Alpha₁-Antitrypsin Phenotype: Transient Cathodal Shift in Serum of Infant Girl with Urinary Cytomegalovirus and Fatty Liver

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Summary

A 2-month-old white girl had severe liver disease (but without signs of hepatic necrosis, infection or cirrhosis), urinary cytomegalovirus, transient reduction of α_1 -antitrypsin concentration and transient abnormal α_1 -antitrypsin phenotype that were not present in her parents. Five serum specimens that were obtained during the 1½ months of acute phase liver disease indicated, by polyacrylamide gel isoelectric focusing (PAG-IEF), acid starch gel and agarose electrophoresis as well as immunofixation, an unusual α_1 -antitrypsin phenotype that we labeled delta (Δ). It migrated adjacent to Z, i.e., cathodal of Z and Zpratt on PAG-IEF; anodal of Z but cathodal of X, S, Zpratt on starch gel. We labeled the girl's complete phenotype M Δ . After clinical recovery, her phenotype was MM and identical to that of her parents. Hepatic electron-microscopy of the acute phase specimen showed dilated bile canaliculi. We observed the following in hepatocytes: clusters of globular inclusions surrounded by myelin sheets that, to a lesser extent, also appeared in the liver of CMV-infected children with phenotype MM; dilated endoplasmic reticulum cisternae that contained floccular material; and marked steatosis. These changes were less severe in the convalescent liver specimen.

Speculation

The patient's transient α_1 -antitrypsin Δ protein may be the result of a postribosomal reduction in sialic acid of M protein triggered perhaps by severe liver disease. If so, we would not expect a further cathodal shift of Pi type Δ after treatment of M Δ serum with neuraminidase. Alternatively, Δ protein might be the product of an α_1 -antitrypsin allele Pi^Δ that was transiently activated by the stress of the liver disease as is the juvenile globin gene in the adult goat by erythropoietic stress. The argument for Pi^Δ would be strengthened if: (1) the identical Δ protein was found in patients with other severe diseases of the liver; (2) the primary structure of Δ protein differed from that of M protein; (3) the material within the cisternae of the patient's hepatocytes was related to α_1 -antitrypsin; and (4) neuraminidase treatment of M Δ serum was followed by a further cathodal shift of Pi type Δ .

Alpha₁-antitrypsin (α_1 -AT) is a protease inhibitor (Pi) encountered in more than 35 allelic variants whose phenotypes can be demonstrated electrophoretically in serum (4). Each phenotype is labeled with the letter that corresponds to the relative migratory position of the respective α_1 -AT protein bands on polyacrylamide gel isoelectric focusing (PAG-IEF). For example, phenotype EM would indicate a set of α_1 -AT bands anodal of F but cathodal of D that are thus labeled E as well as an additional set of α_1 -AT bands anodal of N but cathodal of L that are thus labeled M. Family studies reveal that the phenotypes reflect α_1 -AT alleles inherited in codominant fashion. In our example, EM indicates that the patient possesses the α_1 -AT alleles Pi^E and Pi^M .

We now report an infant girl who had severe liver disease, urinary excretion of cytomegalovirus (CMV) and a transiently abnormal α_1 -AT phenotype that was not demonstrable in her parents. The patient has been briefly mentioned previously (10, 11).

PATIENTS AND METHODS

A white girl was delivered spontaneously after 42 wk of uneventful pregnancy. Weight was 3970 g at birth, 4450 g at 1 month and 4010 g at 2 months of age when she was hospitalized for weight loss and vomiting. She was thin, alert and not jaundiced. The liver edge was 4 cm below the rib cage. Serum transaminases were elevated. CMV antibody titer in serum was 1:64. It had been 1:16 at birth. Urine cultures for CMV were positive. The first needle liver biopsy was done on day 78. Hepatic tissue culture for CMV was negative. Vomiting became worse. After the liver biopsy she had 2 wk of parenteral alimentation. Oral feeding was resumed and the girl went home 3 wk later. She received no other medication for her disease.

Between 12 and 20 months of age, the liver edge was 2 cm below the rib cage. By ultrasound, the size of kidney and spleen was normal but liver size was increased. Serum transaminases were normal. Urine cultures for CMV were positive. The second needle liver biopsy was done on day 586.

