Review

Cryoglobulins: putative effectors of adaptive immune response

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ABSTRACT

Cryoglobulinaemia consists of circulating monoclonal and/or polyclonal immunoglobulins with rheumatoid factor (RF) activity that precipitate at temperatures <37°C. Cryoglobulinaemic syndrome, characterised by clinical signs of systemic vasculitis, is associated with chronic infection of hepatitis C virus (HCV) and might evolve in B-cell malignancies. In about one third of all HCV infection cases, serum autoantibodies are commonly found. This is probably due directly to the transformation of infected B cells but, also, indirectly, to the viral chronic stimulation of a pool of autoreactive B cells. The pattern of IgG subclasses seems to contribute to the worsening progression of HCV infection into lymphoproliferative and/or autoimmune diseases. Many evidences showed that B cells circulating in patients with HCV-associated mixed cryoglobulinaemia (MC) are profoundly abnormal; moreover, in most of cases, normal B cells are replaced by expanded clonal B cells characterised by the low expression of CD21. After viral eradication, these cells persist in circulation and their occurrence does not correlate with serum cryoglobulins nor with vasculitis response or relapse. It is probably due to the persistence of monoclonal B cells producing RF, that in course of MC can be reactivated by circulating immune complexes, highly produced during infections or tumours. Here, we aimed to review current literature focusing the pathogenesis of MC referring to specificity and immunochemical characteristics of the immunoglobulins involved in cryoprecipitation.

Introduction

Cryoglobulinaemia is defined as the presence of circulating immunoglobulins (Igs) that precipitate at cold temperatures and re-dissolve on rewarming. Cryoglobulins (CGs) maintain their solubility when aggregated in optimal thermal condition. A reduction of temperature provokes steric modifications of molecules with exposition of non-polar residua, reduction of solubility and cryoprecipitation: it probably occurs because of the rapid formation of cold-insoluble IgM-IgG immune complexes or simply by a decreasing solubility, resulting from an unfavourable interaction between CGs and solvent at low temperatures (1). When the temperature rewarms at 37°C, molecules return to the initial conformation (2). Typically, CGs precipitate in the skin vessels of the extremities where the temperature more frequently drops below 37°C causing skin lesions and are responsible for renal injury by an immune-complex mechanism (3).

Reversible cryoprecipitability of proteins was observed by Heidelberger and Kendall as a concomitant feature of immune-complex formation (4). Wintrobe and Buell firstly pointed out the clinical importance of CGs in a patient with multiple myeloma (1, 5). The relevance of this phenomenon became apparent when clinical associations with vasculitis and nephritis were described, resembling those observed in experimental serum sickness.

According to the characteristics of the involved Igs, CGs have been classified by Brouet *et al.*: type I is formed of a single monoclonal Ig; type II of a monoclonal Ig and polyclonal Ig; in type III, only polyclonal immunoglobulins are found (6). Type II and III CGs cryoprecipitate, characterised by IgG-antigen/IgM-anti-IgG immune complexes, are often composed by autoantibodies (auto-Abs) and monoclonal RF IgM and/or IgG (7, 8).

Type I CGs occurs in patients with lymphoproliferative disorders such as mul-

tiple myeloma, lymphoma, or Waldestrom's macroglobulinaemia (1) producing symptoms of vasculitis or distal gangrene/necrosis. Type II and type III CGs are detected in patients with a wide variety of diseases, infective and autoimmune disorders, lymphoproliferative and chronic liver diseases, with the main symptoms of purpura, arthralgia, and Raynaud's phenomena. In 1992s, Musset *et al*. observed new microheterogeneity represented by the occurrence of two or more small monoclonal bands of IgM or IgG isotype in sera from patients with cryoglobulinaemia (9). Similar results have been described by Tissot, who characterised some trace amount of CGs with a pattern of polyclonal IgG associated with a mixture of polyclonal and monoclonal IgM (10). The inclusion of this new subtype of CGs, as a transitional step between type II CGs and type III CGs, focused greater attention on clinical, histopathological and follow-up aspects as well as on a more adequate diagnostic and therapeutic definitions, which differ along each and every moment of the evolutionary pathway of the disease (11, 12) (Table I).

