Detection of Circulating and Endothelial Cell Polymers of Z and Wild Type α1-Antitrypsin by a Monoclonal Antibody*

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Globular inclusions of abnormal α1-antitrypsin (AAT) in the endoplasmic reticulum of hepatocytes are a characteristic feature of AAT deficiency of the PiZZ phenotype. Monoclonal antibodies, which contain constant specificity and affinity, are often used for the identification of Z-mutation carriers. A mouse monoclonal antibody (ATZ11) raised against PiZZ hepatocytic AAT was successfully used in enzyme-linked immunosorbent assays (ELISA) and in identification of Z-related AAT globular inclusions by immunohistochemical techniques. Using electrophoresis, Western blotting, and ELISA procedures, we have shown in the present study that this monoclonal antibody specifically detects a conformation-dependent neoepitope on both polymerized and elastase-complexed molecular forms of AAT. The antibody has no apparent affinity for native, latent, or cleaved forms of AAT. The antibody ATZ11 illustrates the structural resemblance between the polymerized form of AAT and its complex with elastase and provides evidence that Z-homozygotes beyond the native form may have at least one more circulating molecular form of AAT, i.e. its polymerized form. In addition, staining of endothelial cells with ATZ11 antibody in both M- and Z-AAT individuals shows that AAT attached to endothelial cells is in a polymerized form. The antibody can be a powerful tool for the study of the molecular profile of AAT, not only in Z-deficiency cases but also in other (patho)physiological conditions.

The capacity to undergo conformational changes is crucial for the physiological function of many proteins; the serine proteinase inhibitors (serpins) are a clear case for such changes having been exploited during evolution as a means of modulating inhibitory activity (1). Serpins possess two structural elements that are conformationally labile and are essential for efficient proteinase inhibition: a reactive center loop and a β-sheet (β-sheet A) that is able to open and accommodate the reactive center loop after its cleavage by the attacking proteases (2–5). The x-ray crystal structures of native, cleaved, cleaved in complex with protease, and polymerized forms of serpins have confirmed the abilities of these proteins to undergo profound conformational changes under certain environmental conditions (6–8). The crystal structure of the α1-antitrypsin-trypsin complex showed complete insertion of the reactive site loop, which substantially increases thermal and conformational stability of the serpin and results in the irreversible inhibition and then the structural destruction of the proteinase (9).

The conformational changes that occur in the serpin molecule during the formation of the enzyme-serpin complexes, interaction with other molecules, polymerization, cleavage, and oxidation lead to the generation of conformation-dependent neoepitopes (10). Immunochemical analysis by means of monoclonal antibodies is a useful approach for study of the formation of new epitopes that occur as a result of the conformational polymorphism of serpins (11, 12). For example, the suggestion that the whole reactive site loop is incorporated as an additional strand into the β-sheet A upon complex formation is supported by immunological evidence obtained using anti-thrombin in which unique epitopes were exposed through binding to proteases and cleavage of the reactive side bond (13). These epitopes were also present in the cleaved serpin, where crystallographic data have revealed full loop insertion. For another serpin, the C1 inhibitor, it was shown that neoepitopes are induced upon interaction with target and non-target proteases, illustrating the structural similarities between its complexed and cleaved forms. (14).

Molecular mobility confers on serpins not only the capacity to bind and entrap their target proteinases in highly stable complexes but also a concomitant propensity to form dysfunctional molecules (15). A change of a single amino acid in certain domains of the serpin molecule can block changes in the structure necessary for normal inhibitory activity and folding and can lead to the polymerization of mutant serpin into intracellular aggregates (16). Our understanding of the lost and altered activities of dysfunctional serpins has been greatly advanced by correlating studies on molecular structure with analysis of the mechanisms of serpins and their target proteinases and of their physical properties (17–19).

