Serine Proteinase Inhibitors as Acute Phase Reactants in Liver Disease

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α₁-Antitrypsin, α₁-antichymotrypsin and angiotensinogen are three genetically homologous acute phase reactants which belong to the super family of serine proteinase inhibitors (serpins). In this report the dissociate expression of these respective genes in various liver diseases or after exposure to estrogen is emphasized. In addition, the influence of abnormal genetic variants on plasma levels of these proteins and on liver cell function is discussed.

(Key Words: Antitrypsin, Antichymotrypsin, Angiotensinogen, Acute phase, Liver disease)

INTRODUCTION

Although the predominant biosynthesis of all glycoproteins which are involved in the acute phase reaction takes place in the hepatocyte, the changes caused by liver disease per se are often quite modest when compared to the more impressive changes in the immunoglobulin patterns. It is sufficient to name the dramatic increases of polyclonal IgG synthesis in chronic hepatitis of autoimmune etiology or the corresponding IgM increase in primary biliary cirrhosis. However, quantitation of plasma acute phase proteins may, as in other non-hepatic diseases, provide the clinician with useful information concerning disease activity, progress and specific complications. An analysis of acute phase proteins may also reveal hereditary deficiency states which, albeit rare, are pathogenetically and etiologically important. In addition both acquired and inborn defects may provide researchers with useful models for studies on regulation of biosynthesis and the post-synthetic fate of proteins. α₁-Antitrypsin (AAT), also known as the α₁-proteinase inhibitor constitutes an excellent example of a positive acute phase reactant which has attracted hepatologists’ attention for several reasons: the plasma level can be used as an activity indicator in various liver diseases and the classical deficiency state (Proteinase Inhibitor Pi, PIZ) provides an interesting model of an abnormal secretory process associated with chronic progressive liver disease. AAT, α₁-antichymotrypsin (ACT) and angiotensinogen (AGT) all belong to a super family of serine proteinase inhibitors referred to as serpins (12). The proteins in this family (Table I) are derived from a common ancestral proteinase inhibitor and exhibit a high degree of sequence homology, similarities in gene structure, functional domains and reactive centers. The amino acid sequence at the reactive center determines the specificity for target proteases. Not all members of the family are acute phase reactants, and no proteinase inhibitory function for AGT has been identified. The present review will focus on the role of AAT, ACT and AGT in liver disease with the specific aim to illustrate the variable expression of these three serpin genes in different clinical and experimental settings. The role of proteinase inhibitors and other acute phase reactants as modulators of the immune response has recently been reviewed (30) and will not be discussed here.

α₁-ANTITRYPsin AND OTHER ACUTE PHASE PROTEINS IN LIVER DISEASE

A basic plasma protein pattern typical of acute liver disease was found in a series of patients with hepatitis B in 1972 (29). In contrast to the usual acute phase reaction seen in inflam-
Table I Some members of the Serpin superfamily

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Conc (μmol/l)</th>
<th>Mass (kD)</th>
<th>Target protease</th>
<th>Acute phase reactant</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1-Antitrypsin (AAT)</td>
<td>25</td>
<td>51</td>
<td>Elastase</td>
<td>+</td>
</tr>
<tr>
<td>α1-Antichymotrypsin (ACT)</td>
<td>7</td>
<td>58</td>
<td>Cathepsin G</td>
<td>+</td>
</tr>
<tr>
<td>Angiotensinogen (AGT)</td>
<td>0.01</td>
<td>51</td>
<td>Unknown</td>
<td>+</td>
</tr>
<tr>
<td>Antithrombin III</td>
<td>2</td>
<td>61</td>
<td>Thrombin</td>
<td>-</td>
</tr>
<tr>
<td>Antiplasmin</td>
<td>1</td>
<td>70</td>
<td>Plasmin</td>
<td>-</td>
</tr>
<tr>
<td>Protein C inhibitor</td>
<td>0.01</td>
<td>57</td>
<td>Prot C (Act)</td>
<td>-</td>
</tr>
<tr>
<td>C1-inhibitor</td>
<td>2</td>
<td>104</td>
<td>Cls, kallikrein</td>
<td>-</td>
</tr>
<tr>
<td>Thyroxin binding globulin</td>
<td>0.2</td>
<td>54</td>
<td>unknown</td>
<td>-</td>
</tr>
</tbody>
</table>

