CHANGES OF IMMUNOREACTIVITY IN \( \alpha_1 \)-ANTITRYPsin IN PATIENTS WITH AUTOIMMUNE DISEASES

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Abstract—Recent studies from this laboratory have shown that a monoclonal antibody prepared against a specific epitope on \( \alpha_1 \)-antitrypsin is a valuable diagnostic marker for autoimmune conditions. In the present study we have further characterized this monoclonal antibody and reassessed its diagnostic value in screening samples from patients with various autoimmune conditions. \( \alpha_1 \)-Antitrypsin was micropurified from patients with selected autoimmune conditions and from normal donors. The purified \( \alpha_1 \)-antitrypsin isolated from patients with autoimmune conditions and normal donors was deglycosylated using both a mixture of exoglycosidases and endoglycosidase F. The immunoreactivity of the native and deglycosylated \( \alpha_1 \)-antitrypsin was examined using both a monoclonal antibody and a polyclonal antibody in enzyme linked immunosorbent assay (ELISA) and radioimmunoassay (RIA), respectively. It was noted that \( \alpha_1 \)-antitrypsin isolated from patients with autoimmune diseases generated a displacement curve dissimilar to \( \alpha_1 \)-antitrypsin purified from normal donors or \( \alpha_1 \)-antitrypsin from patients with autoimmune diseases subjected to deglycosylation when these samples were examined by ELISA using the monoclonal antibody. However, when the polyclonal antibody was used for these studies, no difference was found between the native and deglycosylated \( \alpha_1 \)-antitrypsin suggesting that the monoclonal antibody recognized an epitope not detectable by the polyclonal antibody. We have also assessed the diagnostic usefulness of this monoclonal antibody using a battery of 530 serum samples obtained from patients with different autoimmune diseases and compared to normal human serum (NHS, \( N = 66 \)); these include: systemic lupus erythematosus (SLE, \( N = 149 \)), rheumatoid arthritis (RA, \( N = 64 \)), renal diseases (NP, \( N = 33 \)), liver diseases (HP, \( N = 33 \)), mixed connective tissue disease (MCTD, \( N = 12 \)), diabetes (DB, \( N = 40 \)), Sjögren’s syndrome (SS, \( N = 41 \)), polymyositis (PM, \( N = 20 \)), scleroderma (SCL, \( N = 20 \)), Alzheimer’s disease (AZ, \( N = 11 \)), and patients with elevated levels of carcinoembryonic antigen (CEA, \( N = 41 \)). The results of this study demonstrated that this monoclonal antibody is positively corre-
lated with SLE and SS. The significance of the monoclonal antibody in connection with the pathogenesis of autoimmune diseases was discussed.

INTRODUCTION

The pathogenesis and the mechanism of autoimmune diseases is not yet known (1). It is considered that alteration of self-antigen via protein denaturation and/or glycosylation may lead to a change in the immunogenicity that triggers an autoimmune response. It has been known for the past decades that protein denaturation can lead to exposure of antigenic determinants on polypeptides that are normally hidden (2, 3). Abnormal proteins, probably derived from denaturation processes, have been detected in the sera of patients with autoimmune diseases and in inflamed rats (4). Protein denaturation can also be regarded as the first step of a more complex process of protein alteration. Protein denaturation induces conformational changes, possibly leading to the exposure of highly hydrophobic and reactive domains that are hidden in the protein core. It could also elicit complex enzymatic reactions such as glycosylation. On the other hand, glycosylation can be regarded as a protective physiological mechanism against protein denaturation, since it has been reported that glycosylation increases protein stability (5–9). Such an interpretation is in agreement with the observation that protein glycosylation is elevated in several pathological conditions including patients with severe burns (10, 11). Thus, glycosylation of endogenous proteins could alter their overall antigenic properties that lead to a pathological autoimmune response since abnormally glycosylated proteins in patients with autoimmune diseases have been detected (12–16).

In a previous study we prepared a monoclonal antibody against α₁-antitrypsin isolated from patients with autoimmune diseases. α₁-Antitrypsin is an acute-phase protein of Mr 54,000 with 12–16% carbohydrate and has a normal concentration of about 1.3 mg/ml in the plasma, whose concentration increased by two to fourfold during an inflammatory response (17, 18). Using this monoclonal antibody, designated A2a18b8, we have developed an enzyme-linked immunosorbent assay (ELISA) (19). Using a limited number of samples we have shown that this assay appears to be specific for certain diseases. We postulated that this monoclonal antibody, A2a18b8, recognized specific epitope(s) in proximity of the carbohydrate moiety of α₁-antitrypsin (19). In the present study we have extended our analysis to over 500 samples from patients with different autoimmune conditions. We have compared these results to those obtained with a radioimmunoassay (RIA) using a polyclonal antibody prepared against the entire molecule to detect the changes in concentration of α₁-antitrypsin in these samples. We also have prepared deglycosylated α₁-antitrypsin using purified proteins isolated from both normal donors and patients with