ABNORMAL GLYCOSYLATION OF 
$\alpha_2$-MACROGLOBULIN, A NON-ACUTE-PHASE PROTEIN, IN PATIENTS WITH AUTOIMMUNE DISEASES

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Abstract—Previous studies from this and other laboratories have shown that abnormal glycosylation of several acute-phase proteins can be detected in various pathological conditions including autoimmune diseases. In the present study, we have investigated if abnormal glycosylation is limited to acute-phase proteins. We used the concanavalin A (Con A) blots in conjunction with the peptide mapping techniques to analyze serum samples and cerebrospinal fluids (CSF) obtained from patients with autoimmune diseases: systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), mixed connective tissue disease (MCTD), scleroderma (SCL), Sjögren’s syndrome (SS), and polymyositis (PM); diseases of probable autoimmune origin: hepatopathies (HP); diseases of suspected autoimmune origin: schizophrenia and Alzheimer's disease (AZ); and conditions not related to autoimmunity: pregnancy (PG) and elevation of the carcinoembryonic antigen (CEA), in comparison to normal donors (NHS). We have micropurified two human proteins; $\alpha_2$-macroglobulin, a non-acute-phase protein, and $\beta$-chain of haptoglobin, a known acute-phase protein, from serum samples of individual patients with SLE, RA, MCTD, SCL and SS, and from PG and NHS for analysis. The identity of the purified proteins was confirmed by immunoblots using either monospecific polyclonal or monoclonal antibodies, and by direct N-terminal amino acid sequencing. Peptide maps for each of these proteins were generated using Staphylococcus aureus protease V8, a Glu-C endopeptidase. When the peptide fragments of $\alpha_2$-macroglobulin were resolved by SDS-PAGE and visualized using silver staining, no differences were noted between patient samples and controls. However, when they were examined by lectin blots using Con A, the Con A-reactive fragments increased specifically and significantly in samples derived from patients of SLE, SCL, MCTD, and RA. Similarly when the peptide fragments of the $\beta$-chain

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of haptoglobin were visualized by silver staining, no differences were noted; however, the Con A reactivity of specific fragments increased in SLE, RA, SCL, and SS patients. Analysis of these results indicated that there has been a selective increase in Con A-reactive fragments in both acute-phase and non-acute-phase proteins in autoimmune conditions. Thus, the study of changes in glycosylation patterns in selected serum proteins may be a valuable diagnostic approach to define the pathophysiology of inflammatory and autoimmune disorders.

INTRODUCTION

The pathogenesis of most autoimmune diseases is still unknown. It has been proposed that endogeneous protein alterations such as denaturation can be responsible for pathological autoimmune responses (1-4). In pathological conditions such as inflammation, glycosylation could play a protective biological role to increase protein stability. It is known that glycosylation can increase protein stability (5-9). Glycosylation is also known to alter the antigenic properties of proteins (10, 11) and can possibly trigger an autoimmune response (12, 13). Earlier studies from our laboratories have demonstrated that abnormal protein denaturation and glycosylation can be detected in patients with autoimmune diseases and inflamed rats (4, 12-14). Changes of protein glycosylation in patients with inflammatory disorders have also been reported in other laboratories (15-18). Furthermore, abnormal glycosylation seems to occur in other pathological conditions, such as in patients with severe burns (19, 20).

In the present study, we have examined serum samples and cerebrospinal fluids (CSF) derived from patients with various autoimmune diseases to determine if abnormal glycosylation is restricted to acute-phase proteins. We have investigated samples derived from patients with impaired autoimmunity, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), mixed connective tissue disease (MCTD), scleroderma (SCL), Sjögren’s syndrome (SS), polymyositis (PM); diseases of probable autoimmune origin; hepatopathies (HP); diseases with suspected autoimmune origin; schizophrenia (21-23) and Alzheimer disease (24); and conditions/diseases that are not related to autoimmunity, such as pregnancy (PG) and conditions with elevated levels of carcinoembryonic antigen (CEA), in comparison to normal donors (NHS). We have selected two human serum proteins—α2–macroglobulin, a protease inhibitor that accounts for almost 80% of the total proteolytic inhibitory activity in normal plasma and is not an acute-phase protein in the human, and β-chain of haptoglobin, an acute-phase protein that has a high affinity for hemoglobin (25)—for this study.