

## Hepatitis C Virus, Autoimmunity and Lymphoproliferation

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### Abstract

We summarize clinical, laboratory and pathologic details regarding a patient who presented with extrahepatic disease manifestations of hepatitis C virus (HCV) infection, including cryoglobulinemic leg ulcers due to cutaneous vasculitis, peripheral sensorimotor neuropathy, and recurrent pulmonary infiltrates. The patient had evidence for B-cell lymphoproliferation, diagnosed as extranodal lymphoma on initial (though not subsequent) bone marrow examination, retroperitoneal lymphadenopathy, and the presence of a Type II IgMκ monoclonal rheumatoid factor which became cryoprecipitable on complexing to IgG. Chronic hepatitis was mild on liver biopsy, though fibrotic changes developed over a three-year period of follow-up. She had consistently normal liver function tests, except for a brief rebound effect on discontinuing interferon-alpha, and preterminally. Symptoms were only partially responsive to trials of corticosteroids, cytotoxic agents, plasmapheresis and interferon, and the patient ultimately died at The Mount Sinai Hospital of sepsis. We review current information regarding the spectrum of extrahepatic HCV infection, including pathogenic factors relevant to its overlapping autoimmune, rheumatic and lymphoproliferative disease manifestations. The exact prevalence of these HCV-related syndromes among the 1% of the world population estimated to be infected by this virus remains to be delineated. Chronicity of infection, and lack of efficacy of currently available therapy in effecting sustained clearance of the virus from the host, have made this an important public health problem that is likely to increase in significance. Possible relationships to non-Hodgkin's lymphoma may present novel opportunities to delineate the basis for oncogenesis in HCV infection. **Key Words:** Cryoglobulinemia, hepatitis C virus, non-Hodgkin's lymphoma, autoimmunity, vasculitis.

### Introduction

HEPATITIS C VIRUS (HCV) is an RNA virus that is a member of the *Flaviviridae*, a family which includes members that are both hepato- and lymphotropic, and may be associated with chronic infectious diseases. The genome of this virus was first reported ten years ago using reverse cloning techniques on the blood of a chimpanzee infected by what was then termed "non-A, non-B" hepatitis (1). In the time since that report, much descriptive information has accumulated regarding the persistence of HCV, potential mechanisms

utilized by the virus to escape host immune responses, and limited response to therapies such as interferon-alpha. The availability of accurate serologic tests and assays to directly or indirectly quantitate viral RNA in biological fluids has led in turn to a virtual disappearance of HCV from the nation's blood supply, heretofore a major mode of transmission, and a significant downturn in the number of new infections reported each year. It has also led to an awareness of large reservoirs of chronically infected individuals, many of whom are apparently asymptomatic, or who have disease manifestations that do not clearly imply chronic liver disease (2).

It is estimated that 3.9 million persons in the United States are HCV antibody (HCVAb) positive (i.e., 1.8% of the population; 0.1–0.7% of healthy blood donors), of whom 2.7 million are HCV RNA positive, and therefore considered to be chronically infected (3); this percent increases dramatically if specific populations of patients (e.g., hemophiliacs, intravenous drug abusers, HIV-infected individuals) are surveyed. Of the

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dialysis population, 10–40% are HCVAb positive. This population of patients poses particular diagnostic and therapeutic challenges, because HCV may be a cause of chronic renal disease, and may manifest differently in this setting; also, specific therapeutic issues arise relating to chronic renal insufficiency and potential problems with immunomodulatory therapies in the post-renal transplant patient (4, 5). Although HCV accounts for about 20% of community-acquired acute viral hepatitis, 40–50% of patients give no clear-cut history of infection. Fifty to 80% of infected individuals become chronically infected (versus 2–5% persons infected with hepatitis B virus). About 50% of infected persons have normal or minimally elevated liver function tests (LFTs) (2, 3). Furthermore, elevations in liver function tests may be intermittent, reflecting to some extent spontaneous variations in viral RNA that may change over a range as high as  $\log_6 (10^6)$  variations in viral copy number in serum with time, allowing the possibility that infection may be missed unless multiple samplings are made (6, 7). Of those chronically infected, 20–35% progress to cirrhosis, and a significant number will go on to develop hepatocellular carcinoma; consequently, HCV is now the major cause of these two conditions in the United States. The average time to cirrhosis is 10 years, to hepatocellular carcinoma 29 years. Each year, eight to ten thousand persons die of liver-associated complications of HCV, and one thousand undergo liver transplantation; of those transplanted, approximately 40% will develop a recurrence of hepatitis in the allograft and many will require specific therapy and/or another transplant (8, 9).

More accurate and sensitive testing techniques have also made it clear that extrahepatic disease manifestations may provide subtle complications of chronic infection, or in turn dominate its course. These extrahepatic manifestations of HCV infection include both autoimmune phenomena and frank autoimmune and/or rheumatic diseases, as well as an association with B-cell lymphoproliferation and possibly non-Hodgkin's lymphoma. The following is illustrative of these various aspects of HCV infection.

### Case History

A 70-year-old woman was transferred to The Mount Sinai Hospital for management of pulmonary infiltrates.

Ten years previously, she had first developed recurrent episodes of purpura, occurring mostly below knees and around the ankles, increased by

standing, not related to ambient temperature, which would resolve spontaneously after about a week. Over the year before her initial evaluation, she noted the purpuric lesions to also be associated with numbness and decreased sensation, mostly involving the dorsal foot and lower third of both legs.

Five years previously, she came to the vascular and dermatology clinics for evaluation and treatment of large leg ulcers that had developed around and above both lateral malleoli (Fig. 1A). Biopsy of a purpuric lesion showed leukocytoclastic vasculitis; she was started on corticosteroids, and referred to Rheumatology for further evaluation. Initial work-up at that time included normal blood count and liver function tests (LFTs), urinalysis significant for red blood cells and proteinuria, and a type II monoclonal mixed cryoglobulin present at a concentration of 1.36 mg/mL in serum. Serum values for IgG — 480 mg/dL (normal range, 800–1700); IgM — 247 mg/dL (60–370); IgA — 133 mg/dL (85–450); rheumatoid factor (RF) — 594 IU (<20); antinuclear antibody (ANA) negative; C3 — 105 mg/dL (75–135); C4 — 8 mg/dL (10–40). Analysis of 24-hour urine samples revealed 1013–1300 mg protein; creatinine clearance (Ccr) was 82 mL/minute (normal, 85–175 mL/min); monoclonal IgMk in serum and cryoglobulin, with urine negative for monoclonal proteins by immunoelectrophoresis. HCVAb were positive by recombinant immunoblot assay (RIBA), but hepatitis A and B serologies were negative. HCV RNA was detected in both serum and cryoprecipitate, genotype 1b.