formation, only AAT was seen to rise during the first weeks of hepatitis, with normal levels of α1-acid glycoprotein, fibrinogen and C-reactive protein. Haptoglobin levels were noted to fall below normal, possibly reflecting changes in portal blood flow. In patients with hepatitis A or extrahepatic obstructive jaundice, a more conventional acute phase pattern was seen. Variable patterns are seen in chronic liver disease, reflecting both disease activity and protein synthetic capacity. Thus in clinically inactive but well-compensated cirrhosis, AAT is often normal, but α1-acid glycoprotein and haptoglobin levels tend to be subnormal. In more active disease, e.g. chronic active hepatitis, a selective increase of AAT is often seen. The magnitude of the AAT response reflects the inflammatory activity seen in concomitant liver biopsies (8). These established patterns are of definite value in clinical practice. When present, they indicate a clinically important liver disease and assist in judging disease activity and, in some cases, the effect of therapy.

THE BIOSYNTHESIS OF α1-ANTITRYPSIN IN LIVER DISEASE

The biosynthesis of AAT has been studied by in vitro mRNA translation and in cell culture using both isolated rat hepatocytes and human hepatoma cell lines (11, 9). Although the predominant biosynthesis of plasma AAT is thought to occur in the liver, the AAT gene is also expressed in extrahepatic tissues, e.g. macrophages (3) and multiple other cell types (10). The selective elevation of plasma AAT in active liver disease has generally been assumed to result from augmented biosynthesis in the hepatocytes, but available experimental data do not support this concept. The biosynthesis of a number of other acute phase reactants have been shown to be regulated in the human hepatoma cell lines Hep G2 and Hep 3B by the monokines interleukin-1 (IL-1) and tumor necrosis factor (TNF), but the rate of AAT synthesis remained unaffected in these systems (46). Darlington et al. (17) were unable to influence AAT synthesis in Hep 3B2 cells incubated with supernatants of lipopolysaccharide (LPS) stimulated peripheral blood mononuclear cells. They suggested that the increase of AAT in vivo may be due to post translational mechanisms such as reduced degradation. Schwarzenberg et al. (49) measured specific AAT mRNA in human cirrhotic livers and found decreased levels in spite of normal or raised plasma AAT levels. In contrast, significant increases in expression of AAT have been demonstrated in human monocytes. It has therefore been suggested that the expression of AAT by human hepatocytes is largely constitutive while that of macrophages is regulated (3).

The above reports question the role of AAT as an acute phase reactant. In this context it is interesting to note that some differences in glycosylation pattern of plasma AAT (53) as well as other proteins (36) occur in acute phase situations and in malignant conditions. These changes could conceivably affect the catabolism of AAT, but the turnover rate of AAT during acute phase reactions has never been studied. Increased plasma AAT levels in the acute phase have generally been estimated by immunologi-
cal methods, and the immunoreactive species may include functionally inactive forms. Functionally inert variants have recently been observed in sera from patients with advanced cancer (14).

AAT IN HEREDITARY DEFICIENCY STATES: THE SECRETORY BLOCK

In 1969 Sharp first observed PAS-positive globules, later identified as immunoreactive AAT in the rough endoplasmic reticulum (ER) of hepatocytes (50). Later studies demonstrated the lack of sialic acid in the carbohydrate moiety of intracellular AAT (5). These observations, together with increasing knowledge on the post translational modifications of glycoproteins during export from secretory cells, suggest that PiZ AAT is retained in its core-glycosylated form within the rough ER of cells. Although the amino acid substitution from Glu to Lys at residue 342 (24) in PiZ AAT is expected (15) to cause conformational changes in the PiZ AAT molecule compared to the PiM tertiary structure (39), the tertiary structure of the PiZ protein remains to be determined. An additional substitution recently reported is Val to Ala at residue 213 (44). As this mutation is also present in about 23% of PiM1 alleles, associated with normal plasma AAT levels, it is not expected to participate in the secretory block. Another possible explanation for the secretory block is spontaneous aggregation of the PiZ protein itself, and a tendency for PiZ AAT purified from human plasma to aggregate has been reported (16). Alternatively, conformational changes could prevent recognition by receptors which facilitate transport through subcellular organelles, or possibly cause steric hindrance in necessary interactions with modifying enzymes. To date there is no experimental data to verify any of the above hypotheses. What is known, however, is that transcription of PiZ DNA appears to occur normally (49) and that 10–15% of the synthesized protein, i.e. the mRNA translation product, does make its way through the smooth ER and Golgi and is exported in a form which contains sialic acid in the normally complex-glycosylated carbohydrate structure (20).

