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Review

The cryoglobulins: an overview

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Abstract

Cryoglobulins are cold-precipitable immunoglobulins associated with a number of infectious, autoimmune and neoplastic disorders. Their appearance along with rheumatoid factor (RF) can be considered a normal event in the clearance of immune complexes and rarely produces any symptoms. The association between hepatitis C virus (HCV) and mixed cryoglobulinemia (MC) has been rendered evident since the recognition of serological markers of HCV infection. There is thus every reason to suppose that direct or indirect involvement of B cells on the part of the HCV results in their persistent stimulation, clonal expansion and release of molecules with RF activity. The formation of RF/IgG immune complexes is the key pathogenetic mechanism. The close correlation between HCV infection and MC also throws new light on the interpretation of autoimmune phenomena in the course of viral infection and on the close link between autoimmune diseases and lymphoproliferative disorders. The higher risk of non-Hodgkin's lymphoma (NHL) displayed by HCV positive subjects, especially in the Mediterranean basin, suggests that the HCV's chronic lymphoproliferative drive may progress towards frank lymphoid neoplasia. The presence of MC does not represent an *in situ* or 'occult' NHL, because recent evidences indicate that none of the clones interpreted as predominant displays the molecular features of a true neoplastic process. The cryoglobulinemic syndrome is probably the consequence of pathogenic *noxae* that act upon the immune system of a host in which regulation of the peripheral T cell response appears to be in some way altered.

Keywords Cryoglobulinemia, hepatitis C virus (HCV), lymphoproliferation, rheumatoid factor (RF).

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Introduction

A singular occurrence was first reported by Wintrobe and Buell in 1933 [1]: precipitates of various sizes appeared in some sera stored at a low temperature and then disappeared during incubation at 37 °C. The term 'cryoglobulins' was coined for these cold-precipitable serum proteins by Lerner and Watson in 1947 [2]. Some 20 years later, Meltzer and Franklin [3] provided a nosographical correlation of cryoglobulinemia with a particular clinical triad: purpura, arthralgias and asthenia.

Cryoglobulinemia is a systemic vasculitis that mainly damages the small and medium-calibre arteries and veins. It is thought that the deposition of immune complexes on the vessel wall activates complement and mediates this damage [4].

Indeed, small amounts of cryoprecipitable material in normal human sera are frequently detected, and it is likely that they reflect specific physiological interactions between immunoglobulin (Ig) molecules [5]. On the contrary, cryoprecipitation occurring in cryoglobulinemia is caused by intrinsic characteristics of both the monoclonal and the polyclonal Ig components [6].

The viral origin of cryoglobulinemia was suspected from the outset, but it was not until the beginning of the 1990s

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that the first evidence of its close relationship with hepatitis C virus (HCV) infection appeared [7,8]. Subsequent observational and experimental confirmation of the etiological role of HCV has brought about a substantial revision of the classification of cryoglobulins and suggested new ways of treating cryoglobulinemia.

This article reviews the main clinical hallmark of the cryoglobulinemic syndrome and discusses the potential causative role of HCV. A fuller understanding of the virus-related mechanisms of lymphoproliferation in the cryoglobulinemic patients is explored in view of the unique clinical and biologic opportunity to link viral infection, autoimmunity and benign and malignant lymphoproliferative disorders.

Laboratory detection

When cryoglobulinemia is suspected, blood is drawn with a syringe warmed to 37 °C and left to clot at the same temperature under thermostat control. The centrifuged serum is then placed in a refrigerator at 2–4 °C for one or more days. In positive cases, the cryoglobulins precipitate to the bottom of the test tube. They are separated from the supernatant and purified by washing with buffered physiological saline that has been warmed to remove any serum proteins passively trapped in the precipitate. Centrifugation at 4 °C and 1400 r.p.m. in a graduated Wintrobe tube enables the amount of precipitate to be expressed as a percentage (the cryocrit) of the total serum (Fig. 1).

The next step is immunochemical typing. Electrophoresis on cellulose acetate often (though not always) reveals a homogeneous band, usually in the gamma zone, less frequently in the beta zone. Immunoelectrophoresis shows fusion for most of their course of the two precipitation arcs ascribable to the IgGs and the IgMs, and this illustrates their close interaction in the formation of immune complexes.

Serum complement levels are invariably reduced: the C4 component in particular is sometimes unmeasurable. Some IgMs display anticomplement activity. In addition, the bone marrow often has a characteristic histomorphological picture consisting of focal clumps of lymphocytes often associated with a slight increase in plasma cells. Lastly, the IgMs display high rheumatoid factor (RF) activity even when their serum concentration is normal.

