Concise Report

Hepatitis C virus-associated B-cell proliferation—the role of serum B lymphocyte stimulator (BLyS/BAFF)

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Objective. B lymphocyte stimulator (BLyS) is known to support B-cell proliferation (BCP) in B-cell haemopathies and autoimmune diseases. We assume that BLyS may play a role in the initiation and expression of hepatitis C virus (HCV)-associated BCP. We assessed BLyS serum levels in HCV-infected patients and in various forms of HCV-associated BCP [i.e. mixed cryoglobulin (MC), rheumatoid factor (RF) and systemic vasculitis].

Methods. A total of 76 HCV-infected patients (HCV RNA+) were compared with 13 healthy volunteers. Epidemiological, clinical, immunochimical and virological data were prospectively collected. BLyS serum levels were assessed by an ELISA sandwich method.

Results. Of the 76 patients, 38 females, 38 males, mean age 53 ± 15 yrs; 47 (62%) patients had type II (27 patients) or type III MC (20 patients); 27 (35.5%) patients had HCV-systemic vasculitis. BLyS serum levels tended to be higher in HCV-infected patients than in healthy controls (1.8 ± 0.9 vs 1.5 ± 0.2 ng/ml), were higher in patients with MC than without (2.03 ± 1.02 vs 1.5 ± 0.5 ng/ml; P = 0.008), and even higher in type II than type III MC (2.3 ± 1.2 vs 1.7 ± 0.6 ng/ml; P = 0.03). There was a correlation between BLyS and MC serum levels (R = 0.4; P = 0.004). BLyS serum levels were higher in patients with a positive RF than in those without (2.06 ± 1.09 vs 1.6 ± 0.56 ng/ml, P = 0.035), and with systemic vasculitis than in those without (2.24 ± 1.16 vs 1.6 ± 0.6 ng/ml; P = 0.006).

Conclusion. BLyS serum levels are significantly correlated with B-cell proliferation during chronic HCV infection. These results strongly suggest a role for BLyS in the induction and expression of HCV-BCP.

Key words: BLyS (BAFF), HCV, B-cell proliferation, Mixed cryoglobulaemia.

Hepatitis C virus (HCV) infection, a worldwide disease, is strikingly associated with several extra-hepatic manifestations. Most of these are related to B-cell proliferative disorders. The most common is mixed cryoglobulins (MCs), which occur in about half of the HCV-infected patients [1–4]. The MCs are classified according to the presence or absence of a monoclonal component among other polyclonal immunoglobulins (Igs) in the serum cryoprecipitate: type II MC in the presence of a monoclonal component and type III MC when only polyclonal Igs are present [5]. There is evidence showing that type II MC is characterized by a monoclonal and/or oligoclonal proliferation of B-cells in bone marrow, liver tissue and peripheral blood [6–8]. These clonal B-cells are responsible for the production of monoclonal IgM, which displays anti-IgG rheumatoid factor (RF) activity. The monoclonal IgM-RF is the major component of the MC cryoprecipitate in addition to anti-HCV-antibodies, HCV viral particles and lipoproteins [1, 9]. In addition to MC and RF, HCV infection is also associated with the production of anti-nuclear and anti-cardiolipid antibodies, which reflect chronic non-specific B-cell proliferation and antigen-driven stimulation [4, 10–13]. Peculiar clinical features are also associated with HCV-infection, including B-cell lymphoma [14] and the association with a Sjögren-like syndrome. Although some data suggest that an overexpression of the anti-apoptotic Bcl2 protein family [15–17] or an antigen-driven process [18–20] are involved, the mechanisms through which B-cell proliferation (BCP) may occur during HCV infection remain to be elucidated.

Knowledge of the mechanisms underlying BCP and B-cell survival pathways has been strengthened since the identification of B lymphocyte stimulator (BLyS), also called B-cell activating factor (BAFF) or tumour necrosis factor (TNF)- and Apo-L-related leucocyte-expressed ligand-1 (TALL-1) [21, 22]. BLyS is a 285 amino acid protein encoded by a gene on chromosome 13q32–34 and secreted by myeloid cells including monocytes, macrophages, dendritic and activated B-cells [21, 23]. Three receptors for BLyS have been identified: (i) B-cell maturation antigen (BCMA), (ii) transmembrane activator and calcium-modulating cyclophilin ligand (CAML) interactor (TACI) and (iii) more recently, B-cell-activating factor receptor (BAFF-R). BCMA and BAFF-R are predominantly expressed on B lymphocytes, whereas TACI can be found on B-cells and activated T-cells [21, 24–27]. Several studies have shown strong evidence of the pivotal role of BLyS in BCP and the related haematological and autoimmune diseases. Transgenic mice overexpressing BLyS developed critical BCP in blood and marginal zones of lymph nodes, with the production of high titres of Igs, RF, anti-DNA
antibodies and sometimes cryoglobulin [25, 28–30]. While ageing, these mice also developed lupus-like or Sjo¨gren-like syndromes [30]. In human haematological diseases, a high BLyS expression has been found in non-Hodgkin’s lymphomas (mainly follicular lymphoma) and myeloma [31–33]. Raised serum levels of BLyS have also been demonstrated in autoimmune diseases, such as systemic lupus, systemic sclerosis, rheumatoid arthritis and Sjo¨gren’s syndrome [34–38].