Whereas emphysema in PiZZ AAT deficiency has generally been assumed to result from a proteinase-antiproteinase imbalance at the alveolar level, no explanation exists for the development of liver disease in this condition. Possible pathogenetic mechanisms include proteinase-antiproteinase imbalance in the extracellular milieu, but this seems unlikely due to the absence of known cases of liver disease in the rare Pi null phenotype. Alternatively, the intracellular aggregates of AAT may be hepatotoxic in some way. Electron microscopic studies have suggested an increased peroxisome activity in PiZ livers (23). Experimental studies in Xeno- pus oocytes injected with human PiM or PiZ mRNA have shown protein synthesis in these systems (20) with decreased AAT export from PiZ oocytes. In addition, PiZ but no PiM oocytes demonstrated significantly augmented synthesis of lysosomal enzymes (4). Recently the isolated human genes for PiM and PiZ AAT have been transferred to mouse embryos resulting in transgenic mice (51) bearing multiple human gene copies. These mice have confirmed and extended previous observations concerning sites of synthesis of AAT (10). Globular inclusions of PiZ AAT are readily visible in hepatocytes. In this animal model, however, no increase in lysosomal enzymes can be demonstrated in PiZ mice compared to PiM mice and to normal litter mates (Sifers, Carlson unpublished data). It is hoped that further studies in transformed cell lines and in these transgenic mice may elucidate mechanisms of protein aggregation and liver injury in PiZ subjects.

α1-ANTICHYMOTRYPsin AS AN ACUTE PHASE REACTANT

ACT is a member of the serpin super family which is highly homologous AAT in both gene structure and amino acid composition (2, 13). It displays microheterogeneity that is only partially due to differences in sialic acid content (21). We have shown that the microheterogeneity remaining after desialylation includes two separate amino terminal isoforms which can be separated by a difference in charge (57). On the other hand, we found no differences in molecular mass or isoform pattern of ACT in normal and acute phase plasma (37).

The normal plasma concentration of ACT ranges from 0.3–0.6 g/l with a mean of 0.49 +/− 0.07 g/l (22). In contrast to AAT (35), plasma concentrations of ACT decrease signifi-
cantly after 7–8 weeks of estrogen treatment, but do not change after treatment with progesterone (25), and plasma ACT levels do not seem to increase during pregnancy (28).

ACT is synthesized mainly in the liver, but expression of the ACT gene has also been demonstrated by immunohistochemistry in several epithelial tissues and in neoplasms, particularly in histiocytic tumors (42). ACT has been claimed to be a sensitive marker for hepatocellular carcinoma (45). The plasma concentration of ACT increases rapidly up to fourfold in response to trauma and surgery (1). Monokines such as IL-1 and TNF have increased expression of the ACT gene in human Hep G2 cells (17) suggesting an acute phase response. Prostaglandins also cause an increase in plasma ACT levels in man (55) by an unknown mechanism.

The biological role of ACT is largely unknown. ACT is produced and actively secreted in lung macrophages (7) and it is found in sputa from patients with chronic bronchitis at levels higher than can be explained by transudation from the blood (48), suggesting a pneumoprotective role. ACT inhibits cathepsin G, mast cell chymase, and pancreatic chymotrypsin (33, 52). The inhibition of cathepsin G, a neutrophil proteinase with elastolytic properties, seems to be the most rapid and specific (52). Both mast cell chymase and cathepsin G can convert Angiotensin I to the vasoactive Angiotensin II (47). Cathepsin G can also generate formation of chemotactic activity when incubated with C3 and C5 (54).

ACT IN LIVER DISEASE: AN HEREDITARY DEFICIENCY

We have investigated plasma ACT levels in >200 patients with biopsy verified liver disease (38). The levels were significantly increased in relation to levels in healthy controls (p < 0.001). No tendency toward low ACT levels was found in patients with pronounced hepatocellular dysfunction. There was a significant correlation between plasma AAT and ACT levels (r = 0.50, p < 0.001). Our findings conflict with the findings of Matsuzaki [[et al.]] who reported low levels of ACT in the majority of patients with both compensated and decompensated liver cirrhosis of unreported etiology (41). In a subsample of patients with chronic active hepato-
titis (N = 26) we identified four individuals with a low ACT/AAT ratio and plasma ACT levels below 70% of normal. None had markers for autoimmune or viral disease. In one of these probands we found similarly low levels in first degree relatives indicating heredity.

