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# Suppressor T-Cell Deficiency in Primary Sclerosing Cholangitis

## Case and Family Study

ALAN E. KILBY, MD, EDWARD L. KRAWITT, MD, RICHARD J. ALBERTINI, MD, BETTEJAYNE F. CHASTENAY, BS, and ALEX JOHN, MD

*Primary sclerosing cholangitis is considered to be an autoimmune disease of the liver in which there is an association with the HLA phenotypes B8 and DR3 and in which circulating autoantibodies occur. Abnormalities of immune regulation may be present but whether or not they are primary or acquired is not known. This report is of a patient with primary sclerosing cholangitis who was homozygous for HLA B8 DR3, had a circulating antinuclear antibody, and a defect in nonspecific suppressor T-cell activity despite glucocorticosteroid treatment. Nevertheless, family studies revealed no evidence of an immunoregulatory defect in first-degree relatives despite the presence of Raynaud's phenomenon and malignancy in two sisters.*

**KEY WORDS:** liver disease; primary sclerosing cholangitis; autoimmunity; inflammatory bowel disease; ulcerative colitis; immunology; immunogenetics; immune regulation; suppressor cells.

Although genetic and immunological factors appear to be important in the pathogenesis of primary sclerosing cholangitis (PSC), the interrelationships between these features remain largely undefined. PSC has been shown to be associated with the HLA phenotypes B8 and DR3 (1–3), is often seen in conjunction with inflammatory bowel disease, particularly nonspecific ulcerative colitis, and is accompanied by a variety of abnormalities of immune function including immunoregulatory defects (1–12). As in other autoimmune diseases of the liver, it has been postulated that deficient suppressor T-cell function is important in the pathogenesis of PSC, but whether or not abnormalities of immune regulation are primary or acquired is not known. This report is of a patient with PSC associated with nonspecific ulcerative colitis who was homozygous for HLA B8 DR3, had a circulating antinuclear antibody and a defect in nonspecific suppressor T-cell activity.

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From the Department of Medicine, University of Vermont, Burlington, Vermont.

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Address for reprint requests: Dr. Edward L. Krawitt, Gastroenterology Unit, Given C-317, University of Vermont, Burlington, Vermont 05405.

## MATERIALS AND METHODS

The patient and his family were studied, in order to search for an intrafamily association of HLA phenotypes and autoantibodies and/or alteration of suppressor T-cell function, after having given informed consent according to the guidelines of the Committee on Human Experimentation of the University of Vermont and with the approval of the Clinical Research Center Committee of the University of Vermont. HLA typing, autoantibody determination, display of the T-cell surface markers for CD4 and CD8 and suppressor T-cell activity, which depended upon the suppression of *in vitro* IgG secretion by concanavalin A-stimulated suppressor cells were determined as previously reported (13, 14).

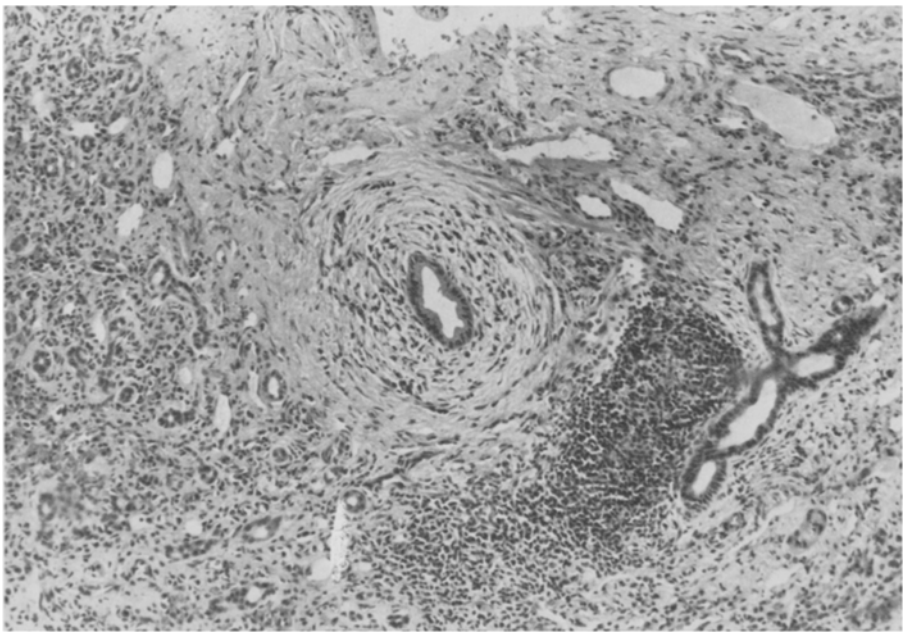

**Fig 1.** Inflammation and periductular fibrosis characteristic of primary sclerosing cholangitis (hematoxylin and eosin ×100).

