Iron loading and oxidative stress in the Atm<sup>-/-</sup> mouse liver

Cameron J. McDonald,1 Lesa Ostini,1 Daniel F. Wallace,1,2 Abraham N. John,3 Dianne J. Watters,2,3 and V. Nathan Subramaniam1,2

1Membrane Transport Laboratory, Division of Cancer and Cell Biology, Queensland Institute of Medical Research; 2Griffith Medical Research College, a Joint Program of Griffith University and the Queensland Institute of Medical Research; and 3School of Biomolecular and Physical Sciences, Eskitis Institute for Cell and Molecular Therapies, Griffith University, Brisbane, Queensland, Australia

Submitted 26 October 2010; accepted in final form 2 February 2011

McDonald CJ, Ostini L, Wallace DF, John AN, Watters DJ, Subramaniam VN. Iron loading and oxidative stress in the Atm<sup>-/-</sup> mouse liver. Am J Physiol Gastrointest Liver Physiol 300: G554–G560, 2011. First published February 3, 2011; doi:10.1152/ajpgi.00486.2010.—Ataxia-Telangiectasia (A-T) is an autosomal recessive disorder resulting in a myriad of abnormalities, including progressive neurodegeneration and cancer predisposition. At the cellular level, A-T is a disease of chronic oxidative stress (OS) causing damage to proteins, lipids, and DNA. OS is contributed to by pro-oxidative transition metals such as iron that catalyze the conversion of weakly reactive oxygen species to highly reactive hydroxyl radicals. Iron-associated OS has been linked to neurodegeneration in Alzheimer’s and Parkinson’s diseases and development of lymphoid tumors (which afflict ~30% of A-T patients). To investigate iron regulation in A-T, iron indexes, regulatory genes, and OS markers were studied in livers of wild-type and Ataxia telangiectasia mutated (Atm) null mice on control or high-iron diets. Atm<sup>-/-</sup> mice had increased serum iron, hepatic iron, and ferritin and significantly higher Hspc compared with wild-type mice. When challenged with the high-iron diet, Bmp6 and Hfe expression was significantly increased. Atm<sup>-/-</sup> mice had increased protein tyrosine nitration and significantly higher Heme Oxygenase (decycling) 1 levels that were substantially increased by a high-iron diet. Ferroportin gene expression was significantly increased; however, protein levels were unchanged. We demonstrate that Atm<sup>-/-</sup> mice have a propensity to accumulate iron that is associated with a significant increase in hepatic OS. The iron-induced increase in hepcidin peptide in turn suppresses ferroportin protein levels, thus nullifying the upregulation of mRNA expression in response to increased OS. Our results suggest that increased iron status may contribute to the chronic OS seen in A-T patients and development of disease pathology.

Ataxia-Telangiectasia; hepcidin; ferroportin; heme oxygenase 1

ATAXIA-TELANGIECTASIA (A-T) is an autosomal recessive disorder resulting in progressive neurodegeneration, ocularcutaneous telangiectasia, cancer predisposition, immunodeficiency, ionizing radiation hypersensitivity, and a myriad of other abnormalities (7). The disease is caused by mutations in the Ataxia telangiectasia mutated (ATM) gene, which codes for a serine/threonine protein kinase. ATM is responsible for the phosphorylation of a large number of proteins with a diverse range of functions, including DNA double-stranded break repair and intracellular redox homeostasis (22, 26).

Because of the role of ATM in redox homeostasis, at a cellular level, A-T results in chronic oxidative stress (OS), which leads to protein and lipid damage, as well as DNA double-stranded breaks and chromosomal instability (22, 32). This damage can then lead to cellular dysfunction, apoptosis, or, in the worst case, result in cancerous cells. The increased OS occurs because of deficiencies in cellular antioxidant defenses, with A-T models showing impaired glutathione synthesis and decreased MnSOD and catalase activities (19). This reduced antioxidant capacity has been linked to increased oxidative damage through rescue experiments in which antioxidant treatments significantly reduced the amount of protein, lipid, and DNA oxidative damage (8, 17, 18, 33). The loss or reduction of these antioxidant defenses in A-T thus places a greater dependence on the remaining antioxidant defense mechanisms of A-T cells.