We hypothesized a potential role for BLyS in HCV-related BCP, and examined the BLyS serum levels in HCV-infected patients. Our results demonstrate that BLyS serum levels in HCV-infected patients are markedly elevated in patients with MC, mainly in those with type II MC, with a positive RF and systemic vasculitis.

Patients and methods

The design of this study was approved by the local ethical committee as conformed to the current standards and a written consent was obtained from all the patients and volunteers. A total of 76 consecutive HCV-infected patients (positive anti-HCV antibodies and HCV RNA) being followed in our department were included. Epidemiological, clinical and biochemical parameters, and sera were prospectively collected. HCV-induced systemic vasculitis was defined by the presence of serum MC and related symptoms, including asthma, skin purpura, arthralgia or arthritis, peripheral neuropathy and in some cases membranoproliferative glomerulonephritis. The control group included 13 healthy volunteers.

Sera from HCV-infected patients and healthy volunteers were harvested and stored at -80°C. BLyS serum levels were appraised using an enzyme-linked immunosorbent assay (ELISA) sandwich method with the Quantikine® Human BAFF/BLyS/TNFSF13B Immunoassay (R&D Systems, # DBLYS0) following the manufacturer’s instructions. There is no proved interference thus far between the BLyS dosage and the presence of a RF, referring to the manufacturer’s data and to a recent study. Which has used the same kit for serum BLyS dosage [34]. Serum cryoglobulin detection and immunochemical typing were performed using a validated immunoblotting method [39]. The cut-off for positivity was 0.05 g/l as was previously published [1]. The RF was measured using an ELISA method with a cut-off for positivity of 15 U/ml.

Categorical variables were compared using Fisher’s exact and chi-square tests, and continuous variables using the t-test or Mann–Whitney U-test when appropriate. All tests were two-tailed and a P-value of <0.05 was considered statistically significant. All statistical analyses were performed using MedCalc® version 7.4.2.0.

Results

The main features of our study population are reported in Table 1. Twenty-seven patients (35.5%) presented with MC systemic vasculitis, related mainly to type II MC (21 patients). Arthralgia was present in 39.5% (30/76) of the patients; cutaneous vasculitis (purpura) in 26.3% (20/76); peripheral neuropathy in 25% (19/76) and glomerulonephritis in 7.9% (6/76).

Forty-seven patients (61.8%) had positive MC, which was type II in 27 patients (57%) and type III in 20 patients (43%). The mean MC serum level was 0.59±0.8 g/l. It was found to be higher in patients with type II MC than in those with type III (0.88±0.98 g/l vs 0.2±0.12 g/l, respectively; P = 0.006). MC serum levels were also higher in patients with systemic vasculitis than in those without (0.85±0.24±0.2 g/l; P = 0.01). RF was found positive in 54.3% (38/70) of the patients. A positive RF was found more frequently in patients with MC than in those without [68.2% (30/44) vs 30.8% (8/26); P = 0.005], and in patients with systemic vasculitis than in those without [73% (19/26) vs 43.2% (19/44); P = 0.029]. RF serum levels were higher in patients with MC than in those without (193±450 vs 41±66 U/ml; P = 0.006), in patients with type II than in those with type III MC (307±585 vs 51±49 U/ml; P = 0.01), and in patients with systemic vasculitis than in those without (274±567 vs 52±63 U/ml; P = 0.03).

BLyS serum levels are higher in patients with MC, mainly in those with type II MC, a positive RF and systemic vasculitis

As shown in Fig. 1, BLyS serum levels tended to be higher in HCV-infected patients than in controls (1.84±0.89 vs 1.47±0.19 ng/ml). In HCV-infected patients, BLyS serum levels were significantly higher in patients with MC than in those without (2.03±1.02 vs 1.5±0.5 ng/ml; P = 0.008), mainly in those with type II than in those with type III MC (2.3±1.17 vs 1.67±0.6 ng/ml; P = 0.027). BLyS serum levels were also highly correlated with MC serum levels (correlation coefficient R = 0.43; P = 0.004) (Fig. 2). BLyS serum levels were found to be higher in patients with positive RF than in those without (2.06±1.09 vs 1.61±0.56 ng/ml; P = 0.035), and in those with systemic vasculitis than in those without (2.24±1.16 vs 1.6±0.6 ng/ml; P = 0.006) (Fig. 1). BLyS serum levels remained higher in patients compared to those without arthralgia (2.19±1.13 vs 1.6±0.59 ng/ml, respectively; P = 0.01), purpura (2.29±1.2 vs 1.67±0.67 ng/ml; P = 0.01), peripheral neuropathy (2.4±1.3 vs 1.6±0.59 ng/ml; P = 0.003) and glomerulonephritis (2.7±1.1 vs 1.8±0.8 ng/ml; P = 0.036).