Classification of cryoglobulins

Brouet *et al* [9] classified cryoglobulins in three types according to their immunochemical composition, and this is still valid on account of its nosographic simplicity and good correspondence with the clinical manifestations of cryoglobulinemia. The three forms are: (a) single or type I; (b) mixed, type II; (c) mixed, type III. Mixed cryoglobulins

Fig. 1 Different levels of whitish cryoprecipitates after keeping Wintrobe's tubes at 4 °C for 48 h and centrifugation at 1400 r.p.m. for 10 min

(MC) are immune complexes composed of monoclonal IgM (MC type II) or polyclonal IgM (MC type III) that have RF activity (IgM-RF) and bind to polyclonal IgGs. There are, however, unusual cryoglobulins whose immunochemical structure cannot be fitted into these three classes. MC formed by oligoclonal IgM and traces of polyclonal IgG, for example, have been described and their inclusion in the Brouet classification as a type II-type III variant has been proposed [10]. This IgM microheterogeneity has been seen as indirect evidence of a possible transition from type III to type II. Transformation of the IgM-RF fraction from polyclonal (type III) to oligoclonal (type II-type III variant) and then to monoclonal (type II) would point to a spectrum of biological continuity on the part of B cell clones whereby their expansion in response to an antigen stimulus results in the prevalence of one clone over the others. It is believed that microheterogeneity of the monoclonal fraction is present in about 10% of cases [11]. A higher frequency of the subclass IgG3 has also been demonstrated [12]. IgG3s have a peculiar cryogenic role of their own, stemming from their ability to assemble themselves through spontaneous Fc–Fc interactions. The high cryogenic potential of the anti-IgG3 IgMs has also been stressed [13].

By contrast with normal serum proteins, which are highly soluble and can remain in aqueous solution in a cold environment even at concentrations of more than 100 mg mL^{-1}, cryoglobulins precipitate or gel when the

temperature falls below 37 °C at pH 7, and even at concentrations of only 0·1 mg mL^{-1} [14]. The precipitation temperature increases in function of the concentration. At low concentrations it is about 4 °C. Cryoglobulins always redissolve when serum is warmed at 37 °C. Very small quantities of cryoprecipitate (about 30 ng mL^{-1}) may, however, be found even in normal conditions [5].

Type I cryoglobulins account for 10–15% of the total. They are almost only observed in Waldenström's macroglobulinemia or multiple myeloma, and are usually monoclonal IgM or less frequently IgG fractions. On the rare occasions when they belong to the IgA isotype, they sometimes behave as pyroglobulins, i.e. proteins that precipitate irreversibly at 50 °C. Type I cryoglobulins may also be solely composed of light chains and be excreted in the urine as Bence Jones cryoproteinuria, or accumulate in the serum in the event of renal failure.

About 6O% of cryoglobulins are MC (IgM-IgG) with a monoclonal IgM fraction (type II). These cryoglobulins sometimes include nonimmunoglobulin protein fractions. Generally speaking, neither the IgG component nor the IgM fraction is cold-precipitable on its own. The IgM fraction, however, behaves like an incomplete cryoglobulin, in the sense that it can also form a cryoprecipitate with IgG from a normal individual, whereas no precipitation takes place when the IgG fraction from a patient with MC is reacted with the IgM of a normal subject [14].

The type II IgG fraction is always polyclonal and thus mounts both kappa and lambda light chains, whereas its monoclonal IgM fraction almost always mounts kappa chains. It is endowed with RF activity, and in many cases anti-idiotypic activity [15]. Most IgM fractions react with both intact IgGs and the $F(ab')_2$ fragment, and also with the Fc fragment of autologous IgGs. These two types of molecular interaction confer greater stability on cryoprecipitating IgM-IgG immune complexes. Type II MC are frequently associated with vasculitis, glomerulonephritis and skin manifestations [16].

Type III MC with their polyclonal IgM and IgG fractions account for the remaining 25–30% of the cryoglobulins.

Clinical manifestations

Our observation of more than 200 patients followed for many years has shown that the clinical picture of cryoglobulinemia is very heterogeneous and protean (Table 1).

As mentioned earlier, type I cryoglobulinemias are almost always diagnosed as immunoproliferative diseases. Patients are not usually distinguishable clinically from those with Waldenström's macroglobulinemia, multiple myeloma or chronic lymphocytic leukemia, where the presence of cryoglobulins together with a high cryocrit may be a casual finding. Signs of peripheral vessel occlusion are not unusual. This is sometimes associated with a hyperviscosity syndrome and hence the presence of purpura lesions and dystrophic manifestations, and the formation of torpid ulcers on the lower limbs.

