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Autoantibodies to neutrophilic proteases in a case of panniculitis by deficit of α 1-antitrypsin

SIR, α 1-Antitrypsin is a serine protease inhibitor whose principal targets are neutrophil elastase, proteinase 3 and cathepsin G [1]. Several mutants of the gene encoding α 1-antitrypsin have been identified; the non-functional Z variant is found in 1–2% of Caucasians of European descent [2]. ZZ homozygotes develop a disease leading to lung emphysema and/or liver cirrhosis [3]. Panniculitis is a rare manifestation of the disease [4]. The pathogenesis probably relates to the lack of inhibition of neutrophil proteases due to α 1-antitrypsin deficiency at the site of neutrophil degranulation [2]. However, the factor(s) determining the variability of clinical manifestations among patients have not been clarified.

Patient PR is a 33-yr-old female. She first came to our notice in July 1995 because she had been affected for 5 yr with recurrent panniculitis (characterized by erythematous, sometimes ulcerated, nodular skin lesions evolving into atrophic scars), malaise and fever. Altered laboratory tests were as follows: decreased C3 complement factor (75 mg/dl); decreased complement haemolytic activity (CH50 = 670 U/ml; normal range 750–1300 U/ml); decreased IgG (550 mg/dl); decreased α 1 serum proteins (1.5%); decreased α 1-antitrypsin (17 mg/l; normal value >2.5 g/l); and increased circulating immune complexes (CIC) (25%). Autoantibodies to different antigens [antinuclear antibodies, extractable nuclear antigens (ENA), phospholipids, smooth muscle cells, mitochondria] were negative, including anti-neutrophil cytoplasmic antibodies (ANCA) to proteinase 3. Phenotypic study of the patient's α 1-antitrypsin showed a condition of homozygosity for Z. The lungs and liver were unaffected. Biopsy analysis revealed granulomatous inflammation of the dermis and hypodermis. Therapy with methylprednisolone (200 mg/day, 3 days per month) later supplemented with dapson (100 mg/day) achieved complete clinical resolution.

We wondered why this patient manifested only panniculitis, a frequent manifestation of systemic autoimmune diseases and vasculitides due to deposition of CIC at the inflammation site. Since the patient showed

decreased serum complement and increased CIC, we supposed that immune complexes might contribute to the onset of tissue lesions. We suspected that disruption of the physiological equilibrium between proteases and their inhibitors might have triggered the onset of autoimmune phenomena. This is reasonable, as peripheral immune tolerance is regulated by the idiotypic network and depends on the concentration of antigen available [5]. Indeed, the existence of an association between ANCA-related vasculitides and deficit of α 1-antitrypsin has been reported [6, 7]. Therefore, before starting treatment, the patient's neutrophils were isolated from heparinized blood by Ficoll gradient centrifugation. Cells were lysed by repeatedly freezing and thawing them in the presence of the protease inhibitor phenylmethylsulphonyl fluoride. The lysate was incubated at 4°C for 4 h with 1 ml of serum from the patient or from one of five healthy subjects (of comparable age and sex) as controls; it was then immunoprecipitated with protein A-conjugated Sepharose beads. After centrifugation the pellet was collected and loaded on a 12% sodium dodecyl sulphate–polyacrylamide gel under non-reducing conditions. A 30-kDa molecular weight band, revealed by silver staining, was observed only after immune precipitation with the patient's serum (Fig. 1). Interestingly, elastase, proteinase 3 and cathepsin G, the targets of α 1-antitrypsin, have a molecular weight of 30 kDa [1]. A double-determinant immune assay was therefore performed to define the specific antigens recognized by the patient's serum autoantibodies. Ninety-six-well polyvinyl chloride microtitre plates were coated with elastase, cathepsin G or human albumin (Sigma, Milan, Italy) (10 μ g/ml in carbonate buffer) by overnight incubation at 4°C. (Proteinase 3 was not used because the absence of anti-proteinase 3 antibodies in the patient's PR serum had been already demonstrated.) After washing, serum samples were added to the wells and incubated for 1 h at 37°C. A peroxidase-conjugated goat anti-human immunoglobulin antiserum (Sigma) (10 μ g/ml) was used as the secondary antibody. Plates were developed with orthophenylenediamine. Optical

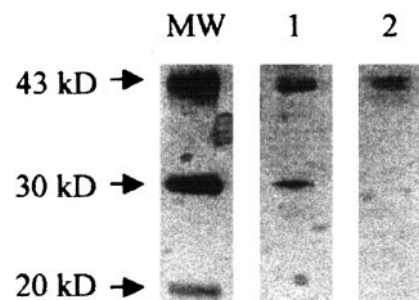


FIG. 1. Migration in sodium dodecyl sulphate–polyacrylamide gel electrophoresis under non-reducing conditions of proteins immunoprecipitated from neutrophil lysate by the patient's serum (lane 1) and a representative control serum (lane 2). A 30-kDa band is evident only in lane 1. None of the control sera tested showed the 30 kDa band (not shown). MW, molecular weight.

density (OD), measured at 490 nm with an ELISA reader, demonstrated that the patient's serum reacted to elastase (OD 0.852) and cathepsin G (OD 0.79) but not to human albumin (OD 0.11). The control sera did not show any reactivity (OD 0.1 for elastase, 0.09 for cathepsin G, 0.08 for albumin). Thus, immune complexes consisting of autoantibodies and neutrophil proteases may exist in patients with α 1-antitrypsin deficiency and participate in inflammatory processes (although a direct demonstration of their pathogenic role in our patient was not achieved because of the ethical impossibility of obtaining a repeat biopsy specimen). The relevance of neutrophil infiltration of the dermis to the induction of α 1-antitrypsin deficiency panniculitis has been demonstrated already [8], as has the regression of panniculitis after the recovery of normal α 1-antitrypsin levels due to liver transplantation [9]. Together, these findings suggest that both the direct enzymatic activity of released proteases and CIC induced by autoimmune reactions may generate panniculitis in the presence of α 1-antitrypsin deficiency. Furthermore, they make it possible to hypothesize that a genetic deficiency causing altered homeostasis between interacting molecules might favour the onset of autoimmune reactions, perhaps due to the breakdown of idiotypic control.

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