

Alpha-1-antitrypsin deficiency: diagnosis and treatment

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Alpha-1-antitrypsin (AT) deficiency was first described in the late 1960s by Laurell and Eriksson [1] in patients with severe pulmonary emphysema. The recognition of AT deficiency as a cause of emphysema then led to what is still the prevailing theory for the pathogenesis of emphysema, the protease-anti-protease theory [2]. This theory is based on the concept that a lack of antiprotease allows protease activity to destroy the connective tissue matrix of the lung. The lack of antiprotease could be genetic, as is the case for AT deficiency, or due to functional inactivation of AT by active oxygen intermediates that are released by phagocytes of smokers. Several years after AT deficiency was first recognized, Sharp and colleagues [3] discovered AT deficiency in an infant with neonatal liver disease. Soon it was found that AT deficiency accounted for a significant number of cases of neonatal liver disease that were previously categorized as idiopathic [4]. We now know that AT deficiency is the most common genetic cause of neonatal liver disease and the most frequent diagnosis necessitating liver transplantation. It has also been shown to cause chronic liver disease, cryptogenic cirrhosis, and hepatocellular carcinoma in adults never previously known to have liver disease in infancy or childhood [5]. The incidence of the classical form of AT deficiency is 1 in 1600 to 1 in 2000 live births in most populations [6], but prospective screening studies in Sweden indicate that

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only ~10% of the affected individuals develop clinically significant liver disease by the time they reach the fourth decade of life [7,8]. These observations indicate that genetic traits unlinked to the AT gene or environmental factors predispose to or protect AT-deficient individuals from liver disease.

Clinical manifestations of liver disease in alpha-1-antitrypsin deficiency

In most cases this liver disease first becomes apparent at 4 to 8 weeks of age because of persistent jaundice (Box 1). Conjugated bilirubin and transaminase levels in the blood are mildly to moderately elevated. The liver may be enlarged but rarely are there symptoms, signs, or laboratory values that suggest severe liver injury. It is very difficult to clinically differentiate these infants from infants affected by many other causes of neonatal liver disease, including infections, metabolic diseases, and inherited hepatobiliary anomalies such as biliary atresia. AT deficiency is usually thought of as one of the causes of a broad diagnostic category termed “neonatal hepatitis syndrome.” Occasionally this diagnosis will be discovered in a newborn with bleeding symptoms such as hematemesis, melena, bleeding from the umbilical stump, or bruising [9]. In some cases there may be a cholestatic picture with icterus, pruritus, and laboratory abnormalities such as hypercholesterolemia. Indeed this subgroup of AT-deficient infants may

Box 1. Alpha-1-antitrypsin deficiency-associated liver disease

Clinical manifestations

Infancy	<ul style="list-style-type: none"> • Prolonged obstructive jaundice • Elevated transaminases
Early childhood	<ul style="list-style-type: none"> • Symptoms of cholestasis • Elevated transaminases • Asymptomatic hepatomegaly
Late childhood/adolescence	<ul style="list-style-type: none"> • Severe liver dysfunction • Chronic active hepatitis • Cryptogenic cirrhosis • Portal hypertension • Hepatocellular carcinoma

Diagnostic features

Diminished serum levels of ATZ (10% to 15% normal levels)
 Abnormal mobility of AT in isoelectric focusing (PIZ)
 Periodic acid–Schiff-positive, diastase-resistant globules in liver cells

have severe biliary epithelial cell damage and even paucity of the intrahepatic bile ducts detected in their liver biopsies [10]. Rarely AT deficiency manifests itself with severe progressive liver disease in the first year of life [11].

The liver disease of AT deficiency may also be diagnosed later in childhood with asymptomatic hepatomegaly, elevated transaminases detected incidentally, or jaundice that develops during an intercurrent illness. Finally, this disease can first present in childhood, adolescence, or adult life with complications of portal hypertension, including splenomegaly, hypersplenism, and gastrointestinal bleeding from varices, ascites, or hepatic encephalopathy. It should be considered in any adult patient with chronic liver disease, cryptogenic cirrhosis, or hepatocellular carcinoma. An autopsy study done in Sweden suggested that as many as 25% of AT-deficient men who die between the ages of 40 and 60 have evidence of injury or carcinoma incidentally detected in their livers [5].

The natural history of liver disease in AT deficiency is quite variable. Most infants that present with prolonged jaundice are asymptomatic by the time they reach 1 year of age. In the majority of these cases there is no further evidence of liver disease for many years. Although this diagnosis has only been known for about 30 years, it is widely believed that many of these individuals never develop clinically significant liver disease. The only prospective data on the course of AT deficiency come from the Swedish nationwide screening study started by Sveger [7] in the early 1970s. In that study, 200,000 newborn infants were screened and 127 were found to have the classic form of AT deficiency. Fourteen of the 127 had prolonged obstructive jaundice and nine of these 14 had clinically significant liver disease. Another eight of the 127 had hepatomegaly with or without elevated bilirubin or transaminase levels. Approximately 50% of the remaining population had elevated transaminase levels alone. The long-term outcome for these infants was last published when their mean age was 18 years of age [8], but unpublished observations by Sveger indicate that this has not changed for this population well into their twenties. There has been no evidence for the development of clinically significant liver disease in any of the patients since the first year of life. This means that only 10% of the population at most has encountered clinically significant liver disease. Of the remaining AT-deficient population, 85% have had persistently normal transaminase levels as they have aged. Liver biopsies have not been done in this study [8], and therefore it is not known whether some of these seemingly unaffected individuals have subclinical histologic abnormalities and will develop clinical signs as they reach the fourth and fifth decades of life.

