Case Report

Persistent Cryoglobulinemic Vasculitis Following Successful Treatment of Hepatitis C Virus

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ABSTRACT. There is a well established link between type II mixed cryoglobulinemia (MC) and hepatitis C virus (HCV) infection, and HCV is believed to be the cause of cryoprotein formation and tissue deposition. Successful treatment of HCV infection has resulted in resolution of cryoglobulinemia and vasculitis. We describe 4 patients who had persistent MC and vasculitis despite successful eradication of HCV with antiviral therapy. (J Rheumatol 2005;32:1164–7)

Key Indexing Terms:
CRYOGLOBULINEMIA HEPATITIS C VIRUS VASCULITIS

Hepatitis C virus (HCV), a worldwide pathogen, is the leading cause for endstage liver disease and indication for liver transplant in the United States. HCV is also associated with a variety of immunologic extrahepatic manifestations, the best studied of which is the presence of elevated cryoglobulins in the serum. While up to 40%–50% of HCV infected patients have elevated levels of circulating cryoglobulins[1,2], only a small proportion develop vasculitic sequelae in target organs such as the skin, peripheral nerve, and kidney[3]. There is strong evidence, particularly from studies of the composition of the cryoglobulins themselves[2] as well as from immunohistologic studies of tissues such as skin[4], that HCV is a cause of the formation of the cryoprotein and ultimately its deposition in the target tissues.

The most common type of cryoglobulinemia in patients with HCV is type II mixed cryoglobulinemia (MC), characterized by the presence of polyclonal Ig in association with a monoclonal Ig (typically IgM or IgG). Treatment of cryoglobulinemia is indicated when progressive organ-threatening disease is present. Prior to the association of MC with HCV, treatment generally consisted of high dose glucocorticoids, cytotoxic agents, and plasmapheresis. While described as effective, this therapy was often only transiently beneficial[5,6]. However, therapy directed against HCV with interferon-α (IFN-α) and more recently combined IFN-α and ribavirin has offered a new strategy for HCV associated cryoglobulinemia. This therapeutic strategy has been reported to be successful at achieving remission for HCV related MC[7-11].

In virtually all reports of successful treatment of HCV associated cryoglobulinemic vasculitis there has been a strong correlation between antiviral response and vasculitic response[11-13]. Particularly with cutaneous vasculitis, a sustained virologic response has been associated with effective and enduring remissions. In contrast, patients who either do not achieve an antiviral effect or who experience a relapse will have incomplete or transient improvement in the vasculitis[7,8,14-17]. Thus recurrence of symptoms attributable to cryoglobulinemia is paralleled by recurrence of detectable HCV RNA in those who have received IFN therapy[7].

We recently identified 4 patients from 3 centers who had persistent symptomatic cryoglobulinemia despite successful and sustained HCV eradication with antiviral therapy. Data from these 4 patients were collected retrospectively and form the basis of this observational report.

CASE REPORTS

Pertinent details for each case are listed in Table 1.

Patient 1. A 49-year-old man was diagnosed with HCV infection (genotype 2b) in 1999. In May 2001 he developed lower extremity petechiae and was found to have type II MC with an IgM kappa monoclonal component. Thrombocytopenia subsequently developed and he was treated with prednisone, which promptly improved the platelet count although petechiae persisted. Before antiviral therapy the viral load by HCV RNA assay (Roche Amplicor HCV Monitor) was > 850,000 IU/ml. He received a 24-week course of pegylated IFN-α and ribavirin starting in October 2002. Lower extremity petechiae resolved and subsequent testing for HCV RNA (Amplicor) revealed no detectable virus (sensitivity of < 600 IU/ml), but persistence of cryoglobulins. The cryocrit prior to antiviral therapy was 23% and remained between 10% and 20% throughout the period of observation. The levels of C4 were < 10 mg/dl (normal 16–38) before and after antiviral therapy. In July 2003, 4 months after cessation of antiviral thera-

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Accepted for publication January 24, 2005.

Table 1. Patient characteristics.