## RESULTS

**Patient.** At the time of investigation the patient was a 39-year-old white married lineman for an electric company who had been seen 11 years previously for bloody diarrhea, abdominal cramping, anorexia, and fatigue. At that time, based on sigmoidoscopic and radiologic findings, a diagnosis of nonspecific ulcerative colitis was made. Serum biochemistries were: albumin 4.0 g/dl, globulin 2.9 g/dl, bilirubin 2.1 mg/dl (total), 0.6 mg/dl (direct), alkaline phosphatase 62 units (normal 35), SGOT 35 IU/liter, SGPT 100 IU/liter.

During the ensuing years he was treated with sulfasalazine and subsequently with glucocorticosteroids. He did well for eight years, at which time, after a six-month course of fatigue and dark urine, he was reevaluated. Sigmoidoscopy demonstrated friable mucosa and a rectal biopsy showed mild edema and inflammatory changes. A liver/spleen scan with Tc-99m sulfur colloid revealed splenomegaly and inhomogeneous uptake of the nuclide in the liver. Serum biochemistries were: albumin 3.0 g/dl, globulin 4.6 g/dl, bilirubin 1.7 mg/dl (total), 0.7 mg/dl (direct), alkaline phosphatase 133 units, SGOT 300 IU/liter, SGPT 374 IU/liter. An operative liver biopsy was performed. The liver was normal in size but was extremely firm and nodular. The microscopic appearance of the biopsy was classical for PSC (Figure 1). The chronic inflammatory infiltrate, which was largely confined to the portal areas, consisted of lymphocytes, plasma cells, and a few eosinophils. Periductular fibrosis was present around the larger bile ducts and cirrhosis was present. Within some of the fibrous bands in the portal areas there was evidence of bile duct proliferation.

For the next three years the patient was treated with glucocorticosteroids, and when seen in the Clinical Research Center was taking 20 mg prednisone daily. Shortly thereafter, because of dysplasia noted on biopsy of the colon obtained at the time of surveillance colonoscopy, a total proctocolectomy was performed. Subsequently, an endoscopic retrograde cholangiopancreatography revealed attenuation of the common hepatic duct and abnormalities of at least three branches of the intrahepatic ducts, characterized by tapering, which returned to slightly greater caliber proximal to the narrow segments. The development of ascites and bleeding esophageal varices has required management with dietary and diuretic treatment and periodic injection of esophageal varices with sodium morrhuate.

**Immunological Studies of the Patient and Family Members.** Results of studies are summarized in the pedigree shown in Fig. 2. The patient is HLA genotype A3B8DR3/A1B8DR3 and therefore homozygous for B8 and DR3. His suppressor cell