Even patients with severe liver disease caused by AT deficiency may have a stable or relatively slowly progressing course. In one retrospective review of a pediatric hepatology experience, nine of 17 patients with AT deficiency and cirrhosis, portal hypertension, or both had a prolonged, relatively uneventful course for at least 4 years after the diagnosis of cirrhosis or portal hypertension [12]. Two of these patients eventually underwent liver transplantation, but seven were leading relatively healthy lives for as long as 23 years while carrying a diagnosis of severe AT deficiency-associated liver disease.

It has not yet been possible to identify specific clinical or laboratory signs that can be used to predict a poor prognosis for liver involvement in AT deficiency. Results of one early study suggested that persistence of hyperbilirubinemia, hard hepatomegaly, development of splenomegaly, and progressive prolongation of the prothrombin time were indicators of poor prognosis [13]. In another study, elevated transaminase levels, prolonged prothrombin time, and lower trypsin inhibitory capacity correlated with a worse prognosis [14]. In my experience (unpublished observations), the first definitive evidence for poor prognosis comes in the form of a complication that affects the overall life functioning of the patient.

It is still unclear whether heterozygotes for the classic form of AT deficiency are predisposed to liver disease. Early studies of liver biopsy collections suggested that there was a relationship between heterozygosity and the development of liver disease [15]. This has been confirmed by later studies of liver biopsy collections; in particular, the liver biopsies from patients who have undergone liver transplantation show a higher than expected prevalence of heterozygosity for the classic form of AT deficiency without another diagnostic explanation for severe liver disease [16]. However, these studies and others like them have an inherent bias in ascertainment. Results of one cross-sectional study of patients with AT deficiency, who were re-examined with recently developed more sensitive and sophisticated diagnostic assays, suggested that liver disease in heterozygotes could be accounted for, to a major extent, by infections with hepatitis C virus or by autoimmune disease [17]. Unfortunately neither type of study [16,17] has provided convincing evidence for or against a predisposition to liver disease in PIMZ individuals. Nevertheless, my experience with numerous protease inhibitor type Z (PIMZ) individuals with severe liver disease and no other plausible explanation leads me to believe that the predisposition does exist.

Liver disease has been described for several other allelic variants of AT. Children with compound heterozygosity for the S and Z alleles are affected by liver disease in a manner similar to that of protease inhibitor type ZZ (PIZZ) children [7,8]. There have been several reports of liver disease in AT deficiency protease inhibitor type M Malton (PIM Malton) [18,19]. This is an interesting association because the abnormal AT Malton molecule has been shown to undergo polymerization and retention in the endoplasmic reticulum (ER) [20]. Because it has only been reported in single patients with other allelic variants of AT [21], it is not clear whether liver disease is causally related to those variants.

Destructive lung disease or emphysema caused by AT deficiency probably does not become clinically manifest until late in the third decade. Although there are a few reports of younger individuals with lung disease, the diagnosis of AT deficiency in these cases was not convincing [21].

Limited information is available regarding the incidence of liver disease in AT-deficient individuals with established emphysema. In one study of 22 PIZZ patients with emphysema, transaminase levels were elevated in 10 patients and cholestasis in one patient [22]. Liver biopsies were not done in this study, so it

might underestimate the extent and incidence of liver disease in adults with emphysema as their predominant clinical problem.

Diagnosis

AT deficiency should be considered in anyone with elevated transaminases, elevated conjugated bilirubin levels, asymptomatic hepatomegaly, signs or symptoms of portal hypertension, signs or symptoms of cholestasis, or bleeding/bruising with a prolonged prothrombin time. It should be considered in adults with chronic hepatitis, cryptogenic cirrhosis, and hepatocellular carcinoma.

The diagnosis is established by means of serum AT phenotype [protease inhibitor type (PI type)] determination in isoelectric-focusing electrophoresis or agarose electrophoresis at acid pH (Fig. 1). Serum concentrations can be used for screening with follow-up PI typing of any values below normal (85 to 215 mg/dL). A retrospective study of all pediatric patients who had both serum concentrations and PI typing done at one center indicated that the serum concentration determination had a positive predictive value of 94% and a negative predictive value of 100% for homozygous PIZZ AT deficiency [23]. However, because of the inherent limitations of retrospectively defining a patient

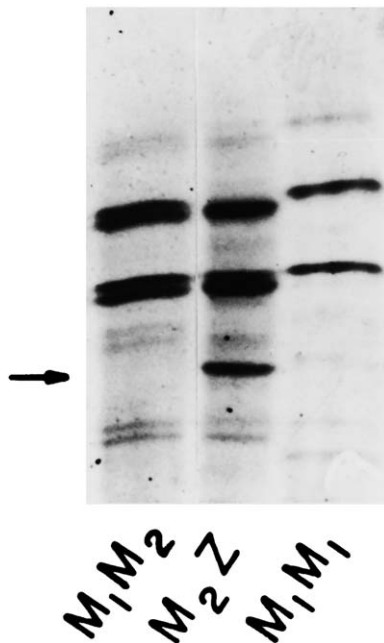


Fig. 1. Isoelectric focusing of serum. The more rapidly migrating Z allele is indicated by the arrow. (Reprinted from Perlmutter DH. α 1-antitrypsin deficiency. In: Snape WJ, editor. Consultations in Gastroenterology. Philadelphia: WB Saunders; 1996. p. 791–3; with permission.)

population for the analysis, the results of the study are not necessarily applicable to each diagnostic situation that might be encountered. It is wise to get both the serum concentration and PI typing when seriously considering this diagnosis. Serum concentrations increase during the host response to inflammation and therein may reach normal levels in heterozygotes and near-normal levels in homozygotes. Both the serum concentration and the PI type will be needed to confirm the homozygous, compound heterozygous, and the heterozygous states that can be present at the AT locus. In some cases, phenotype determination of parents and other relatives is necessary to confirm the diagnosis if there is any discrepancy and to ensure the distinction between the ZZ and SZ allotypes for which isoelectric focusing may not be straightforward. These distinctions will be important for genetic counseling.

