Immune Phenotype of Chronic Liver Disease

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Immune disorders in chronic liver disease may reflect common host propensities or disease-specific factors. Our aim was to determine the principal bases for these expressions. Four hundred fifty-one patients with various chronic liver diseases were assessed prospectively for concurrent immune disorders. Individuals with immune diseases were more frequently women (73% vs 60%, $P = 0.02$) and they had HLA DR4 more often than counterparts with other HLA (46% vs 23%, $P = 0.000008$). The association between HLA DR4 and immune disease was apparent within individual liver diseases and within different categories of liver disease. Women with HLA DR4 had a higher frequency of immune disease than women without HLA DR4 (52% vs 22%, $P \leq 0.000001$), and they also had immune diseases more commonly than DR4-positive men (52% vs 31%, $P = 0.03$). DR4-positive men, however, had higher frequencies of immune disease than DR4-negative men, especially in the nonimmune types of liver disease (26% vs 4%, $P = 0.002$). We conclude that HLA DR4 and female gender constitute an immune phenotype that is an important basis for autoimmune expression in chronic liver disease.

KEY WORDS: autoimmune diseases; female gender; immune phenotype.

Immune-mediated nonhepatic diseases, including autoimmune thyroiditis, Graves' disease, Sjogren's syndrome, and ulcerative colitis, are frequently found in type 1 autoimmune hepatitis (1, 2). A host-dependent genetic predisposition for these manifestations has been proposed and an association has been described with HLA DR4 (2–4). Similar immune manifestations occur in other types of chronic liver disease and an association with HLA DR4 has also been found (5–8). These findings suggest the existence of an "immune phenotype" that is host-dependent and not disease-specific.

Immune response genes reside within the major histocompatibility complex (MHC) in experimental animals, and there is circumstantial evidence that these genes exist in humans (9). Concurrent immune diseases in various chronic liver diseases could reflect this genetic predisposition. The liver disease may release or modify self-antigens that trigger the immune expression or it may present to the genetically predisposed host extrinsic antigens that cross-react with self-antigens (molecular mimicry) (10). Cytokines, such as endogenous interferon, may also alter immunoreactivity in a nonselective fashion (11) and the liver disease itself may impair important immunomodulatory functions, such as suppressor T-cell activity, in relation to its severity (12).

HLA DR4 may have an antigen-binding groove that facilitates the presentation of self-antigens or foreign antigens that resemble self-antigens to circulating immunocytes (13). This presentation may in turn stimulate an immunoreactivity that results in the expression of an immune disease. Not all concurrent immune diseases, however, have an association with HLA DR4 and not all patients with HLA DR4 have immune manifestations (3, 7). Furthermore, many immune diseases in type 1 autoimmune hepatitis and other chronic liver disorders have HLA associations

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outside the context of the liver disease, and they are not HLA DR4-related (7). In all these instances, factors other than HLA DR4 must be invoked to explain the propensity for immune expression, and they may be host-related, disease-specific, host- and disease-related, or coincidental.

In this study, we extend our hypothesis of an immune phenotype based on HLA DR4 to determine other important host-dependent and disease-specific factors that affect the frequency of immune expression in chronic liver disease. We determine the disease-specificity of the immune findings, and we evaluate the importance of differences in disease activity, stage, and nature on their occurrence. Lastly, we assess the role of gender on immune expression in patients with and without HLA DR4, and we determine for the first time the impact of these host-dependent factors on immune expression in different types of chronic liver disease. By counterbalancing host- and disease-related features, the propensities for immune expression are defined and the bases for differences in the prevalence of immune phenomena in various chronic liver diseases described.

MATERIALS AND METHODS

Study Populations. Four hundred fifty-one patients with well-established chronic liver disease were studied prospectively for concurrent immune diseases. Each patient was assessed by one investigator (A.J.C.) in accordance with a predefined protocol that systematically evaluated etiologic factors for the liver disease (viral, drug, genetic, alcoholic, and autoimmune bases), DR human leukocyte antigens (HLA), associated immune diseases, conventional laboratory indices of inflammatory activity and disease severity, and histologic findings (1–7). The patients were chosen from 502 individuals who had been evaluated similarly but in whom the database for classification and analysis was incomplete or nondiagnostic. Our study had been approved by the Institutional Review Board of the Mayo Clinic as part of a program project grant.

