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Review

Extrahepatic disease manifestations of HCV infection: some current issues

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1. Introduction

Productive hepatitis C virus (HCV) infection appears to be primarily, and in most patients almost exclusively, confined to the liver. However, a wide variety of extrahepatic disease manifestations have been reported to be associated with the infection. The prevalence of extrahepatic disease manifestations is not known with certainty but these observations have suggested that HCV is involved in non-hepatic pathological processes.

There are two immunologic features of HCV infection that may predispose to extrahepatic disease manifestations. The first feature is the virus avoids immune elimination. This feature leads to chronic infection, accumulation of circulating immune complexes, and autoimmune phenomena associated with chronic viral infections. The second feature is the virus stimulates production of monoclonal rheumatoid factors (mRF). In the majority of patents mRF is highly restricted and has been dubbed 'WA' mRF. This feature causes the type II cryoglobulinemia associated with HCV infection (HCV-type II cryoglobulinemia) that is responsible for most of the symptomatic cryoglobulinemic vasculitis, or Melzer-Franklin syndrome. Although this manifestation occurs relatively infrequently, as do all the extrahepatic disease manifestations, it is the major extrahepatic disease manifestation of HCV infection due to the increased morbidity and mortality accompanying the disease.

A widely postulated but controversial hypothesis is that extrahepatic disease manifestations are caused by extrahepatic tropisms of HCV, particularly lymphotropism. The latter is thought to be involved in the production of autoantibodies and non-Hodgkin's B-cell lymphoma (B-NHL). In this review, the emphasis will be on some of these topics as well as other current issues. Only a brief overview of all the extrahepatic disease manifestations associated with HCV infection will be given. A comprehensive review of the subject has been published [1].

2. Extrahepatic disease manifestations associated with HCV infection

At least 36 extrahepatic disease manifestations, mainly autoimmune disorders, have been reported to be associated with HCV infection (Table 1) [2–69]. There are conflicting reports for most of these associations and definitive validation is lacking for all except the association with mixed cryoglobulinemia. In most reports relatively small numbers of patients and an inadequate numbers of control subjects were studied. In many studies there was a problem with patient selection bias that can produce false associations especially in endemic regions where coincidental disease is more likely to be observed. The true prevalence of any of these manifestations is not known because studies have not been performed on large numbers of unselected HCV-infected patient populations. In all cases, with the exception of symptomatic mixed cryoglobulinemia, the critical determination of a specific role for HCV in the pathogenesis has not been established.

Despite the limitations of the studies noted above, observations on extrahepatic disease manifestations have been taken as evidence that HCV has tropism for cells other than hepatocytes, particularly lymphocytes. It has been postulated that HCV infection of lymphocytes is the cause of the increased autoimmune disease reported to be associated with HCV infection. However clinical observations dispute this hypothesis. The autoantibodies in patients with HCV infection appear to resemble the physiologic type accompanying many viral infections. The autoantibody titers are low, there is no female gender predominance, there is no association with HLA-DR genes, and there are usually no clinical autoimmune

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Table 1
Reported extrahepatic disease associations with HCV infection [2–69]

Antiphospholipid syndrome	MALToma
Aplastic anemia	Membranoproliferative glomerulonephritis
Autoimmune hemolytic anemia	Membranous glomerulonephritis
Autoimmune thyroiditis	Mixed cryoglobulinemia
Chronic fatigue syndrome	Mooren corneal ulcers
Behcet's syndrome	Multiple myeloma
Carotid atherosclerosis	Non-Hodgkin's lymphoma
CRST syndrome	Neurocognitive Impairment
Dermatomyositis	Pancreatitis
Diabetes mellitus	Polyarteritis nodosa
Fibromyalgia	Polymyositis
Guillain-Barré syndrome	Porphyria cutanea tarda
Hypertrophic cardiomyopathy	Rheumatoid arthritis
Hypocholesterolemia	Sialadenitis
Idiopathic pulmonary fibrosis	Sjögren's syndrome
Idiopathic thrombocytopenia purpura	Systemic lupus erythematosus
IgA deficiency	Uveitis
Lichen planus	Waldenstrom's macroglobulinemia

manifestations. For example, the anticardiolipin antibodies in HCV infection occur in low titers, are not associated with thrombocytopenia or thrombotic events, and the anti β_2-glycoprotein antibodies usually associated with clinical manifestations are not present [2,3]. This difference is also illustrated by the fact that autoantibodies associated with HCV infection are distinct from those found in autoimmune hepatitis, an established clinical autoimmune disease [70].