The PI type is particularly important in the neonatal period because it may be very difficult to distinguish patients with AT deficiency from those with biliary atresia. Moreover, it is not uncommon for neonates with a PIZZ phenotype to have no biliary excretion on scintigraphic studies [24]. There is one report of AT deficiency and biliary atresia in a single patient [25]. We have had several patients with homozygous PIZZ AT deficiency and cholestasis for whom there is no biliary excretion of technetium-labeled mebrofenin, but with more prolonged observation, in each of these cases, cholestasis remitted so that it was then obvious that the patients did not have biliary atresia.

The distinctive histologic feature of homozygous PIZZ AT deficiency, periodic acid–Schiff-positive, diastase-resistant globules in the ER of hepatocytes, substantiates the diagnosis (Fig. 2). According to some observers, these globules are not as easy to detect in the first few months of life [26]. The presence

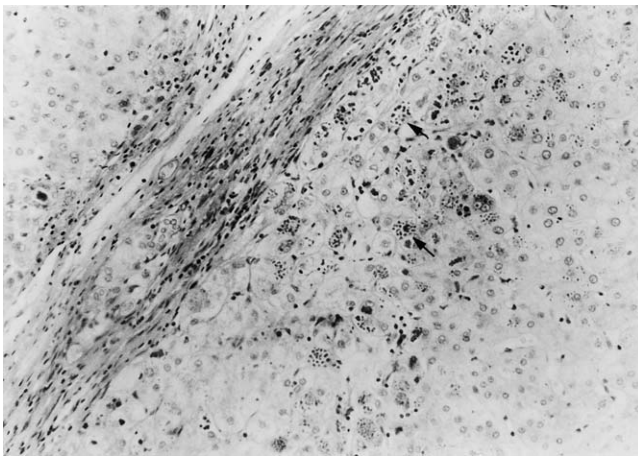


Fig. 2. Liver biopsy from a PIZZ individual. Examples of PAS-positive, diastase-resistant globules are indicated by the arrows. (Reprinted from Perlmutter DH. α 1-antitrysin deficiency. In: Snape WJ, editor. Consultations in Gastroenterology. Philadelphia: WB Saunders; 1996. p. 791–3; with permission.)

of these inclusions should not be interpreted as diagnostic of AT deficiency. Similar structures are occasionally present in other liver diseases [27]. The inclusions are eosinophilic, round to oval, and 1 to 40 μm in diameter. They are most prominent in periportal hepatocytes but may also be seen in Kupffer cells and biliary epithelial cells [28]. The liver biopsy may also be characterized by variable degrees of hepatocellular necrosis, inflammatory cell infiltration, periportal fibrosis, or cirrhosis. There is often evidence of biliary epithelial cell destruction. Our recent studies have also shown evidence for an autophagic reaction and mitochondrial injury, as well [29,30].

Treatment/supportive care

No specific therapy for AT deficiency-associated liver disease exists, and so clinical care involves avoidance of cigarette smoking to prevent exacerbation of destructive lung disease/emphysema, supportive management of symptoms caused by liver dysfunction, and prevention of complications of liver disease. Cigarette smoking markedly accelerates the lung disease associated with AT deficiency, reduces the quality of life, and significantly shortens the longevity of these patients [31].

Progressive liver dysfunction and failure in children with AT deficiency has been managed successfully with liver transplantation, resulting in survival rates well over 92% for 5 years [32,33]. Nevertheless, a number of homozygotes with severe liver disease, even cirrhosis or portal hypertension, may have a relatively low rate of disease progression and lead a relatively normal life for extended periods. With the availability of living-related donor transplantation techniques it may be possible to observe these patients for some time before transplantation becomes necessary. Children with AT deficiency and mild liver dysfunction, elevated transaminase levels or hepatomegaly, and without functional impairment may never need liver transplantation.

Patients with AT deficiency and emphysema have been treated with purified plasma or recombinant AT administered intravenously or by means of aerosol as replacement therapy [34]. This therapy is associated with improvement in serum and bronchoalveolar lavage fluid AT concentrations and neutrophil elastase inhibitory capacity in lavage fluid without significant side effects. Although results of initial studies have suggested that there is a slower decline in forced expiratory volume in patients undergoing replacement therapy, this occurred in only a subgroup of patients and the study was not randomized [35]. This therapy is designed for established and progressive emphysema. Protein replacement therapy is not being considered for patients with liver disease because there is no evidence that deficient serum levels of AT play a role in the development of liver injury.

A number of patients with severe emphysema from AT deficiency are being treated with lung transplantation. Over a 13-year experience, 86 patients with AT

deficiency underwent lung transplantation in St. Louis with an ~60% 5-year survival [36].

Pathogenesis of liver disease in alpha-1-antitrypsin deficiency

AT is the archetype of a family of serum proteins called serpins because most of them are inhibitors of serine proteases [37]. AT is the principal blood-borne inhibitor of destructive neutrophil proteases including elastase, cathepsin G, and proteinase 3 [38]. It is a glycoprotein secreted by liver cells and is considered an acute-phase reactant because its plasma levels increase during the host response to inflammation/tissue injury [39].