After multiple regression test including BLyS serum levels as the dependent variable and type II MC, vasculitis and RF as independent variables, only the presence of a type II MC was found to be associated with high BLyS serum levels (correlation coefficient R = 0.406; P = 0.0005).

The serum levels of γ-globulins (IgA, IgG and IgM) were available for 33 patients and there was no correlation between BLyS serum levels and the IgA (R = 0.2; P = 0.2), IgG (R = 0.08; P = 0.6) and IgM serum levels (R = −0.04; P = 0.8). BLyS serum levels were also not associated with the disease duration (R = −0.2; P = 0.1).

Discussion

In this study, we showed that BLyS serum levels are positively associated with markers of HCV-associated BCP. Serum levels of BLyS were found to be higher in HCV-infected patients with

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<th>Table 1. Main characteristics of the study population</th>
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<td>Age (yrs)*</td>
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<td>Mixed cryoglobulin (MC), n (%)</td>
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<td>Positive rheumatoid factor, n (%)</td>
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<td>MC systemic vasculitis, n (%)</td>
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*Expressed as mean ± S.D.
type II MC, high MC serum levels, a positive RF and associated systemic vasculitis. BLyS serum levels were, after multivariate analysis, mainly found to be associated with the presence of a type II MC. It has been shown that the immunochemical types of MC may vary during the disease course [40]. Type III MC may evolve to an oligoclonal form and finally to type II MC, which features a monoclonal component [40]. BLyS serum levels may follow the MC immunochemical type course and increase while B-cell monoclonality (type II MC) appears. BLyS serum levels were also found to correlate with MC serum levels. This finding suggests a role for BLyS in sustaining a high level of IgG secretion by B-cells, as demonstrated in mice models overexpressing BLyS [29, 30]. However, we have not find a correlation between BLyS serum levels and the serum levels of immunoglobulins IgG, IgM or IgA. A supplemental but expected finding is that BLyS serum levels were also higher in patients

**FIG. 1.** BLyS serum levels in HCV-infected patients with various expression of HCV-associated B-cell proliferation. HCV, hepatitis C virus infection; MC, mixed cryoglobulin; RF, rheumatoid factor; SV, systemic vasculitis.

**FIG. 2.** Correlation between BLyS and MC serum levels. Analysis was based on log10 transformed values. The correlation coefficient was determined by the Pearson correlation coefficient (R). The solid line represents the regression line and the dotted line the 95% confidence interval.
with a positive RF, a major component of the cryoprecipitate that is secreted by the monoclonal B-cell population.

The mechanisms underlying BCP during chronic HCV-infection are not completely known. An antigen-driven process might be involved, given the highly restricted use of genes encoding the variable regions \( (V_{H}, V_{K}) \) known to be preferentially used by B-cells secreting IgM-RF [6, 18–20]. A key role for the anti-apoptotic Bcl2 protein family has been advocated, as 71–86% of HCV-infected patients with MC have overexpression of the anti-apoptotic Bcl2 factor, in close association with a high rate of the related B lymphocyte \( t(14;18) \) translocation. These immunocytological abnormalities disappear when a sustained virological response is achieved after anti-HCV therapy [15–17, 41, 42]. It could be that these two mechanisms are linked through the BLyS pathway. BLyS may stimulate B-cells when they are coactivated via their antigen receptors and, as a consequence, induce expression of Bcl2, a critical anti-apoptotic gene [43].

Our results can also be paralleled to those in transgenic models overexpressing BLyS. The expansion of B-cells in lymph nodes, splenic marginal zones and peripheral blood in transgenic mice, and the consequences of overexpression of BLyS are thought to strikingly include a pivotal role for the Bcl2 family protein pathway [29]. These mice, while ageing, develop clinical and biological manifestations of autoimmune diseases, namely lupus-like syndrome with glomerulonephritis with Ig-deposits and Sjögren-like syndrome with severe sialadenitis [30]. It has been shown that BLyS serum levels are higher in patients with Sjögren’s syndrome and are associated with the serum levels of anti-SSA antibodies, RF and \( \gamma \)-globulins [35]. These findings, together with our data, show further similarities between Sjögren’s syndrome and chronic HCV infection in relation to chronic B-cell expansion [44].

In conclusion, our results reinforce the hypothesis of a critical role for BLyS in the induction and expression of BCP during chronic HCV-infection. They open a new area of knowledge of HCV-BCP, placing B-cells in a pivotal role, and calling for additional research to elucidate the intrinsic mechanisms explaining BLyS overexpression (role for viral proteins, antigen-driven process). They also create an exciting prospect for the use of anti-BLyS overexpression (role for viral proteins, antigen-driven process). They also create an exciting prospect for the use of anti-BLyS antibodies or decoy BLyS receptors in the future therapeutic algorithm of HCV-infected patients with severe MC-associated vasculitis.

P.C. has carried out consultancy and group research from Roche, Schering Plough and Gilead Sciences. The other authors have declared no conflicts of interest.

References


