Frequency and Significance of Antibodies to Asialoglycoprotein Receptor in Type 1 Autoimmune Hepatitis

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Antibodies to asialoglycoprotein receptor have diagnostic specificity for autoimmune hepatitis, but it is uncertain if they are complementary or redundant markers of the disease. Our aims were to assess their frequency and significance in type 1 autoimmune hepatitis and determine their contribution to the evaluation of these patients. Sera from 54 well-characterized patients were evaluated for antibodies to asialoglycoprotein receptor by a radioimmunofiltration assay based on rabbit-derived protein. Forty-four patients (82%) were seropositive. Seropositive patients were distinguished from seronegative counterparts by having higher serum gamma globulin (3.7 ± 0.2 g/dl vs 2.3 ± 0.3 g/dl, P = 0.0007) and immunoglobulin G levels (3707 ± 179 mg/dl vs 2203 ± 263 mg/dl, P = 0.0005) at presentation and a greater frequency of relapse after drug withdrawal (88% vs 33%, P = 0.01). Seropositivity for smooth muscle and/or antinuclear antibodies did not define treatment outcomes and antinuclear antibodies occurred less frequently than the other markers. Concurrent testing for antibodies to asialoglycoprotein receptor and smooth muscle identified all patients. We conclude that antibodies to asialoglycoprotein receptor are common in type 1 autoimmune hepatitis and they identify patients with a high frequency of relapse after corticosteroid withdrawal. Concurrent testing for these antibodies and smooth muscle antibodies has the same diagnostic sensitivity as testing for antinuclear and smooth muscle antibodies but a greater prognostic implication.

KEY WORDS: asialoglycoprotein receptor; autoantibodies; autoimmune hepatitis.

Autoimmune hepatitis is an unresolving inflammation of the liver that is characterized by the presence of periporal or interface hepatitis on histologic examination, hypergammaglobulinemia, and autoantibodies in serum (1–5). There is no immunoserologic marker that is pathognomonic of the disease and multiple non-organ-specific autoantibodies have been associated with the diagnosis (1, 2, 6). Indeed, three forms of autoimmune hepatitis have been proposed based on different immunoserologic reactivities (2, 6–8).

Antinuclear (ANA) and/or smooth muscle (SMA) antibodies characterize type 1 autoimmune hepatitis (1, 2, 8); antibodies to liver/kidney microsome type 1 (anti-LKM1) connote type 2 autoimmune hepatitis (9); and antibodies to soluble liver antigen (anti-SLA) (10) and antibodies to liver–pancreas (anti-LP) (11) have each been proposed as markers for a type 3 autoimmune hepatitis. Unfortunately, none of these autoantibodies is organ-specific and only anti-SLA are disease-specific (9, 12).
Type 1 autoimmune hepatitis is the most common form of autoimmune hepatitis among adults in the United States (1, 2, 13). Its diagnostic markers, however, lack not only liver and disease specificity but also etiologic importance and prognostic significance (14). Anti-nuclear antibodies and SMA occur commonly in other liver diseases (6, 15, 16) and nonhepatic conditions (17–19); anti-nuclear reactivities are diverse and directed against multiple nuclear antigens that do not define clinically distinct subgroups (20, 21); smooth muscle reactivities, including those against actin, are commonly mixed and diagnostically inconclusive (19, 22); and neither ANA nor SMA have been predictive of disease outcome (14, 20, 21). Each autoantibody species lacks pathogenicity, and none indict a candidate autoantigen responsible for the disease (1, 2, 6).