The pathogenesis of liver disease in AT deficiency is entirely different from that of the destructive lung disease. Lung disease results from a loss-of-function mechanism in which the lack of AT permits uninhibited neutrophil-mediated proteolytic damage to the elastic connective tissue matrix of the lung. In contrast, liver injury involves a gain-of-function mechanism whereby retention of the inefficiently secreted, mutant α 1 antitrypsin Z (ATZ) molecule in the ER of liver cells triggers a series of events that are eventually hepatotoxic. The strongest evidence for a gain-of-function mechanism comes from studies in which mice transgenic for mutant human ATZ develop liver injury with many of the histopathologic hallmarks of the human condition [40,41]. Because there are normal levels of anti-elastases in these mice, as directed by endogenous genes, the liver injury cannot be attributed to a loss-of-function mechanism.

The ATZ molecule is characterized by a point mutation that results in the substitution of lysine for glutamate 342 and accounts for defective secretion. This substitution reduces the stability of the monomeric form of the molecule and increases the likelihood that it will form polymers in the ER by the “loop-sheet” insertion mechanism [38]. Indeed, polymers have been detected in the ER of hepatocytes by electron microscopic analysis of a liver biopsy from a PIZZ individual, and in vitro studies indicate that ATZ undergoes polymerization to a certain extent spontaneously and to a greater extent during relatively minor perturbations, such as a rise in temperature. These observations led Lomas and Mahdeva [38] to speculate that increases in body temperature during systemic inflammation might exacerbate this tendency in vivo and that differences in incidence of severity of febrile illness might account for the variation in expression of liver disease among ATZ-deficient hosts.

The strongest evidence that polymerization causes retention of ATZ in the ER comes from studies in which the fate of ATZ is examined after the introduction of additional mutations into the molecule. For instance, Kim et al [42] introduced a mutation, F51L, into the ATZ molecule at amino acid 51. This mutation is remote from the Z mutation, E342K, but was predicted on the basis of structural characteristics to impede loop-sheet polymerization. Indeed, the F51L mutation makes the ATZ molecule less prone to polymerization and more efficient at folding in vitro, and it moderates the intracellular retention properties of ATZ in

microinjected *Xenopus* oocytes [43] and in yeast [44]. However, we have recently found that a novel, naturally occurring variant of ATZ, bearing both the same E342K substitution that is found in ATZ and a carboxyl-terminal truncation, is retained in the ER for at least as long as ATZ, even though it does not polymerize [45]. These results could indicate that mechanisms other than polymerization determine whether mutant ATZ molecules are retained in the ER. An alternative possibility is that polymerization of ATZ is not the cause of ER retention but rather its result.

It is still not entirely clear what proportion of the newly synthesized mutant ATZ molecules is converted to the polymerized state in the ER. In one cell culture model system, we found that $17.0\% \pm 1.9\%$ of ATZ is in the insoluble fraction at steady state [45], but comparable *in vivo* data are not yet available. It is also not known whether polymeric molecules are degraded in the ER less rapidly than their monomeric counterparts or whether polymeric molecules, when retained in the ER, are more hepatotoxic than their monomeric counterparts. Indeed, recent studies on the effect of temperature on the fate of ATZ have indicated the high degree of complexity involved in these issues. Although Lomas et al [46] showed that a rise in temperature to 42°C increases the polymerization of purified ATZ *in vitro*, Burrows et al [47] found that a rise in temperature to 42°C improves secretion of ATZ and decreases its intracellular degradation of ATZ. Consistent with the well-established role that temperature plays in most biochemical processes, these results suggest that changes in temperature have the potential to affect multiple steps in the pathways by which ATZ is translocated through the secretory and degradative compartments, as well as the relative proportions of ATZ in the monomeric and polymeric state. On the basis of these considerations, as well as long-standing clinical experience with AT-deficient children and other children with liver disease, and in the absence of clear epidemiologic evidence, it seems unlikely that there is a simple relationship between febrile episodes and phenotypic expression of liver disease in AT-deficient patients.

Several studies have shown that ATZ is degraded in the ER and that the proteasome is a key component of the degradation pathway [48–51]. Degradation of ATZ is markedly reduced by specific proteasome inhibitors in yeast and mammalian cells [49,50]. There is also evidence for the involvement of ubiquitin-independent proteosomal and nonproteosomal pathways in degradation of ATZ in the mammalian cell-free system [52].

As discussed later, autophagy may represent one nonproteosomal mechanism for degradation of ATZ [29]. Because this finding is based on the effect of chemical inhibitors of autophagy, which have other effects on cellular metabolism, definitive evidence for the role of autophagy in degradation of ATZ will require more detailed, probably genetic studies. Cabral et al [53] have provided evidence for a nonproteosomal degradation pathway that is sensitive to tyrosine phosphatase inhibitors. The relative contributions of proteosomal and nonproteosomal mechanisms to the disposal of ATZ *in vivo* are still unknown.

The mechanism by which the proteasome gains access from the cytoplasm to ATZ on the luminal side of the ER membrane is also uncertain. Although

retrograde translocation from the ER to the cytoplasm has been demonstrated for some luminal substrates of the proteasome, there is very limited evidence for retrograde translocation of ATZ. Werner et al [49] detected ATZ free in the cytosolic fraction of yeast when the proteasome was inhibited, but only a small fraction of the total ATZ in the ER could be detected, and there has been no other evidence for retrotranslocation. Recent studies have provided evidence for extraction of substrates through the ER membrane by the proteasome [54]. The AAA ATPase Cdc 48/p97 and its partners appear to play an important role in this process [55].

