Symposium: Emerging Role of Pathogens in Chronic Diseases Requiring Nutritional Intervention

Viral Induction of Type 2 Diabetes and Autoimmune Liver Disease

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ABSTRACT Cross-sectional studies performed worldwide have shown that hepatitis C virus (HCV) infection is linked with type 2 diabetes, but these endocrine and liver diseases have an insidious onset, and it has been difficult to establish that patients acquire HCV infection before the development of diabetes. It is likely that investigations in small animal models or in vitro systems will be required to determine whether a causal relationship of HCV infection and the development of diabetes can be established. We have developed an in vitro model to study the viral induction of primary biliary cirrhosis (PBC) based on the phenotype of the diseased biliary epithelial cells. PBC patients make antimitochondrial antibodies and express proteins reactive to these antibodies on their biliary epithelium. In coculture studies we have found that normal biliary epithelial cells develop the phenotypic manifestation of PBC in vitro specifically when cultivated with lymph nodes from PBC patients and not with relevant liver disease control subjects. We have also cloned a novel human retrovirus from a PBC biliary epithelium cDNA library and confirmed that the development of the PBC phenotype in vitro coincides with the presence of this virus. In clinical trials using antiretroviral therapy, we have observed a reversal of ductopenia as well as improvements in histology and hepatic biochemistry in patients with PBC. As Koch’s postulates are not readily applicable to chronic diseases, we have used cocultivation viral transmission model in vitro and antimicrobial clinical studies in vivo to help establish a causal relationship with a retrovirus infection and the phenotypic manifestation of disease. J. Nutr. 131: 2805S-2808S, 2001.

KEY WORDS: • diabetes • hepatitis • infection • liver • cirrhosis

There are an increasing number of reports that hint at an infectious etiology for a variety of autoimmune, neoplastic, neurologic and psychiatric conditions as well as nutritional disorders such as type 2 diabetes and obesity. Only a small proportion of these hypotheses are likely to stand the test of time, and even when a causal relationship is convincingly shown, the Herculean task of providing the evidence can often take years to accomplish.

Today, the problem of establishing causal relationships is compounded further with the ease of cloning occult pathogens, mandating the development of new strategies to determine disease associations without wasting time and resources. For example, several novel viruses have been cloned from patients with post-transfusion hepatitis without any obvious link to chronic liver disease. The problem of proving causality is confounded further as Koch’s postulates are not readily applicable to chronic disorders unless the pathogen can be cultured and a small animal model convincingly develops the phenotypic manifestation of disease. Indeed, the discovery of hepatitis C virus (HCV) was notable for the very reason that the cloning was performed without the virus ever being isolated, visualized, grown in tissue culture system or passaged in a small animal model. In this review, we suggest that in vitro coculture models and clinical trials with antiviral therapy can provide sufficient evidence to help establish a causal association for viral induction of chronic idiopathic disorders. Specifically, we evaluate the epidemiologic, pathophysiology and immunogenetic data suggesting an association of HCV infection with type 2 diabetes and suggest the use of vitro coculture systems and clinical trials that have helped to implicate a recently isolated human retrovirus in the etiology of primary biliary cirrhosis.

HCV infection and type 2 diabetes

The notion that HCV infection provides an increased risk of type 2 diabetes comes from epidemiologic studies of patients with liver disease (1–7), type 2 diabetes (6–9) and thalassemia (10), as well as a national survey (11). A 2- to 10-fold increase in diabetes has been reported worldwide (Table 1) in HCV-positive patients compared with liver disease control subjects.
(1–7), although a single study failed to support the association (12). In both of the largest retrospective studies conducted to date, which incorporate >1,000 patients, HCV infection was found to be an independent risk factor associated with type 2 diabetes by multivariate analysis (4,6). Collectively the studies outlined in Table 1 have been criticized for the lack of matching age, sex, BMI, socioeconomic status, race and stage of liver disease (12). However, the association of HCV infection with diabetes has been observed in noncirrhotic populations with thalassemia, for example, who have a fourfold increased risk of diabetes with HCV infection (10), and in a study of liver transplant recipients, in which HCV-positive patients have a reported four- to eightfold increased prevalence of diabetes as compared with patients with other viral or cholestatic liver disease 1 y after liver transplantation (13).

