Virchows Arch (1999) 434:533–535

ORIGINAL ARTICLE

Bruno Bonetti · Maria Scardoni · Salvatore Monaco
Nicola Rizzuto · Aldo Scarpa

Hepatitis C virus infection of peripheral nerves in type II cryoglobulinaemia

Received: 12 October 1998 / Accepted: 3 February 1999

Abstract Peripheral neuropathy is a frequent complication in patients suffering from type II mixed cryoglobulinaemia (mCGII), a sort of vasculitis that is strongly associated with hepatitis C virus (HCV) infection and characterised by high concentrations of anti-HCV antibodies and HCV RNA in the cryoprecipitates. We report the finding of HCV RNA in homogenates of nerve biopsies from five such patients, by reverse transcription-polymerase chain reaction (RT-PCR) amplification of different regions of the viral genome. HCV RNA was localized in epineurial cells by in situ RT-PCR. Our data suggest that HCV infection of nerves plays a major role in mCGII-associated neuropathy.

Key words Mixed cryoglobulinaemia · Hepatitis C virus infection · Peripheral neuropathy

Introduction

Peripheral neuropathy is a frequent complication in patients suffering from type II mixed cryoglobulinaemia (mCGII) [3], a type of vasculitis that is strongly associated with hepatitis C virus (HCV) infection and characterised by high concentrations of anti-HCV antibodies and HCV RNA in the cryoprecipitates [1]. However, the role of cryoglobulins and/or HCV infection in the pathogenesis of peripheral neuropathy have yet to be elucidated [2]. Here we report that HCV may infect peripheral nerves, and that the infection is not restricted to a particular HCV genotype.

B. Bonetti · S. Monaco (✉)[1] · N. Rizzuto
Dipartimento di Scienze Neurologiche e della Visione,
Università di Verona, Italy

M. Scardoni · A. Scarpa
Dipartimento di Patologia-Anatomia Patalogica,
Università di Verona, Italy

Mailing address:
[1] Clinica Neurologica, Policlinico Borgo Roma,
Via delle Menegone, I-37134 Verona, Italy

Materials and methods

Frozen, formalin-fixed and plastic-embedded sural nerve biopsy specimens were obtained from five patients with clinical and electrophysiological signs of peripheral neuropathy associated with mCGII and chronic HCV hepatitis. RNA was extracted, using an IsoQuick extraction kit (Microprobe, USA), from five 10-µm cryostatic sections of nerve biopsies and subjected to reverse transcription-polymerase chain reaction (RT-PCR) using two different sets of primers [8, 10], amplifying sequences of 187 bp [8]and 235 bp [10] of the 5'-untranslated region of the viral genome, respectively. HCV genotypes [9] were determined by using the Line Probe assay [10]. The specific RT-PCR protocols for the different primers used were as detailed in the corresponding literature references [8–10]. The cellular distribution of HCV-RNA was investigated by in situ RT-PCR on 5-µm paraffin sections, following the protocol detailed by Nuovo et al. [7]. Nerve biopsies from 5 HCV-seronegative patients served as negative controls. Samples were judged when consistent results had been achieved in three independent experiments.

Results

Clinicopathological data on the five patients affected by mCGII and the results of our study are summarized in Table 1. Light microscopic examination of plastic-embedded sections showed moderate to marked loss of myelinated axons, coupled with endoneurial microangiopathy, capillary vasculitis and epineurial macrovascular inflammation (Fig. 1A, B).

Examination of serial plastic and paraffin sections disclosed mononuclear inflammatory infiltrates around small and precapillary arterioles in all cases, and epineurial arteriolitis in case D. In cases B and C healed vasculitis of large epineurial arterioles was observed [4].

HCV RNA was found in all five nerve biopsies by RT-PCR amplification of 5'-untranslated regions of the viral genome (Fig. 2), and was characterised as types 1a, 1b and 4/5 in two, two and one cases, respectively (Fig. 3). The same HCV genotypes were found in the matched serum of each patient.