One hundred seventy-seven patients had type 1 autoimmune hepatitis (39%); 5 patients had autoimmune cholangitis (1%); 32 patients had primary biliary cirrhosis (7%); 21 had primary sclerosing cholangitis (5%); 21 had chronic hepatitis B (5%); 133 had chronic hepatitis C (29%); 14 had chronic alcoholic liver disease (3%); 30 had nonalcoholic steatohepatitis (7%); and 18 had cryptogenic chronic hepatitis (4%) (Table 1). The diagnosis of autoimmune hepatitis required satisfaction of the criteria promulgated by the International Autoimmune Hepatitis Group (14), and seropositivity for smooth muscle antibodies (SMA) and/or antinuclear antibodies (ANA) was required for the designation of type 1 disease (15). The diagnoses of the other conditions were based on conventional clinical criteria (7).

Patients were further classified as having immune type and nonimmune type chronic liver disease based on putative pathogenic mechanisms. Two hundred thirty-five patients (52%) were categorized as having immune type disorders, including 177 patients with type 1 autoimmune hepatitis, 5 patients with autoimmune cholangitis, 32 patients with primary biliary cirrhosis, and 21 patients with primary sclerosing cholangitis. Two hundred sixteen patients were categorized as having nonimmune type disorders (48%), including 21 patients with chronic hepatitis B, 133 patients with chronic hepatitis C, 14 patients with chronic alcoholic liver disease, 30 patients with nonalcoholic steatohepatitis, and 18 patients with cryptogenic chronic hepatitis.

Table 1. Frequency of Concurrent Immune Diseases in Chronic Liver Disease

Liver diagnosis	*Total patients* (N)	*Patients with immune diseases** [N (%)]
Type 1 autoimmune hepatitis	177	68 (39)[a,b,c,d]
Autoimmune cholangitis	5	1 (20)
Primary biliary cirrhosis	32	9 (28)
Primary sclerosing cholangitis	21	15 (71)[b]
Chronic hepatitis B	21	4 (19)
Chronic hepatitis C	133	29 (22)[a]
Alcoholic disease	14	1 (7)[c]
Nonalcoholic steatohepatitis	30	7 (23)
Cryptogenic hepatitis	18	2 (11)[d]

* Significantly different from type 1 autoimmune hepatitis at levels of [a] $P = 0.002$; [b] $P = 0.005$; and [c,d] $P = 0.02$.

Patients were also classified into viral and nonviral categories depending on the presumed etiology of their disease. One hundred fifty-four patients (34%) had either chronic hepatitis B (21 patients) or chronic hepatitis C (133 patients), and they were designated as having viral disease. Two hundred ninety-seven patients (66%) lacked serologic markers of viral infection, and they were designated as having nonviral disease.

Concurrent Immune Diseases. Concurrent nonhepatic disorders of an immunologic nature were sought in a uniform fashion in each patient in accordance with previously published guidelines (1–7). The presence of thyromegaly, synovitis, colitis, and skin lesions were recorded systematically. The sensitive thyroid-stimulating hormone level and thyroid antibodies, including thyroglobulin and microsomal antibodies, were determined in each individual. If clinical history, physical examination, or laboratory findings revealed abnormalities, additional studies were performed as indicated.

The diagnosis of autoimmune thyroiditis required the presence of thyroid dysfunction and seropositivity for one or both thyroid antibodies (16). Hyperthyroidism, as indicated by hyperthyroxemia and suppressed sensitive thyroid-stimulating hormone level, in conjunction with thyromegaly, connoted Graves' disease (17). An historical basis for the diagnosis was accepted if thyroid ablation had been undertaken previously. Other nonthyroid diseases were designated as immunologic in nature if their putative pathogenesis included immune mechanisms (4). In this regard, insulin-dependent diabetes mellitus was included among the diagnostic considerations (18).

Forty patients (9%) satisfied criteria for autoimmune thyroiditis. Twenty-three patients had ulcerative colitis; 11 had Graves' disease; 10 had synovitis; 6 had insulin-dependent diabetes; 6 had cutaneous vasculitis; 5 had

asthma; 4 had Crohn's disease; 3 had idiopathic thrombocytopenic purpura; 3 had Sjogren's syndrome; 3 had keratoconjunctivitis sicca; 3 had rheumatoid arthritis; 2 had dermatitis herpetiformis; 2 had erythema nodosum; 2 had lichen planus; 2 had mononeuritis multiplex; 2 had systemic lupus erythematosis; and 1 each had lymphocytic colitis, myositis, glomerulonephritis, pernicious anemia, Coomb's-positive hemolytic anemia, pericarditis, pulmonary fibrosis, systemic sclerosis, or vitiligo. The total number of individuals with concurrent immune disorders was 136 (30%).