**Fig. 1.** Anterior view of patient's legs, showing multiple leg ulcers and palpable purpura at the time of initial evaluation (A). A lateral view several months later (B), following local and systemic therapy, shows complete healing and subsidence of purpuric lesions.



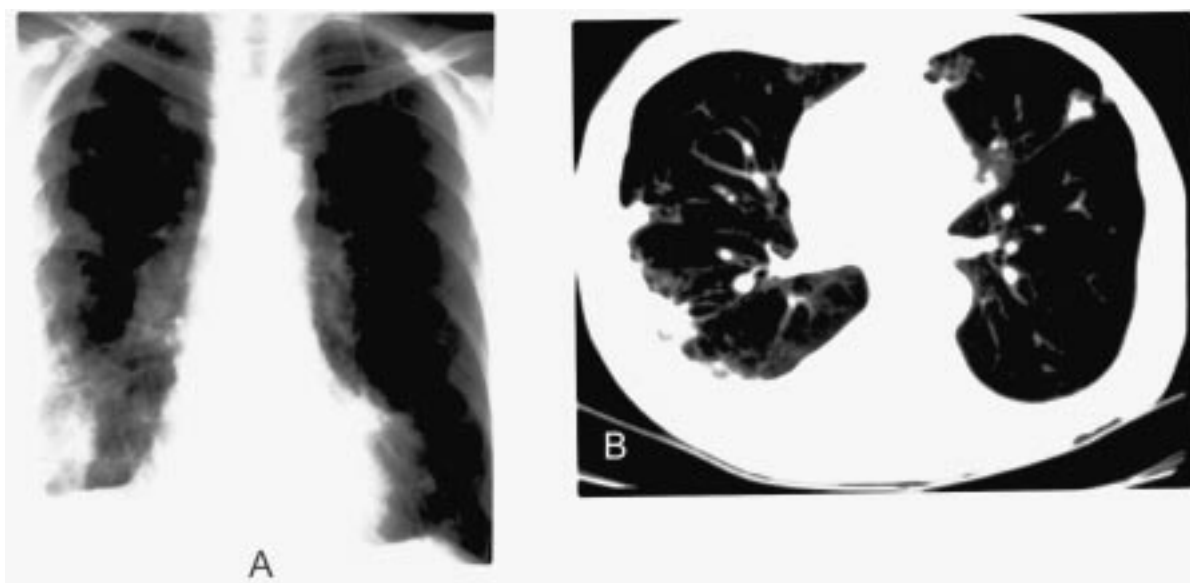
**Fig. 2.** CT of the abdomen, showing a 1.2 cm lymph node (arrowhead) adjacent to the aorta. Also noted on this study were smaller pretracheal, precarinal and periportal nodes (not shown).

Proteinuria and hematuria were initially attributed to a 6.5 x 5.0 cm cyst involving the upper pole of the left kidney, which was seen on ultrasound, with renal scan showing 39% function in the left kidney, and 61% in the right. A bone marrow aspiration and biopsy showed multiple nodules of small lymphoid cells, consistent with lymphoma, small lymphocytic type; as shown in Fig. 6A. The CT scan of the abdomen showed significant retroperitoneal lymphadenopathy, an example of which is shown in Fig. 2. Electrophysiologic testing confirmed a sensorimotor axonal polyneuropathy.

The patient was treated for hypertension, and received care for her leg ulcers; prednisone was continued. A trial of plasmapheresis was poorly tolerated, and she was started on chlorambucil, with subsequent resolution of ulcers (Fig. 1B) and diminution of purpura and cryoglobulin levels. Treatment had been suspended four years previously because of the development of estrogen receptor-positive breast carcinoma, requiring a radical left mastectomy, subsequent chemotherapy, and tamoxifen maintenance therapy.

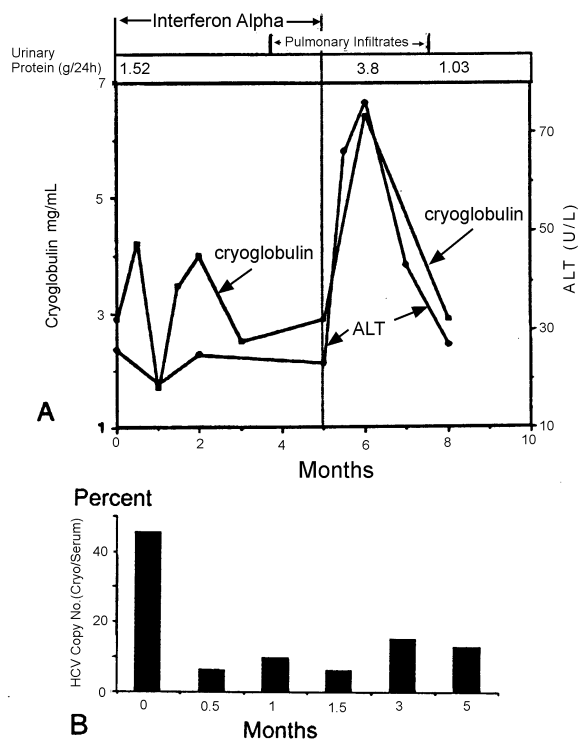
Purpura, cryoglobulinemia (2.1 mg/mL) and leg ulcers had recurred three years previously, at which time IgG levels were found to range from 240–423 mg/dL; IgA — 146–153 mg/dL; IgM — 211–249 mg/dL; C4 — 9 mg/dL; relative serum viscosity — 1.6 (normal range, 1.4–1.8); C3 — 93 mg/dL; LFTs normal; RF — 1208 IU/dL; and 24-hour protein — 1517 mg. CT of the abdomen again showed periportal (1.2 cm), pericaval (1.2 cm) and aortocaval (<1 cm) adenopathy (Fig. 2), along with mild hepatomegaly and a normal-sized spleen. Liver biopsy revealed chronic hepatitis, minimal activity, with no fibrosis; the biopsy was positive for HCV RNA by reverse transcription (RT) *in situ* polymerase chain reaction (PCR) amplification; genotype Ib. Electrophysiologic studies were unchanged from the previous study.

