To the editor:

Can BAFF promoter polymorphism be a predisposing condition for HCV-related mixed cryoglobulinemia?

Mixed cryoglobulinemia (MC) is a benign but prelymphomatous condition whose clinical manifestations are secondary to systemic immune complex–related vasculitis, which is the final step in a complex process initiated by unregulated B-cell expansion. MC is strongly associated with hepatitis C virus (HCV) infection.[1] In fact, a majority of MC patients (> 90%) exhibit HCV markers, and approximately 50% of HCV patients exhibit a wide range of MC markers/symptoms, varying from asymptomatic serum cryoglobulins to MC syndrome (MCS), symptoms of which include weakness, arthralgias, and purpura.[2] Several mechanisms involved in the pathogenesis of HCV-related MC have been proposed, but the reasons why cryoglobulins appear in only half of all HCV patients are still unclear. Suggested key determinants include host genetic background or viral factors.

B cell–activating factor (BAFF, or B-lymphocyte stimulator) is a recently discovered TNF-α family member whose essential role in B-lymphocyte development and survival has been clarified.[3] Impairment of BAFF regulation has been associated with human autoimmune disorders, namely Sjögren syndrome, systemic lupus erythematosus, and rheumatoid arthritis (RA). Recent reports found elevated serum BAFF levels in HCV patients with lymphoproliferative disorders (LPDs; MC and B-cell non-Hodgkin lymphoma [NHL]), but the mechanisms of this phenomenon are still undefined.[4-6]

The polymorphism −871C/T was detected in the BAFF promoter, and the presence of the mutated −871T genotype was associated with higher BAFF mRNA levels in monocytes.[7,8] The presence of this allele was also more frequent in RA patients compared with controls, and in patients with familial LPDs.[7,8] In these studies, a correlation was found between high serum BAFF levels and the presence of the mutated allele, as well as increased transcriptional activity of the promoter with −871T.

Based on this background, we aimed at investigating the role of the polymorphism in HCV-related MCS patients as a genetic contributor to its pathogenesis. We analyzed the presence of the mutation using restriction fragment length polymorphism (RFLP) analysis[7] in 123 HCV patients: 57 with HCV-associated MCS and 66 chronic HCV carriers without any evidence of serum cryoglobulins or other autoimmune/LPDs. The studied groups were comparable for age, liver disease, and virologic parameters, whereas females were more frequent in the MC group, as expected (Table 1). We noted a significantly higher prevalence of T allele homozygosity in the group of patients with MCS ($P < .001$), as well as the presence of the T allele (homozygous TT plus heterozygous TC) in respect to HCV carriers without MC ($P = .004$). This result was consistent with the higher serum levels found in MC patients compared with patients with the sole HCV infection ($P < .001$; Table 1).

Table 1. Clinical and laboratory data for 123 hepatitis C virus (HCV) patients with or without mixed cryoglobulinemia (MC)

	HCV, n = 66	MC-HCV, n = 57	*P*
Age, y	55.6 ± 24.3	58.8 ± 25.2	ns
Sex (male/female), n	43/23	22/35	.003
Histology, n			ns
Chronic hepatitis	52	48	
Cirrhosis	14	9	
ALT (× ULN)	3.42 ± 1.7	3.11 ± 1.2	ns
Viral titer, IU/mL × 10^6	2.5 ± 3.2	1.87 ± 1.64	ns
HCV genotype, n (%)			ns
1	39 (59%)	32 (56%)	
2	14 (21%)	17 (30%)	
3	9 (14%)	6 (11%)	
4	4 (6%)	2 (3%)	
Cryocrit (%)	0	6.9 ± 5.8	< .001
C3, mL/dL*	114.3 ± 62.6	129.7 ± 58.2	ns
C4, mL/dL†	88.6 ± 48.7	13.5 ± 11.6	< .001
Rheumatoid factor, IU/mL‡	12.3 ± 7.2	427.5 ± 1190.4	.005
BAFF serum levels, ng/mL	1.49 ± 0.21	3.22 ± 1.12	< .001
BAFF promoter genotype, %			
T/T	8	34	< .001
C/T	48	46	ns
C/C	44	20	.004
Presence of T allele	56	80	.004

Results are presented as means plus or minus SD except where otherwise noted.
ALT indicates alanine aminotransferase; ULN, upper limit of normal; and ns, not significant.
*Complement C3 normal values range from 83 to 177 mL/dL.
†Complement C4 normal values range from 20 to 150 mL/dL.
‡Rheumatoid factor normal values are < 25 UL/mL.