At 971 days of age, liver size, serum chemistries and psychomotor development including language ability were normal. Urine culture for CMV was negative. CMV antibody titer in serum was 1:64.

Four other infants with positive CMV urine cultures who were available for α_1 -AT phenotyping had MM. Two of these children had diagnostic needle liver biopsies because of hepatomegaly.

Serum of patients and relatives was stored at -70°C after the addition of 2% sodium azide (final conc. 0.02%) for inhibition of bacterial growth. The electrophoretic studies for α_1 -AT were done before and after dithiothreitol was added to the serum (final conc.: 0.03 mM). *Alpha₁-AT concentration* was measured by radial immunodiffusion and was expressed as mg α_1 -AT per ml serum (15). *Trypsin inhibitory capacity* (TIC) was determined as mg trypsin inhibited per ml serum (5). *Pi type* was determined by three techniques: (1) horizontal discontinuous starch gel electrophoresis at pH 4.95 (6); (2) agarose gel electrophoresis at pH 8.6 (3); (3) PAG-IEF (1). We have described the details of our use of these techniques (10). *Crossed immunoelectrophoresis* after PAG-IEF and starch gel electrophoresis (7) as well as *immunofixation* after PAG-IEF were used for the identification of the bands as α_1 -AT (2).

RESULTS

During the patient's clinical illness, serum concentration of α_1 -AT was decreased, transaminases were increased and α_1 -AT phe-

notype was abnormal. Findings in serum of parents and relatives were normal (Table 1).

In figure 1a, the patient's α_1 -AT phenotype on PAG-IEF at age 46, 82 days (M Δ) and after her clinical recovery at age 179 days (MM $\cdot$) are compared with ZZ, MZpratt and MM. Alpha₁-AT phenotype Δ migrates cathodally of Z and Zpratt whereas M cannot be distinguished from M.

In figure 1b, protein bands Δ are identified as α_1 -AT by immunofixation after PAG-IEF. Arrows indicate bands Δ_8 . The arrow head points to an α_1 -AT band ($\square$) that is cathodal of Δ and that was transiently present in serum of another child without clinical liver disease who had asthma. A specimen of this boy's serum obtained one day earlier exhibited MM as did specimens of his serum and those of his parents obtained nine months later.

In Figure 2a, acid starch gel electrophoresis indicates that Δ migrates cathodally of Zpratt but anodally of Z.

In Figure 2b, on acid starch gel electrophoresis, the patient's α_1 -AT phenotype Δ that is present during her clinical illness migrates cathodally of X, S, Zpratt but anodally of Z whereas α_1 -AT phenotype M $\cdot$ of the convalescent specimens is indistinguishable from M.

On agarose electrophoresis at pH 8.6, the patient's α_1 -AT protein moves as a single band like M.

The addition of dithiothreitol to the serum does not alter any of the α_1 -AT electrophoretic banding patterns.

Hepatic light microscopy revealed the following: (1) at 78 days of age, *i.e.*, during the peak of the clinical illness, there is severe macrovesicular fatty infiltration, distortion of hepatic architecture without inflammation, necrosis or fibrosis, and no immunofluorescent evidence for intrahepatic α_1 -AT; and (2) at 586 days of age, the fatty infiltration is minimal. There are no other abnormalities.

Hepatic electronmicroscopy (Fig. 3) revealed the following: at 78 days of age, there are many intracellular lipid inclusions of up to forty times the size of nuclei, dilated bile canaliculi with stunted villi, and focal sites of cytoplasmic dissolution. At these sites, the endoplasmic reticulum is replaced by clusters of globules that are often surrounded by stacks of circular membranes resembling sectors of fragmented myelin sheaths. Some cisternae of endoplasmic reticulum are moderately dilated and contain floccular material. Residual bodies are plentiful in hepatocytes and in Kupffer's cells. At 586 days of age, the fatty infiltration is minimal and the intracellular appearance is near normal.

Hepatic electronmicroscopy of the two CMV excreting children who had MM is similar to that of the patient's convalescent specimen.