For most autoantibodies that occur in HCV-infected patients, the prevalence and significance are in question. In contrast, it is has been widely documented that there is an increased prevalence of polyclonal rheumatoid factors (RF). Production of RF occurs in response to circulating complexes of IgG and occurs in high prevalence predominantly in patients with prolonged chronic infection. Hence, the mechanism is distinct from that involved in the production of other autoantibodies. Production of mRF also appears to be distinct from both the production of RF and other autoantibodies. On B cell clonal analysis mRF sequences are detectable in HCV-infected patients without cryoglobulinemia and do not correlate with the presence of RF [71]. Also consistent with a distinct mechanism for the production of mRF is that the prevalence of other autoantibodies in type II cryoglobulinemia is low [72].

Several associations of extrahepatic disease manifestations with HCV infection other than symptomatic type II cryoglobulinemia are notable. There appears to be a strong association of salivary gland lesions, lymphocytic capillaritis, and lymphocytic adenitis with chronic hepatitis C [73]. The clinical characteristics of patients with these lesions, however, differed from those of patients with Sjögren's syndrome by a predominantly male rather than female sex prevalence, absence of serum antinuclear antibodies, low rather than high association with HLA DR 3, milder histopathology (predominantly grades I–II rather than grades II–IV), and clinical symptoms (only 30% had xerostomia and none had xerophthalmia). Despite several reports of increased prevalence of HCV in patients with Sjögren's syndrome it is clear that the virus is neither necessary nor sufficient to produce this disease [74].

A study of 48 patients who had Sjögren's syndrome without mixed cryoglobulinemia were all positive for SSA/Ro but none had HCV infection [75]. HCV infection is common only in patients with Sjögren's syndrome associated with symptomatic type II cryoglobulinemia. However, the prevalence of Sjögren's syndrome in type II cryoglobulinemia associated with HCV infection is only 6% [1]. There are conflicting results from studies of salivary tissue from patients with HCV infection [76,77]. In HCV infected patients with sialadenitis or Sjögren's syndrome, HCV was detected in epithelial cells of salivary gland but there were no differences in the lesions in HCV infected and non-infected patients [76]. Studies on salivary glands from HCV infection without salivary gland abnormalities were negative [77]. Similarly, in palpable purpura lesions in patients with HCV-type II cryoglobulinemia, HCV was detected in keratinocytes and glandular epithelial cells in the skin in the inflammatory lesions but not in normal skin. Up-regulation of LDL receptors was demonstrated on keratinocytes in the lesional but not the normal skin [78]. Hence, up-regulation of LDL receptors associated with inflammation may result in uptake of HCV since the LDL receptor has been shown to mediate endocytosis of the virus [79].

Of interest in regard to potential mechanisms involved in sialadenitis in HCV infection is that studies in transgenic mice expressing HCV envelope genes found sialadenitis that had features similar to the lesions in humans, i.e. no gender prevalence and negative antinuclear autoantibodies [80]. Despite the non-physiologic over-expression of envelope proteins in this animal model [81], the results suggest that the HCV virus may be directly involved in sialadenitis rather than triggering an autoimmune process. The finding in this mouse model may also relate to the occurrence of lichen planus in HCV infection. As with Sjögren's syndrome it is clear that the HCV is neither sufficient nor necessary for production of lichen planus [74] and a recent study has questioned the increased prevalence of lichen planus among HCV-infected patients that has been reported in endemic areas [82]. However it has been demonstrated that in patients with lichen planus, HCV specific CD4 + and CD8 + lymphocytes are detectible in the lichen planus lesions but not in the blood [83]. Hence, in both Sjögren's syndrome and lichen planus HCV may be a contributing but not a primary pathologic factor.

