

**Editorial****Rituximab: Converging Mechanisms of Action in Non-Hodgkin's Lymphoma?**

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Rituximab, a chimeric IgG1 monoclonal antibody that specifically recognizes the CD20 surface marker present on more than 80% of NHLs,<sup>5</sup> was in 1997 the first monoclonal antibody approved by the Food and Drug Administration for the treatment of human cancer (1). The pivotal study leading to the approval of rituximab demonstrated significant activity in indolent B-cell lymphomas, with a response rate of 50% in relapsed and refractory disease (2). Despite this, complete responses are uncommon (<10%), and median response durations are less than 12 months. The addition of rituximab to combination chemotherapy regimens has resulted in improved response rates in indolent lymphomas (3) and improved response rate and event-free and overall survival in aggressive lymphomas (4) when compared with combination chemotherapy alone. However, the incremental improvement obtained with the addition of rituximab to chemotherapy is not complete, particularly among high-risk patients. Obviously there is a continuing need to identify novel therapeutic combinations and/or engineered antibody molecules to further improve the efficacy of monoclonal antibody therapy for lymphoma and other malignancies.

The ability to improve upon currently used rituximab regimens relies in large part on an understanding of the mechanism of action of rituximab *in vivo*, so that key mechanistic components can be identified and exploited. For example, the determination of an immune effector cell subset necessary for optimal antitumor effect of rituximab could lead to the rational design of a therapeutic combination of rituximab with an immunomodulatory cytokine aimed at increasing either the number or activity (or both) of that specific effector cell population *in vivo*. In addition, it must be recognized that the relevant mechanism of action of rituximab and other antibodies could

vary among different types of B-cell malignancy due to differences in target antigen expression, functional effector cells, and sensitivity to rituximab-induced signaling and apoptosis.

Despite more than 15 years of collected preclinical and clinical experience, the primary mechanism of action of rituximab *in vivo* remains unresolved, with continuing debate over the relative importance of various potential mechanisms, including ADCC, complement-mediated cytotoxicity, and direct induction of apoptosis through an incompletely characterized CD20-mediated signaling pathway. Indeed, all of these possibilities have been demonstrated to be operational in studies using lymphoma cells *in vitro*.

Evidence published by our group and others has demonstrated that primary B-cell CLL cells undergo caspase-dependent apoptosis *in vitro* (5) and *in vivo* after rituximab treatment (6), although a requirement for immune effector cells or complement cannot be excluded in the latter study. In addition, factors that antagonize apoptosis such as overexpression of antiapoptotic proteins (7) or aberrant p53 function (8) are associated with rituximab resistance in CLL. Similar to CLL, a subset of lymphoma cell lines undergoes apoptosis in culture upon the addition of rituximab (9–11); this effect is increased in the presence of cross-linking of the CD20-bound rituximab on the cell surface with antihuman IgG (12). Despite these results, others have failed to demonstrate significant apoptosis of other lymphoma cell lines treated with rituximab *in vitro* (13), and no *in vivo* studies have been performed in NHL similar to those described for CLL (6).

Complement-mediated cytotoxicity has been reported by some as a predominant mechanism of rituximab activity *in vitro* (9, 14, 15). Complement-mediated lysis of primary lymphoma cells treated with rituximab *in vitro* correlates with the level of expression of complement regulatory proteins on the tumor cells (14, 16, 17). However, whereas some have demonstrated that clinical response to rituximab *in vivo* has a similar correlation (18), others have not confirmed this finding in NHL (19) or CLL (7).

Others have demonstrated that ADCC, mediated *in vitro* by NK cells (9) or neutrophils (20), contributes to the activity of rituximab. The study by Cartron *et al.* (21), which demonstrates a correlation between clinical response to rituximab and a specific allelic polymorphism in the FcγRIIIa receptor for IgG, suggests that a FcγRIIIa-mediated mechanism, such as ADCC, is likely to be important *in vivo*.