By in situ RT-PCR, a consistent number of nuclear-stained oblong cells similar in morphology to macro-

Table 1 Clinico-pathological data and results of hepatitis C virus (*HCV*) RNA testing (*MN* multiple neuropathy, *PN* polyneuropathy)

Case	Age/sex	Neuropathy	Epineurial inflammation	HCV			
				Seric	Nerve	In situ PCR	Type
A	61/F	MN	Yes	+	+	+	4–5
B	51/M	PN	Yes	+	+	–	1a
C	59/F	PN	Yes	+	+	–	1b
D	48/M	MN	Yes	+	+	+	1b
E	60/F	PN+MN	Yes	+	+	+	1a

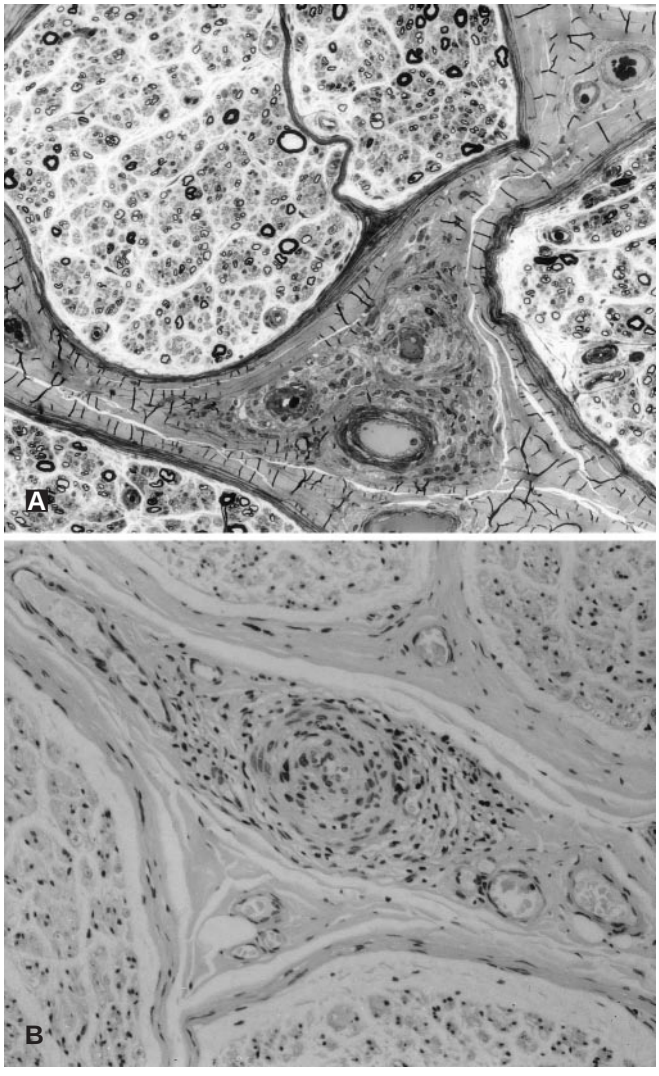

Fig. 1 Pathological features of sural nerves from patients with hepatitis C virus (HCV) infection and cryoglobulinaemia (CG) on (**A**) plastic-embedded and (**B**) paraffin sections. Nerve fascicles show depletion of myelinated axons (A) and large mononuclear infiltrations around epineurial arterioles (**A**, **B**). ×160

phages were detected around epineurial vessels (Fig. 4) in three nerve biopsies. Positive elements were abundant at sites where mononuclear inflammatory cells were present. Endothelial and mononuclear inflammatory cells in the epineurium were negative, as were cells of the endoneurial compartment.

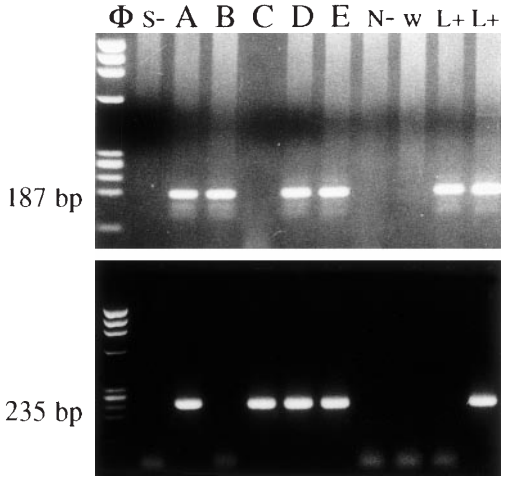

Fig. 2 HCV RNA detection by RT-PCR in peripheral nerve biopsies. *A–E* identify nerve biopsies. *Φ* is Hae III-digested Φx174 DNA marker. *S-* and *N-* are serum and nerve negative controls, respectively. *L+* are two different liver-positive controls; w is the reaction mix without the addition of nucleic acid template. All samples but *C* showed the expected 187-bp RT-PCR product with one set of primers (see ref. 4), whereas a second set of primers allowed the amplification of the expected 235 bp PCR product in all cases but *B*. The different results in cases *B* and *C* may be due to sequence differences between individual viruses in the region recognised by the different primers (see also the first *L+ lane*)