Very high prevalence of HCV infection has been reported among patients with porphyria cutanea tarda in Southern Europe and Japan but not in Northern Europe and Australia suggesting again a possible patient selection bias in endemic regions [84]. Yet a high prevalence was also found in the United States and it has been suggested that HCV is a precipitating factor for porphyria cutanea tarda [85]. The mechanism whereby HCV would precipitate

the disease is unclear since the infection does not decrease activity of hepatic uroporphyrinogen decarboxylase, which is deficient in active porphyria cutanea tarda [86]. Confounding these studies is the high prevalence of alcoholism among patients with porphyria cutanea tarda since alcohol is a known risk factor for precipitating the disease. Therefore, it remains questionable whether HCV alone can trigger porphyria cutanea tarda.

3. Current issues

3.1. Prevalence and clinical significance of mixed cryoglobulinemia

Meltzer et al. described clinical manifestations associated with 'mixed' cryoglobulinemia in 1966. They described a 'syndrome' that consisted of the triad of palpable purpura, arthralgias and weakness plus other clinical features, such as glomerulonephritis, lymphadenopathy, splenomegaly, and hepatomegaly in some of the patients [87]. Cryoglobulinemia in these patients had a 'mixed' composition of IgG and IgM RF. Studies performed by the Franklin group, as well as others, implicated the cryoglobulin components in the immune complex mediated vascular and glomerular lesions that occurred in many of these patients. It is now apparent that the triad of palpable purpura, arthalgias and weakness occurs in a minority of patients and that the main characteristic of the disease is the markedly heterogeneous manifestations of a systemic vasculitis with palpable purpura being the most prevalent.

The later classification of cryoglobulins based on immunoglobulin (Ig) composition by Brouet and colleagues [88], which defined three types of cryoglobulins, was also based on the characterization of the cryoglobulins from patients with disease manifestations. Type I consisted of a single monoclonal Ig; types II and III, or mixed cryoglobulins, consisted of polyclonal IgG and monoclonal (type II) or polyclonal (type III) IgM with RF activity. Because RF was a component of most of the types II and III cryoglobulins, the original Brouet study was essentially a correlation of IgG-IgM RF complexes with manifestations of immune complex diseases, as were the studies by the Franklin group. In recent years there has been widespread study of HCV infected patients without extrahepatic clinical manifestations.

The prevalence of mixed cryoglobulinemia increases with the duration of the hepatitis. Patients with chronic hepatitis C that have mixed cryoglobulinemia have an apparent duration of disease that is almost twice as long as those without cryoglobulinemia [89]. High prevalence of mixed cryoglobulinemia (35–90%) has been reported for patients with HCV infection [90,91]. However, in assessing the prevalence of mixed cryoglobulinemia it must be considered that prevalence has not been determined in populations of unselected HCV infected patients. Hence, reports of high prevalence of mixed cryoglobulinemia may be due to a patient selection bias since most studies come from gastroenterology centers that have patient populations with an increased prevalence of patients with long duration disease, e.g. cirrhosis.

In only a minority of patients with mixed cryoglobulinemia are there extrahepatic clinical manifestations. In asymptomatic patients the significance, if any, of cryoglobulins in the development of extrahepatic clinical manifestations or cirrhosis has been the subject of speculation, particularly regarding cirrhosis. A recent meta-analysis of 19 studies on a total of 2323 patients with chronic hepatitis C found a 44% prevalence of cryoglobulinemia (analysis of the cryoglobulins was not provided) and a highly significant association between cirrhosis and cryoglobulinemia after adjustment for age, gender, and estimated duration of disease [91]. The authors concluded that cryoglobulins should be considered a prognostic indicator for the increased risk of cirrhosis in patients with chronic hepatitis C. This conclusion is obviously flawed since symptomatic type II cryoglobulinemia is associated with a low prevalence of cirrhosis [72]. Because of marked heterogeneity of cryoglobulins in HCV infection precise characterization of cryoglobulins and their specific role in the putative associated lesion must be elucidated to determine pathological significance. Thus far, there have been no studies to determine if there is a distinct type of cryoglobulin associated with cirrhosis. The role of specific cryoglobulins has been demonstrated, in part, only for the mRF in membranous proliferative glomerulonephritis lesions [92] and skin lesions [78] in HCV-type II cryoglobulinemia.