	Patient 1	Patient 2	Patient 3	Patient 4
Age/sex	49, M	48, M	52, F	52, F
Duration of HCV treatment, mo	6	18	12	12
HCV-RNA after antiviral therapy	Negative at 18 mo	Negative at 24 mo	Negative at 12 mo	Negative at 9 mo
Type II cryoglobulin	Polyclonal IgG+ monoclonal IgM kappa	Polyclonal IgG+ monoclonal IgM kappa	Polyclonal IgG+ monoclonal IgM kappa	Polyclonal IgG+ monoclonal IgM kappa

py, he developed multiple painful lower extremity ulcerations that failed to heal despite daily doses of 20 mg prednisone. Serum HCV RNA was negative (Amplicor and NGI assays) and remained undetectable on serial examination over the ensuing months, although high titers of MC persisted. Laboratory studies at the time the ulcers developed revealed a platelet count of 54,000 and urinalysis with 10–50 red blood cells, 5–10 white blood cells, and 2+ protein. A bone marrow biopsy performed to exclude malignancy revealed hypercellularity with benign-appearing lymphoid aggregates. Flow cytometry of the bone marrow showed that the B cells mainly expressed kappa light chains consistent with a lymphoproliferative disorder of B cell kappa lineage. Computerized tomographic (CT) scans of the chest, abdomen, and pelvis were normal. He was referred to the National Institutes of Health (NIH) for enrollment in a trial studying the use of anti-CD20 antibody (rituximab) in the treatment of HCV related MC. The cryoprecipitate tested alone for HCV by branched DNA assay was negative (sensitivity < 615 IU/ml; performed by Dr. M. Sneller, NIH).

Patient 2. A 48-year-old man was diagnosed with HCV in 1997. He was treated with IFN-α monotherapy for 18 months, which resulted in undetectable HCV RNA (Roche Amplicor HCV Monitor, sensitivity < 600 IU/ml). For many years prior to antiviral therapy he had experienced recurrent purpuric lesions mainly on the lower extremities, several episodes of monoarthritis, and a peripheral sensory polyneuropathy. Despite 5 negative serum HCV RNA assays (Amplicor) over a 24 month period, he was found to have increasing titers of type II MC with an IgM kappa monoclonal component. Prior to antiviral therapy, cryoglobulin concentrations were 5000 µg/ml, and rose to 10,260 µg/ml 18 months after antiviral therapy. The C4 level was reduced, at 14 mg/ml (normal 16–64 mg/ml) after antiviral therapy, and the cryoprecipitate tested alone for the presence of HCV RNA (Amplicor HCV v2.0, sensitivity < 50 IU/ml) was negative. Body CT scans were performed to exclude a B cell malignancy and were unremarkable. A bone marrow aspirate was normocellular and revealed 2 lymphoid aggregates consisting of predominantly small morphologically benign lymphocytes. Flow cytometry analysis of the marrow revealed a clonal IgM kappa B cell population. Despite a slow increase in cryoglobulin titers his only symptoms continue to be a mild sensory polyneuropathy and rare intermittent crops of inflammatory purpura.

Patient 3. The diagnosis of HCV in a 52-year-old woman was established in 1999 after she developed lower extremity palpable purpura that was biopsied and found to be leukocytoclastic vasculitis. Type II cryoglobulins were identified with an IgM kappa monoclonal component (titer 1120 µg/ml, normal 0–50 µg/ml), and C4 was < 2 mg/ml (normal 16–64). She received one year of treatment with pegylated IFN-α and ribavirin ending in September 2002. Immediately and 6 months after antiviral treatment she had a negative assay for HCV RNA (Roche Amplicor, sensitivity < 600 IU/ml). One year after antiviral therapy she developed dyspnea, edema, diarrhea, emesis, numbness in the feet and hands, and recurrence of lower extremity purpura. Serum creatinine was elevated and continued to rise over the next 4 weeks to 6 mg/dl, necessitating hemodialysis. A kidney biopsy revealed obliteration of the capillary lumens due to the presence of intracapillary eosinophilic material that was periodic acid Schiff reaction positive. There was focal thickening of the glomerular basement membrane, and immunofluorescence showed 3+ IgG, 4+ IgM, 2+ IgA, and 2+ C3 granular mesangial and peripheral glomerular staining. Electron microscopy revealed electron-dense, nodular material in a subendothelial and intraluminal location consistent with cryoglobulin deposits. Type II cryoglobulins were again identified, with a titer of 1500 µg/ml. The cryoprecipitate alone was analyzed for HCV RNA (Amplicor HCV v2.0, sensitivity < 50 IU/ml) and was negative. A bone marrow biopsy showed a normocellular marrow with focal lymphoid aggregates of benign morphology. Flow cytometry and immunophenotypic studies showed a monoclonal IgM kappa B cell population that accounted for 0.2% of total cells. CT scans revealed small nonspecific lymphadenopathy, but no evidence of malignancy. She continued dialysis and plasmapheresis 3 times weekly. Prednisone was started and intravenous rituximab 375 mg/m^2 was given prior to discharge. The patient was lost to followup.