One such unaffected antioxidant defense consists of the bile pigments biliverdin and bilirubin, which have been shown to have anti-mutagenic properties (9, 13). Biliverdin is produced by the catabolism of heme by Heme Oxygenase (decycling) 1 (Hmox1), which releases biliverdin, iron, carbon monoxide, and water. Biliverdin is then rapidly converted to bilirubin by biliverdin reductase. Both bilirubin and biliverdin are potent antioxidants (13). Ironically, however, the production of these anti-oxidants by Hmox1 also results in the release of iron, a strong pro-oxidant. Transition metals such as iron act as pro-oxidative molecules by catalyzing the conversion of weak reactive oxygen species such as H<sub>2</sub>O<sub>2</sub> to produce highly reactive hydroxyl radicals that cause significant oxidative damage (2). Within normally functioning cells, concurrent with the upregulation of Hmox1 in response to OS is the upregulation in expression of the iron exporter Ferroportin (Fpn) (41). In this OS defense system, the upregulation of Fpn expression leads to increased ferroportin protein at the cell surface, providing increased export of the potentially harmful iron from the cells, leaving the antioxidants to act in cellular defense.

Ferroportin, the only known cellular iron efflux molecule, is strongly regulated in response to whole body iron status (15). Iron balance within the body is maintained by a number of regulatory genes that modulate its absorption, transport, sequestration, and utilization within the body. Hepcidin, the product of the Hamp gene, is regulated in response to body iron status, with its expression downregulated by iron deficiency and upregulated by iron loading (for review, see Ref. 30). Hepcidin then regulates body iron levels via its interaction with ferroportin. Hepcidin binds ferroportin, leading to its internalization and eventual degradation in lysosomes, thus decreasing further cellular iron export (31). This mechanism regulates whole body iron status primarily through limiting or enhancing iron export from enterocytes of the gut and iron recycling through the reticuloendothelial system.
The Atm<sup>-/-</sup> mouse exhibits similar characteristics to that of A-T patients and has been used extensively to study the functions of ATM and the pathogenesis of A-T disease (4, 16, 44). Shackelford and coworkers (35–37) have previously shown that the sera of Atm<sup>-/-</sup> mice contain more labile iron than that of wild-type mice, and that iron chelation in colony-forming experiments increased the colony-forming and survival capacity of A-T cells. Given the significance of OS to A-T disease pathology, and the high capacity of iron to affect OS, it is surprising that the regulation of iron homeostasis within the Atm<sup>-/-</sup> mouse has not been reported previously. The importance of cellular iron regulation is further enhanced in A-T given the increased reliance of A-T cells on anti-oxidant defenses such as the Hmox1/ferroporin system.

To investigate the role of iron homeostasis in the development and presence of the A-T phenotype, we analyzed iron indexes, mRNA expression, and protein levels of iron regulatory genes and markers of OS in the livers of wild-type and Atm<sup>-/-</sup> mice on a normal or high-iron diet. Our studies suggest that iron homeostasis in Atm<sup>-/-</sup> mice is disturbed and that the increased iron status may contribute to the chronic OS seen in A-T patients.

METHODS

Animals. All mice were weaned at 21 days and maintained on standard laboratory chow ad libitum before 6 wk of age. Wild-type littermates and Atm<sup>-/-</sup> mice used in the study were produced by breeding heterozygotes obtained from the laboratory of Martin Lavin, Queensland Institute of Medical Research (Brisbane, Australia), and have been described previously (16). The mice were genotyped according to published procedures (18). Six-week-old male mice were fed a normal iron diet (75 mg/kg iron) or a high-iron diet (20 g/kg; 2% carbonyl iron) for 4 wk (Specialty Feeds, Glenn Forrest, Western Australia). The numbers of animals used were as follows: wild-type and Atm<sup>-/-</sup> on normal diet both n = 5, wild-type on a high-iron diet n = 5, and Atm<sup>-/-</sup> on a high-iron diet n = 4. All animals received humane care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Health and Medical Research Council of Australia. All experimental protocols were approved by the Griffith University Animal Ethics Committee.

Measurement of iron indexes. Transferrin saturation (TS) was measured using an iron and iron-binding capacity kit (Sigma-Aldrich, Castle Hill, Australia). Nonheme hepatic iron concentration (HIC) was measured using the method of Torrance and Bothwell (40).