To determine whether the fate of ATZ is different in AT-deficient hosts susceptible to liver disease (“susceptible hosts”) compared with AT-deficient individuals who are protected from liver disease (“protected hosts”), Wu et al [56] transduced skin fibroblasts from PIZZ individuals, with or without liver disease, with amphotropic recombinant retroviral particles designed for constitutive expression of the mutant ATZ. The PIZZ individuals were carefully selected to ensure appropriate representation. Susceptible hosts were defined as having severe liver disease by clinical criteria. Protected hosts were discovered incidentally and never had clinical or biochemical evidence of liver disease. Human skin fibroblasts were selected because they do not express the endogenous ATZ gene but, presumably, express other genes involved in the postsynthetic processing of secretory proteins. Each fibroblast cell line expressed the human ATZ transgene. Unlike wild-type AT, mutant ATZ protein was selectively retained intracellularly in every case. However, cells from susceptible and protected hosts differed in that the former showed a marked delay in degradation of ATZ after it accumulated in the ER (Fig. 3). Thus, these data provide evidence that alterations in quality control mechanisms, such as the ER degradation pathway, can predispose AT-deficient hosts to liver injury.

Several recent studies have shown that ER retention of mutant ATZ provokes a rather specific cellular response with autophagy as a major feature autophagic. Autophagy is thought to be a general mechanism whereby cytosol and intracellular organelles, such as ER, are first sequestered from the rest of the cytoplasm, allowing them to be degraded subsequently within lysosomes (Fig. 4). This process has been observed in many cell types, especially during stress states, such as nutrient deprivation, and during the cellular remodeling that accompanies differentiation, morphogenesis, and aging. Our studies show that autophagosomes develop in several different model cell culture systems genetically engineered to express ATZ, including human fibroblasts, murine hepatoma, and rat hepatoma cell lines. Moreover, in a HeLa cell line engineered for inducible expression of ATZ, autophagosomes appear as a specific response to the expression of ATZ and its retention in the ER [29]. There is a marked increase in autophagosomes in hepatocytes in transgenic mouse models of AT deficiency and a disease-specific increase in autophagosomes in liver biopsies from patients with AT deficiency (Fig. 5). Mutant ATZ molecules can be detected in autophagosomes by immune electron microscopy, often together with the ER molecular chaperone calnexin. Intracellular degradation of ATZ is partially reduced by

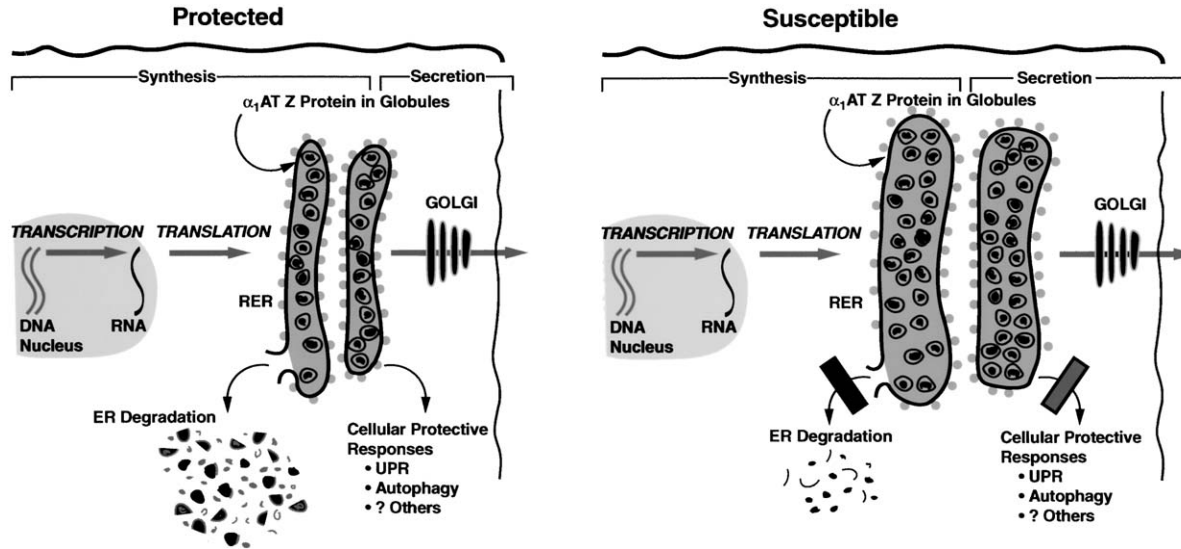


Fig. 3. Differences in the fate of, or response to, α_1 ATZ in protected and susceptible host cells.