CGs testing is often neglected by clinicians, despite its usefulness in patient management. Recent reports stressed the requirement of international guidelines, standardised pre-analytical protocols to maintain samples at right temperature procedures in the 3 phases of laboratory workup (pre-analytical, analytical and characterisation of the cryoprecipitate) (2, 3, 13, 14) (Fig. 1).

Mixed cryoglobulinaemia (MC) is a multifactorial disorder, based on genetic, environmental, and immunogenic factors, occurring in combination in the pathogenesis of the disorder. HCV appears to have a preeminent aetiologic role in MC, since HCV infection can be found in 76–95% of patients with MC. The clonality and HCV specificity of Ig in cryoprecipitates from patients with HCV-related MC were investigated (8). Notably, type I CGs containing IgG3 isotype shows a greater tendency to aggregate. The IgG3 differ from the other subclasses of IgG for non-ionic and hydrophobic interaction that may favourite the auto aggregation (15).

This review is aimed to analyse the current literature focusing the pathogenesis of cryoglobulinaemia referring to specificity and immunochemical characteristics of the Igs involved in cryoprecipitation.

Pathophysiology and biomarkers

Cryoprecipitation takes place thanks to different parameters as specific Ig levels, pH, ionic force, temperature, weak noncovalent interactions as well as the electrical charge that is dependent by the amino acid sequences and sugar moieties contained in Ig (16). Host predisposition and environmental triggers contribute to the pathogenesis. The result is the production of monoclonal and/or polyclonal immunoglobulins, interacting with each other to form immune complexes, most frequently associated with chronic infectious diseases as (HCV and HBV), autoimmune diseases, and B-cell lymphoproliferative disorders (16).

A genome-wide significant association with CGs-related vasculitis was identified with single nucleotide polymorphism near NOTCH4 and MHC class II genes (17). Chronic infection of T, B lymphocytes, and macrophages may be responsible for the proliferation of B lymphocytes producing circulating immune complexes composed of CGs and auto-Abs. Persistent antigenic stimuli could be responsible for the development of autoimmunity by molecular mimicry.

B cell proliferation is an important mechanism in the pathogenesis of cryoglobulinaemia that may induce an overproduction of polyclonal free light chains (FLC). Polyclonal circulating FLCs provide an index of total Ig synthesis and may be considered a biomarker of immune stimulation and inflammation, correlated with disease activity in different autoimmune conditions, an attractive novel target in precision medicine (18-20). Patients at risk of disease flare could be monitored with FLC, to allow early intervention and possibly reduce end-organ damage and mortality. Increased FLC is associated with a presence of CGs. Chronic HCV infection and viral replication may drive B-cell activation and dysfunction, resulting in FLC overproduction (21).

CGs seem to be part of a progressive clonal selection process in which B-cells are stimulated to produce oligoclonal IgG3 with RF activity. The persistence of the antigenic stimulus elicits the production of polyclonal IgM-RF and subsequently the formation of oligoclonal IgG/polyclonal IgM containing CGs. In the last stage, a monoclonal IgM-RF clone is formed which may coexist with a monoclonal IgG3-RF clone (12).

The evidence that RF-IgG, RF-IgM and serum FLC increase whilst C4 is reduced, both in symptomatic and asymptomatic patients, suggests that, even in absence of MC symptoms, the low levels of CGs may represent a trigger of activation for immune system in course of HCV infection. Moreover, the finding of a positive correlation between raised levels of vascular endothelial growth factor with both free k and λ chains in HCV-MC patients could be suggestive that the strong viral-driven B activation would be worsening into lymphoproliferative disorders (22, 23).

Cryoglobulinaemia in non-HCV patients

Current literature on non-HCV-associated cryoglobulinaemia is very limited and little is known about the immunological and serological profile of affected patients. The cryoglobulinaemic syndrome not associated with HCV infection is often found concomitantly with other infections, autoimmune diseases and B-cell lymphoproliferative disorders.