Two years previously, following the liver biopsy, she had been started on interferon-alpha (IFN $\alpha$ ), 3M units three times per week, which she



**Fig. 3.** **A.** Chest X-ray taken to evaluate cough and dyspnea, which developed five months after the initiation of therapy with IFN $\alpha$ , shows new right upper and lower lobe peripheral infiltrates, and a right-sided pleural effusion. **B.** A CT scan of the chest taken shortly thereafter shows bilateral subpleural nodular infiltrates, all of which resolved spontaneously over the following two months.

tolerated for five months before it was discontinued because of the development of cough, dyspnea and a chest X-ray showing a new right upper lobe infiltrate, and pleural and parenchymal changes at the right base (Fig. 3A and B). During this period, there was no significant diminution in HCV RNA viral load, assessed by quantitative PCR (Amplicor™, Roche Molecular Systems, Somerville, New Jersey), or of the cryoglobulin level (Fig. 4A), though a significant redistribution of HCV RNA from the cryoprecipitate to the serum supernatant was noted over this time period (Fig. 4B). Increased LFTs, proteinuria (3805 mg), and cryoglobulinemia (2.4–2.83 mg/mL) were noted over the 2–3 months following discontinuance of the drug (Fig. 4B), along with gradual resolution of the pulmonary lesions on serial CT studies of the chest.

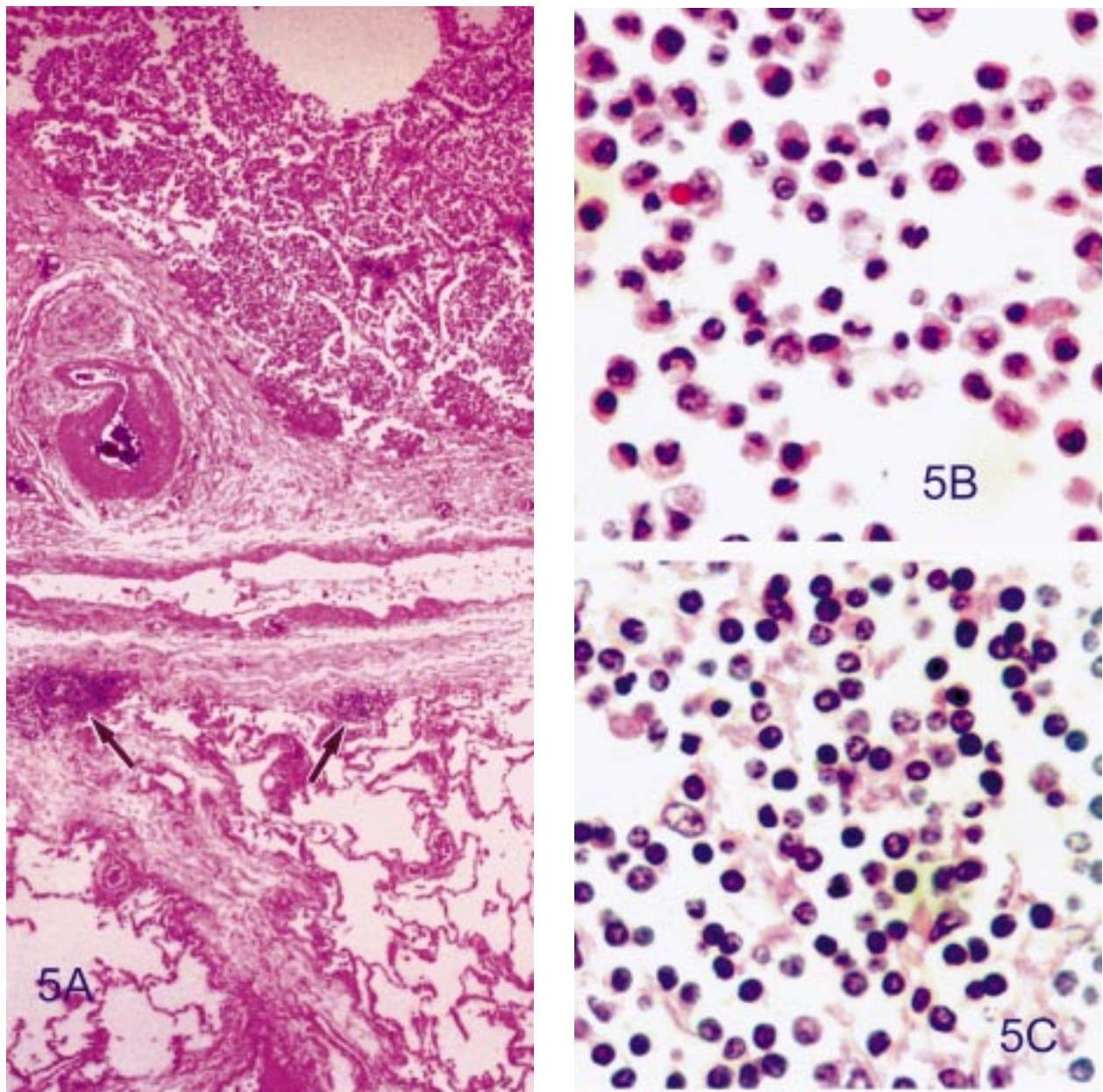


**Fig. 4.** Cryoglobulin quantitation and serum alanine aminotransferase (ALT) levels during and following discontinuance of therapy with IFN (A). Treatment with interferon-alpha was given during the first 5 months, as shown. Although no clinical or apparent biochemical response was seen, there was a striking redistribution of HCV RNA from the cryoprecipitate to the serum supernatant (B), possibly due to altered stoichiometry of cryoprecipitable and noncryoprecipitable HCV-containing immune-complexes. In retrospect, pulmonary infiltrates may have been a manifestation of the disease (86), and the striking increase in proteinuria, ALT levels (which had never been abnormal previously), cryoglobulins, and total HCV copy number (not shown) following discontinuance of IFN $\alpha$  may have been a "rebound" phenomenon (80). Normal levels for ALT are 0–40 IU/L.