α1-Antitrypsin is the major serpin in the human circulation. It is produced predominantly by hepatocytes but also by blood monocytes, macrophages, pulmonary alveolar cells, and intestinal epithelial cells (20, 21). The biological activity of AAT can be affected by point mutations modifying its structure and/or secretion. Several genetic variants of AAT are associated with
low plasma AAT levels (22). Severe AAT deficiency of the homozygous PiZZ phenotype, which differs from the normal M variant in the substitution of Glu\(^{342}\) with Lys, was first recognized as a new hereditary condition predisposing to disease on the basis of low plasma levels (10% normal) of the protein that arise not from the lack of AAT synthesis but from a blockade of its secretion (23, 24). Molecules of the Z variant of AAT are retained in the endoplasmic reticulum of hepatocytes as inclusion bodies that can be recognized by periodic acid–Schiff (PAS) staining (25). PAS-positive intracellular inclusions are the end result of AAT polymerization due to a sequential insertion of the reactive loop from one AAT molecule into a β-sheet of another (26, 27). The retained Z-AAT polymers are cytotoxic for the hepatocytes and can cause liver damage with a variable clinical presentation, from neonatal hepatitis to liver cirrhosis and hepatocellular carcinoma in adults (28).

Individuals with AAT deficiency are also at an increased risk of developing emphysema, which is the most frequent complication of AAT deficiency and is believed to occur because of the decrease in elastase inhibitory capacity normally provided by AAT. However, AAT polymers also occur in the lungs of patients with AAT deficiency (29, 30). Moreover, an association has been shown between Z-AAT deficiency and immunemediated diseases, particularly C-ANCA positive vasculitis disorders (31).

A monoclonal antibody raised against the PiZZ type of AAT purified from the liver of Z-homozygotes was widely used for the recognition of AAT deficiency in ELISA procedures (32). The antibody was shown to recognize specifically the Z type of AAT, and the linear epitope was thought to be located at the mutation site (Lys\(^{342}\) → Glu) in the AAT molecule. This idea was challenged by later studies showing the ability of a single amino acid substitution in a protein molecule to change the shape of the molecule and result in the opening and/or creation of new, conformation-dependent epitopes (11, 13, 34, 35). Typically, the pronounced structural change that occurs during the formation of the enzyme-serpin complex leads to the generation of new epitopes (36). To date, monoclonal antibodies have been described that recognize a conformation-dependent neoepitope in both the activated protein C-protein C inhibitor complex and the cleaved, loop-inserted inhibitor but lack affinity for the native form of inhibitor (10). Such antibodies provide evidence that identical neoepitopes can be present in two different molecular forms of proteolytically modified inhibitor and that they are conformationally dependent.

We hypothesize that the change in protein folding of Z-AAT could cause formation of the multidomain, conformational epitopes but need not necessarily involve the mutation site. Here we have shown that a mouse monoclonal antibody, ATZ11, which has been produced against Z-AAT, recognizes a conformational neoepitope including both polymerized and elastase-complexed forms of AAT but lacks affinity to native, latent, or cleaved AAT.

**Experimental Procedures**

**Specific Reagents**—Native, purified human AAT was a gift from the Department of Clinical Chemistry, University Hospital, Malmo. Eight of the patients had pulmonary emphysema, one had idiopathic pulmonary fibrosis, and two had idiopathic pulmonary arterial hypertension. None of them had clinical signs of liver disease or vasculitis. Plasma samples from 12 normal PiM individuals were used as controls. Z-AAT plasma samples were analyzed immediately after collection and again 2 weeks later.

**Preparation of Cleaved AAT and AAT-Elastase Complexes**—A stock solution of human plasma AAT was prepared in sterile Tris-buffered saline (15 mM Tris, 0.1% NaCl, pH 7.4) at a concentration of 18 mg/ml. Cleaved AAT was prepared as previously described (37). Briefly, native AAT was incubated with porcine pancreatic elastase at a molar ratio of 1.5 for 15 min at 37 °C. The cleaved AAT was separated from the elastase using a centrifugal microconcentrator (Centricon-30, Millipore Corp., Bedford, MA). AAT-elastase complexes were produced by incubating AAT with porcine pancreatic elastase at a molar ratio of 1.2:1 for 15 min at 37 °C. All preparations of AAT were analyzed by 7.5, 10, or 12% PAGE and SDS-PAGE gels followed by staining with Coomassie Blue.