Type I cryoglobulinaemia is associated with the presence of B-cell malignancies, notably Waldenström macroglobulinaemia, myeloma and monoclonal gammopathy of undetermined significance (MGUS), where the capacity to form cryoprecipitates is a feature of the monoclonal protein. Although cryoglobulinaemia can be asymptomatic and CGs serum levels not always correlated with clinical severity, some patients with low levels of CGs may nonetheless show severe symptoms (21, 24).

Saadoun *et al*. described clinical signs and outcomes of a group of patients with non-HCV-related MC. The im-

Table I. Key features of cryoglobulins and cryoglobulinaemic syndromes.

	IG COMPONENT	CLINICAL SIGNS	DISORDERS	BIOMARKER S
CG Type I	**monoclonal** IgM (mainly) IgG2 (mainly), IgG3 IgA (rarely)	**Cutaneous** purpura, acrocyanosis, necrosis, ulcers, livedo reticularis **Extra cutaneous** peripheral neuropathy, renal and joint involvement **Systemic vasculitis**	MGUS, SM, Waldeström macroglobulinaemia MM	**RF-IgM/IgG**: + FLC
MC Type II	**monoclonal** **(RF activity)** + **polyclonal** IgM+IgG IgG+IgG IgA+IgG (rarely)	**Cutaneous** Meltzer's Triad **Extra cutaneous** renal disease, membrano-proliferative glomerulonephritis. neuropathy intestinal ischaemia, alveolar haemorrhage, CNS involvement myocardium failure	**Infectious** HCV, HBV, HIV **Non-infectious** Sjögren's syndrome, LES, RA, B-cell Lymphoma, NHL Solid Tumors Cold-agglutinin Disease **Essential MC** **(unknown cause)**	**AutoAb** ANA, ENA, AMA **Hypo-complementemia** ↓C3, ↓C4, ↓CH50
MC Type III	**polyclonal or** **olygoclonal (RF activity)** + **polyclonal** (microeterogeneous) IgG+IgM IgM+IgG+IgA IgA+IgG	**Cutaneous** **Extra cutaneous** peripheral neuropathy, renal and joint involvement	**Infectious** HBV, HIV, Epstein-Barr, Cytomegalovirus Endocarditis, Spirochetes Fungal infections Parasitosys **Non-infectious** LES, RA Intestinal diseases Biliary cirrhosis Solid Tumors **Essential MC**	**RF-IgM/IgG** Type II: ++ Type III: ++ **FLC** k: ++ λ: +

munological features consisted in low C4 and C3 complement level, RF activity, antinuclear antibodies (ANA), anti-SSA and anticardiolipin antibodies with different frequencies (25).

In many infectious diseases, cryoglobulinaemia has been considered as an epiphenomenon without a clinical significance and related to disease management. Recently, Eble *et al*. show a high prevalence of CGs level ≤0.05 g/L in clinical practice suggesting that non-HCV CGs level ≤0.05 g/L may be responsible for severe renal and neurological complications, leading to high morbidity and mortality in these patients (24). In patients with HBV-related MC the cryoglobulinaemic glomerulonephritis is the main renal lesion (26). In a multicentre study, the effect of HBV antiviral treatment on cryoglobulinaemic vasculitis, a rare complication occurring in HBV patients, was investigated. A decrease of cryocrit level was observed at the end of treatment in all patients. Moreover, a reduction of RF and C4 levels was observed in the same cohort (27, 28). Overall, as concluded in a recent review on HBV-related MC, nucleot(s)ide analogues represent a promising therapeutic option for HBV-related vasculitis (29).

HCV cryoglobulinaemia

In about one third of HCV infected patients, serum auto-Abs are commonly found, mainly anti-smooth muscle (ASMA) and ANA, with an increased prevalence in females (30, 31). This is probably due to direct transformation of infected B cell but also to HCV chronic stimulation of a limited pool of autoreactive B cells (32, 33).

Lenzi *et al*. observed that in chronic HCV-patients, the anti-liver and kidney microsomal antibody type 1 (anti-LKM1) positivity correlates with the activity of liver disease, suggesting a role in the progression of liver damage (34). Gregorio *et al*. showed that the reactivity to the nuclear and smooth muscle peptides homologous to HCV antigens is strongly associated with chronic HCV infection, indicating that these auto-Abs are generated as a specific response to HCV infection and that represent a consequence of cross-reactive immune responses (35).