Three additional families with subnormal levels of ACT have been found by population screening (19). Subnormal ACT levels (< 64% of normal) were present in approximately half of the first degree relatives to each proband, with a probability of this familial distribution occurring by chance of 10^-17. The pattern of inheritance is consistent with autosomal codominant inheritance and the heterozygote frequency based on this screening study is 0.7%. Six of eight ACT deficient individuals over 25 years of age had liver disease and three of eight had lung manifestations, varying from severe disease to subtle laboratory abnormalities.

Plasma samples from patients in an extended liver biopsy series were later analyzed. Of 316 patients, 10 had subnormal ACT levels. Four of these had chronic active hepatitis, three cryptogenic cirrhosis, one primary biliary cirrhosis (PBC), one chronic persistent hepatitis, and one steatosis with fibrosis. All except the patient with PBC lacked autoimmune and viral markers. One patient with cryptogenic cirrhosis is PiZZ and two others are PiMZ.

Preliminary histochemical studies demonstrate no intracellular inclusions on PAS-staining after diastase incubation of liver biopsies from these patients. The more sensitive immuno peroxidase method shows granular staining specific for ACT in the hepatocytes of some of these patients. This pattern was absent in numerous control biopsies from patients with autoimmune chronic active hepatitis and normal or elevated ACT levels. Electronmicroscopy of one of these biopsies has revealed fluffy material in the endoplasmic reticulum of hepatocytes. These findings are highly suggestive of a secretory block analogous to that seen in PiZZ AAT deficiency, and corresponding alterations in the ACT protein are being sought. So far, no molecular abnormalities have been detected by ordinary physicochemical methods in plasma ACT from deficient subjects (38). In these samples, as in normal plasma, there was an aminoterminal dimorphism with a minor
isoform lacking the aminoterminal His-Pro dipeptide. It should, however, be emphasized that the patients we have encountered appear to be heterozygotes for ACT deficiency. In the presence of one normal and one mutant allele corresponding to the AAT Pi MZ phenotype, plasma ACT concentrations resulting from the deficiency allele would be very low.

ANGIOTENSINOGEN IN LIVER DISEASE

Angiotensinogen (AGT) is synthesized in the liver and secreted into plasma. The consecutive proteolytic cleavage by renin and angiotensin converting enzyme transform AGT to the biologically active octapeptide angiotensin II. Several groups have reported molecular cloning and the nucleotide sequence of a human AGT cDNA (26, 32). Analysis of the AGT protein sequence indicated significant homology with the serpin super family (26). Furthermore, an identical structural organization of the AGT, AAT and ACT genes has been demonstrated (2). Differences in nucleotide sequences have been reported (26, 32), suggesting a genetic polymorphism for this protein which has yet to be demonstrated at the protein level. AGT behaves as an acute phase reactant with increasing plasma levels in acute inflammatory conditions (42). Increased AGT mRNA levels have been demonstrated in rat livers after intraperitoneal injection of E. coli LPS (27). Using radio immunoassay of liberated angiotensin I as an indirect determination of renin substrate, several authors have suggested that AGT levels are low in cirrhosis, possibly due to hepatic dysfunction (18). We recently compared plasma AGT levels determined by electro immunoassay in a large series of patients with chronic liver disease with those in patients with active rheumatoid arthritis (RA). AGT levels are high in RA patients in contrast to liver disease subjects whose levels tend to fall, occasionally to < 30% of normal. The decline was found to be unpredictable and unrelated to both type and severity of liver disease. AGT, in contrast to AAT and ACT, is extremely sensitive to estrogen with up to fourfold increases in plasma levels in women using oral contraceptives. It is thus conceivable that plasma levels measured in liver disease are modulated by the endocrine dysbalance that is so often seen in these patients. Our data are consistent with a dissociate expression of the three homologous serpin genes in chronic liver disease and in response to estrogen. It is of interest to compare these findings with a recent report showing decreased AGT mRNA level in HeP G2 cells exposed to the hepatocyte stimulating factor (HSF), a monokine which stimulates the synthesis of various other acute phase proteins (51).

CONCLUSION

During the past few years significant new information has accumulated concerning the genetic structure of the serpin acute phase reactants, the tissue distribution of gene expression, as well as similarities and differences in regulation of protein synthesis and microheterogeneity. We anticipate that clinical observations together with studies in cell culture, and knowledge of nucleotide sequences in regulatory regions of the homologous serpin genes will soon contribute to a better understanding of the molecular basis of the acute phase response.

REFERENCES

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