Rituximab induces remission in refractory HCV associated cryoglobulinaemic vasculitis


Objectives: To report the successful induction of remission with the monoclonal anti-CD20 antibody rituximab in a patient with hepatitis C virus (HCV) associated cryoglobulinaemic vasculitis and a non-Hodgkin’s lymphoma (NHL) resistant to previously advocated conventional treatments.

Case report: The patient was a 45 year old woman with HCV associated cryoglobulinaemic vasculitis, with purpura, arthralgia, constitutional symptoms, and a polyneuropathy. A malignant NHL was found as underlying lymphoproliferative disease. At this stage the disease was refractory to interferon α2b and ribavirin and to subsequent immunosuppressive treatment with cyclophosphamide. Six rituximab infusions targeting the CD20 antigen on cells of the B cell lineage induced remission of the vasculitis. Bone marrow biopsy disclosed absence of the NHL. Remission has subsequently been maintained and HCV eliminated with the new pegylated interferon α2b and ribavirin for nearly one year.

Conclusions: Transition of the underlying “benign” lymphoproliferative disease to a malignant lymphoma may result in difficult to treat HCV associated cryoglobulinaemic vasculitis. Rituximab offers a new possibility for inducing remission in refractory HCV associated cryoglobulinaemic vasculitis and the lymphoproliferative disorder. After remission, HCV may subsequently be eliminated with pegylated interferon α2b and ribavirin.

Hepatitis C virus (HCV) associated mixed cryoglobulinaemia has been referred to as a crossroad between classic autoimmune disease and malignancy. Various autoimmune phenomena and mixed cryoglobulinaemia are a consequence of chronic B cell stimulation, prolonged B cell survival, and poly- or oligoclonal B cell expansion in the presence of chronic HCV infection. A higher than expected prevalence of HCV has been reported for certain low grade B cell non-Hodgkin’s lymphomas (NHLs) and monoclonal gammopathies. Moreover, mixed cryoglobulinaemia has been found in B cell NHL, and B cell NHL has occasionally been found to complicate the clinical course of patients with HCV associated cryoglobulinaemic vasculitis.

Treatment with interferon (IFN)α has been shown to reverse bone marrow monoclonal B cell expansion in patients with HCV associated mixed cryoglobulinaemia. However, the efficacy of IFNα monotherapy in patients with HCV associated mixed cryoglobulinaemia is limited owing to low response rates, high relapse rates, and frequent adverse effects. Virus elimination rates and amelioration of symptoms are improved by a combination of IFNα and the purine nucleoside analogue ribavirin. Patients failing to respond to IFNα and ribavirin treatment or having acute exacerbations with rapidly deteriorating organ function and/or severe vasculitic manifestations have to be treated with cytotoxic agents—that is, usually corticosteroids and cyclophosphamide. However, the prognosis of patients with disease manifestations refractory to immunosuppressive treatment has remained poor so far.

CASE REPORT
A 45 year old woman was admitted to our department in June 1999 with purpura of the legs, arthralgia, paraesthesia of her feet and fingers, Raynaud’s phenomenon, and constitutional symptoms—that is, fatigue and unintended weight loss of 5 kg within a few weeks. Peripheral motor-sensory polyneuropathy was confirmed by electromyography. The patient had received multiple blood transfusions during the birth of her first child. Thus, post-transfusion hepatitis C was assumed.

Relevant laboratory findings were slightly raised transaminases (alanine aminotransferase 32 U/l, aspartate aminotransferase 21 U/l), rheumatoid factor 450 U/ml, and complement consumption with low C3c complement of 0.59 g/l (normal range 0.9–1.8 g/l), C4 complement <0.09 g/l (0.4–0.9 g/l), and total haemolytic complement CH50 of 12 U/l (20–60 U/l). Autoantibodies other than the rheumatoid factor were not detected. Type II mixed cryoglobulinaemia (3426 mg/l, detection threshold <80 mg/l) according to Brouet et al was determined with a monoclonal IgM and a polyclonal IgG component. Serum and plasma samples as well as cryoglobulins were assayed for HCV-RNA, genotype, and anti-HCV antibodies. HCV-RNA assays were used for detection (HCV-Amplicor qualitative assay, Roche) and quantification (HCV-RNA Amplicor Monitor quantitative assay) of the HCV-RNA load. Genotypes were determined with the INNO-LI PA HCV II (Innogenetics, Belgium) test by reamplifying with nested primers of the INNO-LIPA HCV II the amplificates of the HCV-RNA Amplicor Monitor quantitative assay. The concentration of HCV-RNA was between 500 000 copies/ml of the plasma and 700 000 copies/ml of the enriched cryoglobulin preparation containing 2200 copies/ml HCV-RNA. Genotyping reproducibly disclosed an infection with both genotypes 1b and 3. In contrast with the cryoglobulin preparation, genotype 1b was the only genotype to be detected in the plasma and serum samples. The homogenised cryoglobulin preparation was positive for anti-HCV antibodies (Pasteur Monolisa plus). Additionally, the Matrix assay (Abbott Matrix anti-HCV) confirmed this result, disclosing strong positive signals for the core, NS3 and NS5 antigens, respectively. However, no reactivity was detected.