Analysing HCV-patients with rheumatoid arthritis (RA) in comparison with non-HCV RA, we found an increased prevalence of ANA in HCV-RA group, whereas anti-mitochondrial antibodies, ASMA, anti-parietal cell antibodies and anti-LKM 1 (performed by indirect immunofluorescence) were negative. Moreover, ANA were observed above all in cryoprecipitates (82%) compared to the supernatant (48%). The ANA pattern was also different: in non-HCV-RA patients, the coarsely speckled pattern was the quite frequently occurred,

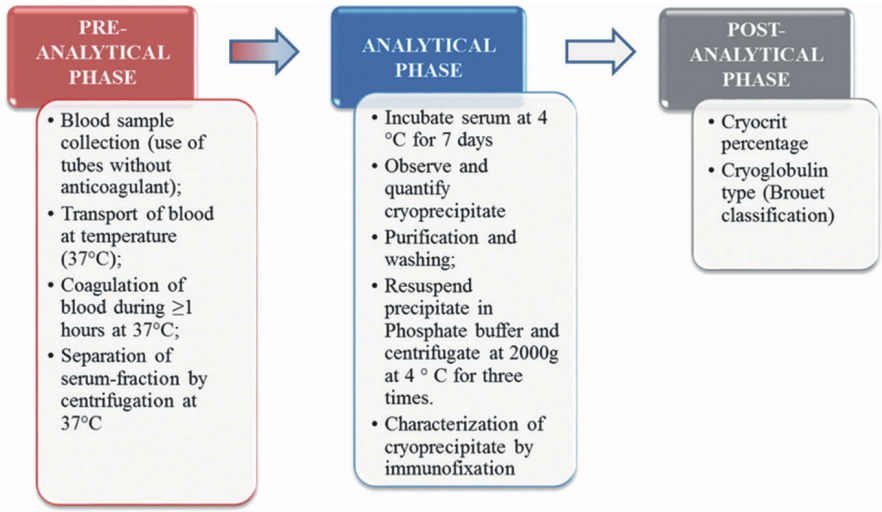

Fig. 1. The phases of laboratory workup: pre-analytical, analytical and characterisation of the cryoprecipitate.

completely absent among HCV-RA patients, while, the finely speckled pattern was mostly frequent in HCV-RA group. The occurrence of non-specific patterns in HCV-positive patients could be linked to a non-specific antibody response, possibly due to the persistent antigenic stimulus of the virus (7). We also found that presence of ANA seems strictly related to type II and type III CGs production, moreover ANA positive patients showed a higher mean percentage of cryocrit (8, 36).

Several factors, often interconnected, seem to contribute to the pathogenesis and progression of lymphoproliferative and/or autoimmune diseases in HCV-infected patients such as different IgG subclasses patterns (12). Human IgG include four subclasses: IgG1 (60-65% total IgG), IgG2 (20-25%), IgG3 (5-10%) and IgG4 (3-6%), which display different chemical and physical properties and whose clinical significance is not completely known. Little is known about the role of IgG subclasses in the pathophysiology of cryoglobulinaemia, but different studies displayed that IgG subclasses may characterised different syndromes and autoimmune disorders (37-39). The analysis of IgG subclasses distribution in patients with HCV-cryoglobulinaemia resulted in the enrichment of certain IgG subclasses, mainly IgG1 and IgG3 frequently monoclonal (40).

Serum distribution of IgG subclasses and their role in patients with cryoglobulinaemia has been evaluated since 1971 when Virella *et al*. reported that IgG3 may trigger concentration-dependent aggregates and the hypothesis of the high interactions towards similar molecules, possibly due to conformational reason, was confirmed by the prevalence of IgG3 proteins among IgG CGs (41). These data agree with the analysis of Ig components in murine CGs that confirmed the auto reactivity of IgG3 and showed the univocal presence of the IgG3 subclass and IgG3 monoclonal CGs with RF activity that might be responsible for extra-hepatic manifestations (42). Human and murine IgG3 display an equal capacity to self-assembly and demonstrate a role in the formation of cryoprecipitate. IgG3 induces effector functions acting as a potent pro-inflammatory antibody, therefore its short half-life may function to limit an abnormal inflammatory response (43).