Immunoserologic Assays for Liver-Related Autoantibodies. Patients were screened at presentation for the presence of SMA, ANA, antibodies to liver/kidney microsome type 1 (anti-LKM1), and antimitochondrial antibodies (AMA) by indirect immunofluorescence on murine kidney and stomach sections as described previously (19–22). A serum titer of 1:40 or higher was considered positive for SMA, ANA, and AMA. A serum titer of 1:10 or higher was considered positive for anti-LKM1.

Four hundred forty-eight patients (99%) were tested for SMA; 449 patients (99%) were tested for ANA; 446 patients (99%) were tested for AMA; and 421 patients (93%) were tested for anti-LKM1.

Immunoserologic Assays for Thyroid Antibodies. Thyroglobulin and microsomal thyroid antibodies were sought in all patients by standard techniques (23–25). A titer of at least 1:100 was considered positive. Forty-four patients (10%) had thyroglobulin antibodies, and 114 patients (25%) had microsomal antibodies. Forty patients (9%) had both antibodies and seventy-eight patients (17%) had only one. Of the 40 patients with autoimmune thyroiditis, 24 patients were seropositive for only one thyroid antibody (thyroglobulin antibodies in one patient and microsomal antibodies in 23 patients) and 16 were seropositive for both thyroid antibodies.

Histologic Assessments. Liver tissue was obtained by needle biopsy in 406 patients (90%) at the time of presentation. Of the 45 patients who did not undergo liver biopsy examination, 27 had chronic hepatitis C, 6 had chronic alcoholic liver disease, 4 had primary biliary cirrhosis, 3 had cryptogenic chronic hepatitis, 2 had primary sclerosing cholangitis, 2 had chronic hepatitis B, and 1 had type 1 autoimmune hepatitis. In each instance, the clinical diagnosis was judged secure without histologic confirmation and the patients were included in the analysis.

Specimens were interpreted under code, and the diagnosis of cirrhosis required fibrosis and the presence of a complete regenerative nodule. One hundred patients who were biopsied (25%) had cirrhosis.

Virologic Assessments. All patients were tested for antibodies to hepatitis C virus (anti-HCV) by a second generation ELISA (Ortho Diagnostic Systems, Inc., Raritan, New Jersey). Hepatitis B surface antigen (HBsAg) was also sought in each patient by ELISA (Abbott Laboratories, North Chicago, Illinois).

HLA DR3 and DR4 Determinations. Four hundred twenty-two patients (94%) were evaluated for the class II (DR locus) antigens. All patients were caucasoid. A standard microlymphocytotoxicity technique was used and multiple locally derived and commercially available sera defined the DR3 and DR4 specificities as described elsewhere (2, 3). Restriction fragment length polymorphism (RFLP) was used to define DR3 and DR4 status in 269 patients (26) and polymerase chain reaction with sequence specific primers (PCR-SSP) was used to define DR3 and DR4 status in 107 patients (27). RFLP and PCR-SSP split DR3 into DR17 and DR18, but for the purposes of this study all findings were expressed as DR3.

Statistical Analyses. Dichotomous variables were compared by the Fisher exact test. The unpaired *t* test was used to evaluate the significance of differences in means for continuous variables. The Mann-Whitney test was used to compare nonparametric variables in independent samples. HLA DR3 and DR4 are known risk factors for autoimmune expression, and only the frequencies of these antigens were analyzed in our study population. Since the variables for comparison had been formulated *a priori* and then assessed systematically in each study group, an unadjusted P value of 0.05 was used to determine statistical significance. Data are presented as the mean ± SEM in tables and text.

RESULTS

Immune Features and Type of Liver Disease. Patients with type 1 autoimmune hepatitis had a higher frequency of concurrent immune disease than patients with other chronic liver diseases, especially those with chronic hepatitis C, alcoholic liver disease, and cryptogenic chronic hepatitis (Table 1). Only patients with primary sclerosing cholangitis differed in this regard mainly because of their high frequency of concurrent inflammatory bowel disease (Table 1).

Type of Immune Disease and Liver Diagnosis. Extrahepatic immune manifestations occurred in all types of chronic liver disease regardless of presumed nature (Table 2). Thyroid disease occurred most commonly, affecting 51 patients (11%) either as autoimmune thyroiditis (40 patients) or Graves' disease (11 patients). Only the small number of patients with alcoholic liver disease (14 patients) lacked this manifestation (Table 2).

