may suggest that the nonresponsiveness to hepatitis B vaccine is due to the immunotolerance or immunosuppression induced by latent HBV infection. It has been demonstrated that HBV antigens such as HBV e antigen could induce immunotolerance and cause chronic HBV infection [6]. Also, HBV itself may cause immunotolerance by directly infecting T and B lymphocytes, resulting in the viral persistence [7]. Immunosuppression may occur during viral infection through different mechanisms, such as the viral-triggered imbalance in immunoregulation with resultant overactivity of suppressor T lymphocytes [8]. However, what mechanism caused the latent HBV infection in the nonresponders in the present study is unclear; further investigation on this issue is being done in our laboratory.

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Effect of Interferon-α2b on Cryoglobulinemia Related to Hepatitis C Virus Infection

Colleagues—Cryoglobulinemia has been reported in association with hepatitis B virus (HBV) [1] and more recently with hepatitis C virus (HCV) infection [2]. There seems to be a relationship between hepatotropic viruses and essential mixed cryoglobulinemia [3]. To support this association, we report the effect of interferon-α2b (IFN-α2b) in two patients with chronic hepatitis due to HCV and mixed cryoglobulinemia.

Patient 1, a 65-year-old white woman with non-insulin-dependent diabetes for 10 years was admitted for thrombocytopenia. Past history revealed polyarthralgia for 3 years before admission. Diabetes was controlled with gliclazide (Diamicron). There was no previous history of blood transfusion or known exposure to hepatitis. Clinical examination revealed mild hepatosplenomegaly. Laboratory findings included erythrocyte sedimentation rate, 40/65 mm/h; platelet count, 72 × 10^9/l; serum bilirubin, 9 μmol/l; aspartate aminotransferase, 130 IU/l; alanine aminotransferase, 188 IU/l (normal, 7–29); alkaline phosphatase, 315 IU/l (normal, 90–250); latent circulating immune complexes, 6 μg/ml (normal, 0–1.5), and C4 level, 0.11 g/l (normal, 0.24–0.5 g/l). Mixed cryoglobulinemia with polyclonal IgG and IgM was present. IgM antibodies to hepatitis A and B surface antigen were not detected.

Patient 2, a 51-year-old white woman, has been previously reported [5]; it included associated recurrent tender nodules over the legs, arthralgias, and Raynaud’s phenomenon. She had been diagnosed 4 years earlier with non-A, non-B hepatitis. Mixed cryoglobulinemia with polyclonal IgG and IgM was present. IgM antibodies to hepatitis A and B surface antigen were not detected. EIA for HCV in serum was positive (optical density, >3) as were recombinant immunoblot assay (second-generation RIBA; Ortho) and polymerase chain reaction (PCR). PCR amplification for HVC RNA in serum was done by modified nested PCR with primers from the 5’ noncoding, highly conserved region of the HCV genome followed by ethidium bromide staining and by hybridization with an internal oligonucleotide probe as previously described [4].

Results of bone marrow aspiration were normal. Liver biopsy disclosed chronic active hepatitis with cirrhosis. Staining for hepatitis B core antigen by immunofluorescence was negative. The patient was treated with IFN-α2b (Intron-A; Schering Plough, Bloomfield, NJ), 3 million units subcutaneously, three times weekly.

After 3 months arthralgias subsided, and the search for cryoglobulinemia was negative. The patient has remained well for the past 12 months, but transaminases have remained elevated.

The clinical presentation for patient 2, a 51-year-old white woman, has been previously reported [5]; it included associated recurrent tender nodules over the legs, arthralgias, and Raynaud’s phenomenon. She had been diagnosed 4 years earlier with non-A, non-B hepatitis. Mixed cryoglobulinemia with polyclonal IgG and IgM was present. IgM antibodies to hepatitis A and B surface antigen were not detected. EIA for HCV in serum was positive (optical density, >3) as were RIBA and PCR.

A quantitative test for antibodies to HCV in serum (anti-HCV EIA; Ortho Diagnostics, Raritan, NJ) was positive (optical density, 2.3; cutoff, 0.452) as were recombinant immunoblot assay (second-generation RIBA; Ortho) and polymerase chain reaction (PCR). PCR amplification for HVC RNA in serum was done by modified nested PCR with primers from the 5’ noncoding, highly conserved region of the HCV genome followed by ethidium bromide staining and by hybridization with an internal oligonucleotide probe as previously described [4].