These results emphasize the potential contribution of the genetic background of HCV-infected patients in the development of LPDs. In this light, specific human leukocyte antigen (HLA) clusters have been previously associated with a higher risk of developing MCS and concomitant NHL.[9] In addition, recent data limiting the importance of virus-specific determinants are consistent with the relevance of genetic and host factors in promoting HCV-related LPDs.[10]

The transcriptional activation induced by the mutated BAFF promoter can be considered one of the mechanisms involved in the pathogenesis of HCV-related autoimmune/lymphoproliferative disorders, and the polymorphism can contribute, possibly in combination with other allelic patterns, to determining a genetic profile characteristic of the cryoglobulinemic phenotype.

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To the editor:

Complement activation impacts B-cell depletion by both type I and type II CD20 monoclonal antibodies

With great interest, we have read the article by Beers et al[1] documenting a potent B-cell depleting ability for type II (or tositumumab-like) CD20 antibodies. The authors conclude that complement-dependent cytotoxicity (CDC) is of little importance for B-cell depletion induced not only by type II, but also by type I (or rituximab-like) CD20 antibodies. Unfortunately, the experimental design chosen by Beers et al does not allow such a conclusion, and the data in our view appear to suggest the reverse.

First, Beers et al show that tositumomab also induces CDC, albeit at a lower level than rituximab. Similarly, we also routinely observe significant complement-mediated lysis of lymphoma cells by tositumomab (Figure 1). Second, to specifically address the role of complement, the authors generated a mouse IgG2a version of rituximab (Rit-m2a) and introduced a lysine-into-alanine mutation at position 322 (K322A) to abrogate C1q binding and CDC. A study by Idusogie et al,[2] however, previously demonstrated this mutation to be insufficient to remove CDC activity, and K322A-mutated rituximab retained more than 60% of its CDC capacity at near-physiologic complement levels. Indeed, we have confirmed that for other CD20 antibodies, such as HuMab 7D8,[3] significant CDC activity remains after mutating K322 (Figure 1). Hence, on basis of their data, Beers et al cannot exclude complement activation as an in vivo mechanism of action for B-cell depletion by either tositumomab or rituximab.

Notably, the authors point out that it is not understood why the genetic background of the hCD20-transgenic mice used, strongly influenced CD20-induced B-cell depletion, with stronger depletion in BALB/c than in C57BL/6 mice. In this context, it is important to note that serum complement activity differs significantly between mouse strains and varies with gender and age.[4-6] In our own experience, BALB/c mice (males in particular) have relatively high levels of complement activity compared with C57BL/6 mice. In our view, this may explain the observed differences and identify complement activation as a significantly contributing factor to the in vivo mechanism of action of CD20 antibodies.

The contribution of complement activation to B-cell depletion by CD20 antibodies is strongly supported by several studies. Kennedy et al have shown that $CD20^+$ cells are depleted concomitantly

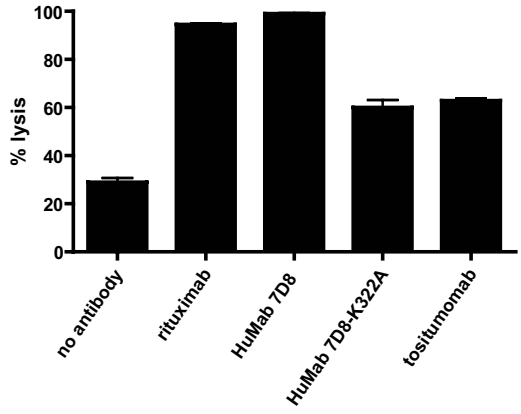

Figure 1. Complement-dependent cytotoxicity of CD20 antibodies. Daudi cells (0.1×10^6) were incubated with CD20 antibodies rituximab, HuMab 7D8, HuMab 7D8-K322A or tositumomab (10 μg/mL) at room temperature for 15 minutes. Normal human serum (final concentration 20%) was added as a source of complement, and cells were incubated for 45 minutes at 37°C. Propidium iodide was added, and cells were analyzed by flow cytometry. Results are shown as percentage of propidium iodide–positive cells proportional to total cell number (% lysis) and are representative of 3 separate experiments.