The hypothesis that with increasing duration of disease type III mixed cryoglobulinemia progresses to type II cryoglobulinemia was derived from the observation that the apparent duration of disease in patients with hepatitis C and type II cryoglobulins was twice as long as those with type III cryoglobulinemia [89]. More sensitive methods used for detection of monoclonal Ig [93] have revealed patients with oligoclonal IgM and has lead to the additional postulation that the IgM RF producing B cells transform from polyclonality to oligoclonality to monoclonality with chronic infection [94]. There have been no studies to test this 'cryoglobulin transition' hypothesis.

The alternative interpretation of the data that underlies the cryoglobulin transition hypothesis is that polyclonal IgM RF, oligoclonal IgM, and monoclonal IgM RF (mRF) arise by distinct mechanisms and type II cryoglobulinemia develops without evolving from type III cryoglobulinemia through oligoclonal IgM RF populations of B cells. The mechanism for the production of mRF in HCV is not known. The majority of these mRF, WA mRF, are encoded by a restricted set of Ig genes that generated a unique antibody binding site epitope or crossidiotype. MRF occur in other infections but the high prevalence of mRF and

presence of the WA crossidiotype may be unique to HCV infection.

In HCV infection there is no molecular evidence that mRF B cells in patients with type II cryoglobulinemia arise from a population of polyclonal RF B cells in patients with type III cryoglobulinemia. On the contrary, recent studies using B cell clonal analysis in patients with chronic HCV infection have shown that the predominant mRF B cell in type II cryoglobulinemia, WA B cells, can be detected in approximately 4% of HCV-infected patients without cryoglobulinemia or RF but not in patients with type III cryoglobulinemia [95].

It is well established from animal model studies that circulating IgG immune complexes stimulates production RF. The oligoclonal IgMs detected in HCV infection have not been shown to be RF. These oligoclonal IgM antibodies may represent a response to viral antigens rather than IgG immune complexes. Oligoclonal IgMs have been documented in other viral infections particularly in cerebrospinal fluid [96]. Moreover, there is no evidence that RF in human immune complex diseases transitions to mRF. In rheumatoid arthritis, the best example of an immune complex disease with RF formation, the development of mRF is an exceedingly rare occurrence.

3.2. Membranoproliferative glomerulonephritis (MPGN) independent of mixed cryoglobulinemia

The key issues regarding MPGN in patients with HCV infection were addressed at a recent NIH sponsored workshop on hepatitis C and renal disease [97]. These include reasons why some cryoglobulins are associated with kidney damage while others are not, and whether non-cryoglobulinemic associated glomerulonephritis can be caused by HCV. Glomerulonephritis, almost exclusively MPGN, is associated with mixed cryoglobulinemia secondary to HCV infection in approximately one third of patients [1]. The MPGN lesion is characterized by distinct morphological features: endocapillary proliferation; monocytic infiltration; double contour membranes; large eosinophilic; PAS-positive intraluminal deposits; and vasculitis of small and medium sized renal arteries [92]. On electron microscopy, deposits, usually subendothelial, are present and may have tubular and crystalline patterns that are also found in the cryoglobulins [98,99]. MPGN appears to occur predominantly with type II cryoglobulins [92,100].

The mRF in the type II cryoglobulins appears to be a critical element in the deposition of these cryoglobulins in glomeruli [92]. There is evidence from studies of MPGN associated with mixed cryoglobulins in patients with systemic lupus erythematosus that this may be a general role of mRF in MPGN lesions [101]. The relatively high prevalence of mRF in HCV infection may be the reason for the predominance of HCV in cryoglobulinemic glomerulonephritis [100].

Johnson and co-workers first proposed that MPGN occurs in HCV infected patients without cryoglobulinemia [102]. However, in that initial study, five patients had cryoglobulinemia and all eight patients had RF in the serum. Moreover, in a second study by the Johnson group four patients that initially had MPGN without cryoglobulinemia developed cryoglobulinemia on follow-up [103]. This issue has become controversial since several studies have not found an association of HCV with MPGN [104–108] in the absence of cryoglobulinemia. Neither of the conflicting arguments is convincing [105,109] since definitive studies to detect and characterize RF in MPGN lesions in the absence of cryoglobulinemia, and B cell clonal analysis that can detect mRF B cells in the absence of cryoglobulinemia [71], have not been performed.