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tient showed fewer skin manifestations, with marked improve­
ment of the cytolysis.

Levo et al. [1] first implicated hepatitis B virus (HBV) as a
causative agent of essential mixed cryoglobulinemia. Subse­
quently, studies have cast doubt on their findings [4, 6, 7]. Re­
cently Galli [3] reported no difference in the frequency of HBV
markers between large groups of blood donors and patients with
essential mixed cryoglobulinemia. The presence of HCV
markers in patients with essential cryoglobulinemia has been
recently described [2]. Whereas in the first report, the specificity
of the anti-HCV test could not be assessed, HCV viremia was
confirmed by RIBA and PCR in subsequent reports [5, 8]. A
possible association of both HCV and HBV with cryoglobulin­
emia had been suggested [8]. Our findings suggest that HBV
may not need to be present; we have previously reported 10
patients with essential mixed cryoglobulinemia in which HCV
was the only etiologic agent for the cryoglobulinemia [9].

The mechanism by which HCV infection may trigger cryo­
globulin production remains to be determined. In our two pa­
tients, the clearance of cryoglobulinemia after IFN therapy pre­
ceded complete normalization of transaminases, supporting a
direct role of HCV in the pathogenesis of cryoglobulinemia
rather than through liver damage or through impairment of liver
clearance activity with subsequent polyclonal activation of B
cells. Recombinant IFN-α therapy is effective in chronic hepato­
tis and may be useful in cryoglobulinemia related to HCV. Fur­
ther controlled studies with larger number of patients are neces­
sary to determine the optimal treatment for cryoglobulinemia
associated with HCV.

**Low Human Immunodeficiency Virus Titer and
Polymerase Chain Reaction False-Negatives**

Colleagues—The polymerase chain reaction (PCR) [1] is being
increasingly used to search for human immunodeficiency virus
type 1 (HIV-1) DNA in clinical specimens. The standard assay
is usually done on ~1 μg of peripheral blood mononuclear cell
(PBMC) DNA (equivalent to 150,000 cells), an amount that is
expected to contain a detectable level of HIV-1 sequences in
infected subjects and to allow optimal amplification [2]. While
PCR has been shown to be a valuable tool for detecting HIV-1
DNA in latently infected seronegative subjects [3, 4], occasional
HIV-1-seropositive PCR-negative individuals have been unex­
pectedly reported both in earlier [5] and recent [6, 7] studies,
apparently in contrast with the propensity for HIV-1 to persist
indefinitely in the infected host. Since different HIV-1 isolates
are likely to diverge significantly [8], such cases have been gen­
erally interpreted as failure of a PCR primer(s) to hybridize to its
altered target sequence(s). Other possibilities have been envi­
visioned but not further investigated, including sequestration of
the virus in tissues, immunologic clearance of infected PBMC,
and extremely low viral load in PBMC.

We recently found evidence that an extremely low amount of
HIV-1 DNA in PBMC may account for a false-negative PCR
table 1). During a 9-month follow-up, a healthy female sex
partner of an HIV-1-infected individual was found to serocon­
vert to HIV-1, as revealed by Western blot analysis (Bio-Rad,
Hercules, CA). Restricted antibody response to p24 and gp41
was detected at month 4, while sera obtained at 6, 7, and 9
months showed broad positivity. HIV-1 p24 antigen was not
detected in any of the three (6, 7, and 9 months) serum samples
tested (Abbott, North Chicago). Despite the serologic evidence
of infection, none of the three PBMC DNA samples obtained (6,
7, and 9 months) scored positive by PCR for the presence of
HIV-1 DNA sequences. DNA samples were prepared by SDS–
proteinase K digestion of PBMC, followed by two phenol /
chloroform extractions and ethanol precipitation. DNA concen­
tration and size were evaluated spectrophotometrically and by
agarose gel electrophoresis. Control amplification of a human
β-globin gene fragment [1] showed that each sample was suit­
able for PCR analysis. HIV-1 DNA amplification reactions were
done on 1 μg of template DNA by a highly sensitive two-step

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