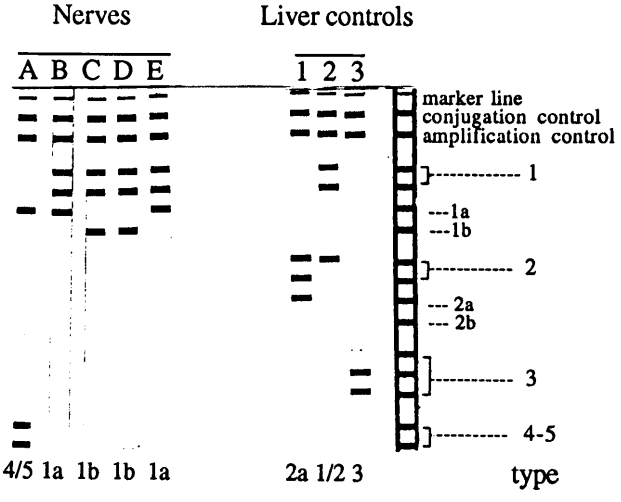

Fig. 3 HCV genotyping by Line Probe assay, which is based on the hybridisation with type-specific oligonucleotide probes of RT-PCR amplified 5'-untranslated region of HCV. The *bar* on the *right* is a schematic note of the expected bands for the different genotypes. HCV type 1a was identified in cases *B* and *E*, type 1b in cases *C* and *D*, and type 4/5 in case *A*

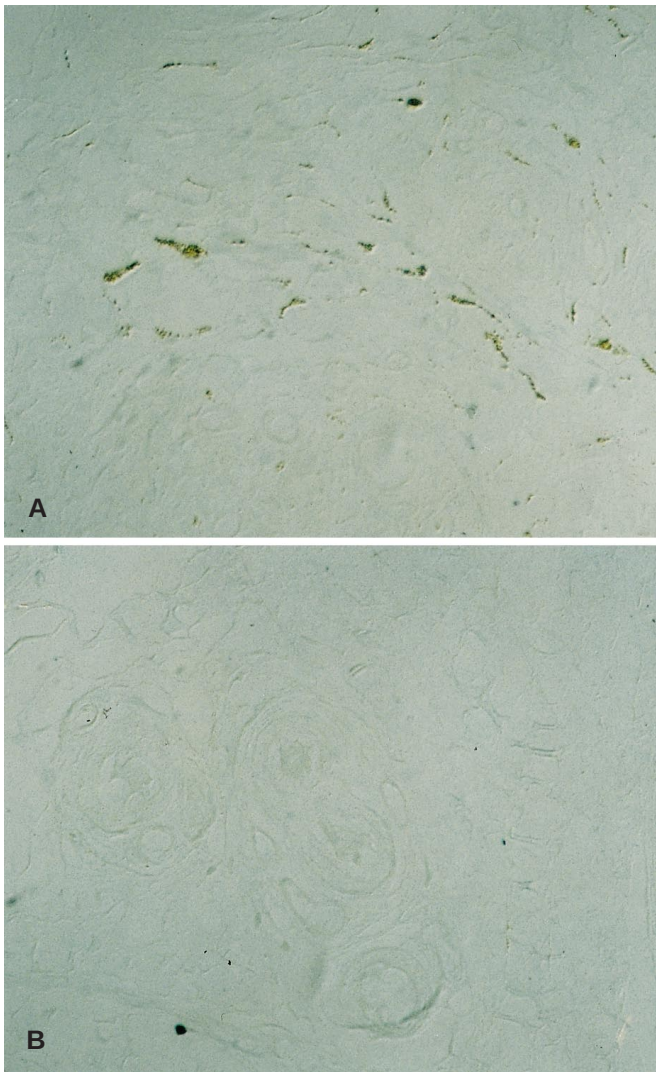

Fig. 4 **A** Distribution of HCV-infected cells by in situ RT-PCR in nerve paraffin section. **B** No signal is detectable in nerves of HCV-seronegative patients. Negative nerve controls showed no reactivity, and no signal was detected in sections pretreated with RNAse or if the RT step was omitted (not shown)