Leg ulcers required increasingly aggressive wound care over the three months before her final admission, during which time period an elevation of  $\gamma$ -glutamyl transpeptidase (GGTP) — 172 IU/L (normal range, 8–69) and increasing IgM (to 525 mg/dL) were noted; HCV RNA — 690, 885 copies/mL; 24-hour protein — 6600 mg; Ccr — 23 mL/min. Two months previously, she was admitted to another hospital for fever to 102°C, chills, cough, anasarca and dyspnea, with new right perihilar and left upper lobe infiltrates and bilateral pleural effusions on chest X-ray and CT of the chest. She was treated initially with ciprofloxacin for a presumed community-acquired pneumonia; however, all cultures were negative. She then received high doses of intravenous corticosteroids, without any favorable response, but with increasing azotemia and hyperglycemia. On transfer to The Mount Sinai Hospital, leg ulcers and active lower extremity purpura were again noted; BUN — 81 mg/dL (normal range, 8–26); AST — 131 IU/L (5–40); ALT — 138 IU/L (0–40); alkaline phosphatase — 419 IU/L (35–125); GGTP — 2409 IU/L; albumin — 2.6 g/dL; IgM — 700 mg/dL; cryoglobulins — 1.29 mg/mL. A follow-up bone marrow aspiration and biopsy, were negative; however, a monoclonal B-cell population was identified by flow cytometry. Abdominal ultrasound and MRI were both consistent with cirrhosis with ascites. Laparoscopic liver and kidney biopsies confirmed active hepatitis, now with septal fibrosis, and hepatic glomerulosclerosis. Steroids were gradually tapered, and plasmapheresis initiated to reduce the level of cryoglobulins. Hospital course was complicated by treatment for purulent drainage of leg ulcers culturing methicillin-resistant *Staph. aureus* and *Pseudomonas*. Preterminally, she became acutely short of breath and was found to have new right upper lobe consolidation on chest X-ray. She died of septic shock, with blood cultures positive for *Pseudomonas*, in spite of broad-spectrum antibiotic coverage, and hemodynamic and ventilator support.

### Autopsy

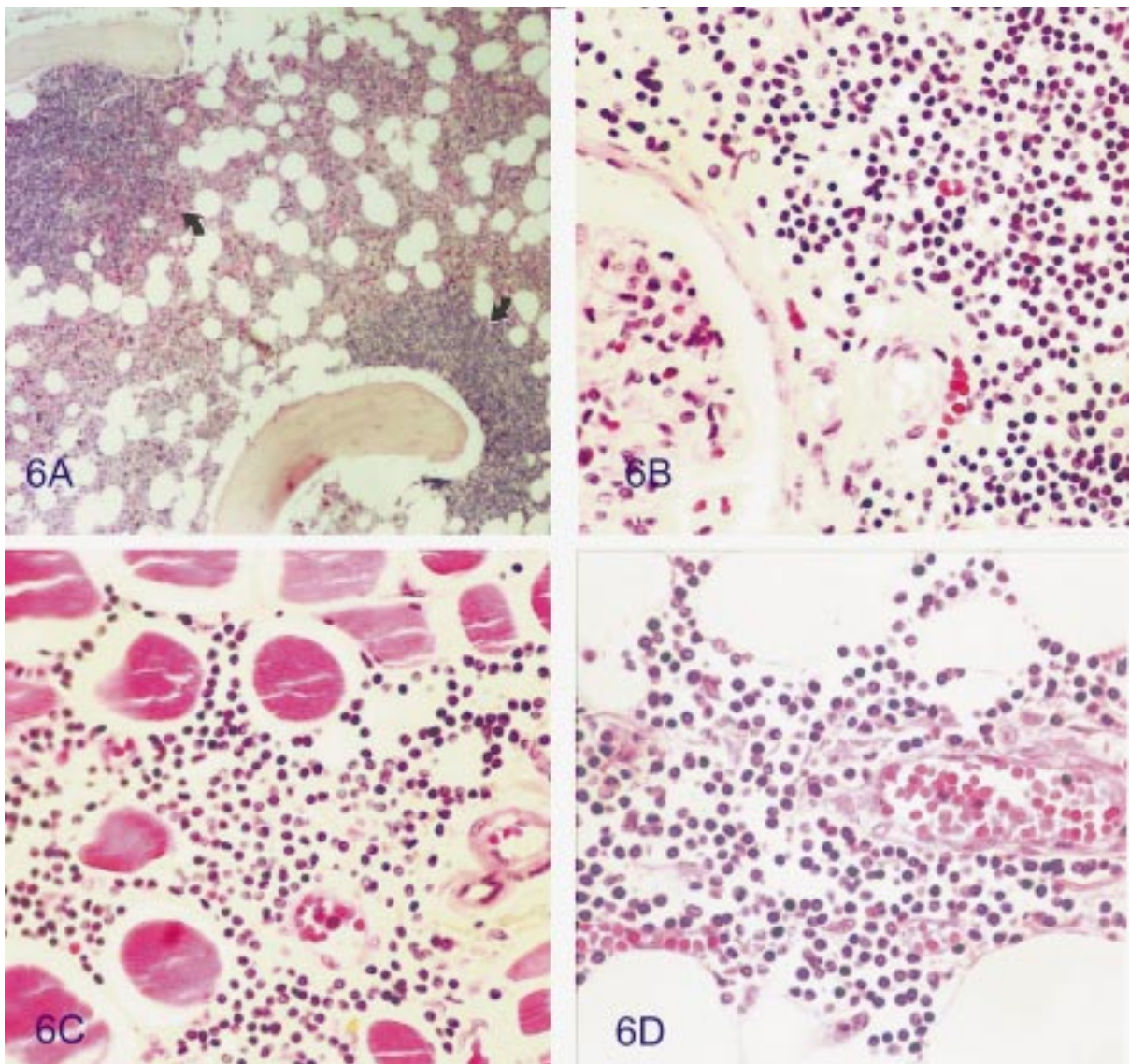
The right upper lobe infiltrate was a severe bronchopneumonia (Fig. 5A) which had disseminated to eventuate in septic shock; no viral inclusions or hyphae were found. In addition to the lobar pneumonia (Fig. 5B), diffuse adenopathy and foci of chronic inflammation, consisting mostly of mature lymphocytes (Fig. 5B), were also noted. Careful review of bone marrow at several sites failed to confirm lymphomatous