The link of diabetes with HCV infection has also been demonstrated in anti-HCV seroprevalence studies in cohorts with diabetes (Table 2). In English type 2 diabetic subjects with abnormal serum aminotransferases, HCV antibody was detected in 28% of patients of African origin, 12% of Caucasians and 8% of Asians (8). In a large U. S. clinic-based population, 4.2% of consecutive patients with diabetes were found to be HCV antibody positive compared with 1.6% in the control group undergoing thyroid scan (6). These seroprevalence studies should also be interpreted with caution because of the lack of data on cirrhosis, ascertainment bias and the difficulty in selecting adequate control groups. However, a cross-sectional study involving nearly 10,000 subjects from the Third National Health and Nutrition Examination Survey addresses some of these concerns (11). Multivariate analysis with matching of known risk factors showed that HCV infection provided a more than threefold increased risk of developing diabetes in individuals aged >40 y and twofold for those aged <40 y, whereas hepatitis B virus infection had no discernible effect (11). None of the patients with type 1 diabetes had evidence of HCV infection, suggesting that the diagnosis of diabetes per se had little impact on the subsequent development of HCV infection. However, no data were available to assess the temporal relationship for the onset of each disease or the role of cirrhosis on the development of diabetes.

Immunogenetic studies also hint at an infectious process in patients with type 2 diabetes and chronic liver disease. In a survey of two separate populations derived from liver transplant recipients at Ochsner Clinic and the National Institutes of Health Liver Transplant Database, the extended DR2,DR51,DQB6 haplotype was found to provide a 2.3-fold increased relative risk for the development of diabetes (14). The same linkage in two independent databases prompts the speculation that type 2 diabetes can occur as a result of viral infection in genetically predisposed individuals with liver disease. From a mechanistic standpoint, HCV-positive patients with diabetes do not develop specific autoantibodies; therefore, autoimmune destruction of the pancreas does not appear to be an important mechanism (2,6). There is evidence that HCV-positive diabetic patients have both peripheral insulin resistance as well as β-cell dysfunction, as indicated by decreased C-peptide levels and limited acute insulin responses (4,15,16). Furthermore, postmortem studies reveal that HCV replicates in the pancreas (17) and clinical trials report improvement in several measures of glucose metabolism after antiviral treatment (16). However, it remains to be determined whether the reversible component of diabetes is attributable to HCV directly perturbing the synthetic function in β-cells in patients with chronic liver disease secondary to HCV infection.

In summary, the epidemiologic association of HCV with type 2 diabetes seems convincing. Coculture studies with clonal β-cells and HCV may answer the question of whether viral infection of the pancreas directly correlates with diminished acute insulin responses seen in patients with HCV infection. A small animal model may ultimately be required to investigate the intricate relationship between liver and endocrine disease to establish whether HCV infection causes diabetes beyond the risk attributable to liver disease alone. It is also possible that transgenic mouse studies using expressed HCV proteins or prospective longitudinal studies assessing endocrine responses to antiviral therapy will provide further insight into the pathogenesis of viral induction of diabetes. However, a multifaceted attack is clearly warranted to investigate a causal association of HCV infection and diabetes to stem the epidemics of both common disorders.

**Table 1**

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<th>Prevalence of diabetes in patients with chronic liver disease</th>
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<td>HCV Liver disease control subjects</td>
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**Table 2**

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<th>Prevalence of anti-HCV in the serum of patients with diabetes and control populations</th>
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LFT, liver function tests.