In our study on naïve HCV patients without clinical signs of autoimmune/lymphoproliferative disorders, we described a strong correlation between ANA positivity and IgG3 subclass and the prevalence of the finely speckled pattern in the IgG3-positive HCV-positive subgroup (36). In addition, most of the RF IgG3 positive patients were ANA positive. IgG3 is indicative of a RF activity against the IgG1-HCV complex, and their autoreactivity may indicate a persistent immune stimulation (36).

RF is an autoantibody against the Fc portion of human IgG enabling them to form immune complexes; this ability is important in the pathogenesis of cryoglobulinaemic vasculitis (33). RF was found in different diseases with immune dysregulation. Moreover, the presence of RF and a reduction of C4 can be observed in most MC patients (12). A cross-reactivity between the HCV, which acts as antigen, and RF IgM or IgG has been demonstrated; many reports showed that the persistence of the virus induces monoclonal (after a transition from oligoclonal) RF. The continuous stimulation of B cells by the virus can induce the development of malignant non-Hodgkin lymphomas as suggested from the evidence that the treatment of infection leads to clinical improvement and lymphoma regression in most patients (44-46) (Fig. 2).

The occurrence of RFs reactive production by HCV stimulus, in patients with genetic abnormalities, may represent the trigger for a neoplastic process leading to the development of lymphoma (47, 48). Sansonno *et al*. reported that B cells, isolated from the liver of infected patients, produced WA, a specific monoclonal RF bearing the WA cross-idiotype, in about 60% of cases of HCV-related type II MC (49, 50). Antiviral therapy induces the decline of WA B cells secreting WA monoclonal RF and cryoglobulinaemia, in parallel with the decrease of viraemia, suggesting that the production of WA monoclonal RF is driven by HCV (51). WA B cells can also be detected in asymptomatic patients with HCV infection; thus, this could be a marker to predict a cryoglobulinaemic vasculitis and to initiate early antiviral therapy (51).

In HCV patients, the measurement of serum FLC can be clinically useful above all as a predictive index of MC and for the diagnostic and prognostic value of the k/λ ratio. High free k serum levels were found in HCV positive patients with type II MC, characterised by a monoclonal component mounting light chains k (52-53), and their serum levels have recently been used for monitoring extrahepatic manifestations caused by HCV (18). The abnormal k/λ ratio correlates positively with the severity of clinical data associated

