Electrophoretic Mobility, Concentration, and Activity of $\alpha_1$-Antitrypsin in Serum of Patients Undergoing Bone-Marrow Transplantation

Richard E. Mullins, Beth Bennett, and Robert L. Hunter, Jr.

We have observed an electrophoretically abnormal, nonfunctional species of $\alpha_1$-antitrypsin in serum from patients who were receiving bone-marrow transplants for treatment of leukemia or aplastic anemia. Three of four patients in whose serum this protein appeared died soon after; the fourth recovered, and the disappearance of the abnormal $\alpha_1$-antitrypsin paralleled his recovery. This suggests that the inability to maintain functional activity of $\alpha_1$-antitrypsin predisposes patients to life-threatening complications during recovery from bone-marrow transplants.

Additional Keyphrases: nonfunctional $\alpha_1$-antitrypsin · leukemia

$\alpha_1$-Antitrypsin (AAT) has been extensively studied because inherited deficiencies of it are associated with severe disease of lung, liver, or both. Several of the more than 20 genetic variants of AAT described in humans have been shown to predispose the carrier to both liver disease and lung disease (1–5). Relatively little attention has been paid to acquired changes in AAT that may arise during disease, even though there is substantial evidence for such changes.

Several investigators have reported abnormal protein bands in the $\alpha_1$ region of serum protein electrophoretograms without characterizing them (6–8). Inokuma (9) recently reported a fast electrophoretic variant of AAT associated with disseminated intravascular coagulation (DIC) and proposed that it was an acquired variant. In addition, Stockley and coworkers (10, 11) and Matheson et al. (12) reported finding an abnormal, functionally inactive, electrophoretically fast AAT in bronchial fluid of some patients with chronic lung disease. They characterized the variant as a split product of AAT, and could produce it from normal AAT by enzymic cleavage in vitro.

We observed abnormal bands in the $\alpha_1$ region of electrophoretograms of sera from several patients who had undergone bone-marrow transplantation during critical phases of their illness. To characterize AAT in these and other patients, we developed several functional and immunochromatographic procedures. Here we describe some of the properties of abnormal AAT and the clinical conditions accompanying its presence.

Materials and Methods

Venous blood was sampled serially from patients admitted for bone-marrow transplantation. Serum samples were analyzed on the day of collection or stored at $-85\,^\circ C$ until analysis. "High-resolution" electrophoresis on agarose gel was performed as described by Jeppson et al. (13). We electrophoresed 4-$\mu$L serum samples on 8 g/L agarose gels in barbiturate buffer, pH 8.6, for 45 min at 20 V/cm and 10–14 °C. The gels were fixed in picric acid and stained with Amido Black. AAT was quantified immunochromatically with a kinetic nephelometer (Beckman Immunochromy System, Fullerton, CA). We determined the trypsin-inhibiting capacity activity of the AAT as previously described, with a Cobas Bio centrifugal analyzer (14). For immunodiffusion as described by Ouchterlony (15) we used antibody to AAT from Meloy Laboratories, Springfield, VA.

Results

From 10/7/81 to 11/15/82, 14 bone-marrow transplants were performed at our institution. We measured the concentration and functional activity of the AAT in serum and carried out protein electrophoresis on serial samples of serum collected from these patients during the period of recovery after transplantation. In 10 patients, the immunochromatically measured concentration of AAT, the serum trypsin-inhibitory capacity, and the $\alpha_1$ band on serum protein electrophoresis all increased in parallel in a pattern usually expected during acute-phase inflammatory response. In the other four patients, however, a distinctly abnormal pattern developed. In these patients, an additional fast band was detected in the $\alpha_1$ region of the serum electrophoretogram. During these periods, the immunochromatically measured concentration and the functional activity of AAT did not increase in parallel, nor was the increase in immunoreactive AAT accompanied by an increase in the trypsin-inhibitory capacity. These abnormalities were transient in one patient but persisted until death in the other three.

The atypical response is typified in the electrophoretic patterns of patient T. W. (Figure 1). On the 18th day after transplantation, an additional band appeared in the $\alpha_1$ region, migrating anodally to the expected AAT band. This abnormal band persisted until the patient died on the 25th day after the transplantation. Figure 2 shows serial protein electrophoretograms for another patient (R. S.) who developed the abnormal band. On days eight through 18, the abnormal band in the $\alpha_1$ region was present. On the 22nd post-transplant day, the abnormal band disappeared, leaving only the usual AAT band in the $\alpha_1$ region. The patient recovered and was discharged on the 29th day after receiving the transplant.

The two protein species observed in the $\alpha_1$ region were eluted from the electrophoresis gels and subjected to Ouchterlony immunodiffusion against anti-AAT. Both of the
The concentration of AAT was sought to be active. Figure 3 illustrates the concentration of AAT in, and the trypsin-inhibiting capacity of, serum samples from patient T. W. The initial increase in AAT was accompanied by an increase in functional activity. However, on day 11, the values for concentration and activity of AAT diverged: the immunoreactive concentration of AAT remained increased while the trypsin-inhibiting capacity decreased. This corresponded to the appearance of the abnormal AAT immunoreactive band in the pattern. This same correlation between appearance of the abnormal AAT band in the electrophoretogram and loss of functional activity was also observed in two other patients.

Figure 3 also shows the AAT concentration and activity measurements over the course of the hospital stay of patient R. S. On day 11, the concentration of AAT as measured by immunoassay increased by approximately 90%, but the trypsin-inhibiting capacity did not increase until day 18. The fast α₁ band was observed during this period of divergence in the two measurements of AAT. On day 22, the α₁ region showed only one protein band, and the immunoclinical and functional measurements of AAT were in better agreement.

Discussion

Inhibition of proteases by AAT is accompanied by formation of an equimolar complex of AAT and the target protease. The prime physiological target of AAT appears to be leukocyte elastase; however, AAT will inhibit most serine proteases to some extent (16–19). In the report (9) of an electrophoretically abnormal AAT in patients with leukemia who developed DIC, Inokuma claimed to rule out the formation of AAT complexes with a serine protease because the trypsin-inhibiting capacity:AAT ratio was the same in DIC patients as in non-DIC individuals. However, we found this ratio to be lower in patients' serum when the electrophoretically abnormal AAT was present. In our study, the formation of AAT–serine protease complexes is one hypothesis that explains the observations. The synthesis of an aberrant AAT by the liver can not be ruled out; however, disturbed liver function was not the common situation in the four patients who exhibited the split α₁ electrophoretic pattern.

Matheson et al. (12) reported the inactivation of AAT through the oxidation of a methionine at the active site by leukocyte myeloperoxidase. After this inactivation, the AAT molecule was susceptible to cleavage by serine proteases. The fragments of this cleavage showed altered electrophoretic mobility very similar to the mobility of the fast α₁ that we observed. An inactivation reaction similar to that reported by Matheson et al. could also explain the observed results in this group of patients.
In summary, we have observed a splitting of the $\alpha_1$ band in serum protein electrophoresis. In addition, we have identified the new fast $\alpha_1$ as a variant of AAT by Ouchterlony immunodiffusion. The fast $\alpha_1$ appears to be a poor prognostic indicator for patient survival, three out of four bone-marrow transplant patients having died soon after the electrophoretic appearance of the fast $\alpha_1$. When the fast $\alpha_1$ is present, we suspect it to be an inactive form of AAT because of the divergence of the immunochemical (i.e., mass) measurements and the functional measurements of AAT.

References