Discussion

Our data suggest that the peripheral neuropathy in patients with mCGII and HCV infection is in part related to the presence of HCV-infected perivascular cells and to the associated lymphocytic infiltrates within the epineurium. The question that arises is whether the detection of HCV RNA in peripheral nerves might reflect tissue contamination by HCV-positive serum. However, results obtained by in situ RT-PCR exclude this possibility. The demonstration of HCV-positive cells in only three nerves of HCV-positive patients in spite of HCV RNA detection in all five biopsies may be explained by focal distribution of the infection.

The axonal degeneration occurring in mCG patients is thought to be due mainly to ischaemia following endoneurial microangiopathy and vasculitis of epineurial arteries [4, 6]. The endoneurial lesion is characterized by the deposition of immunoglobulins and cytolytic complement in endoneurial capillaries [4]. However, epineurial arterioles characterized by the expression of cell adhesion molecules lack immune complex deposition and are surrounded by inflammatory infiltrates containing β2-integrin-positive T-lymphocytes and monocytes [4]. The latter pattern, together with the presence of HCV-infected cells, is reminiscent of the lobular lesion in HCV hepatitis, where the presence of HCV within hepatocytes against the backdrop of a parenchymal T-lymphocytic infiltration suggests a primary role for HCV as a direct or T-cell mediated cause of liver cell damage [5].

Acknowledgements This work was supported by grants from Telethon Italia to S.M., from CNR (no 92.02771.CT04), and by a grant from Verona University to A.S.

References

1. Agnello V, Chung R, Kaplan L (1992) A role for hepatitis C virus infection in type II cryoglobulinemia, N Engl J Med 327:1490–1495
2. Apartis E, Leger J, Musst L, Gugenheim M, Cacoub P, Lyon-Caen O, Pierrot-Deseilligny C, Hauw J, Bouche P (1996) Periperal neuropathy associated with essential mixed cryoglobulinemia: a role for hepatitis C virus infection? J Neurol Neurosurg Psychiatry 60:661–666
3. Authier FJ, Pawlotsky JM, Viard JP, Guillevin L, Degos JD, Gherardi RK (1993) High incidence of hepatitis C virus infection in patients with cryoglobulinemic neuropathy (letter). Ann Neurol 34: 749–750
4. Bonetti B, Invernizzi F, Rizzuto N, Bonazzi M, Zanusso G, Chinaglia G, Monaco S (1997) T-cell-mediated epineurial vasculitis and humoral-mediated microangiopathy in cryoglobulinemic neuropathy. J Neuroimmunol 73: 145–154
5. Dhillon A, Dusheiko G (1995) Pathology of hepatitis C virus infection. Histopathology 26: 297–309
6. Nemni R, Corbo M, Fazio R, Quattrini A, Comi G, Canal N (1988) Cryoglobulinemic neuropathy. A clinical, morphological and immunohistochemical study of 8 cases. Brain 111: 541–552
7. Nuovo G, Lidonnici K, MacConnel P, Lane B (1993) Intracellular localization of polymerase chain reaction (PCR)-amplified hepatitis C cDNA, Am J Surg Pathol 17:683–690
8. Ravaggi, A., Primi, D., and Cariani, E. Direct amplification of HCV RNA from human serum. PCR Methods Appl 1: 291–292
9. Simmonds P, Holmes EC, Cha TA, Chan SW, McOmish F, Irvine B, Beall E, Yap PL, Kolberg J, Urdea MS (1993) Classification of hepatitic C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. J Gen Virol 74:2391–2399
10. Stuyver L, Rossau R, Wyseur A, Duhamel M, Vanderborght B, Heuverswyn HV, Maertens G (1993) Typing of hepatitis C virus isolates and characterization of new subtypes using a line probe assay. J Gen Virol 74:1093–1102