Real-time quantitative PCR. Primer pairs for detecting Hmp1 and Fpm mRNAs have been described previously (28) [Hmox1 (forward: TCCCTCACAGATGGGCTCAC; reverse: TGGACAGATTTACAGGCCCC), Hemojuvelin (Hjv) (forward: TTCGCGGGGCAATCATGGAGAA; reverse: TCCCGAGTATGAGGCTCTTACC)]. The quantitation of mRNA transcripts was determined by real-time PCR using LightCycler 480 SYBR Green Mix in a LightCycler 480 (Roche, Brisbane, Australia) as previously described (28). All targets were normalized to the respective HPRT (forward: GAGATGTTAG-GACAGGA; reverse: GAGGGCCACAATGTGATG) levels using 4Ct.

Western blotting. Liver samples were homogenized in phosphate buffer inhibitor lysis buffer [200 mM Tris, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, 10% glycerol, 1 mM sodium fluoride, 1 mM sodium orthovanadate, 1 mM sodium pyrophosphate, 1:100 protease inhibitor cocktail (Sigma), and 2 mM phenylmethylsulfonyl fluoride, containing 10 μg/ml DNase]. Thirty micrograms of total liver homogenates were electrophoresed on a 10% SDS-PAGE and then transferred to a Hybond-C<sup>+</sup> membrane. Blots were blocked in 10% skim milk powder and 0.5% Tween 20 in PBS (blocking buffer) for 2 h at room temperature (RT) and then incubated with the following primary antibodies: anti-ferritin (1:12,000; Sigma-Aldrich), anti-prohenecin (1:800; see Ref. 43), anti-ferroportin (1:2,000; Alpha Diagnostic, San Antonio, TX), anti-transferrin receptor (TI) R (1:1,200; Invitrogen, Mulgrave, VIC, Australia), anti-TFR2 (1:20,000; see Ref. 42), and anti-GAPDH (1:150,000; Millipore, North Ryde, NSW, Australia) overnight at 4°C in blocking buffer. Blots then underwent three washes in 0.1% Tween 20 in PBS. Secondary antibodies, anti-rabbit and anti-mouse horseradish peroxidase, were incubated with the blot at 1:10,000 in blocking buffer. Blots were washed extensively, and Immobilon Western Chemiluminescent HRP Substrate (Millipore WSBLKS0500) was applied for 5 min to the blot and then exposed to film. Band volume and density were quantitated using SynGene GeneTools version 4.0 software (Synoptics, Cambridge, UK).

Immunohistochemistry. Mouse livers were fixed in 10% buffered formalin, mounted in paraffin, and sectioned. The sections were deparaffinized, rehydrated in an ethanol series, and then incubated in 3% H<sub>2</sub>O<sub>2</sub>/MeOH for 10 min to block endogenous peroxide activity. Slides were then placed in 1 M trisodium citrate, pH 6.0, and microwaved for 20 min to promote antigen retrieval. After a PBS wash, sections underwent blocking in 20% donkey serum/donkey fluorescence dilution buffer (5% FBS, 5% normal donkey serum, and 2% BSA in PBS containing 1 mM CaCl<sub>2</sub> and 1 mM MgCl<sub>2</sub>, pH 7.6) for 1 h at RT and were then incubated in 2 μg/ml anti-nitrated tyrosine (Alpha Diagnostic) or anti-rabbit IgG (Sigma) overnight at 4°C. Sections were then washed 15 min washes in PBS, incubated with Vector Imprint anti-rabbit polymer secondary (Vector Laboratories, Burlingame, CA) for 30 min at RT, and were then exposed to diamobenzidine substrate (Dako, Campbellfield, VIC, Australia) and counterstained with hematoxylin. After being mounted, sections were examined under light microscopy and photographed at magnification ×400.

Statistical analysis. Variables were compared between groups using one-way ANOVA and Student’s t-test using GraphPad Prism 5 (GraphPad Software, San Diego, CA). Student’s t-test was used to compare within the two genotypes on the same diet. An F-test was used to compare variance within each group, and, in instances where variance was found to be significantly different, Welch’s correction was applied. A P value of <0.05 was considered statistically significant. One-way ANOVA with Bonferroni’s Multiple Comparison with selected pairs post hoc test was used to compare the two genotypes across diets. Again, a P value of <0.05 was considered statistically significant.

