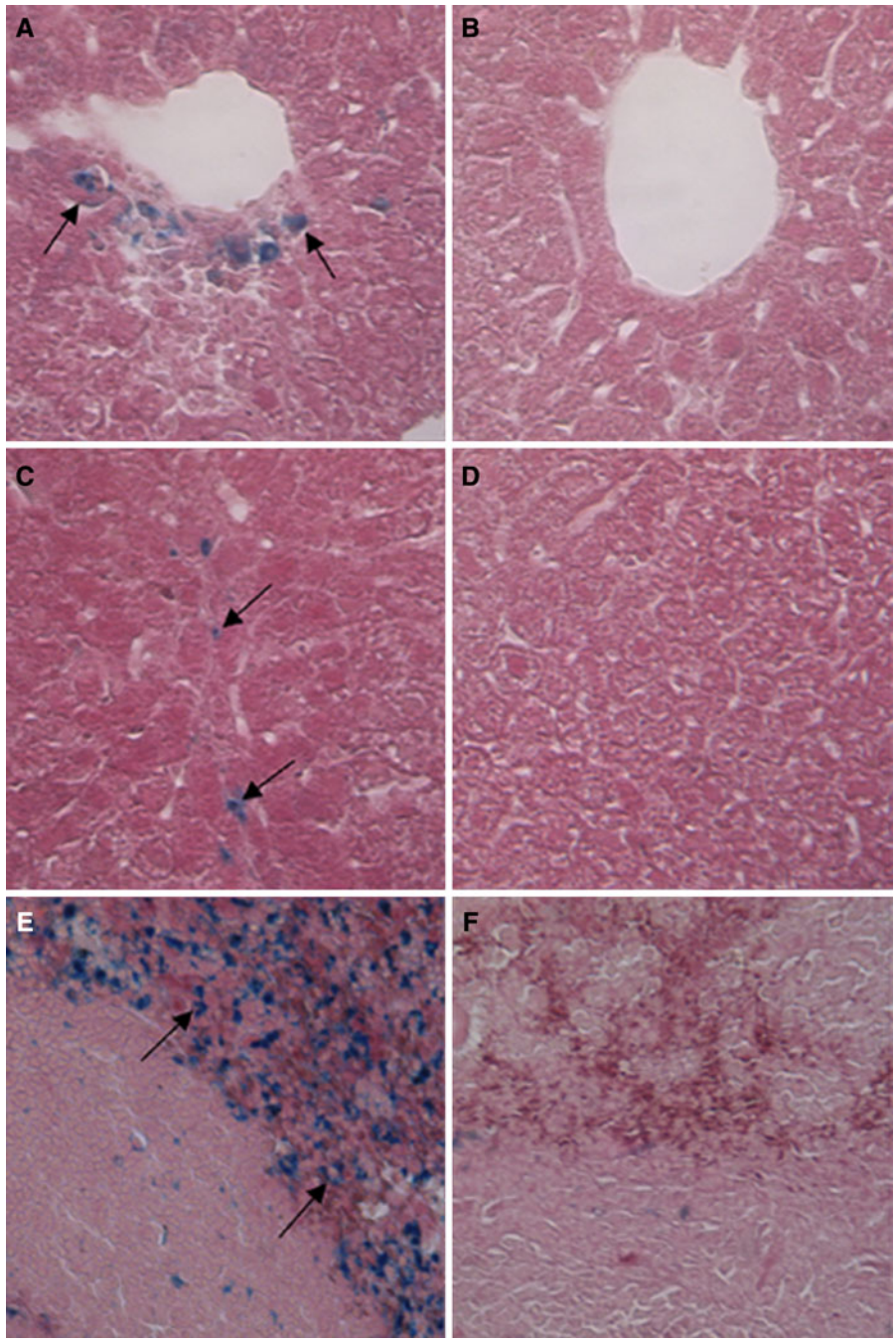

Fig. 6 Histochemical analysis of iron in liver and spleen sections derived from wild-type and *mk/mk* mice. Perl's stain on liver (**a**–**d**) and spleen (**e**, **f**) sections demonstrated higher iron content in hepatocytes (*arrows* in **a**), kupper cells (*arrows* in **c**) and splenic macrophages (*arrows* in **e**) in wild-type mice than that in *mk/mk* mice (**b**, **d**, and **f**)