Table 1 Symptoms most frequently observed in 'essential' mixed cryoglobulinemia

Signs and/or symptoms	Approximate percentage
Purpura	90
Hepatomegaly	70
Arthralgia	60
Asthenia	60
Splenomegaly	50
Raynaud's phenomenon	40
Polyneuropathy	36
Arterial hypertension	35
Leg ulcers	30
Papules, pustules	10
Lower-limb edema	8
Pericarditis	4
Pleural effusion, pulmonary fibrosis	3
Cold urticaria	3
Hyperviscosity syndrome	2
Intestinal vasculitis with abdominal pain	1

Type II cryoglobulins are found in:

1 some patients with Waldenström's macroglobulinemia whose monoclonal IgM fraction displays anti-IgG activity and complexes with the patient's polyclonal IgGs ('rheumatoid macroglobulinemia');

2 patients with connective tissue and other autoimmune diseases;

3 patients with acute and chronic liver diseases;

4 infectious (usually chronic) diseases.

In most cases, however, there is no distinct picture and the term 'essential mixed cryoglobulinemia' (EMC) is usually employed. EMC is more frequent in Southern Europe than in Northern Europe and North America [17]. Its clinical expression varies greatly from one patient to another and ranges from nonspecific asthenia and arthralgia to frank vasculitis, glomerulonephritis and sensorymotor neuropathy. When reliance is placed on the clinical data alone, the correct diagnosis is often missed or delayed. Correct withdrawal and separation of serum to reveal the precipitate are of crucial importance in suspected cases.

Because the most frequent manifestations of EMC are recurrent purpura eruptions, patients are often seen by the dermatologist when purpura is accompanied by torpid leg ulcers, Raynaud's phenomenon, edemas and urticaria [18].

Purpura is undoubtedly the main clinical sign of cryoglobulinemia. Its lesions are palpable, isolated (at least in the initial stages of an eruption episode) and primarily appear on the lower limbs, less frequently on the buttocks and trunk, and very rarely on the face. Cryoglobulinemic purpura is typically intermittent. Eruptions varying greatly in frequency and not usually triggered by a particular cause are separated by periods of apparent remission. Lower-limb purpura is usually preceded by paresthesia or local pricking sensations rather than frank

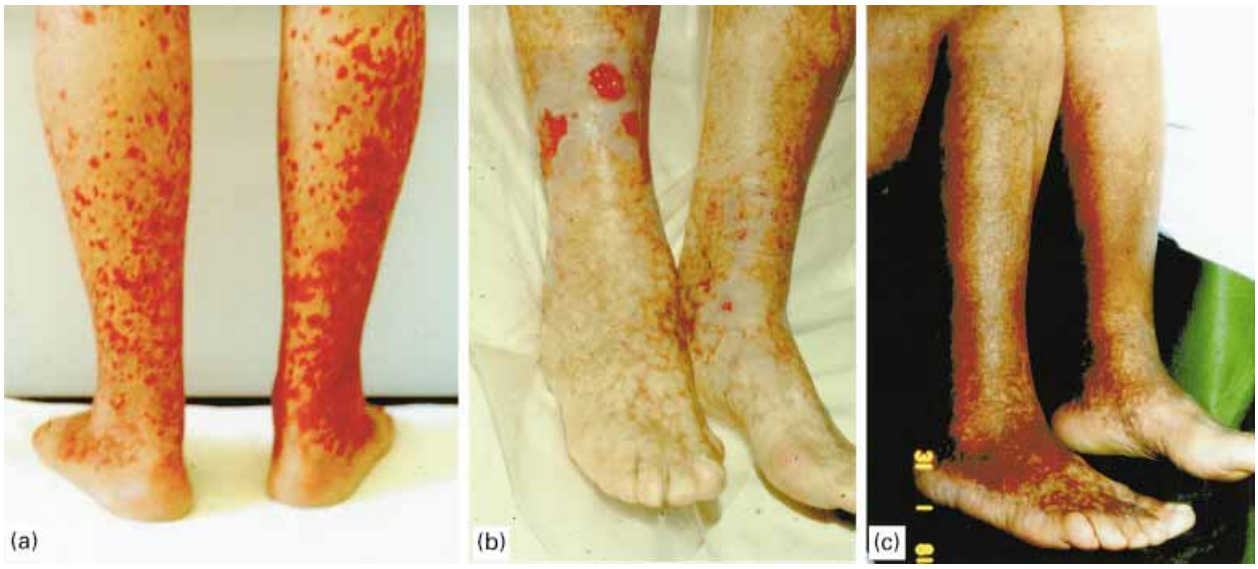

Fig. 2 Various stages of cutaneous vasculitis in type II cryoglobulinemia. (a) Acute phase of purpuric eruptions involving lower legs and thighs; (b) large and deep cutaneous ulcers crop up in dyschromic skin of the legs; (c) a bootleg-like extension of skin brownish pigment resulting from chronic hemosiderin deposits in a 20-year-lasting cryoglobulinemia.

concomitant pain. Ulcers usually appear above the malleoli. The lesions regress spontaneously leaving discoloured skin patches formed of hemosiderin deposits (Fig. 2).