RESULTS

The regulation of iron homeostasis and its relationship to OS was assessed in Atm<sup>-/-</sup> mice on normal iron or high-iron diets. Six-week-old wild-type littermates and Atm<sup>-/-</sup> mice were fed the diet for 4 wk before death. Atm<sup>-/-</sup> mice show increased hepatic and serum iron. The iron status of wild-type and Atm<sup>-/-</sup> mice was assessed by analysis of serum TS, quantification of both hepatic iron and ferritin, and quantification of splenic iron. On both normal iron and high-iron diets, wild-type mice had lower TS compared with Atm<sup>-/-</sup> mice fed on the same diet, although these differences did not reach statistical significance (Fig. 1A). Feeding with the high-iron diet produced significantly higher TS in both wild-type and Atm<sup>-/-</sup> mice (P < 0.05). HIC was significantly higher in Atm<sup>-/-</sup> mice fed a normal iron diet compared with wild-type mice on the same diet (P = 0.02; Fig. 1B). Wild-type and Atm<sup>-/-</sup> mice fed the high-iron diet showed extreme hepatic iron loading that resulted in significantly higher HIC for both genotypes (P < 0.001). Quantification of Western
blotting for hepatic ferritin revealed the same iron-loading pattern as measured by HIC (Fig. 1C and Supplemental Fig. S1A (Supplemental data for this article may be found on the American Journal of Physiology: Gastrointestinal and Liver Physiology website.)). Splenic iron concentration was also examined, with no difference identified between wild-type and Atm−/− mice fed a normal iron diet (see inset for visualization of the difference on a normal iron diet). Both wild-type and Atm−/− animals showed extreme iron loading when fed a high-iron diet. C: hepatic ferritin showed the same iron-loading pattern as seen in HIC (B). D: no difference in spleen iron content was observed between genotypes, although feeding with a high-iron diet significantly increased spleen iron. *P < 0.05 between genotypes on the same diet. **P = 0.058 between genotypes on the same diet. ***P < 0.05 between diets with the same genotype. Error bars indicate ± SE.

Liver sections from wild-type and Atm−/− mice on both diets, which represented the median HIC, were subjected to Perl's Prussian blue staining for iron and hematoxylin and eosin (H&E) staining. The levels of Perl's staining reflected the differences seen in hepatic iron between diets and showed no difference in the gross or cellular pattern of iron distribution within the liver (data not shown). H&E staining also revealed no identifiable difference in the gross or cellular architecture or structure between genotypes or diets (data not shown).

Fig. 1. Ataxia telangiectasia mutated (Atm) homozygous (−/−) mice show increased iron parameters. A: Atm−/− mice show increased transferrin saturation compared with wild-type (WT) animals on both diets, although this does not reach statistical significance. The high-iron diet resulted in increased transferrin saturation in both strains. B: the hepatic iron concentration (HIC) of Atm−/− mice shows significantly more iron compared with wild-type mice fed a normal iron diet (see inset for visualization of the difference on a normal iron diet). Both wild-type and Atm−/− animals showed extreme iron loading when fed a high-iron diet. C: hepatic ferritin showed the same iron-loading pattern as seen in HIC (B). D: no difference in spleen iron content was observed between genotypes, although feeding with a high-iron diet significantly increased spleen iron. *P < 0.05 between genotypes on the same diet. **P = 0.058 between genotypes on the same diet. ***P < 0.05 between diets with the same genotype. Error bars indicate ± SE.

Fig. 2. Atm−/− mice have increased hepatic hepcidin. The key iron regulatory gene hepcidin is increased in response to the increased hepatic iron in Atm−/− compared with wild-type mice. A: on a normal iron diet, Hamp1 mRNA is expressed at a higher level in Atm−/− mice compared with wild-type mice. A high-iron diet resulted in increased expression in both groups. B: the gradient of increasing Hamp1 expression translates to the same gradient of increasing hepcidin protein. C: Bmp6 expression, which has a regulatory role for Hamp1, follows the same gradient of increasing expression, as does Hfe (D). *P < 0.05 between genotypes on the same diet. **P < 0.05 between diets with the same genotype. Error bars indicate ± SE.