Patient 4. The patient was a 52-year-old white woman who was diagnosed with chronic HCV infection (genotype 1A) in the early 1990s. Over the years she experienced intermittent arthralgias, Raynaud's phenomenon, and a recurrent purpuric rash on her lower extremities that was treated with short courses of prednisone. In September 2000 her symptoms worsened markedly and her serum was found to be positive for MC (no titers available). Analysis of the cryoprotein showed polyclonal IgG and monoclonal IgM kappa. She was treated with thrice weekly IFN and ribavirin for 12 months. Before antiviral therapy, HCV RNA was > 500,000 IU/ml (Roche Amplicor, sensitivity < 600 IU/ml). During treatment, her arthralgias and rash completely resolved and HCV RNA became undetectable. After antiviral therapy the leukocytoclastic vasculitis returned. Testing for serum HCV RNA was persistently negative, but cryoglobulins remained positive. Levels of C4 were < 10 mg/ml (normal 10–50) on 2 occasions. She was referred for malignancy investigations and bone marrow biopsy but was lost to followup.

DISCUSSION

We describe 4 patients who had persistence of symptomatic HCV related cryoglobulinemia despite successful treatment of the virus with antiviral therapy. There was no serologic or clinical evidence to suspect an underlying connective tissue disease in these patients. It is notable that these 4 patients were identified from 3 separate academic medical centers after the emergence of a pattern of persistent cryoglobulinemic vasculitis following successful HCV therapy. All data were collected retrospectively, and as a result some pertinent information was not obtained and is not available. We describe these cases in spite of this limitation because it does not appear that this clinical presentation has been previously reported.

The cause of cryoglobulinemia in HCV infection is unknown, but several lines of evidence favor a direct role of HCV infection. These include the concentration of virus and

antibody in the cryoglobulin compared to serum, evidence from clinical trials that both cryoglobulins and cutaneous skin lesions diminish with reduced viral load as a result of effective antiviral therapy, and relapse of both cryoglobulin and cutaneous vasculitis with virologic relapse[15]. A role for lymphoproliferation has also been suggested and is supported by the observation that most patients with HCV associated cryoglobulinemic vasculitis have a clonal component in their cryoprotein generally consisting of a monoclonal IgM or IgG rheumatoid factor (type II cryoglobulin). These monoclonal proteins and the clonal B cells they are derived from often recede with successful IFN-α therapy[18].

One explanation for the clinical presentation in these patients is that residual virus was present and that HCV infection was not eradicated. Although we cannot rule this out, all 4 patients fulfilled the standard criterion for sustained virologic response that is defined as nondetectability of HCV in serum 6 months after completion of antiviral therapy[19]. We were not able to detect HCV in these patients by sensitive RNA assays over a period of time ranging from 9 to 24 months post-antiviral therapy. Since HCV RNA has been found to be concentrated within the cryoglobulins[2], the cryoglobulins were tested for HCV RNA after antiviral therapy in 3 patients and found to be negative. Recent studies have suggested the possibility of occult infection by even more sensitive assays as well as direct examination of hepatic tissue[20]. Although these more stringent studies were not performed in our patients, the negative HCV RNA results over a prolonged period of time are consistent with the standard definition of a sustained virologic response.

Another explanation for the clinical presentation in these patients is that they manifested a B cell lymphoproliferative disorder that was autonomous from the influence of ongoing HCV infection. HCV, independent of its relationship with cryoglobulinemia, is known to be a potent B cell stimulator and is associated with B cell non-Hodgkin's lymphoma (NHL)[21]. HCV related B cell NHL tends to be of a diffuse large-cell histotype and can develop without a history of low grade B cell malignancy or bone marrow involvement[22]. Cryoglobulins and autoimmune features are common in these cases and HCV infection is strongly associated with type II MC, which itself represents a de novo lymphoproliferative disorder[23-25]. A small proportion of patients with cryoglobulinemia (< 10%)[26,27] may go on to develop overt B cell malignancy, although the potential for progression is considered low[21,23-25]. Splenic lymphoma with villous lymphocytes has also been associated with HCV infection, and treatment with IFN has been shown to lead to lymphoma regression[28,29].