Raynaud's phenomenon is often part of the clinical overture and generally involves the hands and feet, lips, ears and the tip of the nose. Arthralgia is common. The proximal interphalangeals of the hands, the metacarpophalangeals, the knees and the hips are often affected. The kidneys are involved in about 25% of cases. Renal failure arrives slowly after years during which the only signs are usually slight proteinuria and/or microhematuria. Hypertension indicative of impaired renal function may appear during the course of the cryoglobulinemic syndrome. The most frequent histological diagnosis is membranoproliferative glomerulonephritis [19].

Liver involvement is observed in about two-thirds of EMC patients [20]. Histopathological examination of transcutaneous liver biopsy specimens reveals a very broad spectrum of disorders ranging from nonprogressing pictures of microsteatosis, portal lymphoid aggregates, hyperplasia of sinusoidal cells, intralobular foci of lymphomonocyte aggregates and sporadic intralobular liver cell necrosis to less frequent pictures of frank active chronic hepatitis, with or without cirrhosis [8].

The incidence of nervous system involvement varies in the literature and reaches almost 60% of cases [21]. Involvement of the peripheral nervous system presents as sensory-motor neuropathy, especially of the lower limbs, where paresthesias, often painful, with loss of strength are reported by the patient. Involvement of the central nervous system with transient dysarthria and hemiplegia is an unusual finding and confusional states are rare [22]. Late signs include abdominal pain indicative of intestinal vasculitis [14].

As can be seen in Fig. 3, there is an inverse relation between vasculitis and the cryocrit: the lower the cryocrit, the greater the frequency of signs of vasculitis, especially in type II mixed cryoglobulinemia. This is a still unexplained phenomenon, but it seems to be related to the intrinsic capacity of immune complexes to activate complement *in situ*. From the current data in HCV-related cryoglobulins it appears that the relative concentrations of virions and immunoglobulins (IgG with anti-HCV activity and IgM with RF activity) are selectively formed *in situ*. The finding that the cryoglobulins dissociate almost completely to monomeric IgM and monomeric IgG at 37 °C is consistent with the notion that circulating high molecular weight complexes are not found in circle and are rapidly removed [23].

Histological examination shows that cryoglobulinemic vasculitis may or may not be accompanied by inflammatory infiltrates, typically composed of lymphocytes. Palpable purpura is more frequent in patients with a low cryocrit, whereas hyaline thrombosis with intravascular deposition of PAS-positive material is more common in type I cryoglobulinemia associated with a high cryocrit. This lesion is clinically silent, but may eventually be complicated by nephropathy. Lastly, the postinflammatory histological picture of the skin is characterised by necrosis, fibrosis, hemosiderosis and repair tissue.

Pathophysiology

Hepatitis C virus

Investigation of the relationship between EMC and virus infections, especially on the part of the so-called 'hepatotropic' viruses, began in the second half of the 1970s.

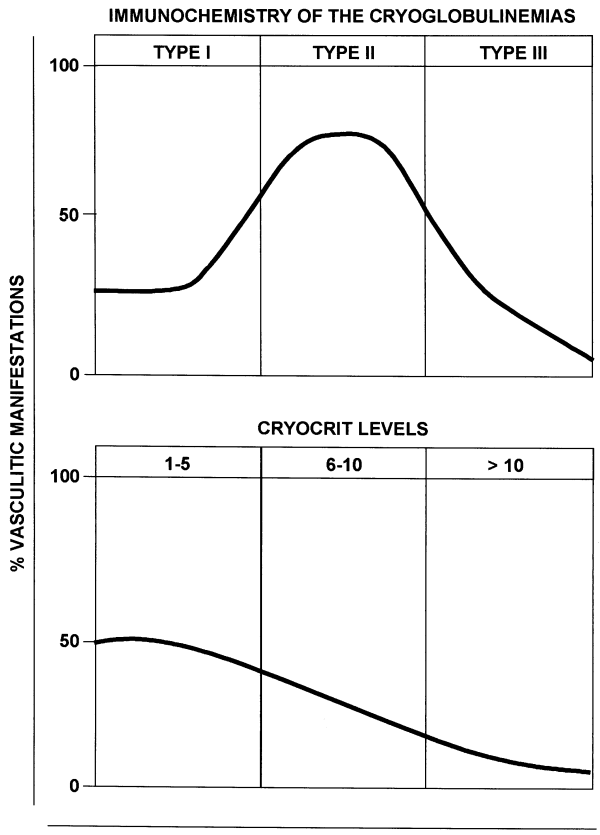

Fig. 3 Vasculitic manifestations are the most frequent percentage-wise in type II mixed cryoglobulinemia and inversely related with the cryocrit.

