To the editor:

Complement: help or hindrance?

There remains considerable uncertainty regarding the role of complement as an effector system for anti-cancer monoclonal antibodies (mAbs) such as rituximab and alemtuzumab. We were, therefore, interested to read the intriguing findings of Wang et al. indicating that complement activation can be detrimental to antibody immunotherapy. They suggest that breakdown products of the complement component C3 block anti-lymphoma mAb activity and render natural killer (NK) cells less active in antibody-dependent cellular cytotoxicity and the treatment of the 38C13 lymphoma with anti-idiotypic mAb. Clearly, these are important observations, well supported by in vitro experiments. However, we feel it is important to stress that the beneficial effect of complement inactivation in vivo is not the general experience with therapeutic mAb, where the absence of complement typically either reduces efficacy or makes no difference (reviewed in Lim et al).

By way of example, Figure 1 shows that depletion of normal B cells with anti-CD20 mAb is equally effective regardless of whether the mice are deficient in C1q or C3 or the mAb is engineered not to engage C1q.

Several possibilities exist to explain these apparently contradictory findings. The first is that the anti-Id/38C13 lymphoma model is unusually dependent on NK cells, which, in turn, are particularly sensitive to blocking by C3b, consistent with the interpretation given by Wang et al. However, this seems unlikely, since previous work from this group has shown that granulocytes also play a role in this model. In addition, successful mAb immunotherapy in mice, including those with anti-CD20 mAb, commonly require an intact mononuclear phagocytic system, but not NK cells (reviewed in Lim et al).

This leaves 2 further potential explanations. The first is that idiotype may be an atypical target for mAb. In support of this, Golay et al have demonstrated that therapy of a hCD20-variant of 38C13 with CD20 mAb was completely dependent upon complement activity, with no role for NK cells or neutrophils. These findings are clearly counter to the results described by Wang et al, perhaps indicating that impact of complement on therapy is determined by the degree of complement activation, with the high levels evoked through CD20 mAb usually being beneficial to therapy, and lower levels, as occur with anti-idiotypic mAb, being detrimental as observed. Second, it must be considered that cobra venom factor (CVF) treatment has functions other than simple complement depletion. Rather than removing complement passively, CVF acts as an unregulated analog of C3b, resulting in uncontrolled activation of C3 with systemic release of breakdown products that impact on innate and acquired immune activation. Hence, CVF treatment provides a useful model of acute respiratory distress syndrome where it leads to acute organ damage. The release of cytokines and chemokines and accumulation of neutrophils after CVF are precisely the inflammatory conditions that might influence the growth of a small number (5000) of passaged tumor cells, particularly with an immunogenic lymphoma such as 38C13. In accordance with this supposition, the results from Wang et al show that CVF alone slows the growth of 38C13 to that seen after treatment with anti-Id mAb. Therefore, the synergistic activity of anti-Id mAb and CVF leading to long-term survival is consistent with the development of acquired immunity. Thus, we feel that an adjuvant effect of CVF must be considered as a plausible alternative explanation of the current in vivo data, especially given the weight of prevailing evidence showing that complement is not deleterious to direct targeting antibody immunotherapy.

Figure 1. Complement does not affect the ability of anti-CD20 mAb to deplete B cells in vivo. (A) BALB/c human CD20 transgenic mice (WT or C1q KO) received 250 μg of anti-human CD20 mAb (Rit m2a) or mAb lacking C1q binding activity (K322A mutation) intravenously on day 0. The number of circulating B cells was then assessed by flow cytometry for CD19 and B220. The results are expressed as percentage of B cells observed at time 0. (B) Experiments show adoptive transfer of hCD20 Tg (target: high carboxyfluorescein succinimidyl ester [Sigma-Aldrich]) and WT (nontarget: low carboxyfluorescein succinimidyl ester) splenic B cells into recipient mice carrying various effector defects [C1q KO, C3 KO, Gamma KO, Clodronate (Sigma-Aldrich), treated]. Twenty-four hours later, mice received control or Rit m2a mAb (10 μg, intravenously), and 16 hours later splenic B cells were analyzed by flow cytometry to determine the target:nontarget ratio. Bars represent mean ± SD, n = 3; each condition is representative of at least 2 independent experiments. The data clearly demonstrate that Rit m2a only deletes target cells (low target:nontarget ratio), and that activatory FcR (absent in Fcγ chain−/− mice) and macrophages (deleted in clodronate-treated mice), but not complement components C1q or C3, are important for target-cell deletion.

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Response

Complement in antibody therapy for lymphoma: both a help and a hindrance?

Beers, Cragg, and Glennie have made major contributions to our understanding of the mechanisms of action of anti-CD20 antibodies, and we appreciate their thoughtful response to our manuscript. As they have cited, there are animal models that suggest complement-dependent lysis contributes to the therapeutic effects of anti-lymphoma antibodies. The primary point of our paper was to raise the possibility that, in some circumstances, complement may hinder antibody-dependent cellular cytotoxicity (ADCC). Thus, when it comes to antibody therapy for lymphoma, we agree it is unclear whether complement is a “help or hindrance.”

Several points deserve specific comment. Beers et al refer to our prior work demonstrating that granulocytes contribute to the antitumor effects of antibody therapy in the 38C13 model. In that publication, we reported that both granulocytes and NK cells contribute to the therapeutic response. Activated natural killer (NK) cells produce cytokines that secondarily activate granulocytes. Thus, enhancing NK-cell activation may improve the therapeutic response even if granulocytes play a role.

Beers et al correctly point out that cobra venom factor (CVF) may have effects other than “simple complement depletion” and can cause inflammatory lung damage. This effect has been shown to be mediated by the C5a anaphylatoxin, which binds and activates neutrophils, leading to sequestration in the lungs. HC3-1496 was more effective than CVF in our model, yet, in contrast to CVF, HC3-1496 does not activate C5. In primate studies, intraarterial injection of high doses HC3-1496 resulted in no impairment of pulmonary function. These data suggest that induction of an inflammatory response was not responsible for the observed effects in our model. Nevertheless, we are currently exploring depletion of C3 using other approaches and in other models. Rechallenge of cured mice demonstrated no protection (S. Wang, G.J.W., unpublished data, June 2009); thus, we do not believe the therapeutic response we observed with the combination of CVF or HC3-1496 and antibody was due to development of an active immune response.

The data presented by Beers et al demonstrate that FcγR, but not complement, is necessary for B-cell clearance. This speaks to the importance of FcγR, but does not specifically address the role of complement in ADCC. Depletion of B cells by anti-CD20 in their studies was complete in both wild-type and C1q knockout mice; thus, any enhanced effect in the absence of complement could have been lost. Cure of a highly aggressive lymphoma, such as 38C13, may require a greater degree of NK-cell activation than is needed to demonstrate transient clearance of benign B cells from the spleen.

It may well be that the answer to the “helps or hinders” question depends upon the scenario, with complement-mediated lysis being more important in some circumstances and complement-inhibiting ADCC more important in others. The real answer will come both from further studies in animal models and from clinical trials.

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