From studies in Italy [105], the United States [100,102,103], and Japan [107], MPGN associated with type II cryoglobulinemia is the predominant type of glomerulonephritis clinically associated with HCV infection. The prevalance is of MPGN in HCV-type II cryoglobulinemia is approximately 30% [1]. However, from autopsy studies on 188 Japanese patients with HCV infection, predominantly with cirrhosis, the prevalence of histologic glomerular deposition of immune complexes was considerably higher than the prevalence of symptomatic glomerulonephritis [110]. The prevalence of histologic glomerulonephritis was 54.8%; the prevalence of MPGN, the most common type, was 11.2%. However, only 12.2% of patients, mainly with MPGN, had clinical manifestation of glomerulonephritis in the year prior to death. This finding is consistent with the higher prevalence of cryoglobulinemia among patients with advanced hepatitis C [89]. Considering the marked heterogeneity of immune complexes in HCV infection [111], a variety of immune complexes are most likely deposited in glomeruli, but only a portion of these, possibly those with mRF, appear to be nephrotoxic.

3.3. Infection of B cell and development of lymphomas

A widely held hypothesis is that the development of B cell malignancies in HCV-infected patients are related to infection of B cells by the virus. From the few studies reported on HCV in lymphoma cells, there is no definitive evidence that the virus is present in the malignant cells. In a study of eight HCV-infected patients with extranodal B-NHL, HCV was not detected in the lymphoma cells [112]. In a salivary gland lymphoma, the virus was found not in the lymphoma cells but in the non-malignant glandular epithelial cells [113]. In one of nine primary liver lymphomas in HCV-infected patients, HCV was detected in the lymphoma cells but not in surrounding hepatocytes using an in situ hybridization assay for HCV that had not been validated on known positive samples [114]. In a recent study on a patient with HCV infection, type II cryoglobulinemia and a monocytoid B cell lymphoma, evidence for replication of the virus, was demonstrated in the spleen and in a cell line developed

from the spleen. Since the cells were immortal in cell culture without employing hybridoma technology, the cell line was probably developed from the lymphoma cells. However identity of the cultured cells with lymphoma cells from the patient was not demonstrated [115].

In opposition to a direct role of HCV in lymphomagenesis is that only 4–6% of patients with HCV-type II cryoglobulinemia develop frank B cell malignancies in long-term follow-up studies [116,117]. Broader studies on the association of B-NHL with HCV infection are conflicting. Eight studies, performed mainly in Italy and Japan, found increased prevalence of HCV infection in patients with B-NHL (9–32%) compared to controls (0–3.1%) [118–123] but ten studies done mainly in the United Kingdom and North America did not find an association [124–131]. However in a longitudinal study of 2162 chronic hepatitis patients in Osaka between 1957 and 1997, the region having the highest prevalence of HCV infection in Japan, only four patients with B-NHL were detected [122]. Hence, HCV infection may be associated with B-NHL but not as prominently as suggested by some of the studies from Italy and Japan. The very high prevalence detected in the latter studies may be due to confounding patient selection bias because of the endemic regions in Italy and Japan. On the other hand, the low prevalence in the non-endemic areas may be due to inadequate patient sample sizes considering the low HCV prevalence in these regions.

A role for HCV in lymphomagenesis is not readily apparent since HCV is not an oncogenic virus. However, such a role cannot be excluded since the HCV core protein has been reported to be involved in transformation of cells to malignant phenotypes [132]. In addition, hypermutation of immunoglobulin and oncogenes in HCV-infected cells has been demonstrated in a preliminary report [133]. The recent finding that regression of splenic lymphoma associated with HCV infection occurs synchronously with decline in viremia with anti-viral therapy strongly suggests a role for HCV in the development of malignancy [134]. However, thus far, no specific HCV lymphomagenic mechanisms have been delineated.