Mistaken over-emphasis of the etiological role of the hepatitis B virus [24] has since been replaced by the gathering of evidence in favour of the close relation between EMC and the HCV, as shown by the very high percentage of HCV infection markers (anti-HCV and HCV RNA) in the serum of EMC patients [20,25–28].

The incidence of HCV infection in EMC, in fact, ranges from 40% to 100% in the reported case series and in different geographical areas, though improved diagnostic techniques have shown increasing prevalence rates in the same area over the course of time. Almost all cases of EMC in the Mediterranean area are HCV positive [29].

The three main lines of evidence for the etiological role of the HCV in cryoglobulinemia are set out below:

1 The concentration of HCV RNA in the cryoprecipitate is from 10- to 1000-fold greater than in the supernatant. This correlation cannot be demonstrated for the anti-HCV antibody titres, which are about the same in the two phases. Because the antibody concentration is not correlated with the cryoprecipitation, it may be inferred that there is an excess of antibodies with respect to the viral particles and virus-related proteins. The cryoprecipitate also contains most of the known antigens (core, E1, E2, NS3, NS4, NS5) and their corresponding antibodies [30];

2 Examination of the soluble and cryoprecipitating immune complexes suggests that the IgG component to which the IgM-RF fraction binds is directed against the HCV proteins. It is interesting to note that the anti-HCV activity in the immune complexes is directed against both the surface proteins of the virus and the proteins masked by the capsid [31];

3 Viral proteins have been found in the damaged tissue, namely in the skin of patients with cryoglobulinemic vasculitis [32] and the kidney of those with cryoglobulinemic membranoproliferative glomerulonephritis [23].

Mechanisms of cryoprecipitation

Production of IgM-RFs represents the crucial point in the pathophysiology of the cryoprecipitating process. IgM-RFs production probably starts from a limited number of genes of the germinal line. Their analysis in HCV-associated cryoprecipitates shows that more than 90% express the WA cross-idiotype (WA being the initials of the patient in whom it was identified) [33]. It may thus be concluded that the antibody repertoire is rather limited and that IgM-RFs are coded for by a few genes, probably in response to very similar antigenic stimuli. These genes are also active in normal subjects and high membrane expression of RF receptors is also observed on their lymphocytes [34]. It is believed, in fact, that the normal role of RF includes the capture, processing and presentation to T cells of antigens trapped in immune complexes. This would explain the intense RF production observed in many autoimmune diseases and some lymphoproliferative disorders [35].

Immune complexes are a significant biological feature of acute and chronic HCV infection [31]. Their production is constant and does not depend on the severity of the liver damage. They reach the lymph nodes via the afferent lymphatics and mainly distribute themselves in the mantle zone with its abundant RF-producing cell precursors [36], where they presumably act as antigen-presenting cells, i.e. cells that present the HCV antigen in the context of the major histocompatibility complex. Further support for this assumption is provided by the fact that HCV-related proteins are mostly identifiable in the extrafollicular areas, including the mantle zone, of the lymph nodes of HCV-positive patients [37].

The WA cross-idiotype is associated with fetal B cell phenotype IgM-RFs. IgMs expressing the WA cross-idiotype, however, are also observed in immune complexes devoid of rheumatoid activity. They do not cryoprecipitate and are not associated with vasculitis. The nature of WA-RF cells, indeed, is still uncertain [38].

One peculiar histological feature of HCV infection is the combination, e.g. in the liver, of mononuclear inflammatory cells in follicle-like formations. Immunophenotyping of the cells comprising the so-called intraportal lymphoid nodules found in the liver biopsies of cryoglobulinemic HCV positive patients shows that they are mostly B cells expressing IgM and (in some cases) CD5 antigen [39]. Co-expression of CD5 by WA cells, and HCV stimulation

of the production of IgM-WA molecules by B cells, are two plausible deductions from these findings.

Lymphocytes from the liver of HCV positive patients have provided particularly interesting data. Production of RF is associated with oligoclonal or monoclonal expansions of intrahepatic B cells [40]. Topographic investigation by microdissection suggests that the B cell clones are derived from different cells of the inflammatory polyclonal population in the infected liver.