Liver sections from wild-type and Atm−/− mice on both diets, which represented the median HIC, were subjected to Perl's Prussian blue staining for iron and hematoxylin and eosin (H&E) staining. The levels of Perl's staining reflected the differences seen in hepatic iron between diets and showed no difference in the gross or cellular pattern of iron distribution within the liver (data not shown). H&E staining also revealed no identifiable difference in the gross or cellular architecture or structure between genotypes or diets (data not shown).
Hepatic hepcidin is increased in Atm<sup>−/−</sup> mice. The key iron regulatory peptide hepcidin is, under normal conditions, the dominant controlling factor of body iron levels. Given the increased HIC in Atm<sup>−/−</sup> mice, we investigated the expression of Hamp1 and the levels of prohepcidin protein. Atm<sup>−/−</sup> mice on the normal iron diet were found to have significantly higher levels, with almost four times the expression level of Hamp1 (P < 0.02; Fig. 2A) compared with wild-type mice. Atm<sup>−/−</sup> animals on the high-iron diet also showed a higher level of Hamp1 expression than the wild-type animals, although the difference was not statistically significant. Wild-type and Atm<sup>−/−</sup> mice on the high-iron diet showed significantly higher Hamp1 expression than mice on the normal iron diet (P < 0.001). To confirm that the higher levels of Hamp1 expression were reflected at the protein level, we quantified hepatic prohepcidin levels by Western blotting (Fig. 2B and Supplemental Fig. S1A). This showed the similar gradient increase in hepcidin levels from normal iron diet wild-type mice to high-iron diet Atm<sup>−/−</sup> mice as seen for Hamp1 expression. The Hfe and Bmp6 genes are involved in the upstream regulation of Hamp1 in response to iron. Both Hfe and Bmp6 also showed a similar gradient of increasing expression, with both being significantly increased by the high-iron diet and Atm<sup>−/−</sup> mice significantly higher than wild-type on the high-iron diet (Fig. 2, C and D). Hjv and Tfr2 are also both involved in the maintenance of body iron status and are able to exert regulatory

![Fig. 3. Atm<sup>−/−</sup> mice have increased hepatic oxidative stress. A: Heme Oxygenase (decycling) 1 (Hmox1) expression is increased in both genotypes when fed a high-iron diet; however, Atm<sup>−/−</sup> mice increase Hmox1 to a significantly higher level than wild-type mice. *P < 0.01 between genotypes. **P < 0.05 between diets. Error bars indicate ± SE. B: liver sections from wild-type and Atm<sup>−/−</sup> mice were subjected to immunohistochemistry to detect protein tyrosine nitration, a marker of oxidative stress (OS). Images shown correspond to the highest levels of Hmox1 expression. The liver sections from wild-type mice showed lower levels of tyrosine nitration than Atm<sup>−/−</sup> liver sections on both the normal iron (i and ii, respectively) and high-iron (iii and iv, respectively) diets. The high-iron diet also increased the amount of detectable tyrosine nitration in wild-type (i–iii) and Atm<sup>−/−</sup> (ii–iv) mice. The coloration visible in tissue gaps in part i is believed to be background staining. Magnification ×400, scale bar represents 100 μm.](http://ajpgi.physiology.org/)

![Fig. 4. Ferroportin mRNA expression is upregulated in response to OS. A: Ferroportin expression is upregulated in Atm<sup>−/−</sup> mice compared with wild-type mice on both diets, with a significant difference on the high-iron diet. High-iron diet resulted in upregulation in both genotypes. *P < 0.01 between genotypes. **P < 0.05 between diets. Error bars indicate ± SE. B: the significant upregulation of Ferroportin mRNA expression in Atm<sup>−/−</sup> mice correlates to upregulation of Hmox1 (P = 0.009, r = 0.80). C: there is no correlation between the upregulation of Ferroportin mRNA expression in wild-type mice and Hmox1 (P = 0.272, r = 0.38). B and C: 95% confidence intervals are shown as broken lines.](http://ajpgi.physiology.org/)

AJP-Gastrointest Liver Physiol • VOL 300 • APRIL 2011 • www.ajpgi.org
effects on the expression of hepcidin. Neither Hjv nor Tfr2 showed any significant differences in mRNA expression or protein levels, respectively (Supplemental Fig. S1A–C).