Fig. 1. a, Pattern of polyacrylamide gel isoelectric focusing of the patient's serum specimens obtained during her acute disease (M Δ) is compared with the pattern after her recovery (MM $\cdot$) and with that of controls, *i.e.*, MZpratt, ZZ and MM. Microheterogeneous bands_{2,4,6,7,8} are indicated for Pi type M (see text for details). b, Immunofixation (see text for details).

Table 1. Serum examinations

	Age (days, d)	Total bilirubin mg/100 ml	SGOT I.U./ liter	SGPT I.U./ liter	α_1 -AT concentration mg/ml	TIC mg/trypsin inhibited/ml	α_1 -AT phenotype
Normal		<1.0	16-74	16-50	2.74 $\pm$ 0.5	0.77 $\pm$ 0.2	PiMM
Patient	46 d						M Δ
	65 d	0.4	120	76	1.3		M Δ
	78 d	0.5	574	264	1.8	0.95	M Δ
	79 d						M Δ
	82 d		132	110			M Δ
	102 d		58	28			M Δ
	179 d				3.0		MM
	315 d		48	30	2.8		MM
	316 d				2.4		MM
	586 d	0.1	36	22		0.61	MM
	971 d	0.1		24	2.3	0.69	MM
Father	20 yr	0.5	18	6	3.0	0.73	MM
Mother	17 yr	0.4	20	14	3.9	0.69	MM
Paternal aunt	19 yr	0.4	10	4	3.8	0.96	MM
Paternal grandmother	38 yr	0.5	14	8	4.5	1.04	MM

Fig. 2. a, Starch gel electrophoresis (see text for details). b, Starch gel electrophoresis; findings explained in text.

DISCUSSION

The α_1 -AT Nomenclature Committee lists the following criteria for an unusual α_1 -AT banding pattern to be accepted as the phenotypic expression of a variant α_1 -AT allele (4). The unusual pattern must be present in sequential serum specimens of the proband and in specimens of related individuals, preferably of two generations. The pattern must not be affected by reducing agents. Although the addition of dithiothreitol to the serum specimens had no effect on the Pi type of our patients, we did not further study the boy's unusual bands depicted in Figure 1b since they appeared in only one serum sample stored in one tube.

However, the girl's unusual Pi type Δ (phenotype $M\Delta$) persisted in five serum specimens obtained during the 36 days acute phase of her liver disease. Thereafter, her Pi type was M while her liver disease improved clinically and histologically. Steatosis was no longer present in hepatocytes of the convalescent biopsy specimen; intracellular clusters of globules remained but they were smaller and fewer. The clusters seemed related to CMV infection but not

to Δ protein because we saw them in hepatocytes of children with CMV excretion and MM phenotype who did not receive parenteral alimentation or other medication. The extreme macrovesicular fatty infiltration of acute phase hepatocytes perhaps was related to Pi type Δ ; however, α_1 -AT phenotypes was unchanged in other diseases with marked hepatic fatty infiltration such as in Reye's syndrome or in deficiency of hydroxymethylglutaryl CoA lyase (personal observation).

Acquired anodal shifts of Pi type M have been observed on acid starch gel electrophoresis of serum from eight Japanese patients with leukemia, solid tumors or septic shock when they also had disseminated intravascular coagulation during the endphase of their disease. The finding was thought to be related to abnormalities in coagulation and fibrinolysis (13).

The "normal" α_1 -AT allele Pi^M is present in more than 90% of the population. Alpha₁-AT allele Pi^Z (present in 3% of Caucasians) conveys an increased risk for pulmonary emphysema (5) and hepatic cirrhosis (16) and is thus of clinical importance. The corresponding α_1 -AT protein Z is reduced in the serum probably

Fig. 3. Electronphotomicrographs of the patient's needle liver biopsy specimens. *a*, Acute phase specimen indicates dilated bile canaliculus (B) with few and stunted microvilli, very large lipid droplets (L) and clusters of globular inclusions surrounded by stacks of lamellae that resemble myelin sheets (inset: detail of (I)); (Bars: 3 μ m; inset: 0.5 μ m).