Recent studies have indicated that antibodies to asialoglycoprotein receptor (anti-ASGPR) have a high specificity for autoimmune hepatitis and that they are present in all types of the disease (23–26). Indeed, anti-human anti-ASGPR occur in 88% of patients with autoimmune hepatitis compared to frequencies of 7% in chronic hepatitis B, 8% in alcoholic liver disease, and 14% in primary biliary cirrhosis (23, 24). Additionally, these antibodies are present concurrently in 82% of patients with ANA and/or SMA, 67% of patients with anti-LKM1, and 67% of patients with anti-SLA (23, 24). The combination of liver and disease specificity suggests that anti-ASGPR have greater diagnostic value than ANA and SMA in the evaluation of patients with type 1 autoimmune hepatitis. Their occurrence in all types of autoimmune hepatitis also indicates that they may be generic markers for the disease.

Asialoglycoprotein (ASGPR) is a liver-specific transmembrane glycoprotein that can capture, display, and internalize potential antigens, induce T-cell proliferation, and activate cytotoxic T cells (24). These functions and its location on the hepatocyte surface allow it to participate in immunoreactions (24, 27). Circulating (28–30) and intrahepatic (31, 32) lymphocytes from patients with autoimmune hepatitis have been shown to be sensitized to ASGPR; autoantibodies that react with ASGPR are typically present in high titer in patients with the disease (23–26); and a genetically determined functional defect in the antigen-specific T-cell response to ASGPR has been described in individuals with autoimmune hepatitis (28, 29). These observations support the candidacy of ASGPR as an important autoantigen in autoimmune hepatitis, and they suggest that anti-ASGPR may reflect pathogenic mechanisms as well as have diagnostic relevance.

In this report, we assess the frequency of anti-ASGPR in patients with type 1 autoimmune hepatitis, and we compare patients with and without these antibodies to determine if they have clinical and prognostic differences. We also evaluate the associations among anti-ASGPR, ANA, and SMA and determine the contributions of each immunoserologic marker alone and in combination with others to the diagnosis of type 1 autoimmune hepatitis. Our goals are to determine if testing for anti-ASGPR is complementary or redundant to testing for ANA and SMA and if superfluous markers can be replaced.

MATERIALS AND METHODS

Study Population. Fifty-four patients who satisfied pre-established criteria for severe type 1 autoimmune hepatitis comprised our study population (1–5) (Table 1). These individuals were selected from 152 patients who had been similarly evaluated, diagnosed, and enrolled in our Mayo Clinic Chronic Hepatitis Program. Selection was based on the availability of a pretreatment serum sample adequate for anti-ASGPR testing.

Each patient denied illicit drug use, homosexual activity, contact with hepatitis, family history of liver disease, excess alcohol consumption, and exposure to hepatotoxic medications or chemicals. All had been screened for hepatitis B and hepatitis C virus infections by second-generation immunosassays and for hereditary diseases, including Wilson disease, hemochromatosis, and α1-antitrypsin deficiency, by conventional tests. Eighteen patients (34%) had SMA seropositivity; 5 patients (9%) had ANA seropositivity; and 31 patients (57%) had both SMA and ANA seropositivity (Table 1). Each serum sample was obtained after informed written consent, and it was stored frozen at −40°C in a facility designed for its maintenance (33). Only specimens obtained prior to the administration of corticosteroids were analyzed.

Clinical Assessments. Complete physical examinations and standard laboratory tests of liver inflammation and function had been performed in all patients at accession by one investigator (A.J.C.). Follow-up assessments were conducted every six months until clinical stability was achieved. Patients then underwent annual examinations.

Immunoserologic Assessments. Smooth muscle antibodies and ANA were assessed in all patients by indirect immunofluorescence as described previously (13, 14, 20) and titers of at least 1:40 were considered positive. Antibodies to liver/kidney microsome type 1 were also evaluated in 39 patients (72%) by indirect immunofluorescence (13), and a positive test required a titer of at least 1:10. None of the tested patients was seropositive for anti-LKM1. In these instances, the diagnosis of type 1 autoimmune hepatitis was further substantiated.

Virologic Assessments. All patients had been tested for antibodies to hepatitis C virus (anti-HCV) by a second-generation ELISA (Ortho Diagnostic Systems, Inc., Raritan, New Jersey) and in each instance, seronegativity had