We extended our investigation on hepatic and splenic iron to the cellular level by performing Perl's staining on tissue sections derived from *mk/mk* and wild-type mice. The results (Fig. 6) demonstrated higher iron content in both liver and spleen in wild-type (Fig. 6a, c, e) than in *mk/mk* (Fig. 6b, d, f) mice,

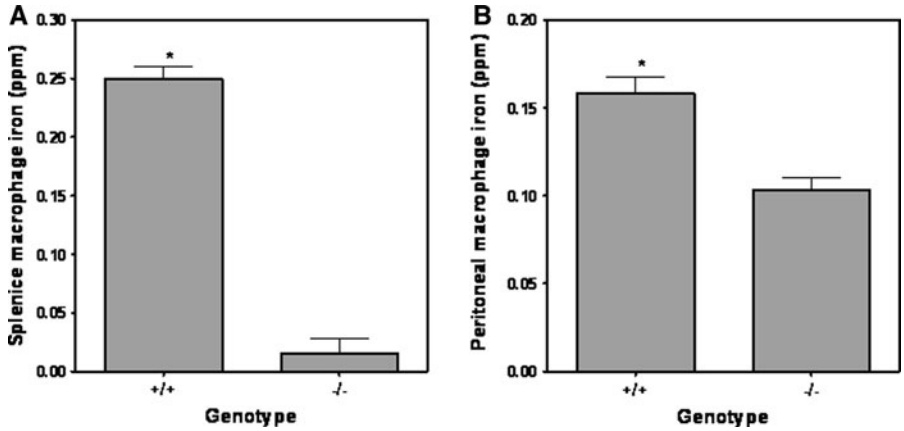

Fig. 7 Comparison of iron content in splenic and peritoneal macrophages derived from wild-type (+/+) and *mk/mk* (−/−) mice. Iron content in enriched pool of splenic macrophages (**a**) and peritoneal macrophages (**b**) from wild-type mice was significantly higher than that from *mk/mk* mice. * Significantly higher in one genotype than the other

however, the difference between these two group of animals is particularly obvious in hepatocytes (Fig. 6a, b), Kuppfer cells (Fig. 6c, d) and splenic macrophages (Fig. 6e, f). When cellular iron content was measured, enriched splenic macrophages derived from wild-type mice had about six times more iron than that from *mk/mk* mice (Fig. 7a). Peritoneal macrophages isolated from wild-type mice also had significantly higher iron than that from *mk/mk* mice (Fig. 7b). Iron accumulation by alveolar macrophages, but not by other macrophages, in *mk/mk* mice suggests a distinct role of alveolar macrophages in iron scavenging.

Discussion

Relative to the wild-type animals, the *mk/mk* mice demonstrated a greater increase in lavage concentrations of both total protein and albumin after bleomycin exposure. Comparable to these two indices of lung injury, neutrophil number and the pro-inflammatory mediator MIP-2 were significantly elevated in the lavage from *mk/mk* mice after bleomycin relative to wild-type mice. This evidence of a greater inflammatory lung injury in the *mk/mk* mice after bleomycin instillation supports a role for DMT1 in the response to this chemotherapeutic agent. Deficiencies of iron transport by DMT1 (with a failure ultimately to sequester the metal in ferritin) in *mk/mk* mice could potentially affect tissue damage. This association between DMT1 and inflammatory lung injury after bleomycin instillation is comparable to increased levels of this metal transporter in hypotransferrinemic mice and diminished injury after particle exposure (Yang et al. 1999). In further support of a relationship between iron transport and lung injury, decreased DMT1 activity in Belgrade rats was related to increased damage after ozone exposure (Ghio et al. 2007).

Following bleomycin exposure, *mk/mk* mice demonstrated greater disruptions in iron homeostasis, relative to the wild-type mice. Lavage iron was increased after bleomycin exposure in both types of mice but these elevations were significantly greater in the *mk/mk* mice. The capacity of bleomycin to complex and accumulate host metal can contribute to elevations of lavage iron. Higher iron concentrations in the *mk/mk* mice indicate that, without normal expression of DMT1, their ability to transport and sequester that metal is diminished after bleomycin exposure. In both the *mk/mk* and wild-type mice, lavage ferritin increased following bleomycin instillation and, like lavage iron, concentrations were significantly higher in the *mk/mk* mice. One scenario which might explain this observed sequence of changes among wild-type mice would be that bleomycin complexes available iron in the lower respiratory tract to present both an oxidative stress and a potential for tissue injury. The host then decreases iron availability limiting injury associated with bleomycin. Iron can be imported by DMT1, sequestered, and then translocated to the reticuloendothelial system (e.g., liver) where its storage presents less of a threat to the host.