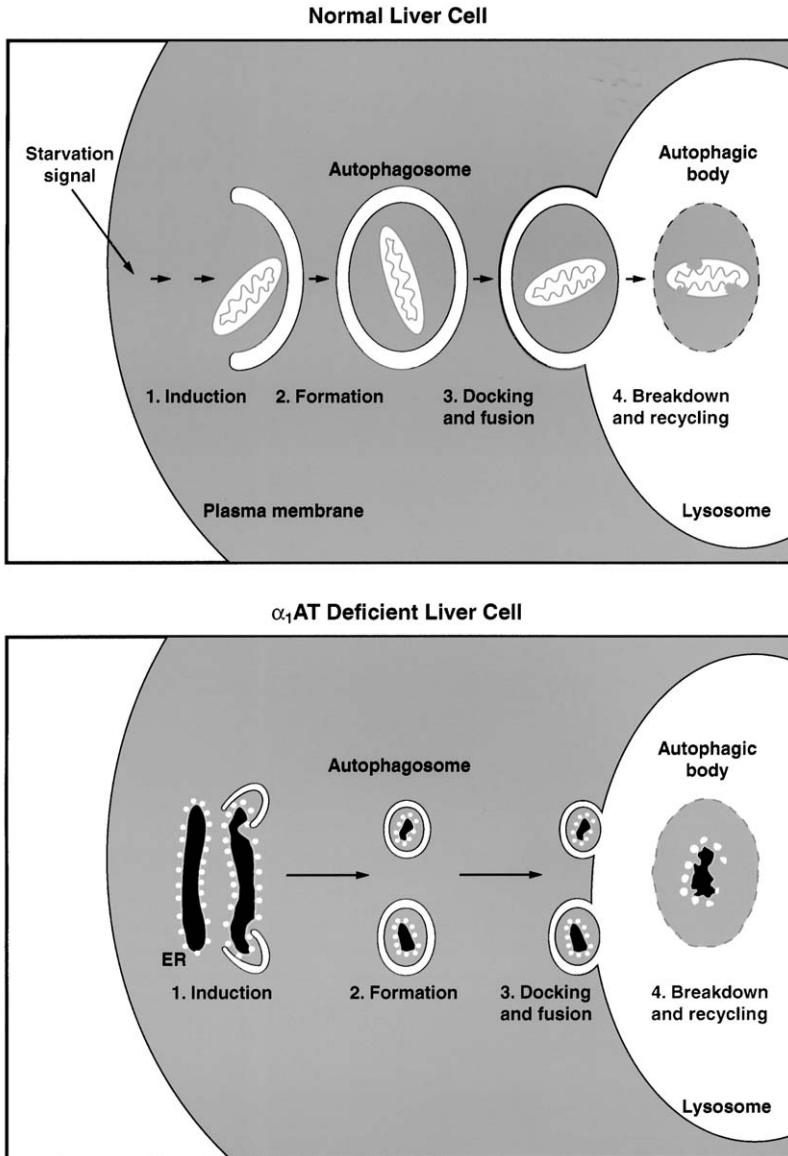


Fig. 4. Autophagy in normal and alpha-1-antitrypsin-deficient liver cells.

chemical inhibitors of autophagy, suggesting that autophagy also contributes to the quality control mechanism for disposal of ATZ [29].

Taken together, these results have suggested that the autophagic response is induced to protect liver cells from the toxic effects of aggregated ATZ retained in the ER. We have also speculated about the role of autophagy in protecting liver cells from tumorigenesis. Several recent studies have shown that autophagic

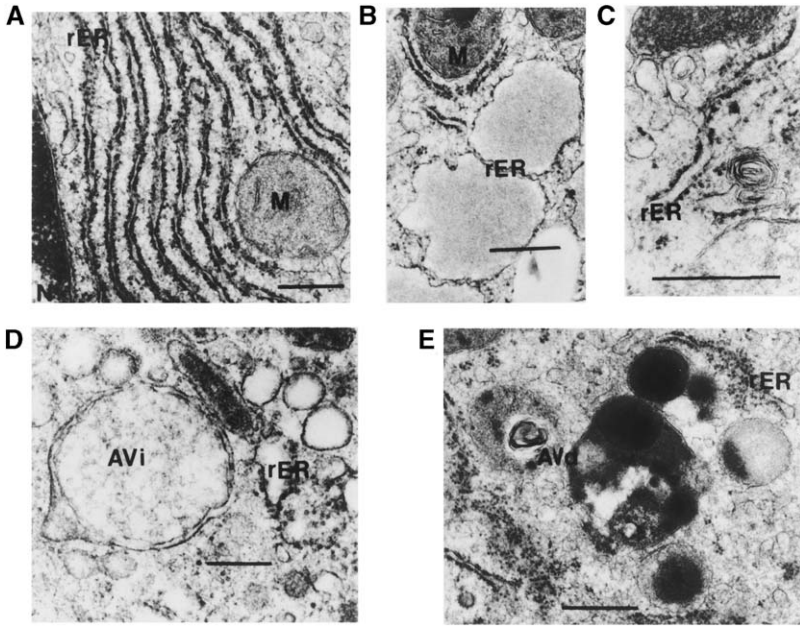


Fig. 5. Electron microscopy of liver from a PIZZ individual. (A) Normal endoplasmic reticulum; (B–E) autophagosomes. (Reprinted from Teckman JH, Perlmutter DH. Retention of the mutant secretory protein α 1-antitrypsin Z in the endoplasmic reticulum induces autophagy. *Am J Physiol* 2000;279:G961–74; with permission.)

activity is decreased in tumors and that reconstitution of autophagic activity inhibits tumorigenesis *in vivo* [57,58]. In our studies, autophagosomes are predominantly found in liver cells with dilated ER in both human and transgenic mouse liver [29]. Previous studies in transgenic mouse models of AT deficiency have shown that hepatocarcinogenesis evolves within nodular aggregates of hepatocytes that are negative for AT expression by immunofluorescent staining [59].