Autoimmune thyroiditis (15% vs 5%, $P = 0.0006$) and Graves' disease (5% vs 1%, $P = 0.008$) were each more frequent in type 1 autoimmune hepatitis than in other conditions, and both forms of autoimmune thyroid disease occurred more often in type 1 autoimmune hepatitis than in chronic hepatitis C (20% vs 7%, $P = 0.001$). Vasculitis was common in chronic hepatitis C, but its occurrence was not higher than that in other chronic liver disorders, including type 1 autoimmune hepatitis (4% vs 1%, $P = 0.09$).

Immune Features and Clinical Findings. Patients with concurrent immune diseases were more commonly women than patients without these manifestations, and they also had SMA more frequently (Table 3). As recognized previously, individuals with concurrent immune diseases were DR4-positive more often

TABLE 2. TYPES OF CONCURRENT IMMUNE DISEASE ACCORDING TO LIVER DISEASE

Liver disease	*Immune disease*	N *(%)*
Type 1 autoimmune hepatitis	Autoimmune thyroiditis	26 (15)
	Ulcerative colitis	11 (6)
	Graves' disease	9 (5)
	Synovitis	3 (2)
	Asthma	2 (1)
	Systemic lupus erythematosus	2 (1)
	Dermatitis herpetiformis	1 (1)
	Coomb's anemia	1 (1)
	Crohn's disease	1 (1)
	Diabetes mellitus	1 (1)
	Erythema nodosum	1 (1)
	Pernious anemia	1 (1)
	Rheumatoid arthritis	1 (1)
	Others*	8 (4)
Autoimmune cholangitis	Graves' disease	1 (20)
Primary biliary cirrhosis	Keratoconjunctivitis sicca	3 (10)
	Autoimmune thyroiditis	1 (3)
	Diabetes mellitus	1 (3)
	Lichen planus	1 (3)
	Sjogren's syndrome	1 (3)
	Synovitis	1 (3)
	Vitiligo	1 (3)
Primary sclerosing cholangitis	Ulcerative colitis	10 (47)
	Crohn's disease	3 (14)
	Asthma	1 (5)
	Autoimmune thyroiditis	1 (5)
Chronic hepatitis B	Autoimmune thyroiditis	1 (5)
	Glomerulonephritis	1 (5)
	Rheumatoid arthritis	1 (5)
	Ulcerative colitis	1 (5)
Chronic hepatitis C	Autoimmune thyroiditis	8 (6)
	Synovitis	5 (4)
	Vasculitis	5 (4)
	Asthma	2 (1)
	Dermatitis herpetiformis	1 (1)
	Diabetes mellitus	1 (1)
	Erythema nodosum	1 (1)
	Graves' disease	1 (1)
	Idiopathic thrombocytopenia	1 (1)
	Lymphocytic colitis	1 (1)
	Neuritis	1 (1)
	Rheumatoid arthritis	1 (1)
	Ulcerative colitis	1 (1)
Alcoholic disease	Diabetes mellitus	1 (7)
Nonalcoholic steatohepatitis	Diabetes mellitus	2 (7)
	Autoimmune thyroiditis	1 (3)
	Idiopathic thrombocytopenia	1 (3)
	Pericarditis	1 (1)
	Sjogren's syndrome	1 (1)
	Synovitis	1 (1)
Cryptogenic hepatitis	Autoimmune thyroiditis	2 (11)

* Others = single cases of iritis, idiopathic thrombocytopenic purpura, myositis, mononeuritis multiplex, pulmonary fibrosis, systemic sclerosis, Sjogren's syndrome, and vasculitis.

than patients without this antigen (Table 3). In contrast, concurrent immune diseases were not associated with HLA DR3, age, the presence of cirrhosis, or the laboratory indices of liver inflammation or immunoreactivity (Table 3).

TABLE 3. FEATURES AT PRESENTATION OF PATIENTS WITH AND WITHOUT IMMUNE DISEASES*

	Immune diseases	
Clinical features	*Present (N = 136)*	*Absent (N = 315)*
Age (yr)	49 ± 1	48 ± 1
Male:female	37:99[d]	123:192[d]
AST (nl: ≤31 units/liter)	292 ± 30	237 ± 18
Bilirubin (nl: ≤1.1 mg/dl)	2.9 ± 0.4	2.3 ± 0.2
γ-globulin (nl: 0.7–1.6 g/dl)	2.4 ± 0.1	2.2 ± 0.1
Immunoglobulin G (nl: 700–1500 mg/dl)	2191 ± 105	1934 ± 63
SMA ≥ 1:40	61/135 (45)[c]	97/313 (31)[c]
ANA ≥ 1:40	64/135 (47)	141/314 (45)
AMA ≥ 1:40	17/135 (13)	34/311 (11)
Cirrhosis	31/123 (25)	69/283 (24)
DR3+/DR4−	28/123	96/299
DR4+/DR3−	53/123[b]	63/299[b]
DR3+/DR4+	16/123[e]	17/299[e]
DR4+/DR3− or DR3+	69/123[a]	80/299[a]
DR3+/DR4− or DR4+	44/123	113/299