Until recently, *in vivo* data on the mechanism of rituximab activity have been lacking. However, Clynes *et al.* (22) have described a series of experiments in FcR-deficient mice, demonstrating that the FcRγ chain, which is common to and required for the function of FcγRI and FcγRIII in the mouse, is required for optimal therapeutic efficacy of rituximab in a nude mouse model of human lymphoma. Furthermore, the fact that genetic deficiency of the inhibitory Fcγ receptor, FcγRII (analogous to FcγRIIB in humans, and not present on NK cells), led

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<sup>5</sup> The abbreviations used are: NHL, non-Hodgkin's lymphoma; ADCC, antibody-dependent cellular cytotoxicity; CLL, chronic lymphocytic leukemia; NK, natural killer.

to improved survival after rituximab therapy in this model suggests that non-NK cells are important for this effect.

Di Gaetano *et al.* (23) have recently provided evidence for the importance of complement for the activity of rituximab *in vivo*. In their study, using a nonimmunodeficient mouse model of a syngeneic lymphoma (EL4) expressing the human CD20 protein on its surface, a single dose of rituximab led to long-term protection from lethal tumor growth. Depletion of murine NK cells, neutrophils, NK cells and neutrophils, or T cells before tumor cell inoculation did not affect the activity of rituximab *in vivo*. However, similar experiments performed in mice genetically deficient in C1q, the first component of the classical complement pathway, demonstrated a critical requirement for complement in mediating the therapeutic activity of rituximab. This was true despite the fact that C1q<sup>-/-</sup> mice were not deficient in ADCC activity.

In this issue of *Clinical Cancer Research*, Hernandez-Illizaliturri *et al.* (24) report that murine neutrophils contribute to the *in vivo* efficacy of rituximab. Using a severe combined immunodeficient mouse model of disseminated Raji lymphoma, the authors show that rituximab therapy protects against lymphoma progression and death. This protection is lost when murine neutrophils, NK cells, or both are depleted *in vivo* before the initiation of rituximab therapy. Comparing the two independent survival studies, the detrimental effect of isolated neutrophil depletion appears to be as great as, if not greater than, isolated NK depletion, suggesting that neutrophils contribute significantly to the *in vivo* mechanism of rituximab activity in this model system. The results presented are provocative. Could there be another explanation besides the conclusion that neutrophils, in this model system, contribute to ADCC of rituximab-coated tumor cells? It is conceivable that by depleting the various effector cell populations via *in vivo* administration of antibody, one somehow temporarily “exhausts” the hosts’ intrinsic FcR-mediated mechanism of eliminating antibody-coated tumor cells. The definitive experiment to prove this unlikely scenario would be to antibody-deplete a seemingly irrelevant population of cells *in vivo* and show that the antitumor efficacy was still intact or to eliminate neutrophils without the *in vivo* administration of an antibody and demonstrate persistent loss of antitumor efficacy.

The *in vivo* results from these three model systems suggest the possibility that, in NHL, complement activation and FcγR-mediated immune mechanisms, including those mediated by neutrophils, cooperate in rituximab’s antitumor effect. This notion has been suggested by others (14, 23); it is further supported by recent investigations in Mac-1-deficient mice. In these experiments, the complement receptor 3 (Mac-1; CD11b/CD18) is absolutely required for FcR-mediated neutrophil cytotoxicity against a tumor target, suggesting a direct interaction between complement and ADCC (25, 26).

Thus, it is becoming clear that these multiple potential mechanisms of rituximab activity, including complement activation, apoptotic signaling, and FcγR-mediated effects, are not independent and mutually exclusive but are likely to be interactive and potentially cooperative components of antibody-mediated therapy. In addition, select mechanisms of action may vary in importance among different CD20-positive lymphoproliferative disorders, emphasizing the importance of disease-

specific investigation of antibody mechanism of action. Understanding the integration of the complement pathway, FcR systems, and rituximab signaling pathways among different select B-cell malignancies will provide yet other opportunities for strategic improvement of rituximab therapy.

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