**Fig. 5.** Sections of the right lung taken at autopsy: (A) At low power, portions of the upper lobe (top) and middle lobe (bottom) are separated by the sparsely cellular interlobar septum (horizontal band extending across the middle of the figure). The upper lobe alveoli contain a dense infiltrate of acute inflammatory cells and debris (H&E, 20x). Normally, expanded alveoli in the middle lobe are filled with air. (B) At higher magnification, the intra-alveolar inflammatory cells are almost exclusively neutrophils, with multilobated nuclei and ample cytoplasm; (H&E, 400x). By contrast, foci of chronic inflammation seen within the interlobar septum (see arrows in A) are composed of lymphocytes, with a single round nucleus and a narrow rim of cytoplasm (H&E, 400x). (C) In contrast, foci of chronic inflammation in the interlobar septum (arrows in 5A) consist of lymphocytes with a single round nucleus and a narrow row of cytoplasm (H&E, 400x).

involvement that had been seen previously (Fig. 6A). The spleen was congested without evidence of malignant transformation; however (consistent with the previous CT findings), several lymph nodes were enlarged, with effacement of normal architecture by mature-appearing lymphocytes, collections of which were also noted at several other sites (Figs. 6 B–D). The left kidney had a 6

x 4 x 4 cm retention cyst in its upper lobe; renal vasculature was diffusely involved by moderate arterial and arteriolar intimal thickening with hyaline deposition and focal fibrinoid necrosis. Glomeruli showed mild mesangial widening with pale reticulated matrix and lobulation, consistent with hepatic glomerulosclerosis. The liver weighed 2,300 grams and showed diffuse conges-



**Fig. 6.** (A) A bone marrow biopsy done at the time of initial evaluation in 1993 revealed dense aggregates of darkly staining cells (arrows) adjacent to the bony trabeculae (H&E, 40x). Higher magnification revealed small mature lymphocytes, consistent with a diagnosis of chronic lymphocytic leukemia/small lymphocytic lymphoma. Sections taken at autopsy reveal dense infiltrates of lymphocytes in a variety of tissues, including kidney (B), skeletal muscle (C), and mesenteric soft tissue (D) (all H&E, 200x).

tion and steatosis; on cut surface, there was interstitial fibrosis, but not nodular cirrhosis; microscopically, portal spaces were found to be involved with dense collections of mature-appearing lymphocytes (Fig. 7A), in addition to incomplete bridging portal fibrosis and bile ductular proliferation (Fig. 7B).

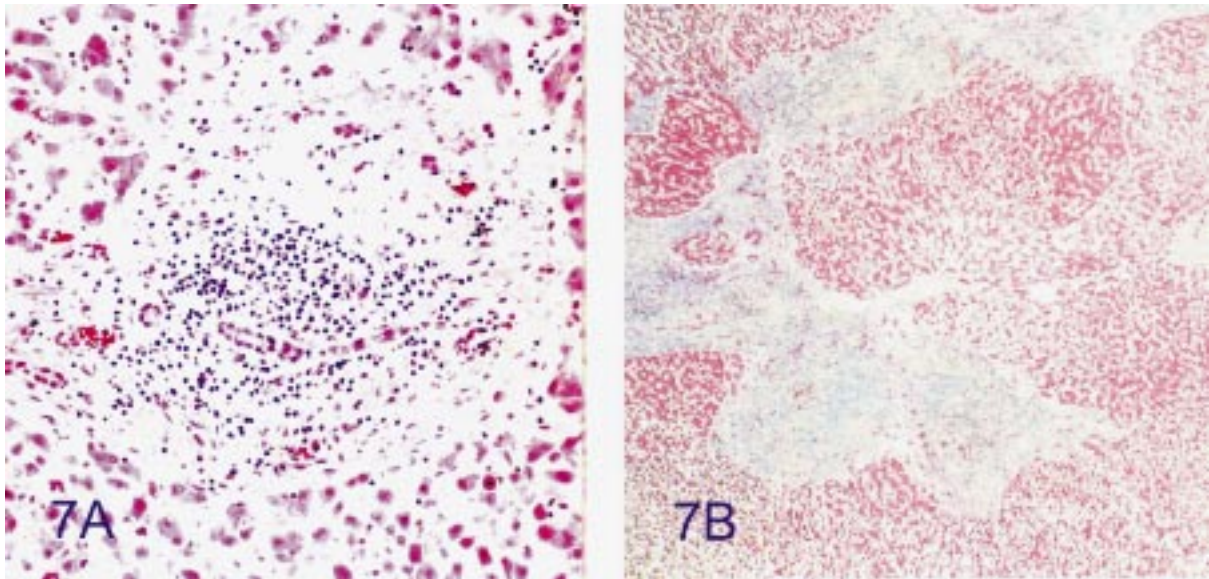
### Comments

Salient clinical, laboratory and pathologic features of the syndrome of mixed cryoglobulinemia (MC) (10) for our patient are summarized in Table 1. Salient features of her case history include:

**TABLE 1**  
*Mixed Cryoglobulinemia*

- Cutaneous vasculitis-purpura/leg ulcers
- Arthralgia/arthritis
- Immune complex nephritis
- Neuropathy
- Inflammatory liver disease
- IgM rheumatoid factor
- Hypocomplementemia
- Type II mixed cryoglobulins

1. A diagnosis of cutaneous vasculitis and cryoglobulinemia was only made five years after



**Fig. 7.** Liver sections taken at autopsy: (A) A portal tract shows enlargement with fibrosis and a lymphoid aggregate surrounding a bile duct (H&E, 200x). (B) Under lower magnification, fibrous septa are seen as blue bands amid the red hepatic parenchyma, bridging adjacent portal tracts (Masson's trichrome, 20x).