A pathogenetic mechanism whereby an initial response to the HCV results in the production of IgM-WA with no rheumatoid activity, and that this is then acquired through somatic mutations accompanying cell proliferation due to persistance of the antigenic stimulus, may thus be proposed. A prolonged stimulus may be supposed to restrict the oligoclonal foci and induce the emergence of a single clone capable of producing monoclonal IgM-WA. The conclusion that the production of cryoglobulins is correlated with the duration of infection as a result of this mechanism has been drawn from the observation that their frequency is higher when cirrhosis typical of long-standing liver disease is present [27,41]. The production of IgM-RFs would probably induce the formation of type III cryoglobulins that eventually change into type II cryoglobulins when the RF becomes monoclonal. However, there is no clinical support for this mechanism, and the shift is still a rare biological event after many years of close observation.

Cryoglobulinemia is probably not dependent on HCV infection, but a disorder that may arise in a subgroup of patients lacking the physiological mechanism needed to inhibit the production of IgMs with high affinity and a high pathogenic potential [42]. A mechanism of this kind would rest on the failure of T cell binding of CD40 with the appropriate ligand, which is probably lacking in cryoglobulinemic subjects [43]. In short, HCV's chronic stimulation of B cells results in somatic mutations generating high-affinity RF molecules and inadequate control of their production.

Non-Hodgkin's Lymphoma (NHL)

It has been shown that EMC, which could be more accurately called 'HCV-correlated MC', is based on a lymphoproliferative disorder whose molecular features have been determined in both the bone marrow [44] and the liver [40].

Lymphocyte activation and the ensuing proliferative spurt have suggested the possibility of a correlation between chronic HCV infection, cryoglobulinemia and non-Hodgkin's lymphoma (NHL). So far, however, the evidence has been solely drawn from clinical and epidemiological observations and several biological considerations, namely: (a) an increased prevalence of HCV infection in B-cell NHL [45–52]; (b) the high frequency of B-cell NHL in HCV target organs [53–57]; (c) an increased prevalence of B-cell NHLs in hepatopathic patients [58–62]; (d) the frequent observation of monoclonal gammopathies in HCV positive patients with liver diseases [63–67]; (e) the presence of genome sequences and viral proteins in the neoplastic and reactive lymphadenopathies of patients with chronic HCV infection [37]; (f) reduction or disappearance of signs of lymphoproliferation after eradication of the HCV [68,69].

Two types of B-cell NHL associated with HCV infection have been identified:

1 NHLs that arise in cryoglobulinemic patients, involve the bone marrow and usually display a low-malignancy phenotype, though they occasionally evolve to a high-malignancy phenotype;

2 NHL not associated with cryoglobulinemia that usually do not involve the bone marrow and frequently express a high-malignancy phenotype from the start.

It is thought that frank NHL arises in 5–10% of HCV positive cryoglobulinemia patients. Its symptomatology is generally indistinct, though the concomitant autoimmune phenomena may worsen or become complicated by hemolytic anemia, thrombocytopenia and granulocytopenia [33].

The mechanisms whereby the B cell clones that determine the evolution of NHL into a malignant lymphoproliferative disease progress have not been fully defined. There is a general conviction that the viral agent persists indefinitely in the host in a latent or low-replication phase. Evidence to this effect is provided, for example, by data from our laboratory showing specific genome sequences in the absence of viral proteins in B cells from the lymph nodes of cryoglobulinemic patients, whereas genomic sequences and proteins were both absent in those of noncryoglobulinemic subjects. This indicates that these cells do not permit HCV infection and replication, and hence that their malignant progression is not directly correlated with the virus [70].

Our findings that hemopoietic stem cells can support HCV replication is of particular interest [71]. We have, in fact, demonstrated that $CD34^+$ hemopoietic progenitor cells are positive for viral genome sequences and proteins in about 80% of chronic HCV carriers with a between-patient positivity of 5% to 30%. Because $CD34^+$ cells are capable of originating all the blood cell lines, including B and T lymphocytes, monocytes and macrophages, they may be a prime site for infection and transmit it to their direct progeny, such as B and T cells and macrophages, while their ability to self-regenerate would ensure its perpetuation.

The demonstration of the presence of $CD34^+$ stem cells in the liver is of equal interest [72]. This close connection between $CD34^+$ cells and the hepatocytes suggests that the stem cells may be initially infected in the liver and then migrate to form an extrahepatic virus reservoir in the bone marrow, or into the thymus, where they could act as dendritic or antigen-presenting cells. This negative selection results in removal of autoreactive T cells and host tolerance of neutralising viral epitopes. A similar mechanism can be advanced to explain the inability of some apparently immunocompetent persons to eradicate HCV infection.

