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

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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
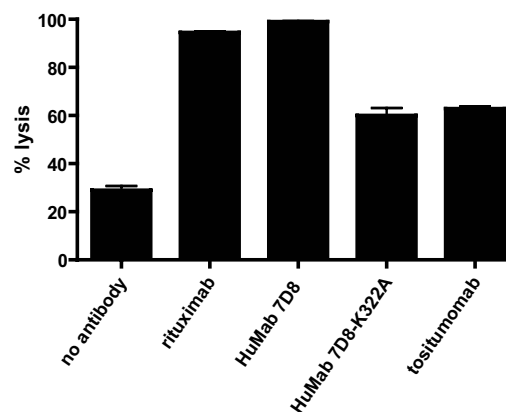
Figure 1. Complement-dependent cytotoxicity of CD20 antibodies. Daudi cells (0.1 × 10^{6}) were incubated with CD20 antibodies rituximab, HuMab 7D8, HuMab 7D8-K322A or tositumomab (10 µg/mL) at room temperature for 15 minutes. Normal human serum (final concentration 20%) was added as a source of complement, 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.

with the consumption of complement in patients during treatment with rituximab.[7] Golay et al demonstrated that therapeutic activity against established B-cell tumors by rituximab required complement.[8] Furthermore, Gong et al[9] showed depletion of resident B-cells, such as those in the splenic marginal zone, to be critically dependent on complement. Notably, as above, the magnitude of complement-mediated B-cell depletion differed between mice of distinct genetic backgrounds. Circulatory B-cells (such as peripheral blood, lymph node, and splenic follicular B-cells), in contrast, can also be effectively cleared by Fc-receptor–mediated mechanisms in the absence of complement.[9,10] The observations by Beers et al, which document depletion of circulating (CFSE-labeled) B-cells in mice deficient for C1q serves to confirm this notion.

We conclude that the data presented by Beers et al do not support conclusions that minimize a role for complement in B-cell depletion by CD20 antibodies. In contrast, the increased B-cell depletion in complement-sufficient BALB/c mice in fact supports a critical contribution. Therapeutic CD20 antibodies may engage multiple mechanisms to deplete B-cells, of which Fc-mediated mechanisms seem sufficient for clearance of circulatory cells, whereas depletion of resident normal and tumor B-cells appears to be critically dependent on complement.

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Response

Superior B cell–depleting activity of type II anti-CD20 mAb is not due to activation of complement

Our paper focuses on reporting that tositumomab (a type II anti–human CD20) greatly outperforms rituximab and other type I anti-CD20 monoclonal antibody (mAb) in depleting human CD20-expressing B cells in a transgenic mouse. This, despite the fact that the mAb were all of the same mouse IgG2a isotype, selected for maximum effector function, and recognized the same cross-blocking epitope on CD20.[1] Previous data in xenograft models has suggested that a type II mAb might have higher efficacy,[2] but our paper is the first to demonstrate this comprehensive difference in a fully syngeneic system using unmanipulated target cells in vivo.

The main effector difference between type I and II CD20 mAb is that type I, which translocate CD20 to lipid microdomains, activate complement very efficiently, whereas type II do not.[3] Extensive investigations in previous work[2-4] and the current paper[5] show that rituximab is at least 25 times more active at evoking complement-dependent cytotoxicity (CDC) than tositumomab (B1) in the presence of rat or human serum. The relatively low activity of tositumomab is confirmed by the data shown by Beurskens et al against highly sensitive targets under optimal conditions. This low activity prevents type II mAb, such as tositumomab, from mediating effective CDC against B-cell targets that are protected by complement regulatory proteins, such as CD55 and CD59.[6,7] Taken together, it is clear that if complement is an important effector mechanism, as

we[2,3,8] and many others[9-13] have shown, it does not explain why tositumomab is so much more effective in the huCD20-transgenic mice. One potential explanation is that, in certain settings, complement is actually deleterious for therapy as recently suggested.[14] To test this idea, we transferred huCD20-transgenic B cells into C1q-deficient mice and made the K322A mutant of Ritm2a (and 1F5; a second type I mAb), a substitution which is known to significantly reduce C1q recruitment in mouse IgG2 antibodies.[15,16] In answer to the question raised by Beurskens et al regarding the effectiveness of the K322A mutation in ablating CDC, we agree that the case for human IgG1 is unclear.[17] In fact, the paper they cite[18(p4178)] states, "Our results demonstrate that the previously described C1q binding motif in murine IgG2b constituting residues E318, K320, and K322 is not applicable to a human IgG1′." Thus, we do not agree that the K322A substitution in mouse mAb is not removing CDC and our results in Figure 4A of our article clearly support this conclusion. Furthermore, this is an ideal modification for our work as it does not interfere with other effector functions, such as IgG binding to FcγR and FcRn.

Regardless of whether this mutation ablated or partially removed CDC activity, our results show that it made little difference to the efficacy of Ritm2a and did not convert its performance into that of a type II mAb. Therefore, we conclude that in this model, complement neither helps nor hinders efficacy of CD20 mAb and does not explain the