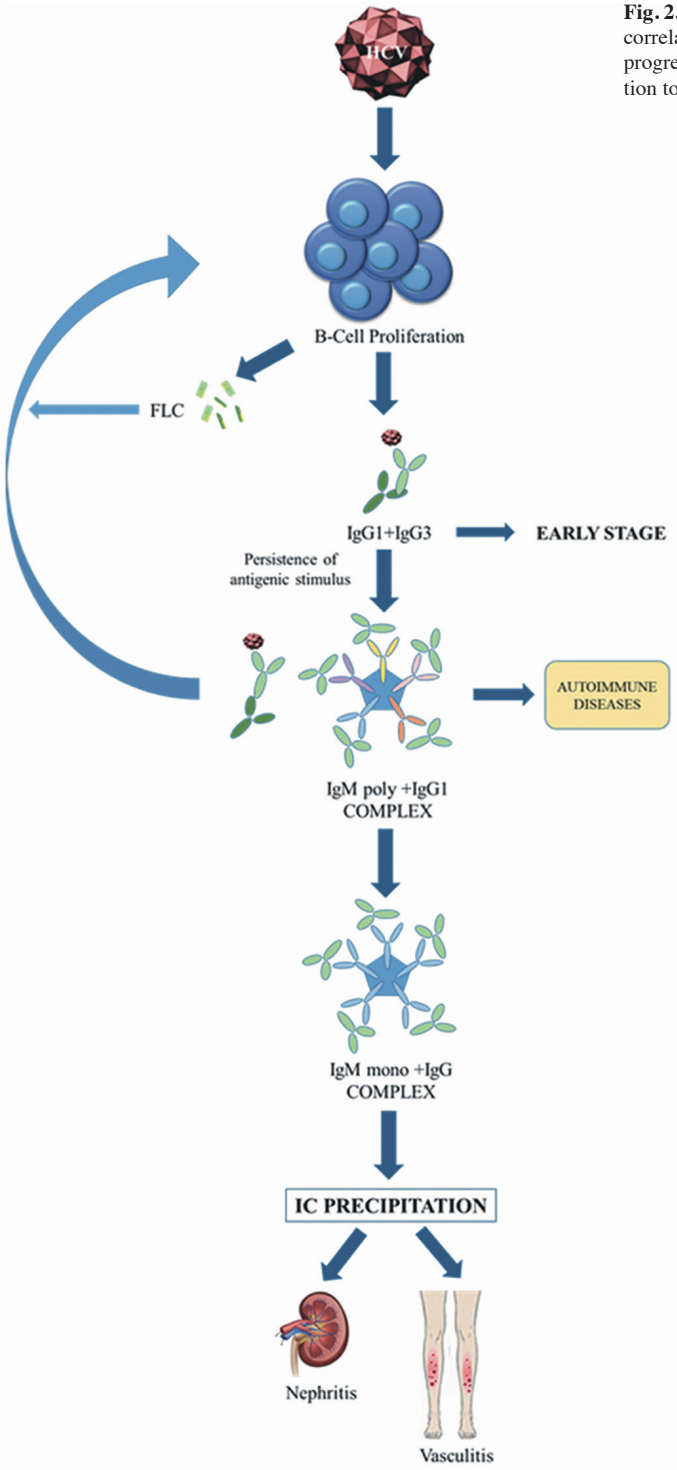

Fig. 2. Clinical and laboratory correlates characterising the progression from HCV infection to cryoprecipitate.

with the lymphoproliferative disorders related to HCV infection, and represents an early index of activation of the lymphoproliferative mechanism in the follow-up of patients with infection HCV (53).

Our group recently observed that FLC assessment could be used as a diagnostic marker of MC vasculitis/syndrome. Cryoglobulinaemia is associated exclusively with B cell proliferative diseases (33), due to different pathogenetic factor which leads to aberrant B cell functions. Pre-treatment FLC ratio values may be considered an important, cost-effective, and easily accessible parameter for predicting patient response to expensive treatments, such B cells depletion with anti-CD20 monoclonal antibody (54).

The pathogenetic correlation between MC and HCV suggests that the virus may be responsible of a multistep process that supports the progression for a wide range of extra-hepatic manifestations, independently from the development of infection and it may be responsible for the proliferation of B lymphocytes which trigger the production of circulating immune complexes composed of CGs and auto-Abs.

Mixed cryoglobulinaemia and B cells

Many evidences showed that circulating B cells in HCV-MC patients are profoundly disturbed and in most of the cases an expansion of $CD21^{low}$ B-cells replace the normal B cell pool (55-57). $CD21^{low}$ B-cells in MC are very similar to the $CD21^{low}$ B-cells found in other immunological and chronic infectious diseases (58-61), characterised by B cell hyperactivation. Phenotypically, expanded $CD21^{low}$ B-cells in MC resemble marginal zone B-cells by the co-expression of IgM and CD27 and are characterised by a peculiar pattern of homing receptors including: CD11c, a typical marker of dendritic cells; CXCR3, CCR7, CD62L, CXCR4, and CXCR5, and inhibitory receptors, as CD22, FcγRIIB (CD32b), CD72, CD85j, CD85k, CD95, LAIR-1, Siglec-6 and Fc receptor-like 4 (FCRL4), an inhibitory receptor that was first described in a subset of human tonsillar B cells with low CD21 expression (58, 62-64).

In 20% of MC patients clonal $CD21^{low}$ B-cells express a B cell receptor (BCR) encoded by the VH1-69 and Vk3-20 heavy and light variable genes. This BCR displays RF activity and is putatively directed to an unknown HCV antigen (65, 66).