At present, however, there are no long-term observational studies from which the real impact of the HCV on B-cell NHL unassociated with cryoglobulinemia could be defined. Its role, in fact, is principally based on the prevalence of infection in B-cell NHL patients. Studies showing a

prevalence of as much as 42% in the Mediterranean area and Japan [45–50,52] are countered by findings of percentages similar to those in the general population in Northern Europe [73–75]. These differences could be geographical in origin, i.e. ascribable to the diffusion of the virus in a given area, or ethnic as suggested by the 22% reported for Hispanics in the United States [51].

Interpretation of these data must also take account of the fact that in nearly all the studies cited it is not made clear whether HCV infection preceded the onset of NHL or was acquired via transfusion or blood derivatives. Immunosuppression induced by NHL and aggravated by chemotherapy undoubtedly sets the stage for infection. Many of these studies were also conducted without appropriate controls.

A study [55] carried out in association with a North Italian oncological centre showed that 17 out of 35 noncryoglobulinemic B-cell NHL patients (48·6%) had definitely been anti-HCV antibody positive from 1 to 4 years prior to their diagnosis, and the biochemical and clinical data in the other 18 were also indicative of chronic liver disease that had arisen many years earlier. In addition, comparison with HCV-negative patients showed that NHL was mainly located outside the lymph nodes in the liver and major salivary glands, usually displayed a large-cell histotype and was not associated with clinically significant autoimmune manifestations.

Viral proteins were never found in the lymphomatous tissues, showing that at all events in this disease stage the HCV is not directly involved in the maintenance and/or progression of NHL.

Mention may also be made of the association of the HCV with other lymphoproliferative processes, such as the monoclonal gammopathies of undetermined significance (MGUS), whose prevalence in patients with chronic HCV infection ranged from 2% to 15% in some studies [63–67]. In our experience, MGUS have a prevalence of 8%, run a fully benign course and do not alter the therapeutic response to interferon.

About 20% of HCV-positive patients without clinical manifestations usually display not very high titres of polyclonal RF that does not activate complement nor cause extrahepatic damage. By analogy with the MGUS, we have called this condition 'lymphoproliferative disorder of undetermined significance' (LDUS) [70]. It too presents as a clinically benign form that does not tend to progress to a frankly malignant picture. Long-term prospective observational studies will obviously be required to secure a more precise clinical and biological definition of these patients.

Treatment

Mixed cryoglobulinemias (type II and III)

The effectiveness of interferon-α (IFN-α) in the management of MC was recognised prior to the demonstration of its close relationship with the HCV. In 1987 in fact, Bonomo *et al.* [76] obtained a therapeutic response in 77% of patients treated with 3 MU day^{-1} IFN-α for three months. Two subsequent controlled studies [77,78] showed that 2 MU day^{-1} for one month and then on alternate days for 5 months, or 1·5 MU day^{-1} for one month followed by 3 MU three times a week for 23 weeks produced significant clinical effects and reduced the signs of cutaneous vasculitis.

Our group has conducted a controlled randomised trial with 3 MU three times a week for one year [79]. This treatment reduced the activity of 'HCV-correlated MC' in about 50% of patients. Association of IFN-α with 16 mg prednisolone (PDN) on the non-IFN-α days did not increase the percentage of clinical responses (42%), but did induce an earlier and stable response, and thus delayed recurrences. These two effects may be due to PDN's modulation of the proinflammatory chemokines induced by IFN-α [80]. Lastly, it has been shown that the frequency of the remissions PDN induces on its own is the same as that of a placebo.

The therapeutic effect of IFN-α is closely associated with inhibition of viral replication. Reduction of the HCV RNA to nonmeasurable levels usually precedes transaminase normalisation and reduction of the cryocrit. Inhibition of the virus by IFN-α presumably means that fewer antigens are available for the formation of immune complexes. In addition, there may be a more active clearance of such complexes by the reticuloendothelial system, one reason being improvement of liver cell function. Decreased production and release of IgM-RFs and improved T cell function combine to reduce cryoprecipitation.

By analogy with the treatment of chronic HCV infection, low HCV RNA levels alone are predictive of a favourable response to IFN. Other parameters, such as the cryocrit, the RF titre and the frequency of vasculitis spurts, are of no value in this respect. In terms of clinical response, the effectiveness of IFN-α in the treatment of 'HCV-correlated MC' is comparable to that observed in the management of active chronic disease of the liver without cryoglobulinemia [81].