The evidence on HCV lymphotropism is conflicting [59,135]. It has not been established that HCV has an unrestricted tropism for lymphocytes. The data supporting lymphotropism is extensively reviewed in reference [59]. The sum of the evidence suggests that replication of HCV occurs only in a small number of peripheral blood mononuclear cells (PBMC), and probably only under abnormal conditions, i.e. immunosuppression [136,137], cell transformation [115]. The finding, in several studies, that the quasispecies of HCV present in PBMC were not present in the serum is consistent with this assessment [137–139]. If HCV infection of lymphocytes was as productive as that of hepatocytes then the virus produced by these cells would constitute a significant part of the viremia since the blood lymphocyte compartment is 1×10^{10} and lymphocyte traffic through the blood is 5×10^{11} cells. The latter number equals the number of lymphocytes contained within the whole human body and is 2.5 times the number of hepatocytes in the liver [140].

Chromosomal translocation has been suggested as a possible mechanism for the role of HCV infection in lymphomagenesis. A high prevalence of *t*(14;18) translocation in patients with HCV infection and an even higher prevalence in patients with HCV-type II cryoglobulinemia have been reported [141–143]. This translocation is thought to predispose to malignancy because the Bcl-2 over-expression that may accompany the translocation confers longevity on cells. However, thus far, *t*(14;18) translocation and Bcl-2 over expression in HCV infected patients have not been demonstrated to occur in the same cells and more specifically in the mRF producing B cells [141,144]. This is a critical point since Bcl-2 over expression may be a characteristic of monoclonal B cells in patients with HCV-type II cryoglobulinemia [145] and thus far, all WA mRF cells studied have shown Bcl-2 over-expression without *t*(14;18) translocation [146,147]. Since Bcl-2 over expression does not always accompany *t*(14;18) translocation [148], B cells with *t*(14:18) translocation without Bcl-2 over-expression and mRF B cells that overexpress Bcl-2 without *t*(14:18) may present in the blood. The two populations could not be discerned without isolation studies that thus far, have not been performed.

A review of 854 lymphomas reported in patients with HCV infection (Table 2) [37,38,43,47,112,118–121,126,128,149–182] illustrates the predominance of B-NHL in these patients. Seventy-nine per cent of patients tested for cryoglobulinemia (42% were not tested) were positive suggesting a higher prevalence of B-NHL among patients with than without cryoglobulinemia. In a multicenter case-control study included in Table 2, the HCV-attributable risk for developing B-NHL was estimated at 4.6%, i.e. approximately one in twenty B-NHL in Italy are associated with HCV infection [183].

The *t*(14:18) translocation, first discovered in patients with follicular cell lymphoma, is present in most patients with this lymphoma. However, *t*(14;18) translocation may occur in high prevalence in normal individuals, increase in prevalence with aging, and occur with greater frequency in proliferating cells [184–188]. Hence, *t*(14;18) translocation in HCV infection does not necessarily have implications for lymphomagenesis. If this transformation does have a role, an increase percentage of B-NHL would be expected to be follicular lymphoma especially among patients with HCV-type II cryoglobulinemia. Table 3 addresses this question. The percentage of each subtype of B-NHL in the various groups of HCV-infected patients from 45% of the patients in Table 2 (55% of B-NHL were not subtyped) are compared to distribution of these subtypes in the general population obtained from an international lymphoma study [189]. Contrary to expectation, immunoplasmacytic lymphomas/Waldenstrom macroglobulinemia and not follicular lymphoma appears to be the most common B-NHL among

Table 2
Predominance of B-NHL lymphomas in HCV-infected patients with and without cryoglobulinemia [37,38,43,47,112,118–121,126,128,149–182]

Lymphoma type	Symptomatic type II cryo	Type II cryo	Type III cryo	Cryo not typed	Cryo negative	Cryo not tested
Lymphoplasmacytic/Waldenstrom	12	10	0	23	4	2
Chronic lymphocytic/small cell	3	0	0	0	0	0
Marginal zone cell						
Nodal	2	2	3	11	3	13
Extranodal	2	0	0	6	36	34
Splenic	0	0	0	7	2	0
Follicular	2	0	0	14	17	5
Mantle cell	0	1	1	0	1	0
Diffuse large B cell	7	4	7	16	22	32
Plasma cell myeloma	0	0	0	0	0	5
Burkitt's	0	0	0	0	1	0
Hairy cell leukemia	0	0	0	2	0	0
Hodgkin's	0	0	0	0	1	1
NK	0	0	0	0	0	0
T-cell	0	0	0	2	1	0
B-NHL, unclassified	19	0	0	267	44	158
Unclassifiable	0	0	0	0	0	3