Abbreviations: HCV, hepatitis C virus; IFN, interferon; NHL, non-Hodgkin’s lymphoma
for the NS5 antigens. These anti-HCV reactivities were confirmed with a second blood sample drawn two weeks later.

A chronic hepatitis with mild grade I portal inflammation and fibrosis and grade 0 lobular inflammation was seen in a liver biopsy. A skin biopsy showed a leucocytoclastic

Figure 1  (A) Bone marrow trephine stained for NACE (×40). Two small lymphoid aggregates were found next to bone trabeculae (→). The lymphoid cells were mainly small lymphocytes with dense chromatin structure and small cytoplasm. Some lymphoplasmacytoid cells were intermingled as were rare blasts. (B) High power view of a Giemsa staining of these lymphoid aggregates. Note the small lymphocytes and lymphoplasmacytoid cells (→). Some of the nuclei show small indentations, rarely a small nucleolus can be found. (C) Bone marrow trephine after treatment with rituximab. NACE staining (×40). No lymphoid aggregates were visible, the haematopoeisis is regularly distributed and all three mature lineages are represented. (D) Immunohistochemical staining of the lymphoid aggregates from the bone marrow trephine before treatment using CD20 antibody, the ABC method DAB (×200). Note: ill defined B cell aggregates which slightly replace the regular haematopoietic cells. (E) CD20 staining of the bone marrow after treatment (×200). Rare CD20 positive B cells were scattered throughout the marrow. (F) Transverse section through the colon sigmoideum with an ulceration of the mucosa (upper right) and an increase in the number of inflammatory cells in the lamina propria and submucosa. There were irregularities of the glands at the margins of the ulcer. In the lower right of the figure a vascular occlusion is shown (×25). Masson trichrome staining. (G) Section of (F). Moderate vascular inflammation with fibrosis and central fibrinoid necrosis is demonstrated. Small to medium sized muscular arteries were involved, resembling cryoglobulinaemic vasculitis. Typically, the inflammatory infiltrate consists of lymphocytes, neutrophilic granulocytes, some eosinophils, and monocytes/histiocytes. Haematoxylin and eosin (H&E) staining (×100).
vasculitis. A bone marrow biopsy disclosed lymphoid CD5–CD20– B cell infiltrates with some CD79+ cells interspersed, consistent with a low grade lymphocytic NHL according to the Kiel classification (figs 1A–E and 2).

Treatment with IFNα2b (3 × 106 IU three times a week subcutaneously; Intron A, Essex Pharma, Munich) and ribavirin (1000 mg/day by mouth, Rebetol, Essex Pharma, Munich) did not eliminate HCV or ameliorate vasculitis activity. Prednisolone was additionally given at a dose of 5 mg/day per os. The patient was admitted again after three months with progressive purpura, arthralgia, and polyneuropathy. Type II mixed cryoglobulinaemia had increased to 6.262 mg/l. Plasmapheresis three times a week (3–4 litres exchange) was administered for two weeks. Subsequent intermittent intravenous cyclophosphamide pulses (800 mg every 3 weeks intravenously; Endoxan, Baxter Oncology, Frankfurt a. M.) did not control the vasculitis activity in the longer term. Cryoglobulinaemia declined transiently and symptoms improved, but fatigue, night sweat, and unintended weight loss continued to be a problem. Exacerbations of vasculitic activity, including an inflammatory colon stenosis due to vasculitis (figs 1F and G), and a flare of the polyneuropathy followed attempts to ease treatment intensity and extend treatment intervals to four or six weeks in July 2000 and August 2001. Thereafter we added the humanised chimeric monoclonal anti-CD20 antibody rituximab (500 mg every three weeks intravenously; Mabthera, Roche, Grenzach-Wyhlen). Remission of the vasculitis was successfully induced after six rituximab infusions. Another bone marrow biopsy did not show any evidence of the NHL. Cryoglobulinaemia has been reduced to its lowest level so far. Remission has subsequently been maintained with pegylated IFNα2b (80 μg/week subcutaneously; Peg Intron, Essex Pharma, Munich) and ribavirin (1000 mg/day by mouth; Rebetol, Essex Pharma, Munich) for nearly 12 months (fig 3).