**Preparation of AAT Polymers and Latent AAT**—AAT polymers were prepared according to Dafforn et al. (38) by incubating AAT (final concentration 1 mg/ml) with 2 mg/ml of elastase for 2 h at room temperature. Polymer formation was confirmed by non-denaturing 7.5% PAGE and a concomitant loss of inhibitory activity against pancreatic elastase.

The latent AAT was prepared as described previously (39) by incubating protein (final concentration 1.2 mg/ml) in 0.7 mM citrate buffer at 65 °C for 24 h. The citrate was removed by dialysis against 20 mM Tris, pH 8.6, at 4 °C. The latent AAT preparations were then heated at 56 °C for 3 h to convert any residual active AAT to polymers. Latent AAT was assessed by 10 or 12% SDS and non-denaturing PAGE.

**Electrophoresis and Western Blotting**—PAGE was performed in a Mini-PROTEAN II electrophoresis cell (Bio-Rad) on 7.5, 10, or 12% homogenous gels.

Electrophoretically separated samples were transferred to a polyvinylidene difluoride membrane (Immobilon™-P, Millipore Corp., Bedford, MA) in transfer buffer (25 mM Tris, 190 mM glycine, 20% methanol) using a semidybl blot electrophoretic transfer system (Trans-Blot S.D., Bio-Rad). After the transfer, the membranes were blocked overnight with 5% nonfat dried milk in Tris-buffered saline containing 0.1% Tween 20 at 4 °C. Blots were developed for 2 h using primary polyclonal rabbit antibodies against human AAT (1:800) or mouse monoclonal antibodies against human Z-AAT (ATZ11) (1:250). In some experiments ATZ11 antibodies were preincubated with the mutation site peptide for 24 h at 4 °C prior to use. The protein bands were visualized by incubation with horseradish peroxidase-conjugated secondary antibody against rabbit or mouse immunoglobulins (1:800), respectively. DAB (3,3-diaminobenzidine tetrahydrochloride, Sigma) was used as a peroxidase substrate.

**Sandwich ELISA Assay**—Nunc-Immuno plates (Nalge Nunc International, Denmark) were routinely coated with 100 µl of plasma samples diluted 1:100 in coating buffer (0.05 mM NaCO\(_3\), pH 9.6) or different monoclonal antibodies against human Z-AAT (ATZ11) (1:250). In some experiments ATZ11 antibodies were preincubated with the mutation site peptide for 24 h at 4 °C prior to use. The protein bands were visualized by incubation with horseradish peroxidase-conjugated secondary antibody against rabbit or mouse immunoglobulins (1:800), respectively. DAB (3,3-diaminobenzidine tetrahydrochloride, Sigma) was used as a peroxidase substrate.
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added and incubated for 2 h on a shaker at room temperature. After washing as described above, 100 µl of peroxidase-conjugated rabbit antibody to mouse immunoglobulins, diluted 1:1000, was added for 2 h at room temperature. Finally, the wells were washed as before, and 200 µl of ATZ11 antibody, diluted 1:1000, was added for 2 h on a shaker at room temperature. After washing, 200 µl of peroxidase-conjugated rabbit antibody to mouse immunoglobulins was added and incubated for 2 h at room temperature. Control slides were incubated with the buffer or non-immunized mouse IgG (1:1000, negative control). The second peroxidase-labeled antibody was applied and incubated for 30 min at room temperature. The absorbance was estimated after 15 min at 405 nm in an ELISA reader. All samples were analyzed in triplicate. For the control, washing buffer was substituted for the monoclonal antibody.