- the onset of symptoms, when the patient sought care for leg ulcers (Fig. 1A),
2. Sensorimotor neuropathy was clinically subtle and did not show progression on serial electrophysiologic studies.
3. HCV infection was demonstrated serologically, as well as by PCR testing of serum, isolated cryoprecipitates, peripheral blood mononuclear cells, and by RT *in situ* PCR of liver tissue. HCV infection was not due to intravenous drug abuse, co-existent HIV infection, or known prior blood transfusions. It progressed to early fibrosis over a three-year period, and there was no history of alcohol abuse.
4. There was no biochemical or virologic response to a trial of IFN $\alpha$ , which however had to be discontinued because of pulmonary abnormalities (Figs. 3 and 4A) that may in retrospect have been due to underlying disease.
5. It is important to carefully evaluate individual patients for underlying renal disease. In this case, proteinuria and progressive deterioration of renal function turned out to be due to hypertension, a retention cyst, and was preterminally aggravated by supervening hepatic glomerulosclerosis. There was no evidence for immune-complex glomerulonephritis or cryoglobulin deposition by immunohistology or electron microscopy.
6. B-cell proliferation was clonal on initial bone marrow examination (Fig. 6A) and evidenced in the persistent presence of an IgM $\kappa$  monoclonal cryoprotein. It was also oligo- or polyclonal in the presence of mature lymphocytes, positive for both kappa and lambda light chain determinants, at multiple sites on autopsy (Fig. 6B–D).

### HCV and Autoimmunity

The acute phase of viral hepatitis infections may be associated with a significant elevation in IgM levels, which in part reflects early IgM antibody responses to various viral antigens. In chronic HCV infection, a polyclonal increase in IgM may be seen, which is due in part to antibody production, now including autoantibodies, most notably IgM antiglobulins (i.e., RFs) reactive with the Fc portion of IgG. Some of these RFs form cryoprecipitable immune complexes on binding to their antigen (i.e., IgG), which may result in type II (monoclonal IgM) or type III (polyclonal IgM) mixed cryoglobulins (11–13).

The overall incidence of cryoglobulinemia in chronic HCV infection has ranged from 13–54% in different series (14–16). Although a correlation between the presence of cryoglobulins, duration of disease, severity of liver pathology, and the presence of extrahepatic manifestations of disease has been made, it is inexact and may be violated in individual cases. The distinction between Types II and III cryoglobulins is generally made by

immunofixation analysis of isolated cryoprecipitates, looking for signature clonal Ig  $\mu$  heavy chain and  $\kappa$  light chain bands; this type of analysis still allows for oligoclonality that might be revealed by more sensitive techniques such as two-dimensional gel electrophoresis or direct sequencing of separated heavy or light chain proteins (17). Although Type II cryoglobulins have been correlated with more severe disease, and particularly with the syndrome of mixed cryoglobulinemia (above), careful longitudinal studies to establish the evolution of a polyclonal IgM antiglobulin response to the selective V-region gene usage characteristic of monoclonal cryoprecipitable IgM RFs have yet to be carried out (18, 19).

Autoantibody, and in particular RF, activity may be seen in the course of various chronic viremias (notably Epstein-Barr), and may complicate the interpretation of these tests, which are often erroneously thought to be diagnostic of specific connective tissue diseases. In some series, chronic HCV infection, in particular, has been associated with a high incidence of other serologic indices of autoimmunity and immune-complex formation (Table 2), in most instances not correlating with overt extrahepatic disease (11, 13, 20–24).

The mechanisms responsible for the expansion of autoantibody-producing B-cells in chronic HCV infection remain conjectural. Possibilities include: (a) attenuation of dominant T-cell suppression of B-cells producing autoantibodies during chronic infection, as has been suggested for Epstein-Barr virus; (b) expansion of autoantibody-producing B-cells due to dysregulation of anti-idiotypic networks; (c) molecular mimicry between viral and self-antigens; (d) direct modulation of immune responses by immune complexes or fragments of Ig (e.g., Fc) or complement components that may be generated in the course of infection; (e) dysregulation of cytokine networks skewing regulatory T-cells to a Th2 phenotype, which may be associated with enhanced

humoral immune responses and autoantibody production; (f) direct infection of specific subsets of B- or T-lymphocytes and/or other mononuclear cells; and (g) host genetic factors (e.g., HLA) which may influence the ability of the host to clear virus or sustain humoral or cell-mediated immune responses.

### The Spectrum of Extrahepatic HCV Infection

A number of recent reviews have summarized an expanding spectrum of disorders, including several recognized rheumatic diseases (Table 3), with which HCV has been associated (25–27). Few approximate the association seen in the syndrome of MC, in which 60–80% of patients can be shown to be HCVAb and/or HCV RNA positive (28). MC, membranoproliferative glomerulonephritis, cutaneous vasculitis, and peripheral neuropathy provide overlapping syndromes in which HCV should be considered in planning diagnostic work-ups, and in which cryoglobulins may be detected in blood. In other instances, reports remain largely anecdotal or have not been uniformly confirmed. Only rarely has a defined rheumatic disease been reported to clearly follow acute HCV infection (29). Conversely, the prevalence of clinically apparent extrahepatic disease among patients chronically infected by HCV, though estimated to be less than 5% (2), has yet to be carefully delineated.