* Numbers in parentheses are percentages. Significantly different from each other at levels of [a] $P < 0.000001$; [b] $P = 0.000008$; [c] $P = 0.005$; and [d,e] $P = 0.02$. AST = serum aspartate aminotransferase level; SMA = smooth muscles antibodies; ANA = antinuclear antibodies; AMA = antimitochondrial antibodies.

Immune Features and Effects of Gender Within and Between Diagnostic Categories. Women had immune-type chronic liver diseases (62% vs 33%, $P < 0.0001$) more commonly than men, and men had viral-type chronic liver diseases (50% vs 25%, $P < 0.0001$) more frequently than women. In each diagnostic category and in each gender, immune diseases occurred more commonly in association with HLA DR4 (Table 4).

Women had a higher frequency of concurrent immune diseases than men in viral type and nonviral

TABLE 4. IMMUNE FEATURES AND EFFECTS OF GENDER WITHIN DIAGNOSTIC CATEGORIES

	Immune features	
Category of liver disease	*Men [N (%)]*	*Women [N (%)]*
Immune type		
DR4+	4/8 (50)	36/72 (50)[d]*
DR4−	15/39 (38)	27/94 (29)[d]
Nonimmune type		
DR4+	9/34 (26)[b,e]	20/35 (57)[a,e]
DR4−	3/70 (4)[b]	9/70 (13)[a]
Viral		
DR4+	10/77 (13)[f]	21/77 (29)[f,g]
DR4−	2/51 (4)	5/48 (10)[g]
Nonviral		
DR4+	5/16 (31)	40/83 (48)[c]
DR4−	16/58 (28)	31/116 (27)[c]

* Significantly different from each other at level of [a] $P = 0.000004$; [b] $P = 0.002$; [c] $P = 0.003$; [d] $P = 0.006$; [e] $P = 0.01$; and [f,g] $P = 0.02$.

type chronic liver diseases, indicating the potentiating effect of female gender in each disease category (Table 4). Furthermore, women who were HLA DR4 positive had higher frequencies of concurrent immune features in all disease categories than female counterparts who were HLA DR4 negative (52% vs 22%, $P \leq 0.000001$), and they also had immune diseases more commonly than DR4-positive men (52% vs 31%, $P = 0.03$) (Table 4). Similarly, DR4-positive men had concurrent immune diseases in all disease categories more commonly than DR4-negative men, especially in the liver diseases of a nonimmune nature (26% vs 4%, $P = 0.002$) (Table 4).

DR4-positive men had similar frequencies of concurrent immune disease regardless of disease category (Table 4), whereas DR4-positive women with nonviral types of chronic liver disease had a greater occurrence of immune manifestations than DR4-positive women with viral types of chronic liver disease (48% vs 29%, $P = 0.009$) (Table 4). These findings indicated that among men the HLA phenotype was more important for immune expression than the nature of the liver disease, whereas among women the nature of the liver disease as well as the HLA phenotype were important (Table 4).

DISCUSSION

Our study indicates that concurrent immune diseases are present in all types of chronic liver disease and that an individual immune manifestation does not compel a particular liver diagnosis. Autoimmune thyroid diseases occurred more frequently in type 1 autoimmune hepatitis, but they were present in all of the diagnostic categories except alcoholic liver disease. In this latter condition, the prevalence of autoimmune thyroiditis and/or Graves' disease may have been affected by the small number of patients and their male predominance (12 of the 14 patients were men).

