(4) Finally, we agree with the statement that the immediate translation of hMASC use from the in vitro assays to clinical settings is, at present, less than prudent. Confidence on their safety and usefulness will require extensive in vivo animal studies, such as those that are ongoing in our laboratory. Therefore, before expressing a priori preconceptions, it will be better to wait for in vivo data.

Carlo A. Beltrami

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Correspondence: Carlo A. Beltrami, Dipartimento di Ricerche Mediche e Morfologiche, Istituto di Anatomia Patologica, Udine 33100, Italy; e-mail: beltrami@uniud.it.

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To the editor:

Association between persistent lymphatic infection by hepatitis C virus after antiviral treatment and mixed cryoglobulinemia

Hepatitis C virus (HCV) is closely related to the development of mixed cryoglobulinemia (MC). Occult HCV infection in sustained virologic responders (SVRs) after antiviral treatment has been shown,[1,2] but MC patients have never been investigated.

We studied 102 HCV patients (64 males, mean age 50.8 ± 12.1 years), who were SVRs after interferon-based anti-HCV therapy, consecutively recruited at our Center from July 2003 to July 2004 and followed-up until July 2007. Patients included 13 subjects with MC syndrome (group A, Table 1) and 89 patients without MC (58 males, mean age 50.2 ± 12.5 years; group B). Blood samples were collected at least twice a year.

Positive-strand and negative-strand (replicative intermediate) HCV RNA was detected by highly sensitive, previously described methods: transcription mediated amplification (TMA; Bayer Healthcare, Tarrytown, NY); reverse transcriptase–polymerase chain reaction [RT-PCR]-nucleic acid-hybridization assay, Real-time PCR, 5′-UTR-HCV RNA negative-strand PCR with Tth polymerase, with appropriate controls.[2,3] Peripheral blood mononuclear cells (PBMC) were cultured with mitogens as previously described.[2,4] T(14;18) was determined in PBMC by MBR bcl-2/J_H PCR as described.[5]

In all patients, serum samples were persistently HCV RNA-negative. HCV RNA was repeatedly detected in stimulated cells (mainly lymphocytes) from 12 patients (8 group A, Table 1, and 4 group B; $P < .001$), whereas posttreatment liver biopsies scored HCV RNA-negative. Negative-strand HCV RNA was shown in PBMC from MC cases.

PBMC infection was shown in 5 patients with persistent MC syndrome and in no subject in whom MC syndrome completely disappeared. Persistence of t(14;18)-positive B-cell clones was associated with persistence of MC syndrome ($P = .021$; Table 1).

We, and others, previously detected positive- and negative-strand HCV RNA in PBMC, and observed the increased detection of viral sequences after mitogen stimulation.[4,6,7] HCV lymphotropism is generally interpreted as a key factor in HCV-related lymphoproliferative disorders, but this hypothesis was never confirmed, probably due to the difficulty in enucleating the role played by lymphatic infection in patients also with liver infection and circulating HCV. In this study, persistence of HCV infection was observed in PBMC (mainly lymphocytes) in the absence of serum or liver HCV-positivity and was significantly associated with MC syndrome. This isolated PBMC infection may be explained by previous data showing that HCV compartmentalization may occur, in which HCV is confined to a given "compartment" not able to "infect" other compartments.[8] Of note, serum samples were thoroughly mixed and warmed to resolubilize cryoglobulins before HCV RNA testing.[9] Further studies are needed to clarify the mechanisms possibly linking HCV lymphatic infection with MC. The association between persistence of t(14;18) and lymphatic infection and, in turn, between persistence of t(14;18) and MC syndrome add value to the hypothesis of a pathogenetic role also played by t(14;18).

From a clinical point of view, this study emphasizes the relevance of a complete eradication of HCV for the resolution of MC syndrome, even if the presence of (one or more) "point of no return" in the natural history of such lymphoproliferative disorder, with progressive independence from the etiologic agent, cannot be excluded. Actually, cases of persistent syndrome in spite of viral eradication have been described,[10] including some personal observations. If this interpretation is correct, the current indication for an early etiologic treatment of HCV-positive MC[11] will be clearly reinforced.

Carlo Giannini, Antonio Petrarca, Monica Monti, Umberto Arena, Patrizio Caini, Vera Solazzo, Laura Gragnani, Stefano Milani, Giacomo Laffi, and Anna Linda Zignego

C.G. and A.P. contributed equally to this work.

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Correspondence: Anna Linda Zignego, MD, PhD, Professor of Medicine, Department of Internal Medicine, University of Florence, Viale Morgagni 85 50134 Florence, Italy; e-mail: a.zignego@dmi.unifi.it.