### HCV and Lymphoproliferation

It has been recognized for a number of years that monoclonal mixed cryoglobulins, as well as clinical features of the syndrome of mixed cryoglobulinemia, may be associated with lymphoproliferative disorders, notably including Waldenström's macroglobulinemia, chronic lymphocytic

**TABLE 2**  
*Immunologic Manifestations of Hepatitis C Infection*

- IgM-containing immune complexes
- Cold-dependent activation of complement
- Rheumatoid factor (~70%)\*
- Antitissue antibodies (40–50%)
- Lymphocytic sialoadenitis\* (~50%)
- Anticardiolipin antibodies\* (~20%)

\* In most instances not associated with clinical rheumatoid arthritis or Sjögren's syndrome, and very rarely with antiphospholipid antibody syndrome

**TABLE 3**  
*HCV and Immunologic Diseases*

- Autoimmune hepatitis
- Membranoproliferative glomerulonephritis
- Mixed cryoglobulinemia
- Polyarthritides
  - rheumatoid-like
  - adult stills disease (†)
- Polyarteritis nodosa (\*)
- Sjögren's syndrome (\*)
- Behçet's disease (†)
- Polymyositis (†)
- Vasculitis (†)

(\*) Controversial  
(†) Case Reports

leukemia, angioblastic lymphadenopathy, and non-Hodgkin's lymphoma (NHL) (30). Molecular analysis of Ig variable region sequences in some of these disorders has demonstrated selective patterns of usage that are also seen in patients with the clinical syndrome of MC (see above). At the antigenic level, selective V-region gene usage is manifest as shared idiotypes (i.e., cross-reactive idiotypes [CRIs]) that occur on both the heavy and light chains of the IgM RFs in the mixed cryoglobulins. These CRIs also occur with increased frequency in the serum of patients with chronic lymphatic leukemia (CLL) and NHL (18). A high incidence of multifocal lymphoid aggregates on bone marrow examination, often with a paratrabecular localization, that appear frequently to be immunohistologically monotypic, correlating with the clonality of Type II cryoglobulins, has been reported among MC patients (31, 32). The overall incidence of NHL among MC has been reported to be as high as 38.7% in one Italian series, virtually all HCV RNA-positive (33). By contrast, MC is rarely associated with other malignancies, notably including hepatocellular carcinoma. These observations suggest that HCV may be a linking and potentially etiologic factor for these disorders.

An increased prevalence of HCVAb and/or HCV RNA among patients with NHL was first reported in 1994 from Italy (34), where HCV and MC were known to be endemic. It was subsequently confirmed in small series from a number of different geographic regions of Italy. In an early review of the Italian reports, 434/2274 (19.1%) cases of B-cell NHL were HCV-positive, and 41.2% of those analyzed were associated with cryoglobulins (35). By contrast, HCV was not significantly associated with Hodgkin's disease or T-cell NHL. Associations with multiple myeloma or monoclonal gammopathy of undetermined significance (MGUS) have been found in some, but not all, series (36, 37). Linkage between HCV and NHL have subsequently also been reported from Japan, the United States, France and Turkey (38–41), but have not been confirmed in other series from the United Kingdom, the Netherlands, the United States and Canada (42–45).

Among patients with NHL found to be HCV-positive, more specific associations with low-grade disease, the lymphoplasmacytoid lymphoma/immunocytoma phenotype, and extranodal localization have been found (46–48). Reports of an increased prevalence of HCV among mucosa-associated lymphoid tissue (MALT) lymphomas (49), although reinforcing the concept that progression to lymphoma may be antigen-dri-

ven and oligoclonal by analogy to emerging information regarding MALT tumors associated with *Helicobacter pylori* infection (50), have not been universally confirmed (51). Conversely, as seen in the follow-up of our patient, chronic HCV infection and mixed cryoglobulinemia may manifest as clonal B-cell proliferation by flow cytometry of bone marrow, even in the absence of morphology indicative of NHL (52). More generally, NHL and HCV-infected patients without cryoglobulinemia have evidence of clonal B-cell proliferation (53), selective heavy and light chain V-region usage, and somatic hypermutation, assessed by Ig gene rearrangement and direct sequence analysis of V-region genes in patients with immunocytomas (18, 54).

The mechanisms that may be responsible for a relationship between HCV and NHL remain uncertain. A number of studies have described localization of HCV to peripheral blood mononuclear cell (PBMC) populations, both in MC patients (55, 56) (including our patient) and those with chronic HCV without cryoglobulinemia (57); specific concentration of viral RNA in CD19-positive B-cells (56, 58) and pluripotent hematopoietic CD34-positive stem cells (59) has been reported. Although these observations have been widely cited as substantiating HCV to be a lymphotropic virus, non-specific adhesion of viral RNA and vicarious endocytosis of HCV-containing immune complexes have not been rigorously excluded as relevant mechanisms (60). Recently, specific binding between HCV envelope protein E2 and CD81, a surface marker present on several peripheral blood monocytic cell (including B-cell) populations, has been reported (61). Similarly, although positive findings have been reported for *in situ* hybridization of PBMC (62), viral RNA has not been shown to be specifically enriched in malignant lymphocytes, and the presence of replicative intermediates within selected cell populations has only been shown in limited studies (60, 63).

The possibility remains that some genotypes (e.g., 2a) (64, 65) or quasi-species of HCV may be more selectively lymphotropic (also explaining the relative lack of clinical hepatitis among the HCV-positive NHL patients) than others. Liver damage in patients with MC may reflect intrahepatic B-cell clonal expansions, and cytokine-mediated inflammatory effects of the often striking lymphocytic infiltration of liver, rather than hepatonecrosis (66, 67). Proliferating lymphocytes have been reported to be infected with HCV and to selectively express mixed cryoglobulin cross-reactive idiomotype (MC CRI), reinforcing in turn an etiologic linkage between the two (66). Exactly how

HCV may in fact trigger oncogenic transformation of B-cells remains a critical issue. Of particular interest in this regard is a series of recent studies that have overexpressed HCV core protein in cell lines, and demonstrated a variety of concomitant biological effects (Table 4) that link inhibition of apoptosis and oncogenic potential (68–71). The relevance of these intriguing observations to natural infection remains to be established.