$CD21^{low}$ B-cells resemble exhausted virus-specific T cells, described in HIV and HCV infections for the expression of inhibitory receptors and the low proliferative potential in response to different stimuli, leading to their definition as exhausted cells (67). Indeed, $CD21^{low}$ B-cells fail to proliferate after stimulation of TLR 7 and 9 and BCR, whereas it seems that co-stimulation with CD40L, BCR, in the presence of

IL-10 and IL-2 induces an increased proliferative response (56-58, 62). Stimulation of TLR9, however, enhances secretion of TNFα and RF antibodies by these cells suggesting partial unresponsiveness (68). In addition, $CD21^{low}$ B-cells appear to be anergic as they fail to flux calcium upon B-cell receptor triggering (69), display high constitutive expression of the active phosphorylated form of extracellular signal–regulated kinase (pERK) and are prone to die by apoptosis (57, 69-70). These functional signatures usually characterise murine B cells made anergic by continual BCR engagement by antigen (71). In accordance, dysregulated expression of apoptosis and anergy-related genes has been found in MC-derived clonal B cells and high expression of DEC1/STRA13, a negative regulator of B cell activation, seems to be a common trait (62).

Recently the occurrence of clonal B cells in MC after eradication of HCV, achieved with direct acting antivirals (DAA), has been studied (72). DAAs offer a unique opportunity to analyse the B cell functional properties after removal of chronic antigen stimulation as they lack the immunomodulatory effect, typical of interferon (IFN)-based therapies. It was shown that the anergic features of clonal B cells, as pERK overexpression and accelerated apoptosis, rapidly reverted after disengagement from HCV, while phenotypic and functional features of exhaustion persist in peripheral blood after sustained viral response for several months, suggesting that other extracellular signals might be responsible for their survival. In the long-term immunological and clinical outcomes of patients with mixed cryoglobulinaemic vasculitis (MCV) treated with DAAs, with or without non-Hodgkin's lymphoma (NHL), circulating B-cell clones persisted in most of patients with MCV or with MCV associated with indolent NHL for up to 2 years after the cure of HCV infection (73). The persistence of B-cell clones did not correlate with serum CGs or vasculitis response to therapy or relapse after viral eradication. In this view it is noteworthy that some MC patients, after HCV eradication, experience a vasculitis' relapse associated with the occurrence of infections, vaccination, and tumours (74, 75). It can be speculated that RF bearing monoclonal B cells in MC might be reactivated by circulating immune complexes produced in high quantities in condition such as infections or tumours. Therefore, as suggested by Kaplan *et al*., the persistence of clonal exhausted B cells in MC, might explain the relapse of MC vasculitis despite clearance of the chronic antigen stimulation provided by HCV (76). Minafò *et al*. recently investigated the Ig heavy and light chain variable regions of monoclonal B-cells from patients with HCV-associated lymphoproliferative disorders and found that distinctive light chain stereotyped signatures either endowed with homology with anti-HCV E2 antibodies or with RF sequences might help distinguishing HCV-dependent lymphoproliferative disorders from those autoantigen driven, accidentally occurring in HCV infected individuals (77). These observations strengthen the hypothesis that other stimuli, than infective ones, might contribute for the survival and transformation of B cell clones in HCV-associated MC.

Mixed cryoglobulinaemia and therapeutic strategies

Considering the pathogenesis of MC, different therapeutic strategies have been developed in recent years and are available for patients suffering from this vasculitis. Some of these treatments are curative whereas other have a limited effect (78, 79). Effectiveness of IFNα was observed empirically in 1987 and after the demonstration of the pathogenic role of HCV in MC, treatment with IFNα became a rational therapeutic strategy, although sustained virologic response and vasculitis disappearance was observed in less than half of the patients (80). Recently the advent of new direct acting anti-viral changed the scenario of HCV-MC achieving sustained virologic response in almost 100% of the cases, together with vasculitis disappearance (81). After this initial enthusiasm, long-term follow-up of MC patients showed that vasculitis relapse was a possible, and not so rare event (82) and the persistence of peripheral B cell clones might be involved in vasculitis reactivation (73, 74). In this view the addition of anti-CD20 treatment could be ideally the optimal therapeutic strategy as DAA achieve sustained virologic response and the depletion of B cells could prevent future relapse. Efficacy of rituximab, a B-cell depleting anti-CD20 monoclonal antibody, has been widely demonstrated both for HCV and essential MC, but its effect is limited, and retreatments are usually necessary (83). If circulating B cell clones reappear after B cell depletion and virus eradication has not been investigated so far.