Immunohistochemistry—Specimens of temporal artery and aorta were obtained by biopsy and autopsy, respectively. The specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. After cutting, sections were deparaffinized and developed in the immunostaining system, TechMate™ 500 Plus (Dako). Blocking antibody was applied for 20 min at room temperature. The primary antibody ATZ11 (1:1000), monoclonal anti-AAT (1:1000), and monoclonal anti-neutrophil elastase (1:1000) were added and allowed to react for 90 min at room temperature. Control slides were incubated with the buffer or non-immunized mouse IgG (1:1000, negative control). The second peroxidase-labeled antibody was applied and incubated for 30 min at room temperature. After washing, the sections were stained with DAB. The sections were deparaffinized and developed in the immunostaining system, TechMate™ 500 Plus (Dako). Blocking antibody was applied for 20 min at room temperature. Finally, the wells were washed as before, and 200 µl of ATZ11 antibody, diluted 1:1000, was added for 2 h on a shaker at room temperature. After washing, 200 µl of peroxidase-conjugated rabbit antibody to mouse immunoglobulins was added and incubated for 2 h at room temperature. Control slides were incubated with the buffer or non-immunized mouse IgG (1:1000, negative control). The second peroxidase-labeled antibody was applied and incubated for 30 min at room temperature. The absorbance was estimated after 15 min at 405 nm in an ELISA reader. All samples were analyzed in triplicate. For the control, washing buffer was substituted for the monoclonal antibody.

Statistical Analysis—The difference of the means in the experimental results was analyzed for statistical significance by Student’s two-sample two-sided t test and/or one-way analysis of variance combined with a multiple comparison procedure (Scheffe multiple-range test), with an overall significance level of α = 0.05.

RESULTS

Detection of Various Molecular Forms of AAT by the Monoclonal Antibody ATZ11—Different molecular forms of AAT, such as native, proteolytically cleaved, polymerized, complexed with elastase, and latent were subjected to 7.5% or 10% non-denaturing and SDS-PAGE, respectively, and immunoblotted with monoclonal antibody ATZ11. First, to investigate the specificity of ATZ11 antibody while conserving protein conformation, non-denaturing (native) PAGE immunoblotting analysis was used. We found that ATZ11 reacts equally well with AAT-elastase complex and with polymerized AAT (Fig. 1) but does not recognize non-inhibitory latent and cleaved forms of AAT. These data confirm that the ATZ11 antibody does not interact with the inhibitory active, so-called “stressed” conformation of this protein. Analysis of the latent, cleaved, and AAT-complexed with elastase forms by 10% native PAGE followed by Western blotting confirmed that the ATZ11 antibody reacts strongly only with AAT-elastase complex (Fig. 2) but does not recognize non-inhibitory latent and cleaved forms of AAT. These data show that the ATZ11 antibody reacts with the AAT-enzyme complex and AAT polymerization induces an immunologically similar conformation, which is detectable by the ATZ11 antibody. Analysis of the same samples by SDS-PAGE and Western blotting showed no interaction between ATZ11 and any molecular form of AAT (Fig. 3). This implies that the epitope recognized by ATZ11 is not determined by the
AAT sequence, i.e. is non-linear. Furthermore, preincubation of ATZ11 with the peptide carrying the Z mutation failed to inhibit ATZ11 interaction with AAT polymers and AAT-elastase complex (data not shown). Thus, these experiments further supported the specificity of ATZ11 for a conformational but non-linear epitope.

**Specificity of the Monoclonal Antibody Studied by the ELISA Method**—The specific ability of polymerized and elastase-complexed AAT, but not native or cleaved AAT, to inhibit binding of the monoclonal antibody to immobilized antigen was demonstrated by competitive ELISA experiments. Microtiter plates were coated with Z-AAT plasma (1:200) with polymerized AAT or Z-AAT patient plasma. After overnight incubation, the mixtures were added to antigen-coated microtiter plates. ELISA assay was performed as described under “Experimental Procedures.”