Patients with B cell proliferation may be at particular risk for the development of overt B cell neoplasms. Despite extensive investigation using clinical, histologic, immunochemical, and molecular techniques, we were unable to identify a neoplastic state in these patients. The 3 patients who underwent bone marrow examinations had no features of an overt B cell malignancy, but all had evidence of a clonal B cell population that was consistent with the monoclonal component of the mixed cryoglobulin. However, differentiation of patients with insipid or low grade lymphoproliferative disease from those with overt B cell malignancies is not always straightforward and in some cases it is somewhat subjective[22,25]. The bone marrow is frequently a site of florid B cell proliferation in MC[22]. De Vita, *et al* have described that in the majority of patients with MC, monoclonal and oligoclonal B cell expansions can be identified in the bone marrow and that the presence of histological features of low grade B cell lymphoproliferation is not predictive of the development of overt B cell neoplasms[30].

Sustained clearance of the virus with antiviral therapy has been the ultimate treatment goal of HCV related cryoglobulinemia, and viral elimination almost invariably leads to disappearance of the clinical manifestations of vasculitis[7–11]. The response of type II MC has been closely linked to the antiviral response in trials of IFN-α monotherapy, standard IFN-α plus ribavirin, and more recently pegylated-IFN and ribavirin[7-9]. Failure of the viral infection to respond has been linked to failure of vasculitis to respond (particularly cutaneous disease), and virologic relapse has been linked to flare of vasculitis[15]. Patients with type II MC who have a successful and sustained virologic and vasculitic response continue to be negative for cryoglobulins, and have regression of B cell monoclonal infiltrates in the bone marrow one year after completion of successful therapy[18]. It can be argued that it takes a period of some months for meaningful declines in rheumatoid factor and cryoglobulin concentrations following successful antiviral therapy. However, the patients in this report were followed from 9 to 24 months after the completion of antiviral therapy, and in all, the cryoglobulin titers were either the same or were increasing.

Failure of regression of both clinical lesions and cryoglobulins after successful antiviral therapy is unusual, but as shown by our cases it may be more common than previously thought. Therapy for the vasculitis in these patients presents a difficult challenge. Recent reports suggest that B cell-targeted therapy with anti-CD20 monoclonal antibody may be appropriate for these patients[31,32].

REFERENCES

1. Cacoub P, Renou C, Rosenthal E, et al. Extrahepatic manifestations associated with hepatitis C virus infection: a prospective multicenter study of 321 patients. Medicine Baltimore 2000; 79:47-56.
2. Lunel F, Musset L, Cacoub P, et al. Cryoglobulinemia in chronic liver diseases: role of hepatitis C virus and liver damage. Gastroenterology 1994;106:1291-300.
3. Cacoub P, Poynard T, Ghillani P, et al. Extrahepatic manifestations of chronic hepatitis C. Arthritis Rheum 1999;42:2204-12.
4. Agnello V, Abel G. Localization of hepatitis C virus in cutaneous vasculitic lesions in patients with type II cryoglobulinemia. Arthritis Rheum 1997;40:2007-15.