Other therapeutic interventions include plasmapheresis that is usually used for life-threatening forms of vasculitis and allows the temporary removing of the cryoprecipitating proteins (84).

Mixed cryoglobulinaemia, COVID-19 and endothelitiis

The viral aetiology in MC vasculitis is well defined in most of cases. Given the potential impact of inflammatory and immunopathological complications in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease 2019 (COVID-19), here we speculated about a potential link between MC and COVID-19 based on very recent literature. Even if at now reports describing the occurrence of MC vasculitis in COVID patients are still lacking, at least two mechanisms can be envisaged to hypothesise a damage of Coronavirus on endothelial cells. Indirectly, as it has been reported that in COVID-19 vasculitis a life-threatening escalation of a dramatic immune dysregulated response, with a switch from a pathogenetic mechanism of type 2 T-helper immune response (humoral immunity) to a type III hypersensitivity (immune complex disease), takes place (85). The deposition of immune complexes induces an amplification of the inflammatory state evolving to cytokine storm syndrome. During SARS-CoV-2 infection, type III hypersensitivity might occur for an excess of soluble antigens determining an accumulation of immune complexes. These antigen-antibody complexes may precipitate

inside tissues, in blood vessels, inducing a severe inflammatory state by the action of complement anaphylatoxins (C3a and C5a) and tissue damage. The result of this process inside the walls of blood vessels is a vasculitis sustained by a mechanism of type III hypersensitivity, that is depicted by an acute necrotising vasculitis with neutrophilic infiltration, karyorrhexis and fibrinoid necrosis (85).

Directly, it has been demonstrated that SARS-CoV-2 can infect engineered human blood vessel organoids *in vitro* (86). The angiotensin converting enzyme 2 (ACE2) receptors, used by the SARS-CoV-2 to infect the host, are largely expressed by endothelial cells which traverse multiple organs; evidence of direct viral infection of the endothelial cells *in vivo* and diffuse endothelial inflammation (endothelitiis) in several organs has been reported in post mortem histological analysis of different tissues (87).

The combination of these two postulated mechanisms (supported by demonstrated evidence) let open the possibility that SARS-CoV-2 triggered dysregulated-immune response (that becomes independent from virus itself) may impair blood vessels resulting in a vasculitis (as observed in HCV-MC). The worsening progression is widely influenced by both endothelial-induced damage to vital organs and co-morbidities associated with underlying diseases.

Conclusions

The dysregulation of immune system, involved in cryoprecipitation, was here reviewed. MC displays a double face being a lymphoproliferative and autoimmune disease as well, and CGs can be considered as the result of the adaptive immune response, induced by the persistence of endogenous (autoimmune diseases) or exogenous stimuli (virus).

Since their occurrences increase co-morbidities and reduce survival, it is important to detect serum CGs in all patients with immunological alterations. The early diagnosis of a cryoglobulinemia and the worsening evolution from a mild form of vasculitis to a severe and life-threatening disease up to the development of non-Hodgkin lymphomas requires a careful analysis and integration of all clinical, laboratory and histological data. Even in the era of DAA that achieve HCV eradication in almost 100% of patients, relapse of MC vasculitis or non-response to treatment have been described (74). For these reasons, the deeply comprehension of underlying mechanisms together with the definition of biomarkers indicative for unresponsiveness to therapy or predictive for disease relapse are widely desired and might ameliorate patients tailored treatments.

The expansion of abnormal clonal B cells producing cryoglobulins could become independent to the trigger and virus eradication. Cryoglobulinaemia could represent a biological model of a new systemic disease induced by a virus as trigger of a dysregulated immune response that, in the end, becomes independent from the virus itself.

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