Cells resident in the lower respiratory tract have the capacity to import iron. The increased concentrations of intracellular iron will then affect an

elevated expression of ferritin. The synthesis of ferritin is regulated by a post-transcriptional mechanism (Cairo and Recalcati 2007). A hairpin sequence at the 5′-untranslated end of ferritin mRNA called the iron responsive element (IRE) binds iron regulatory proteins (IRPs). Available iron reacts with IRPs to decrease their binding to the mRNA. Translation of ferritin proceeds and the ensuing storage of iron within this protein limit its capacity to generate free radicals and confers an antioxidant function (Cozzi et al. 1990).

Ferritin is produced intracellularly and, following bleomycin exposure, lavage concentrations of this metal storage protein in the *mk/mk* mice were elevated relative to the wild-type control. Therefore, some cell type in the lower respiratory tract must have a capacity to import iron, in the absence of DMT1, to affect the production of this storage protein. Macrophages can transport iron intracellularly using pathways other than DMT1 mediated uptake (Barton et al. 1999) and uptake of this metal could proceed following bleomycin even in *mk/mk* mice. Alveolar macrophages appeared to be iron-loaded in the unexposed *mk/mk* mouse and such accumulation of the metal was unique to this cell type. This elevation in cell non-heme iron contrasts lung, liver, and splenic tissues and splenic and peritoneal macrophages which all showed diminished concentrations of this metal in the *mk/mk* mouse. Iron metabolism in alveolar macrophages is distinct from that in structural tissues and other macrophages. In the lung, these phagocytes are constantly exposed to non-host iron from the atmosphere. Alveolar macrophages respond to this potential oxidative stress by importing, and scavenging, the metal (Olakanmi et al. 1993). Several different mechanisms can mediate uptake of iron by the macrophage including the transferrin and lactoferrin pathways, NRAMP1 (Barton et al. 1999), and DMT1 (Nguyen et al. 2006). Despite a capacity to release metal (via FPN1, transferrin, and ferritin), alveolar macrophages accumulate high iron concentrations (Corhay et al. 1992). Continued delivery of iron to the macrophage appears to overwhelm the capacity of ferritin to sequester the metal resulting in an oxidative injury to the cell and a production of hemosiderin. This product stains positively in the Perl's Prussian blue reaction (i.e., the sideromacrophage or the siderophage). Both ferritin and hemosiderin were evident on the stains of these macrophages from unexposed *mk/mk* mice reflecting an abnormal iron homeostasis in these animals. Hypotransferrinemic mice were previously demonstrated to increase DMT1 expression (Yang et al. 1999). In a similar manner, it is possible that *mk/mk* mice modify expression of alternative transport pathways for iron in specific cells. In the alveolar macrophage of the *mk/mk* mice, this could include elevated expression of NRAMP1 or proteins involved in lactoferrin-mediated transport (lactoferrin and its cognate receptor).

A chronic accumulation of iron in the lower respiratory tract, even in ferritin, is unfavorable since oxygen concentrations are greatest here relative to all other sites in the body. Following bleomycin, both types of mice were successful in transporting the metal away from the lung with significant accumulations in liver resulting. The *mk/mk* mice appeared unable to accomplish this transport; the lack of DMT1 and resulting deficiencies in transport may contribute to this.

We conclude that DMT1 expression can affect the inflammatory lung injury following instillation of bleomycin in an animal model. This is in agreement with all previous investigation which demonstrated increasing expression of DMT1 in the lower respiratory tract decreased iron-dependent injuries following particle exposures (Yang et al. 1999) and ozone (Ghio et al. 2007). In tissues which do not function to meet the nutritional requirements of a living system, DMT1 can take part in cell transport of iron focused on the sequestration of the metal in a less reactive state (e.g., intracellular transport to ferritin). Such transport benefits the host through a control of oxidative stress and dependent tissue damage. Following exposure to either iron or chelates which accumulate iron, cell expression of DMT1 will increase. The control of expression of this transporter allows an increased uptake of the metal, its storage in ferritin, and may expedite a release and movement to a more appropriate repository for metal (i.e., the reticulo-endothelial system). This coordinated handling of iron diminishes the potential for oxidative stress, and therefore injury, associated with the metal.

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