Atm−/− mice have increased levels of OS in the liver. It is well documented that, at a cellular level, A-T is a disease of chronic OS (11, 21, 25, 32). To examine the level of OS in the livers of Atm−/− mice and investigate the contribution of increased iron, Hmox1 mRNA levels were assessed along with levels of protein tyrosine nitration in liver sections. No significant difference was identified in the levels of Hmox1 mRNA between wild-type and Atm−/− on the normal iron diet in spite of the differences in HIC. However, when Hmox1 expression was compared between wild-type and Atm−/− mice on the high-iron diet, Atm−/− mice showed significantly higher levels of expression than the wild-type mice (P = 0.007; Fig. 3A).

We also examined oxidative damage by analyzing protein tyrosine nitration by immunohistochemistry in liver sections of wild-type and Atm−/− mice on both diets. Liver sections from Atm−/− mice showed greater levels of tyrosine nitration than wild-type livers on both the normal iron and high-iron diets (Fig. 3B). The high-iron diet also increased the amount of detectable tyrosine nitration in both Atm−/− and wild-type mice.

Ferroportin’s response to OS is suppressed at the protein level. It has been reported previously that Fpn expression is upregulated concurrently with Hmox1 expression in response to OS and that this upregulated expression translates to increased protein levels (41). Echoing the upregulation of Hmox1 expression, Fpn mRNA expression also showed significantly higher levels in Atm−/− mice than in the wild-type animals on the high-iron diet (P = 0.008; Fig. 4A). If this increase in Fpn expression was a result of the increased OS, then a correlation between Hmox1 and Fpn expression would be expected. When this was examined, Atm−/− mice showed a significant positive correlation between the levels of Fpn and Hmox1 mRNA (P = 0.009, r = 0.80; Fig. 4B). Wild-type mice, however, showed no correlation between the levels of Fpn and Hmox1 mRNA (P = 0.272, r = 0.38; Fig. 4C).

Cellular ferroportin protein levels, however, can be decreased by the binding of hepcidin peptide, which results in its internalization and degradation. Given that hepcidin peptide was found to be significantly increased in the Atm−/− mice, we investigated the levels of ferroportin protein in the liver. Interestingly, the levels of ferroportin protein were unchanged in spite of the significant increase in Fpn mRNA expression, suggesting that the increased hepcidin expression resulted in a concomitant degradation of ferroportin protein (Fig. 5, A and B).

DISCUSSION

We have shown that Atm−/− mice develop iron loading in the liver by 10 weeks of age and that this loading is accompanied by equivalent changes in expression of the iron regulatory genes. We have also demonstrated that, when challenged with increased dietary iron, Atm−/− mice develop disproportionately large increases in the expression of genes of the iron regulatory pathway as well as in levels of OS response markers compared with wild-type mice. We suggest that a possible iron feedback effect amplifying the OS may result from increased hepcidin protein suppressing the normal iron export augmentation by ferroportin as part of the OS protective mechanism.

This possible mechanism is likely to result from the competition of cellular vs. whole body iron regulatory processes. The increased intracellular iron produced by the upregulation of Hmox1 in response to OS is, under normal conditions, compensated for by an increase in Fpn expression, resulting in greater cellular export of the free iron. Conversely, increased hepatic iron levels stimulate an increase in hepcidin that acts systemically, causing internalization and degradation of ferroportin protein to minimize the dietary uptake of iron and the recycling of iron from the reticuloendothelial system. This systemic action, however, also blocks the cell’s ability to export pro-oxidant iron. In the case of A-T, this may then lead to cellular accumulation of pro-oxidant iron and potentially increased generation of ROS.
been shown to be significantly upregulated in the cerebellum of neurons in A-T as potential link has been identified between increased iron and the progression of cell damage in these models (35–37). A demonstrating that intracellular iron is a contributing factor to the development of disease pathologies in cell viability as a direct result of iron chelation, using A-T cell models have also shown significant improvement in clinically relevant iron indexes. In vitro iron chelation studies have detailed iron studies in A-T disease (35–37). A study by Shackelford and coworkers (35) has reported nontransferrin bound iron, a highly reactive form of free iron, to be increased in A-T patients and Atnm+/− mice. Our data support this identification of increased body iron in A-T and further enhance the importance of this by showing significant changes in clinically relevant iron indexes. In vitro iron chelation studies using A-T cell models have also shown significant improvements in cell viability as a direct result of iron chelation, demonstrating that intracellular iron is a contributing factor in the progression of cell damage in these models (35–37). A potential link has been identified between increased iron and neurons in A-T as Hmox1, an enzyme involved in OS defense that produces labile iron as a byproduct (14, 39) and which has been shown to be significantly upregulated in the cerebellum of 3-mo-old Atnm+/− mice (3). In the present study, we have shown that Hmox1 is significantly and substantially upregulated in the livers of iron-challenged Atnm+/− mice, suggesting a significantly heightened sensitivity of Hmox1 to OS resulting from intracellular iron. Combined with the negative regulation of iron export by the identified increase in hepcidin, we propose that this system may lead to the rapid accumulation of potentially harmful free iron in neurons and contribute to the neurodegeneration seen in A-T.