Recently, we examined the autophagic response to ER retention of ATZ *in vivo* by testing the effect of fasting on the liver of the PiZ mouse model of AT deficiency [60]. Starvation is a well-defined physiologic stimulus of autophagy, as well as a known environmental stressor of liver disease in children. The results show that there is a marked increase in fat accumulation and in ATZ-containing, ER-derived globules in the liver of the PiZ mouse induced by fasting. These changes were particularly exaggerated at 3 to 6 months of age. Three-month-old PiZ mice had a significantly decreased tolerance for fasting compared with nontransgenic C57 Black mice. Although fasting induced a marked autophagic response in wild-type mice, the autophagic response was already activated in PiZ mice to levels that were more than 50% higher than those in the liver of fasted wild-type mice, and they did not increase further during fasting. These results indicate that autophagy is constitutively activated in AT deficiency

and that the liver is unable to mount an increased autophagic response to physiologic stressors.

In the course of our ultrastructural studies of the liver of the PiZ mouse and of patients with AT deficiency, we have recently been struck by the degree of mitochondrial autophagy induced [30]. A comparison of the livers from four AT-deficient patients with livers from eight patients with other liver diseases and four normal livers showed a marked significant increase in mitochondrial autophagy associated with AT deficiency (Fig. 6). Even more interesting is the observation that many mitochondria that are not surrounded by autophagic vacuolar membranes are nevertheless damaged or in various phases of degeneration in liver cells from AT-deficient hosts. This damage is characterized by the formation of multilamellar structures within the limiting membrane, condensation of the cristae and matrix, and, in some cases, dissolution of the internal structures, often leaving only electron-dense debris compressed into a thin rim at the periphery of the mitochondrion.

Mitochondrial autophagy and injury are also marked in the liver of the PiZ transgenic mouse model of AT deficiency. Immunofluorescence analysis shows the presence of activated caspase-3 in the PiZ mouse liver [30]. Because cyclosporine A (CsA) has been shown to reduce mitochondrial injury [61] and inhibit starvation-induced autophagy [62], we examined the effect of CsA on PiZ mice. We found that it significantly reduces hepatic mitochondrial injury,

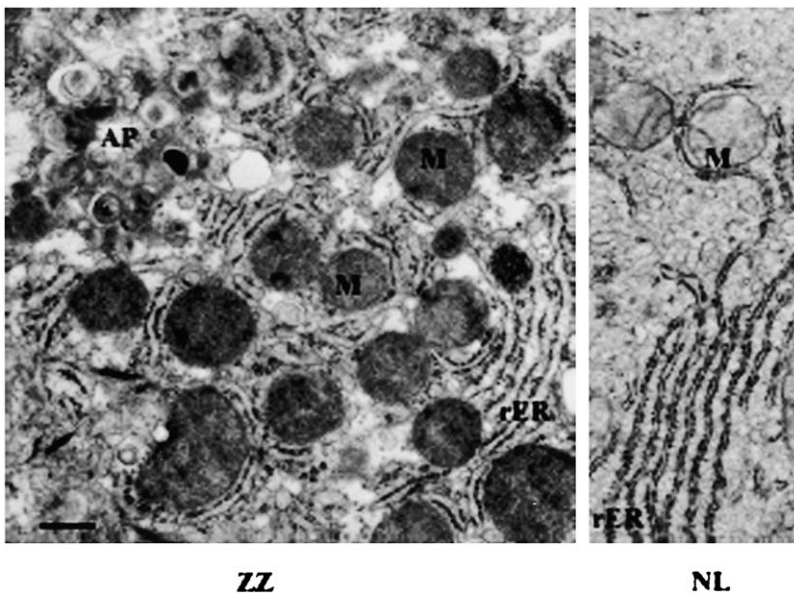


Fig. 6. Mitochondrial autophagy in liver from a PiZZ individual (left) compared with a normal (NL) liver biopsy specimen (right). (Reprinted from Teckman JH, An J-K, Blomenkamp K, Schmidt B, Perlmutter DH. Mitochondrial autophagy and injury in the liver in alpha-1-antitrypsin deficiency. *Am J Physiol* 2004;286:G851–62; with permission).

decreases activation of caspase-3, and improves the animals' tolerance of starvation [30]. These results provide evidence for the novel concept that mitochondrial damage and caspase activation play a role in the mechanism of liver cell injury in AT deficiency. Although this analysis suggests that there is mitochondrial injury that is separate from the autophagic process, the possibility that autophagy plays some role in mitochondrial damage cannot be completely excluded. One model of mitochondrial damage in this deficiency holds that accumulation of ATZ in the ER is responsible for mitochondrial dysfunction, and indeed, there is now ample evidence in the literature for functional interactions between mitochondria and closely apposed ER cisternae [63,64]. Recent studies show that specific signals are transmitted between these two intracellular compartments [65,66] and that mitochondrial dysfunction, including release of cytochrome C and caspase-3 activation, is associated with the ER dilatation and stress induced by brefeldin A, tunicamycin, or thapsigargin [67,68]. It is not yet known, however, whether mitochondrial dysfunction in the latter cases is due to ER dilatation or ER stress or to independent effects on mitochondria by these experimental drugs. A second possible explanation, not necessarily incompatible with the first, envisages mitochondrial dysfunction as a result of the autophagic response to ER retention of ATZ. In this scenario, mitochondria are recognized nonspecifically by the autophagic response, which is constitutively activated to somehow remove and degrade areas of the ER that are distended by aggregated mutant protein. Although our data indicate that CsA inhibits hepatic mitochondrial injury *in vivo*, this benefit could reflect the drug's known effects on the mitochondrial permeability transition [61], on autophagy [62], or both.