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Table 1. Main clinical and laboratory findings of 13 HCV-positive patients with mixed cryoglobulinemia achieving sustained virological response (group A)

Pt. no.	Age at treatment, y	Sex	Duration of MC, y	Liver histologic findings	Other clinical features‡	Cryocrit, %	Cryoglobulin composition	Rheumatoid factor, IU/mL*		Complement C3/C4 level#		HCV genotype	PRE-HCVRNA (serum), IU/mL	Posttreatment HCVRNA°			Presence of t(14;18), yes/no		MC syndrome, yes/no	
														PBMC						
														Uncultured	Cultured with mitogens					
								PRE	POST	PRE	POST				lymph.	macr.	PRE	POST	PRE	POST
1	54	male	5	Chronic hepatitis	Sicca syndrome, peripheral neuropathy	1	IgG+IgM(k)	35	22	64/18	104/18	2c	1.43×10^4	−	+	−	no	no	yes	no
2	39	male	7	Chronic hepatitis	Peripheral neuropathy	1.5	IgG+IgM(k)	57	<20	71/20	111/20	3a	10×10^5	−	+	−	yes	yes¶	yes	yes
3	65	female	15	Cirrhosis	Peripheral neuropathy, sicca syndrome, renal involvement§	11	IgG+IgM(k)	107	92	68/1	68/1	2c	1.3×10^6	+	+	−	yes	yes¶	yes	yes
4	44	female	8	Chronic hepatitis	Sicca syndrome, fever	1	IgG+IgM(k)	36	<20	90/13	12718	1b	8×10^5	+	+	−	yes†	yes¶	yes	yes
5	63	female	9	Chronic hepatitis	Sicca syndrome	8	IgG+IgM(k)	54	21	125/6	147/21	2c	1.17×10^5	−	−	−	yes	no	yes	no
6	50	female	18	Chronic hepatitis	Peripheral neuropathy	10	IgG+IgM(k+λ)	289	90	99/16	153/30	2a	9×10^5	−	−	−	yes	no	yes	no
7	50	female	15	Chronic hepatitis	Peripheral neuropathy	1	IgG+IgM(k)	30	<20	10118	101/25	1b	5.08×10^5	−	+	−	no	no	yes	no
8	49	male	5	Cirrhosis	Peripheral neuropathy, sicca syndrome	1	IgG+IgM(k)	147	27	1252	169/24	1a	1.7×10^4	−	−	−	yes†	no	yes	no
9	38	male	4	Chronic hepatitis	Raynaud phenomenon	5	IgG+IgM(k)	39	<20	121/24	121/24	1a	5.5×10^5	−	+	−	yes	yes¶	yes	yes
10	32	male	3	Chronic hepatitis	Peripheral neuropathy, sicca syndrome	1	IgG+IgM(k+λ)	244	26	8617	107/31	2c	7.5×10^5	−	−	+	yes	yes¶	yes	yes
11	49	female	7	Chronic hepatitis	Sicca syndrome, dermatomyositis	2	IgG+IgM(k)	100	50	138/17	175/32	4c/d	7.47×10^5	−	−	−	yes	yes¶	yes	yes
12	41	female	5	Chronic hepatitis	Dermatomyositis	1.5	IgG+IgM(k)	40	<20	103/13	110/30	1b	1.4×10^6	−	−	+	yes	yes¶	yes	yes
13	60	female	11	Chronic hepatitis	Peripheral neuropathy, Raynaud phenomenon, renal involvement§	2	IgG+IgM(k)	4380	130	167/11	148/25	1b	6.5×10^5	−	−	−	no	no	yes	no

Pt. no. indicates patient number; PRE, pretreatment; POST, posttreatment; lymph., lymphocytes; macr., macrophages; −, negative; and +, positive.
*Normal value are < 25 UL/mL.
#Normal values are 83 to 177 mL/dL for complement C3 and 20 to 150 mL/dL for complement C4.
†T(14;18) determined in both peripheral blood and bone marrow mononuclear cells.
‡Other clinical features: MC-related manifestations observed in addition to the classic Meltzer syndrome.
§Renal involvement was in both cases shown by microscopic hematuria, proteinuria below the nephrotic range (<3 g/24 h), with normal or only fairly reduced renal function (creatinine <1.5 mg%).
¶Identical N-segments in the pre- and posttreatment samples were observed.
°Posttreatment HCVRNA in serum and liver samples was persistently negative.

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