5. D'Amico G, Colasanti G, Ferrario F, Sinico RA. Renal involvement in essential mixed cryoglobulinemia. Kidney Int 1989;35:1004-14.
6. Frankel AH, Singer DR, Winearls CG, Evans DJ, Rees AJ, Pusey CD. Type II essential mixed cryoglobulinemia: Presentation, treatment and outcome in 13 patients. Q J Med 1992;82:101-24.
7. Misiani R, Bellavita P, Fenili D, et al. Interferon alpha-2a therapy in cryoglobulinemia associated with hepatitis C virus. N Engl J Med 1994;330:751-6.
8. Ferri C, Marzo E, Longombardo G, et al. Interferon-alpha in mixed cryoglobulinemia patients: A randomized crossover-controlled trial. Blood 1993;81:1132-6.
9. Zuckerman E, Keren D, Slobodin G, et al. Treatment of refractory, symptomatic, hepatitis C virus related mixed cryoglobulinemia with ribavirin and interferon-alpha. J Rheumatol 2000;27:2172-8.
10. Naarendorp M, Kallemuchikkal U, Nuovo GJ, Gorevic PD. Longterm efficacy of interferon-alpha for extrahepatic disease associated with hepatitis C virus infection. J Rheumatol 2001;28:2466-73.
11. Cacoub P, Lidove O, Maisonobe T, et al. Interferon-alpha and ribavirin treatment in patients with hepatitis C virus-related systemic vasculitis. Arthritis Rheum 2002;46:3317-26.
12. Rossi P, Bertani T, Baio P, et al. Hepatitis C virus-related cryoglobulinemic glomerulonephritis: long-term remission after antiviral therapy. Kidney Int 2003;63:2236-41.
13. Alric L, Plaisier E, Thebault S, et al. Influence of antiviral therapy in hepatitis C virus-associated cryoglobulinemic MPGN. Am J Kidney Dis 2004;43:617-23.
14. Dammacco F, Sansonno D, Han JH, et al. Natural interferon-alpha versus its combination with 6-methyl-prednisolone in the therapy of type II mixed cryoglobulinemia: a long-term, randomized, controlled study. Blood 1994;84:3336-43.
15. Vassilopoulos D, Calabrese LH. Hepatitis C virus infection and vasculitis. Arthritis Rheum 2002;46:585-97.
16. Casato M, Agnello V, Pucillo LP, et al. Predictors of long-term response to high-dose interferon therapy in type II cryoglobulinemia associated with hepatitis C virus infection. Blood 1997;90:3865-73.
17. Agnello V. Therapy for cryoglobulinemia secondary to hepatitis C virus: the need for tailored protocols and multiclinic studies. J Rheumatol 2000;27:2065-7.
18. Mazzaro C, Franzin F, Tulissi P, et al. Regression of monoclonal B-cell expansion in patients affected by mixed cryoglobulinemia responsive to interferon alpha therapy. Cancer 1996;77:2604-13.
19. Lindsay KL. Therapy of hepatitis C: overview. Hepatology 1997;26:71S-77S.
20. Lerat H, Hollinger FB. Hepatitis C virus occult infection or occult HCV-RNA detection? J Infect Dis 2004;189:3-6.
21. Musto P. Hepatitis C virus infection and B-cell non-Hodgkin's lymphomas: more than a simple association. Clin Lymphoma 2002;3:150-60.
22. De Vita S, Sacco C, Sansonno D, et al. Characterization of overt B-cell lymphomas in patients with hepatitis C virus infection. Blood 1997;90:776-82.
23. Pozzato G, Mazzaro C, Crovatto M, et al. Low grade malignant lymphoma, hepatitis C infection and mixed cryoglobulinemia. Blood 1994;84:3047-53.
24. Monteverde A, Sabattini E, Poggi S, et al. Bone marrow findings further support the hypothesis that essential mixed cryoglobulinemia type II is characterized by a monoclonal B-cell proliferation. Leuk Lymphoma 1995;20:119-24.
25. Rasul I, Shepherd FA, Kamel-Reid S, Krajden M, Pantalony D, Heathcote EJ. Detection of occult low-grade B-cell non-Hodgkin's lymphoma in patients with chronic hepatitis C infection and mixed cryoglobulinemia. Hepatology 1999;29:543-7.
26. Trejo O, Ramos-Casals M, Lopez-Guillermo A, et al. Hematologic malignancies in patients with cryoglobulinemia: association with autoimmune and chronic viral diseases. Semin Arthritis Rheum 2003;33:19-28.
27. Dammacco F, Sansonno D, Piccoli C, Tucci FA, Racanelli V. The cryoglobulins: an overview. Eur J Clin Invest 2001;31:628-38.
28. Hermine O, Lefrere F, Bronowicki JP, et al. Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. N Engl J Med 2002;347:89-94.
29. Saadoun D, Suarez F, Lefrere F, et al. Splenic lymphoma with villous lymphocytes, associated with type II cryoglobulinemia and HCV infection: a new entity? Blood 2005;105:74-6.
30. De Vita S, De Re V, Gasparotto D, et al. Oligoclonal non-neoplastic B cell expansion is the key feature of type II mixed cryoglobulinemia. Arthritis Rheum 2000;43:94-102.
31. Zaja F, De Vita S, Russo D, et al. Rituximab for the treatment of type II mixed cryoglobulinemia. Arthritis Rheum 2002;46:2252-4.
32. Sansonno D, De Re V, Lauletta G, Tucci F, Boiocchi M, Dammacco F. Monoclonal antibody treatment of mixed cryoglobulinemia resistant to interferon alpha with an anti-CD20. Blood 2003;101:3818-26.