**Retroviral etiology of primary biliary cirrhosis**

Primary biliary cirrhosis (PBC) is considered an autoimmune disease, because 95% of patients develop antimitochondrial antibodies (AMAs) reactive to pyruvate dehydrogenase complex E2 (PDC-E2) (18). The development of progressive granulomatous destruction of bile ducts usually leads to cirrhosis, but it is questionable how autoantibodies reactive to a ubiquitous intracellular organelle can cause a disease limited to biliary epithelium. It is also important to note that the role of autoimmunity plays in causing PBC is controversial because autoantibody levels and immunosuppression have little impact on the disease process (19). Of interest, immunohistochemistry studies using AMAs demonstrate a protein resembling the
mitochondrial autoantigen, PDC-E2, on PBC patients’ bile ducts and perihilar lymph nodes, which may be responsible for precipitating the formation of autoantibodies in the first place. This aberrant expression of the AMA reactive proteins in diseased tissues is unique to PBC patients and is considered a phenotypic manifestation of PBC.

There are several lines of evidence to suggest an infectious etiology for PBC (20). Reports have documented the clustering of disease in nonrelated family members and specific geographic locations as well as an increased incidence of PBC in migrant populations moving to higher prevalence countries and disease recurrence in the hepatic allograft after liver transplantation (20). To investigate a potential infectious etiology of PBC, we have used a combination of immunologic, cloning and electron micrograph techniques to identify a putative agent. In initial studies, we found that the majority of PBC patients had demonstrable antibody reactivity to the human intracisternal A-type particle, a retrovirus isolated from Sjogren’s syndrome patients, and we also observed virus-like particles by electron microscopy in extracted biliary epithelial cells (BECS) from PBC patients (21). Subsequently, we cloned a novel exogenous retrovirus from a BCA biliary epithelium cDNA library and used reverse transcriptase–polymerase chain reaction (RT-PCR) and immunohistochemistry to demonstrate that the viral infection was specifically detected in perihilar lymph nodes of patients with PBC (22).

We have also established an in vitro model for PBC by coculturing normal BECs with perihilar lymph nodes from patients with chronic liver disease. The BECs cocultured with PBC lymph nodes were found to express increased levels of PDC-E2 autoantigen by Western blot and immunocytochemistry after 5 d (23). Induction of the PBC phenotype occurred specifically with PBC lymph nodes and not with other liver disease controls. The supernatants from the PBC cocultures also induced PDC-E2 expression in fresh BECs, and this was abrogated by gamma irradiation (24). Electron microscopy studies revealed virus-like particles in the BECs and supernatants derived from the PBC cocultures and reverse transcriptase activity in PBC supernatants processed on sucrose gradients ranging from 1.14 to 1.17 g/mL, where enveloped retroviruses codominate (24). Using RT-PCR, we found that the novel viral sequences were detected in the BECs and supernatants cocultivated with PBC samples (22).

Further support for a retroviral etiology of PBC has been provided by pilot studies with antiretroviral therapies. One year of therapy with Lamivudine 150 mg/d in 10 PBC patients resulted in a temporary improvement in liver biochemistry tests, a significant reversal of bile duct loss and an improvement in histologic stage was observed in three patients (25). In a second pilot study, 5 of the 10 PBC patients have been treated for 6 mo on Combivir twice daily with significant reductions in serum aminotransferases and alkaline phosphatase levels, and 3 patients have completely normalized their aminotransferases and alkaline phosphatase activity in PBC supernatants processed on sucrose gradients ranging from 1.14 to 1.17 g/mL, where enveloped retroviruses codominate (24). Using RT-PCR, we found that the novel viral sequences were detected in the BECs and supernatants cocultivated with PBC samples (22). In summary, we have identified an agent with the morphologic, hydrodynamic, enzymatic and genomic features of an exogenous retrovirus that is detected in PBC patients’ lymph nodes in vivo and is associated with the development of the PBC phenotype in vitro. The establishment of a causal association of infection and disease is supported by the preliminary antiviral studies and the modified in vitro postulates with the development of the PBC phenotype in BECs. The specificity of PBC phenotype could be enhanced further using data from a microarray study showing specific activation of host pathways in PBC patients’ hepatic cDNA (26), with evaluation of infected and uninfected BECs for the specific microarray transcriptional fingerprint. Also, the use of antiviral therapy or neutralizing antibodies to prevent the development of the phenotype in vitro would also strengthen the hypothesis considerably. Certainly more flexible and feasible models for determining microbial causality are eagerly awaited.

LITERATURE CITED