Alongside its contribution to neurodegeneration, increased intracellular iron and the resultant OS can also potentiate tumor development (10, 12, 23, 24, 29, 38). Ironically, after contributing to the insult that initiated tumor development, increased cellular iron is then a critical element required for the continued growth of tumors in a number of different cancer types (10, 12, 23, 24, 38). A-T patients are predisposed to a variety of cancers, with one of the most significant clinical features of A-T disease being the incidence of lymphoid tumors that develop in ~30% of A-T patients (for review, see Ref. 26). Although increased intracellular iron has not been directly linked to the initiation of lymphoid tumors, the use of iron chelation in combination with anti-TIR antibodies significantly inhibits lymphoid tumor growth, demonstrating a direct contribution of iron to progression of the disease (23, 24). Furthermore, iron chelation has recently been shown to provide significant cellular protection against ionizing radiation by reducing the resulting OS and oxidative damage (5). Given the hypersensitivity of A-T patients to ionizing radiation, and its likely contribution to their increased cancer susceptibility, our identification of elevated iron status in Atnm+/− mice identifies a factor likely to contribute to both the severity of the damage caused by ionizing radiation and the later progression of lymphoid tumor development.

The mechanism through which the observed iron loading is occurring is as yet unknown. One potential source could be the increased recycling of iron resulting from the increased Hmox1 activity; however, given that Atnm+/− mice on a normal iron diet had increased hepatic iron without any change in Hmox1 expression, it is unlikely that the iron released by Hmox1 activity is responsible for the loading. Given that it has previously been shown that hepatocytes from Atnm+/− mice are more susceptible to environmental toxins and have a reduced liver regenerative capacity (27), it is possible that the defective protein phosphorylation in Atnm+/− mice has either direct or secondary effects on the hepatic intracellular signaling pathways related to systemic iron homeostasis. In the presence of iron loading, the observed increase in Hmmp1 expression suggests that the iron regulatory system is still sensing body iron levels correctly and responding accordingly, however, is unable to prevent the continued accumulation of hepatic iron. This paradox, along with further investigation into the degree to which the increased iron load is contributing to the Atnm+/− OS, will be important questions for future study.

Together, these data and the literature implicating iron’s contribution to diseases with similar neurodegeneration and cancer susceptibility suggest that direct investigation into the role of iron in the development of A-T pathology will be an important step in furthering our understanding of the underlying mechanism of disease progression. Developing an understanding of this contribution to A-T pathology has the potential to influence dietary iron recommendation for patients with A-T and may provide a means of limiting the exacerbation of the A-T phenotype.

ACKNOWLEDGMENTS

We thank Martin Lavin, Dr. Nuri Gueven, and Dr. Sergei Kozlov for helpful discussions.

GRANTS

This work was supported, in part, by a Program Grant from the National Health and Medical Research Council (NHMRC) of Australia (339400) to V. N. Subramaniam, a NHMRC R. D. Wright Career Development Award (443026) to D. F. Wallace, and a Griffith Medical Research College Research Collaboration Grant to V. N. Subramaniam, D. J. Watters and D. F. Wallace.

DISCLOSURES

The authors declare no conflicting interests.

REFERENCES