**Immunohistochemistry**—Temporal artery and aortic specimens taken from non-Z individuals were immunostained for polymerized AAT, and the AAT-elastase complex. Preincubation of ATZ11 with Z-AAT plasma samples in dilutions from 1:2 to 1:32 inhibited ATZ11 binding to immobilized antigen in a concentration-dependent manner from 91 to 30% \((p < 0.01)\). Similarly, preincubation of ATZ11 with various concentrations of either polymerized AAT or AAT-elastase complex showed an effective competition for binding of ATZ11 to immobilized antigen. In contrast, native, cleaved, and latent forms of AAT showed no ability at all to compete with the ATZ11 antibody binding to immobilized antigens (data not shown).

**ELISA assays illustrating the binding (expressed as A405) of monoclonal antibody ATZ11 to immobilized antigen.** Z plasma (A) and polymerized AAT (B) alone and in the presence of different dilutions of polymerized AAT or Z-AAT patient plasma. Each point represents a mean of eight repeats \((p < 0.01)\). The experiments were carried out by mixing a fixed dilution (1:300) of antibody ATZ11 with a dilution series of polymerized AAT or ZZ plasma. After overnight incubation, the mixtures were added to antigen-coated microtiter plates. ELISA assay was performed as described under “Experimental Procedures.”
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**Fig. 6. Localization of AAT in temporal artery.** Specimens stained with polyclonal anti-human AAT (1:1000) show immunoreactivity on the endothelial lining and in the smooth muscle cells (a). Monoclonal antibody ATZ11 (1:1000) shows AAT immunoreactivity only in the endothelium layer (indicated by arrow) (c). The endothelial layer is not stained by anti-elastase antibody (1:500) (b) or by non-immunized mouse IgG antibody (1:500) used as a negative control (d) (original magnification ×100).

**Fig. 7. 7.5% Native PAGE electrophoretic analysis of plasma samples from normal (controls) and PiZ homozygotes followed by Western blotting.** The blots were developed using monoclonal antibody ATZ11 (A) and polyclonal anti-AAT antibody (DAKO) (B). A, lanes 1 and 2, M-AAT plasma; lanes 3–5, deficiency Z-AAT plasma from patients with emphysema; lane 6, polymerized native AAT; lane 7, AAT-elastase complex. B, lane 1, AAT-elastase complex; lane 2, polymerized AAT; lanes 3–5, Z-AAT plasma; lanes 6 and 7, M-AAT plasma.

AAT with polyclonal anti-AAT, polyclonal anti-neutrophil elastase, monoclonal ATZ11 antibodies, and with non-immunized mouse IgG antibody (negative control) (Fig 6, a–d).

Endothelial cell lining stained intensely positive for AAT in all specimens using monoclonal ATZ11 antibody (Fig. 6, c). The polyclonal AAT antibody showed a nearly identical staining pattern of endothelial cell layers compared with the monoclonal ATZ11 antibody (Fig. 6, a), but in addition polyclonal anti-AAT showed AAT immunoreactivity in the smooth muscle cell layer. The polyclonal antibody to elastase as well as control mouse IgG showed no endothelial staining at all (Fig. 6, b and d).

**Identification of the Molecular Profile of AAT in Plasma Samples from Z homozygotes—**Plasma samples from PiMM (n = 12) and PiZZ (n = 12) homozygotes were analyzed by 7.5% non-denaturing PAGE electrophoresis followed by Western blotting. The blots were performed using monoclonal antibody, ATZ11, and commercial polyclonal anti-AAT antibody. Plasma samples were freshly obtained and analyzed at once. Immuno- blots that were developed using polyclonal anti-AAT antibodies showed similar AAT profiles in both Z and M plasma. In contrast, the fact that the same immunoblots developed by using ATZ11 antibodies shows that Z, but not M, plasma samples contain a remarkable amount of polymerized forms of AAT (Fig. 7A). AAT profiles in the analyzed samples were compared with those obtained from the purified, AAT-elastase complex and polymerized AAT (Fig. 7A, lanes 6 and 7; Fig. 7B, lanes 1 and 2). Z-AAT plasma samples, kept at −20 °C and analyzed after 2 weeks, showed an identical polymer profile (data not shown), suggesting that the formation and disintegration of plasma AAT polymers are not very sensitive with regard to sample manipulation. Moreover, as shown in Fig. 7A, one of the Z plasma samples, in addition to the polymeric AAT, showed a protein band occurring at the same position as the AAT-elastase complex prepared in vitro (Fig 7A, lanes 5 and 7). Because Z-AAT molecules are known to have a lesser ability to maintain a stable complex with elastase (27), it is reasonable to believe that storage of the Z plasma samples, as well as multiple refreezing, might result in the dissociation of AAT-elastase complexes. Further studies are needed to confirm that Z-homozygotes, except polymerized AAT, also contain circulating AAT-elastase complexes. Plasma from M samples showed no polymerized and/or complexed AAT as determined by the ATZ11 antibody (Fig. 7A, lanes 1 and 2).