Fig. 3b. Acute phase specimen exhibits mild dilatation of cisternae (C) of endoplasmic reticulum with floccular material that is speculatively interpreted as protein Δ . Inset indicates the stacked lamellae surrounding one of the inclusions ($\downarrow$). These membranes reveal myelin-like periodicity (Bars: 3 μ m; inset: 0.5 μ m).

Fig. 3c. Post-recovery specimen indicates normal bile canaliculi, scant and small lipid droplets (L) and infrequent clusters made up of few globular inclusions located usually near bile canaliculi. Although not normal, the appearance is that of liver in mild CMV infections of other children with α_1 -AT type MM. Inset exhibits normal bile canaliculus ($\uparrow$), several Golgi complex (GC), globular inclusions and normal α -glycogen (gl) (Bars, 3 μ m; inset; 0.5 μ m).

because it is no longer secreted from the liver but is trapped within dilated endoplasmic reticulum of hepatocytes (9). A glutamic acid in M protein is replaced by lysine in Z (14). The intrahepatic trapping of Z protein or its precursor (12) might be a result of this amino acid substitution. At one time, the cathodal migration of Z was attributed to a reduced number of sialic acid residues but several investigators have since shown that the sialic acid content of Z is the same as that of M (8, 14, 18, 20). Thus, one might

speculate that the dissimilar migration of M and Z is another result of the amino acid substitution.

The α_1 -AT protein is further modified after its primary protein structure has been synthesized. These "postribosomal" modifications are reflected in the microheterogeneous bands_{2,4,6,7,8} into which the α_1 -AT protein is separated during isoelectric focusing (Fig. 1a). This microheterogeneity is markedly reduced after treatment of M or Z with neuraminidase when the remaining

bands migrate cathodally of untreated M or Z (14). Vaughan and Carrell (19) chromatographically separated the major microheterogeneous bands_{4,6} into two "isoforms" of M. The isoforms contained branched oligosaccharide side chains that differed in the number of sialic acid end groups. Consequently, band₄, which was part of Type II isoform and had seven sialic acid residues, migrated anodally of band₆, which was part of Type I isoform and had six sialic acid residues.

In keeping with these observations on the primary structure and its subsequent "postribosomal" modification of the α_1 -AT protein, one might speculate that the transient Pi type Δ of our patient reflects either (1) a transiently reduced number of sialic acid residues attached to an otherwise normally structured protein M or (2) an α_1 -AT protein with an altered primary structure.

The first speculation postulates that the banding pattern Δ is not the expression of a variant allele Pi^{Δ} but results from postribosomal modifications of protein M that are related to the liver disease and temporarily alter the gene product of Pi^M . In this case the unusual Δ pattern would not qualify as an α_1 -AT phenotype according to the rules of nomenclature (4). The rules do not cover transient phenotypes. Hence, we labeled the unusual protein with a Greek rather than a Latin letter to state only that Δ protein is the transient phenotypic expression of some α_1 -AT allele.

The alternative speculation on the genesis of Δ protein is also possible. During the existence of Δ protein when the patient's phenotype was M Δ , the concentration of α_1 -AT in her serum was reduced to that observed in patients with phenotype MZ. Simultaneously, hepatocytes of her acute phase biopsy specimen exhibited occasional cisternae of endoplasmic reticulum that were mildly dilated by floccular material, *i.e.*, they exhibited similar changes as in phenotype ZZ but to a lesser degree (9). If the floccular material in Pi type Δ is related to Δ protein as is the floccular material in Pi type Z related to Z protein, and if Z-like protein is trapped in hepatocytes because of the amino acid substitution between proteins M and Z, then the intrahepatic trapping of Δ protein or its precursor might also be associated with an amino acid substitution between proteins M and Δ . Hypothetically, a structurally altered Δ protein could be the product of α_1 -AT allele Pi^{Δ} that was switched on in the patient (but not in her parents) by the stress of her liver disease in a similar manner as is the juvenile globin gene in the adult goat by erythropoietic stress (17). One can begin to examine these alternative hypotheses by assessing whether neuraminidase treatment of M Δ serum is followed by a cathodal shift of Pi type Δ .

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