### Treatment

The clinical course and outcome of the patient reported above raises several issues as to appropriate therapy targeted specifically to eradication of HCV and lymphoproliferation in this setting. These include:

1. MC may be an indolent disease, with a long survival (up to 45 years from onset in our experience) even without any treatment (30, 72).
2. Overall, renal involvement, in the form of membranoproliferative glomerulonephritis, has a negative impact on survival, though kidney disease may remain stable over long periods of time, especially when hypertension is adequately controlled (30, 72). Although proteinuria and hematuria initially suggested the possibility of glomerulonephritis in our patient, this was not corroborated pathologically.
3. Necrotizing vasculitis may be entirely cutaneous in some patients; a more bland obliterative vasculopathy has also been described (30). Although vasculitis of the vasa nervorum has been shown in some patients with neuropathy, in other instances different mechanisms (including cellular immune reactions) may result in neuronal damage (73, 74).
4. Although corticosteroids and cytotoxic agents may be effective treatments for some of the manifestations of MC, they pose a problem

with regard to eradication of HCV because of the potential for elevating viral count while masking hepatic inflammation by normalizing liver function tests (75). Immunodeficient persons (e.g., co-infected by HIV) have a higher incidence of HCV viremia, higher viral titers, and in some series a higher incidence of cryoglobulinemia (76).

5. Biological efficacy of IFN $\alpha$  in MC may be due to its antiviral, antiproliferative or immunologic effects. The latter include upregulation of HLA antigens, direct effects on Ig production by B-cells, induction of regulatory T Helper (Th1-like) cells (associated with protective antiviral immune responses), and activation of natural killer (NK) cells (77). The importance of the antiproliferative effect may be reflected in the regression of B-cell proliferation in some patients with MC, in response to this agent (78).
6. Problems with IFN $\alpha$  as monotherapy for HCV infections include:
  - a. Lack of response in some patients, possibly due to viral or host genetic factors. Thus, HCV genotyping as 1b in our patient may have predicted a relatively poor response to IFN based on past studies, even though 70–80% of the North American infected population are genotypes 1a/1b (79). Although she appeared not to respond clinically, or with regard to cryoglobulin quantitation or HCV copy number (Fig. 4A), it is likely that IFN was having some suppressive effects, evidenced in a redistribution of viral RNA from the cryoprecipitate to the serum supernatant (Fig. 4B), and a striking “rebound phenomenon” following discontinuance of the drug (Fig. 4A). It has previously been noted that a significant proportion of patients who start out with normal ALT levels may develop elevations in LFTs *de novo*, either during or after treatment with IFN $\alpha$  (80). Other host factors that have been implicated as affecting responsiveness to IFN $\alpha$  include expression of specific receptors in liver (81), interleukin-10 promoter polymorphisms (82), and specific major histocompatibility complex (MHC) Class II alleles (83).
  - b. Uncovering of autoimmune phenomena (e.g., anti-insulin, antithyroid or antinu-

**TABLE 4**

*Biological Properties of HCV Core Protein*

- 
- Inhibits cisplatin and c-myc-mediated apoptotic cell death
  - Represses p53 transcription
  - Transforms primary rat embryo fibroblasts to a tumorigenic phenotype
  - Interacts with cytoplasmic tail of lymphotoxin- $\beta$  receptor
  - Inhibits TNF-alpha-mediated apoptosis
  - Associates to cytoplasmic lipid storage droplets and ApoAII
  - Inhibits Fas and tumor necrosis factor alpha-mediated apoptosis via NF- $\kappa$ B activation
-

clear antibodies) or frank autoimmune disease (e.g., lupus erythematosus) in other patients (84, 85), some of which may mimic the clinical manifestations of MC. As with our patient, one previous report has recorded lethal pulmonary vasculitis as a manifestation of MC unresponsive to IFN treatment, with evidence of a striking lymphocytic vasculopathy at postmortem (86)

- c. Other toxicities (84).
- d. A high rate of relapse, which may be due in part to escape of viral mutants, or quasi-species of the virus, from reservoirs in serum, liver, cryoprecipitates, or PBMC (57, 87). The escape results in turn from poor editing by the viral RNA-dependent RNA polymerase, reflected in a mutation rate of  $10^{-3}$  to  $10^{-4}$  base substitutions per genome site per year (88).

Plasmapheresis provides an adjunctive therapy for MC that should be combined with more specific treatment of the underlying disease, and may be complicated by rebound on discontinuance. Cryopheresis is still employed in some centers as a potentially more effective modification of this therapeutic modality (89). Alternative therapies that have been used for other forms of vasculitis, such as colchicine, antimalarials, or thalidomide, either have not been effective or remain untested. As noted above, antiviral agents, such as IFN and ribavirin, may have significant immunomodulatory effects (77, 90) that influence HCV pathology, and have been used in combination therapy with promising results as an initial treatment, for patients failing IFN alone, and in MC (91–93). Other therapies (Table 5) remain investigational (7).

### Conclusions

The syndrome of MC is an interface between autoimmunity and lymphoproliferation that is sig-

nificantly associated with chronic infection by HCV. The hallmark of the B-cell response in this disorder is clonality, specifically involving the IgM isotype, and measured in part by the identification of RFs that become cold-precipitable when complexed with IgG. By contrast, the peripheral blood T-cell response in this disorder appears grossly to be normal. HCV could potentially drive this process by chronic antigenic stimulation, which may undergo subtle variations over time, reflecting the virus' high mutation rate; it may also stimulate polyclonal B-cell proliferation or directly infect specific PBMC populations. In some cases, this eventuates in frank NHL, which may be demonstrated early by bone marrow examination, or become apparent after prolonged periods of infection. This outcome of chronic HCV infection appears to be distinct from the progression to fibrotic liver disease, frank cirrhosis and hepatocellular carcinoma. Eradication of virus would appear to be a desirable goal that will reduce both adverse outcomes, though a significant role for immunomodulatory effects of agents such as IFN and ribavirin has not been discounted.

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**TABLE 5**  
*Investigational Therapies*

<ul style="list-style-type: none"> <li>• Various IFN preparations/regimens</li> <li>• Ribavirin/combination therapy</li> <li>• Protease/helicase inhibitors</li> <li>• Antisense oligonucleotides</li> <li>• Glycosylated E1/E2 complexes or recombinant capsid surrogates for vaccination</li> <li>• DNA vaccines</li> <li>• Adjunctive cytokine therapy</li> </ul>
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