DISCUSSION

This is the first case report to show that the induction of remission in HCV associated cryoglobulinaemic vasculitis and an NHL refractory to conventional treatment can be achieved by the addition of rituximab. So far, there has been only one report demonstrating the efficacy of rituximab in the treatment of HCV associated mixed type II cryoglobulinaemia. In contrast with our patient, no underlying NHL was found in the report of Zaja et al.12

It has been shown that older age, low level cryoglobulinaemia, and low viral load are associated with a favourable response to IFNα treatment in HCV associated mixed cryoglobulinaemia. Coinfection by two or more genotypes has been reported in chronic post-transfusion hepatitis C.13 HCV-RNA has also been demonstrated in the cryoprecipitate.2 We found a coinfection with genotypes 1b and 3 in the cryoglobulin, but only genotype 1b in serum and plasma samples. To our knowledge, a divergence between genotypes detected in serum and plasma and in the cryoprecipitate has not been reported previously. Coinfection with two genotypes and binding of HCV-RNA in the cryoprecipitate may have further contributed to antigenic variation and escape mechanisms of the HCV from a specific immune response and may have added to the risk factors for an unfavourable response to antiviral treatment in our patient.

B cell expansion with oligo- or monoclonal proliferation of B cells in peripheral blood, bone marrow, and liver is a key feature in HCV associated mixed cryoglobulinaemia. Prolonged B cell survival may expose these cells to subsequent genetic aberrations, leading to malignancy.
malignant lymphoma that eventually develops during follow up may stem from a B cell clone other than those sustaining the so-called “benign” lymphoproliferative disease in chronic HCV infection.\(^4\)\(^5\) B cell proliferations may present as B cell chronic lymphocytic leukaemia—that is, low grade lymphocytic NHL, as seen in our patient, or immunocytoma. They tend to remain unmodified for years and have been coined “monotypic lymphoproliferative disorder of undetermined significance (MLDUS)”.\(^6\)\(^7\) Although HCV itself may be insufficient to drive transition into a malignant lymphoma, environmental cofactors and an interaction of the HCV core protein with cellular proto-oncogenes may favour uncontrolled B cell proliferation.\(^8\) Regression of HCV associated splenic lymphoma and mixed cryoglobulinaemia has been shown in nine patients treated with IFN\(^a\) and ribavirin recently.\(^9\) In contrast with our patient, all nine patients responded favourably to a standard dose IFN\(2b\) (3\(\times\)10\(^6\) IU three times a week subcutaneously) or IFN\(2b\) and ribavirin (1000–1200 mg/day by mouth). In one patient HCV-RNA became detectable again and the patient relapsed.\(^1\) In our patient HCV-RNA was demonstrated again and the patient relapsed.\(^1\) Although HCV itself may be insufficient to drive transition into a malignant lymphoma, environmental cofactors and an interaction of the HCV core protein with cellular proto-oncogenes may favour uncontrolled B cell proliferation.\(^2\) Regression of HCV associated splenic lymphoma and mixed cryoglobulinaemia has been shown in nine patients treated with IFN\(^a\) and ribavirin recently.\(^4\) In contrast with our patient, all nine patients responded favourably to a standard dose IFN\(2b\) (3\(\times\)10\(^6\) IU three times a week subcutaneously) or IFN\(2b\) and ribavirin (1000–1200 mg/day by mouth). In one patient HCV-RNA became detectable again and the patient relapsed.\(^1\) In our patient the combined standard dose IFN\(a\) and ribavirin as well as subsequent cyclophosphamide treatment failed to control B cell proliferation and its sequelae—that is, cryoglobulinaemic vasculitis. Because the monoclonal anti-CD20 antibody rituximab is effective in the treatment of low grade B cell NHL,\(^1\) we decided to add rituximab to the cyclophosphamide treatment. The CD20 antigen is expressed by all cells of the B cell lineage and more than 95% of B cell lymphomas but not by stem cells. CD20 is involved in B cell activation, regulation of B cell growth, and transmembrane calcium flux. Killing of CD20 positive cells, mediated by rituximab, is caused by several different mechanisms, including complement mediated cytotoxicity, antibody dependent cell mediated cytotoxicity, and induction of apoptosis.\(^1\)\(^5\) In our patient remission of the vasculitis was successfully induced and cryoglobulinaemia reduced to its lowest level after six rituximab infusions. Another bone marrow biopsy did not show any evidence of the NHL. Pegylated IFN\(2b\) has become available in the meantime and thus, after the induction of remission with rituximab we successfully used pegylated IFN\(2b\) and ribavirin to eliminate HCV and maintain remission. Thus, treatment with rituximab is an effective option for the induction of remission in refractory HCV associated cryoglobulinaemic vasculitis and the underlying lymphoproliferative disease. Elimination of the HCV may subsequently be achieved.

ACKNOWLEDGEMENTS

Supported by grants SFB367/A8 (PL, WLG) and SFB367/A11 (JV) from the German Research Society (Deutsche Forschungsgemeinschaft/DFG), and grant No 02.2.2 from the Verein zur Foerderung der Erforschung und Bekaempfung rheumatischer Erkrankungen Bad Bramstedt e.V. (PL).

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doi: 10.1136/ard.2002.004929

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