**DISCUSSION**

A monoclonal antibody, ATZ11, has been prepared after immunization of mice with globular AAT inclusions purified from a ZZ liver (32). Screening of a large number of plasma samples with ATZ11 using a double sandwich ELISA procedure allowed rapid and easy identification of individuals with homozygous PiZ or intermediate PiMZ, SZ, and FZ AAT deficiency. There was no overlapping observed between plasma from subjects lacking the Z allele and those that were PiZ homozygotes or heterozygous. No false positive plasma samples have been found (40–42). The antibody was also utilized for the identification of PiZ gene carriers in liver tissue sections (43). Together these studies have suggested that the ATZ11 antibody is of great value for the identification of PiZ gene carriers at both plasma and tissue levels.

Recently, however, Fischer et al. (44) have shown that the ATZ11 antibody reacts with endothelial cells of portal capillaries and larger blood vessels from both PiM and PiZ individuals.
They have suggested that this is due to a cross-reaction of the antibody with an epitope on endothelial cells. We have confirmed these findings by showing that various non-Z specimens of vessel endothelium stain intensely positive for AAT with monoclonal ATZ11. The observation that ATZ11 antibody is unable to distinguish between the normal and the deficiency variant of AAT on endothelial cells led us to predict that the ATZ11 antibody recognizes a conformation-dependent, presumably non-linear, epitope of the AAT molecules, not necessarily involving the Z mutation per se. Because a mouse monoclonal antibody ATZ11 was developed against hepatic inclusion bodies isolated from a Z-homozygote, one can assume that the AAT protein used as an antigen was in a polymerized form. In both the native PAGE immunoblot analysis and ELISA systems, polymers prepared in vitro from purified M-AAT clearly showed that the ATZ11 antibody recognizes a polymeric form of AAT. We also found that, in addition to AAT polymers, the antibody reacts with AAT-elastase complex but shows no reaction with the native, inhibitory form of AAT or its non-inhibitory forms, viz. cleaved or latent. A synthetic peptide covering the Z mutation of AAT (Glu342 → Lys) failed to inhibit the antibody reaction with polymerized and complexed AAT, providing further evidence that a neoepitope recognized by the ATZ11 antibody does not involve the mutation site of the Z-AAT molecule.

An interaction between AAT and its target enzyme results in a conformational transition of AAT that allows the opening of β-sheet A and insertion of the cleaved reactive site loop (45). The insertion of the reactive site strand into β-sheet A can also be induced under mild denaturation conditions and during intermolecular polymerization of AAT (46). The structural transitions associated with loop insertion expose epitopes that can stimulate antibody production. Recently a monoclonal antibody has been identified that recognizes a neoepitope covering both complexed and cleaved forms of the protein C inhibitor but shows no affinity for the native form of the serpin (10). It is generally assumed that small conformational differences between the cleaved serpin complexed with an enzyme and cleaved serpin without complex formation limit the possibilities to produce an antibody that recognizes only one of these inactive forms (12). However, for the C1 inhibitor at least one antibody has been described which recognizes a neoepitope exclusively exposed on the cleaved inhibitor and not on the complexed form of the protein (14).