Classification, ref. [149].

patients with type II cryoglobulinemia. Follicular lymphoma that constitutes 22% of all B-NHL was not increased in any of the HCV infected groups, but rather there was a significant decreased percentage in the groups with cryoglobulinemia. Also of note, there was an increased percentage of extra-nodal marginal zone cell lymphomas, in particular, among HCV-infected patients without cryoglobulins.

The increased percentage of extra-nodal marginal cell lymphomas in HCV infection illustrated in Table 3 and the report of more than a 10-fold greater risk for developing B-NHL of the liver and salivary gland than the risk of lymphomas at other sites is consistent with the hypothesis that B-NHL arise selectively from marginal zone B cell [190]. The extra-nodal marginal zone cell lymphomas are considered to be derived from organized lymphoid tissue that accumulate in response to infection or as a component of autoimmune disease at a site beyond the lymph node [191]. There is evidence that the monoclonal B cells in type II cryoglobulinemia arise predominantly in the pseudo-follicle in the liver [145,192]. Also consistent with this hypothesis are studies on a patient with partial trisomy 3 and HCV-type II cryoglobulinemia. Phenotypic markers characteristic of marginal zone cells were present on the WA B cells present in the blood in this patient [147]. The patient had features of splenic lymhoma and, like the monoclonal B cells patients with splenic lymphoma and HCV-infection, the WA B cell regressed with antiviral

Table 3
Percentage of B-NHL subtypes among patient in Table 2: comparison with International Lymphoma Study [189]

Lymphoma type	International Lymphoma Study	Table 2		
		Sym type II	All cryo	Neg cryo
Lymphoplasmacytic/Waldenstrom	1.2	43******	33***	5
Chronic lymphocytic/small cell	6.7	11	1*	0**
Marginal zone cell				
Nodal	1.8	7	13******	3
Extranodal	7.6	7	9	42******
Splenic	<1	0	5***	2
Follicular	22.1	7*	12****	20
Mantle cell	6.0	0	1*	1*
Diffuse large B cell	30.6	25	25**	26*
Burkitt's	<1	0	0	0
Total patients	1069	28	137	85

Two proportion analysis (PASS 2000 software): $P = * < 0.05$, ** < 0.01, *** < 0.001, **** < 0.0001, ****** $< 10^{-6}$.

therapy. The over-represented region of chromosome 3, bands 3q11–29, that were present in this patient is commonly involved in marginal zone B cell lymphomas. The prevalence of this chromosomal transformation in HCV infection has not been determined.

Approximately 25% of B-NHLs in patients with HCV-type II cryoglobulinemia appeared to arise from WA B cells [150,193]. Based on the reactivity of HCV envelope E2 protein with CD81 and homologies of Ig V genes sequences of lymphoma cells with mRF and anti-E2 sequences, it has been postulated that E2 drives proliferation of the monoclonal B cells in patients with HCV-type II cryoglobulinemia that undergo malignant transformation to B-NHL [150,194]. However, the rationale for this hypothesis is faulty since none of the WA mRF sequences, the major mRF in HCV-type II cryoglobulinemia, have any significant homology with the cloned anti-E2 envelope antibodies [193] and the expressed receptors of the with IgG [195]. Moreover, it has been demonstrated that WA mRF does not cross-react with E2 (Elfahal M, Agnello V, unpublished observations).

At present there are more questions than answers regarding the association of B-NHL with HCV infection. The critical question is whether virus replication occurs in normal B cells and is directly lymphomagenic or is lymphomagenesis a stochastic process accompanying HCV-driven proliferation of B cells. Unequivocal evidence of replication of HCV in transformed B cells has been presented [115], however, there is no unequivocal evidence that replication occurs in normal B cells. The low prevalence of B-NHL in long term follow-up studies on patients with HCV-type II cryoglobulinemia favors the stochastic hypothesis [116,117].

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