Individuals developing extrahepatic immune disorders were characterized mainly by their female gender and the presence of HLA DR4. Women have long been known to have a greater immunoreactivity and propensity for autoimmune manifestations than men (28, 29), and this predisposition was evident in all forms of chronic liver disease included in our study (Tables 3 and 4). Women develop higher serum immunoglobulin levels than men after exposure to a common antigen load (30–32), and they have higher natural frequencies of various autoantibodies (33, 34). Estrogens promote B-cell-dependent immune-complex-mediated disease (35); they modulate release of tumor necrosis factor from macrophages, which may in turn affect monocyte cytotoxicity and the chemotaxis of leukocytes (36); they inhibit suppressor T-cell function and thereby activate the immune response (37); they affect $CD4^+$ T-lymphocyte activity, especially antigen-specific cytotoxic T lymphocyte response (38); and they influence cytokine production, especially that of interleukin-1 and interferon-γ (39, 40). An immunomodulatory gene on the X chromosome has also been proposed (41, 42), and the increased immunoreactivity in women may reflect a "double dose" of this gene. Furthermore, other hormones, including androgens (43) and prolactin (44), may affect the vigor of the immune response by counterbalancing estrogen effects (43, 45) or by enhancing antigen processing through direct trophic actions on the immunocytes (44).

Importantly, female gender alone was insufficient to distinguish patients with and without immune manifestations and HLA DR4-negative men and women had similar occurrences of immune disease regardless of the liver diagnosis (Table 4). Both female gender and HLA DR4 positivity were required to enhance the propensity for immune expression, and these findings suggested that female hormones and/or immunoregulatory genes (39, 41–43) potentiated the immunoreactivity to self-antigens displayed by HLA DR4. Interestingly, HLA DR4-positive men had greater frequencies of immune manifestations than HLA DR4-negative men in all categories of liver disease, especially in the nonimmune types (Table 4). This observation indicated that HLA DR4 was an important risk factor for immune expression independently of gender.

The role of the liver disease in facilitating immune expression is unknown. The occurrence of immune disorders was not associated with conventional indices of liver inflammation and function or with the presence of cirrhosis (Table 3). In HLA DR4-positive men, immune manifestations occurred with similar frequency in all categories of liver disease, and there was no etiologic predilection for a particular immune feature. In these individuals, the immune disorder may have been coincidental to the liver disease or the liver disease may have had a facilitative effect that was not disease-specific. In this regard, the liver condition *per se* may have disturbed cytokine production (11) or suppressor T-cell function (12).

In contrast, HLA DR4-positive women who had nonviral types of chronic liver disease had a greater frequency of immune diseases than counterparts with viral types of chronic liver disease. In these individuals, the nature of the liver disease may have influ-

enced the immune propensity by affecting the type and/or amount of autoantigens processed. Alternatively, the susceptibility factors for both the nonviral liver diseases and the immune features may have been similar and their clustering may have reflected the pluripotent nature of the risk factors (2–4, 7).

Alleles within the DR4 loci encode particular amino acid sequences in the DRβ polypeptide that determines the ability of each class II molecule to bind and present antigens to T cells (46, 47). Similar antigen-presenting mechanisms in different chronic liver diseases may facilitate expression of the same immune manifestation. Homologies between different triggering antigens are also required. Molecular mimicry has already been recognized between certain viral genomes and autoantigens (10, 48), and it is possible that some autoantigens have homologous epitopes that trigger similar clinical manifestations in different liver diseases. The female gender may promote this antigenic cross-reactivity by heightening immune responsiveness (28, 29, 39, 43).

In our study, HLA DR4 defined a general propensity for immune expression rather than a susceptibility to a particular disease, and this propensity was enhanced by female gender. Since many of the concurrent immune diseases had HLA associations other than HLA DR4, their clustering in chronic liver disease in association with HLA DR4 suggested that more than one allele was responsible for their expression (49). In the context of chronic liver disease, HLA DR4 may be a secondary, but necessary, co-factor for the development of concurrent immune diseases (49).

The majority of immune disorders described in our patients were autoantigen driven (Table 2) (8). Other immune disorders may be viral antigen driven, and these conditions, including those associated with cryoglobulinemia, may have greater disease specificity than host dependence (8, 50, 51). Cryoglobulinemia was not routinely sought in our patients, and we cannot determine if immune complex diseases have the same risk factors for expression as conditions mediated mainly by immunocytes. Future studies must categorize the immune manifestations according to putative pathogenic mechanisms and define the risk factors associated with each type of immune expression.

In summary, concurrent immune diseases occur in all types of chronic liver disease, and they do not connote a particular liver diagnosis. Female gender affects the propensity for immune expression, especially in patients with HLA DR4, and these host-related factors constitute an immune phenotype. Disease factors influence the nature and the frequency of immune expression in some patients, especially in HLA DR4-positive women with nonviral types of chronic liver disease, but the propensity for immune expression is mainly host-related.

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