To date no antibody has been described covering a neoepitope on both AAT complexed with enzyme and polymerized AAT. The finding that ATZ11 antibody reacts specifically with two non-inhibitory forms of AAT, polymerized and complexed with elastase but not with cleaved or latent forms, implies that the dislocations of structural elements in the AAT molecule caused by the insertion of the active site strand into the β-sheet during polymerization and complex formation are distinct from those occurring in the latent and proteolytically cleaved forms of AAT. The structures of intact, latent, cleaved, and polymerized serpins and serpins complexed with enzyme confirm the polymorphic character of the reactive site strand, which to varying extents can undergo an insertion into β-sheet A (5, 47–49). The ATZ11 antibody that can discriminate conformational states associated with reactive site insertion into β-sheet A may provide great help in elucidating the structural features of AAT as well as in detecting molecular forms of AAT in vivo.

In the classic form of AAT deficiency, phenotype PiZ, the mutant Z-AAT molecules are retained within the endoplasmic reticulum of the liver cells due to an aberrant protein polymerization (50, 51). We have demonstrated here that plasma freshly obtained from patients with homozygous Z deficiency also contains a significant amount of AAT polymers. This observation provides good evidence that Z-AAT polymerization occurs not only within hepatocytes but also in the circulation. Non-Z carriers were found to have very low levels or a total lack of plasma AAT polymers, which is also consistent with the high sensitivity and specificity of the ATZ11-based ELISA system used for the detection of Z carriers in earlier studies (40, 41).

Considering the potential cytotoxicity of Z-AAT polymers to hepatocytes and their occurrence in the circulation, it is relevant to discuss the potential pathogenic role of these polymers in extrahepatic clinical manifestations of AAT deficiency, such as emphysema and vasculitic disorders. The accompanying plasma deficiency predisposes the Z-homozygote to early onset emphysema, particularly in cigarette smokers (52–54). Emphysema is thought to be caused by uninhibited proteolysis in the lung tissue (23, 55), although Z-AAT polymers have also been identified in the lungs of Z-AAT homozygotes with emphysema (56). However, at present we can only speculate about the putative role of AAT polymers in the pathogenesis of emphysema. The polymers of Z-AAT molecules, either locally formed de novo or deriving from the circulation, may favor proteolytic events but also provide as yet unknown biological activities.

The intrinsic property of the wild type AAT to undergo loop-sheet polymerization is much less pronounced than for mutant Z-AAT. To date, in vivo, wild type AAT polymers have been described only in human bile, where AAT interaction with the hydrophobic microenvironment is supposed to enhance polymer formation (57). The observed reaction of ATZ11 antibody with vascular endothelial cells from non-Z AAT individuals clearly demonstrates that endothelial-bound AAT is in a polymerized form. Neutrophil elastase staining of endothelial cells turned out to be negative, arguing against any significant contribution of AAT-elastase complexes to the observed staining pattern.

Expression of AAT in human islet, microvascular endothelial cells has been demonstrated (58), and endothelial binding of AAT and α1-antichymotrypsin derived from plasma has been identified (59). It has been suggested that the loss of endothelial serpins predisposes to vascular injury (60, 61). The mode of biosynthesis and/or uptake mechanisms of AAT in endothelial cells, however, needs further investigation. Moreover, our findings highlight the need for the elucidation of the putative role of AAT in large vessel diseases and also in small vessel disorders, particularly Wegener’s granulomatosis, a characteristic C-ANCA entity. Many recent studies have shown a firm relationship between the presence of a Z allele and anti-proteinase-3-positive systemic vasculitis (31, 33).

Taken together, our data describe a mouse monoclonal antibody that recognizes a conformation-dependent neoepitope in polymerized AAT and in the AAT-elastase complex but that has no affinity for the native form of inhibitor or for the non-inhibitory cleaved or latent forms. We have also shown that a fraction of plasma AAT in Z-homozygotes exists in a polymerized form and that a polymeric wild type AAT is an endothelial cell marker. The ATZ11 antibody can be a valuable tool for further conformational studies of AAT and for various clinical applications.

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