The demonstration that mean HCV RNA levels are increased by treatment with PDN alone is of particular interest, as it suggests that corticosteroids favor replication of HCV as in other viral diseases.

The improvement achieved with IFN-α, however, is generally short-lived. About 80% of responders relapse within six months after its suspension. There is thus a need to establish the optimum doses and more effective protocols.

Few data are available with regard to the effectiveness of IFN-α plus ribavirin in the management of cryoglobulinemia. In the light of what has been said so far, it may reasonably be supposed that this combination enhances and improves the virological response as in patients with chronic HCV-correlated hepatitis without cryoglobulinemia [82,83]. Some information can be gathered from a study [84] showing that administration of the combination

to 17 patients in relapse and 4 who had not responded to IFN-α resulted in a stable response in about 50% of those in relapse, but no improvement in the nonresponders. These results have been confirmed by a recent study [85], in that 7 out of 9 (78%) patients unresponsive to IFN-α achieved a good clinical response to combination therapy.

A new formulation of IFN, called 'pegylated IFN-α' (PEG-IFN-α), may offer significantly better results than the standard formulation [86]. It is chemically modified by the covalent attachment of a branched methoxy polyethylene glycol moiety. It results in substantial changes in the metabolism of the drug, with prolongation of half-life to maintain effective levels in the blood, thus making feasible its administration once-a-week. This sustained action reduces the viral replication that occurs on days without treatment during the standard thrice-weekly regimen of unmodified IFN.

These studies certainly illustrate the utility of IFN-α. However, they do not show whether its efficacy also extends to other clinical features of this protean syndrome. Little is known, for example, about the responses of cryoglobulinemic patients with neuritic or nephropathic complications or active skin ulcers. Here, indeed, IFN might precipitate or aggravate renal failure and neuropathies and delay ulcer healing. It has been suggested that cryoglobulinemia-related ischemic lesions may worsen, presumably through a decrease in inflammation-induced angiogenesis. The antiangiogenic activity of IFN may delay the appropriate healing of ischemic lesions [87].

Corticosteroid boluses usually improve renal function values and have a significant effect on proteinuria, but do not alter disease progression percentages [88]. The natural history of HCV-positive glomerulonephritis and its progression to chronic renal failure and dialysis are not yet clear. The evidence from renal transplant patients suggests that membranoproliferative glomerulonephritis recurs in most HCV-positive patients and immuno-mediated organ damage may thus be inferred [89,90].

Symptomatic, refractory mixed cryoglobulinemia should be considered as an indication for plasmapheresis. Indeed, cryofiltration apheresis represents an effective and selective way to remove cryoproteins [91]. Improvement in MC-related symptoms can be achieved even without biochemical or virologic response. In combination with cytotoxic agents, it has been used in treating patients with rapidly progressive cryoglobulinemic membranoproliferative glomerulonephritis [92] and other severe symptoms, including demyelinating neuropathy [93].

Many questions still remain unanswered: how should approximately 5% of cryoglobulinemic patients unrelated to HCV infection be treated? HCV-unrelated MC represents a cohort of patients for whom the term 'essential' should be maintained. Recently, a role for HIV infection has been proposed [94]. However, no stringent diagnostic criteria have been provided and the possible pathogenetic mechanisms of HIV must be confirmed. Thus, treatment of HCV-negative MC patients poses a considerable challenge. Interferon therapy should not be given as a first intention, because additional autoimmune disorders or exacerbation of cryoglobulinemic vasculitis may occur [95].

The main conclusion that can be drawn is that there are no available consensus guidelines concerning how and how often cryoglobulinemic patients should be treated. It is clear that the optimal therapeutic outcome lies in the primary prevention of hepatitis C acquisition.

Innnovative therapeutic strategies, applied in a large number of patients, will hopefully provide long-awaited solutions. In this context, good promises seem to derive from our preliminary results with the use of rituximab, a chimeric monoclonal antibody specifically directed to CD20 antigen. This treatment possibly represents a reasonable option in HCV-negative cryoglobulinemia (unpublished data).

Single cryoglobulinemias (type I)

As type I cryoglobulins are found almost exclusively, as we have already mentioned, in patients with malignant lymphoproliferative disorders (multiple myeloma, Waldenström's macroglobulinemia, chronic lymphocytic leukemia), their treatment is mainly directed at the underlying cause and usually includes the combination of alkylating agents (such as chlorambucil and melphalan) and prednisone. In these patients, especially in those with Waldenström's macroglobulinemia, an increased frequency of hyperviscosity syndrome has been described. They, indeed, benefit of plasma exchange treatment, which results in improvement of retinal vessels and sensory and motor neuropathy [96].

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