The CsA findings are also noteworthy for their therapeutic implications. They indicate that CsA can prevent mitochondrial damage even under circumstances in which ATZ continues to accumulate in the ER. Thus, they provide a proof-in-principle for mechanism-based therapeutic approaches to liver disease in AT deficiency—pharmacologic intervention directed at distal steps in the pathobiologic pathway that leads to liver injury (the mitochondrial step, for instance), rather than at the primary defect or the early events in the pathway.

Novel chemoprophylactic/therapeutic strategies

In addition to CsA, other strategies for prevention or treatment of target organ injury in AT deficiency have been suggested recently. For instance, several studies have shown that a class of compounds called chemical chaperones can reverse the cellular mislocalization or misfolding of mutant plasma membrane, lysosomal, nuclear and cytoplasmic proteins including CFTR Δ F508, prion proteins, mutant aquaporin molecules associated with nephrogenic diabetes insipidus, and mutant galactosidase A associated with Fabry disease [69–71]. These compounds include glycerol, trimethylamine oxide, deuterated water, and 4-phenylbutyric acid (PBA). We recently found that glycerol and PBA mediate a marked increase in the secretion of ATZ in a model cell culture system [47].

Moreover, oral administration of PBA was well tolerated by PiZ mice (transgenic for the human ATZ gene) and consistently mediated an increase in blood levels of human ATZ, reaching 20% to 50% of the levels present in PiM mice and normal humans. PBA did not affect the synthesis or intracellular degradation of ATZ. The ATZ secreted in the presence of PBA was functionally active in that it could form an inhibitory complex with neutrophil elastase. Because PBA has been used safely for years in children with urea cycle disorders as an ammonia scavenger and because clinical studies have suggested that only partial correction of the deficiency state is needed for the prevention of both liver and lung injury in ATZ deficiency [72–74], PBA is an excellent candidate for chemoprophylaxis of target organ injury in AT deficiency.

Several iminosugar compounds may be useful for chemoprophylaxis of liver and lung disease in AT deficiency. These compounds are designed to interfere with oligosaccharide side chain trimming of glycoproteins and are now being examined as potential therapeutic agents for viral hepatitis and other types of infection [75,76]. We have examined several of these compounds initially to determine the effect of inhibiting glucose or mannose trimming from the carbohydrate side chain of mutant ATZ on its fate in the ER. To our surprise we found that one glycosidase inhibitor, castanospermine (CST), and two α mannosidase I inhibitors, kifunensine (KIF) and deoxymannojirimicin (DMJ), actually mediate increased secretion of ATZ [77]. ATZ secreted in the presence of these drugs is partially functionally active. KIF and DMJ are less attractive candidates for chemoprophylactic trials because they delay degradation of ATZ in addition to increasing its secretion and therefore have the potential to exacerbate susceptibility to liver disease. However, CST has no effect on the degradation of ATZ and may therefore be an appropriate target for development as a chemoprophylactic agent. The mechanism of action of CST on ATZ secretion is unknown. An interesting hypothesis for the mechanism of action of KIF and DMJ has mutant ATZ interacting with ERGIC-53 for transport from ER to Golgi when mannose trimming is inhibited.

Alternative strategies for at least partial correction of AT deficiency may result from a more detailed understanding of the fate of the ATZ molecule in the ER. For instance, delivery of synthetic peptides to the ER to insert into the gap in the A-sheet or into a particular hydrophobic pocket of the ATZ molecule [78] and prevent polymerization of ATZ might result in release of the mutant ATZ molecules into the extracellular fluid and prevent accumulation in the ER. Although it is not yet entirely clear, there is some evidence from studies on the assembly of MHC class I molecules that synthetic peptides may be delivered to the ER from the extracellular medium of cultured cells [79]. There is also evidence that certain molecules may be transported retrograde to the ER by receptor-mediated endocytosis [80]. Even if polymerization results from, rather than causes, the secretory defect, this strategy may be effective if peptide insertion leads to a change in conformation that is associated with translocation competence. Second, elucidation of the biochemical mechanism by which abnormally folded ATZ undergoes intracellular degradation might allow pharmaco-

logic manipulation of this degradative system, such as enhancing proteasomal activity with interferon γ in the subpopulation of the PIZZ individuals pre-disposed to liver injury.

Replacement of AT by means of somatic gene therapy has been discussed in the literature for a number of years [73]. This strategy is potentially less expensive than replacement therapy with purified protein and may avert the need for weekly or even monthly administration. As a form of replacement therapy, however, this strategy will only be useful for lung disease in AT deficiency. Significant issues must be addressed before gene replacement therapy becomes a realistic alternative [81]. The most important prerequisite will be demonstration that replacement therapy with purified plasma AT is truly associated with an ameliorative effect. Several novel types of gene therapy, such as repair of mRNA by trans-splicing ribozymes [82,83], chimeric RNA/DNA oligonucleotides [84–86], triplex-forming oligonucleotides [87], small fragment homologous replacement [88], or RNA silencing [89,90], are theoretically attractive alternative strategies for management of liver disease associated with AT deficiency because they would prevent the synthesis of mutant ATZ and ER retention.

Other studies have shown that transplanted hepatocytes can repopulate the diseased liver in several mouse models [91,92], including a mouse model of childhood metabolic liver disease termed hereditary tyrosinemia. Replication of the transplanted hepatocytes occurs only when there is injury or regeneration in the liver. The results provide evidence that it may be possible to use hepatocyte transplantation techniques to treat hereditary tyrosinemia and, perhaps, other metabolic liver diseases in which the defect is cell autonomous. For instance, AT deficiency involves a cell-autonomous defect and would be an excellent candidate for this strategy.

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