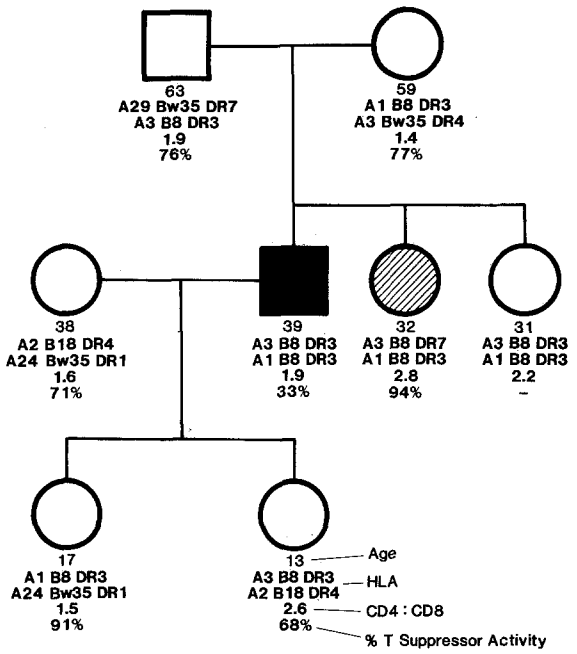


**Fig 2.** Pedigree for a family of a man with primary sclerosing cholangitis (filled square). Cross-hatched circle represents a sister with Raynaud's phenomenon. CD4:CD8 represents ratio of peripheral blood lymphocytes positive for the cell surface marker OKT4 (helper cells) and OKT8 (suppressor/cytotoxic cells). Suppressor cell activity was determined by an assay dependent on *in vitro* concanavalin A-induced suppression of IgG synthesis by peripheral blood lymphocytes.

function of 33% is below the mean for normals in our laboratory (normals 82% ± 4%, $X$ ± SE). Antinuclear antibody was positive at a titer of 1:80. Antimitochondrial and anti-smooth muscle antibodies were not present. The CD4/CD8 ratio however of 1.9 is normal for our laboratory (normals 2.1 ± 0.2, $X$ ± SE).

One sister with Raynaud's phenomenon is partially HLA identical to the patient, except for a recombinant paternal haplotype for DR7/DR3. She therefore is homozygous for B8 but heterozygous for DR3/DR7. A second sister, who is free of autoimmune disease, but has cervical uterine cancer, is HLA identical to the patient. No relative studied had abnormalities of serum biochemistries reflecting liver dysfunction; circulating antinuclear, antimitochondrial, or anti-smooth muscle antibodies; alterations in CD4/CD8 ratios; or depressions of concanavalin A-induced nonspecific suppressor T-cell activity.

## DISCUSSION

An association between PSC and the HLA phenotype B8 has been reported from population-based studies. However, there have been no associations of that HLA phenotype with elevations of circulating immunoglobulins, with the presence of antinuclear, anti-smooth muscle, or anticolon antibodies or with the distributions of $T_\gamma$ or $T_\mu$ cells (1, 2, 9). The patient classification used by Schrumpf et al (2), however, showed an HLA B8 association for patients classified as having ulcerative colitis with hepatobiliary disease, while no such association was seen for ulcerative colitis patients without hepatobiliary disease. A strong association for HLA DR3 also was seen in the ulcerative colitis patients with hepatobiliary disease but a weak association was seen in ulcerative colitis patients without hepatobiliary disease.

Studies of cell surface phenotypes in patients with PSC have demonstrated no difference in $T_\gamma$ or $T_\mu$ cell distribution (2), but a decrease in the number of circulating suppressor T-cells (CD8), resulting in an increase in the CD4/CD8 ratio, has been reported (7, 10, 11). However, cell surface phenotypes of circulating lymphocytes do not necessarily reflect immunoregulatory function and functional studies of concanavalin A-induced suppressor cell activity have revealed conflicting results. Suppressor cell activity was not reduced in a group of patients in which concanavalin A-induced suppression was measured using an *in vitro* blastogenesis assay with autologous responder cells (11). In studies of concanavalin A-induced suppression of *in vitro* immunoglobulin synthesis, however, an impairment was seen in PSC patients (3). An association with the HLA phenotypes DR3 and B8 was seen only in patients with PSC unaccompanied by ulcerative colitis.

The patient reported herein is a man with ulcerative colitis and PSC, who is homozygous for HLA B8 DR3, has a circulating antinuclear antibody and a defect in nonspecific suppressor T-cell activity, using an assay measuring the suppression of *in vitro* IgG secretion. This suppressor cell defect was present despite therapy with prednisone, which is of interest because *in vitro* suppressor cell activity has been shown to be corrected by glucocorticosteroid therapy in patients with autoimmune chronic active hepatitis (15).

Because of the coexistence of the HLA B8 DR3 genotype, the circulating autoantibody, and a defect in suppressor T-cell activity, we thought it would be of interest to search for a genetic abnormality of immune regulation in his family to determine if a putative immunoregulatory locus linked to an HLA or autoantibody locus would become apparent. As is

evident from the pedigree illustrated in Figure 2, there was no evidence of immunoregulatory defects in family members, or for the presence of circulating autoantibodies, although one sister had Raynaud's phenomenon and the other a malignancy. These data, therefore, provide no evidence that the suppressor cell function is primary in this patient, despite its persistence during glucocorticosteroid treatment.

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