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Translational Medicine in Action: Anti-CD20 Therapy in Lymphoma

Sean H. Lim and Ronald Levy

The introduction of rituximab for B cell lymphoma in the late 1990s inaugurated a new era of cancer therapy showcasing mAbs. mAbs are in principle an amalgamation of two characteristics of a perfect anticancer drug. First, rituximab is a therapy targeted to the tumor cell, but it carries fewer side effects than does chemotherapy. Second, with its ability to directly engage the host immune system, it could potentially elicit longer lasting anticancer immunity, although this remains to be proven. This review highlights the fundamental scientific discoveries that allowed the development of clinically successful anti-CD20 mAbs. Since the approval of rituximab, a considerable amount of work has been undertaken by different groups trying to understand the workings and limitations of anti-CD20s. All of these efforts will be critical in designing new mAbs to CD20 and other targets and, ultimately, of anticancer mAbs that will improve on, or even replace, chemotherapy. *The Journal of Immunology*, 2014, 193: 1519–1524.

Lymphoma is predominantly a cancer of B lymphocytes, and nearly 70,000 new cases are diagnosed in the United States each year (1). Depending on the subtype of B cell lymphoma, the disease can pursue an indolent course spanning years, such as in follicular lymphoma (FL), or it can present aggressively during weeks or months, as in diffuse large B cell lymphoma. Although it is acceptable to adopt a “watch and wait” approach in asymptomatic patients with indolent disease, patients with more aggressive subtypes require immediate treatment. For more than three decades the only therapeutic options available had been chemotherapy and/or radiotherapy. Response rates of 60–70% were achieved but attempts to improve therapy by intensifying the number of chemotherapeutic agents or their doses led only to more side effects (2). Researchers had long recognized that novel therapies were required, and immunologists were particularly interested in the idea of a “magic bullet” (3), a drug that could specifically identify and kill cancer cells, thereby limiting toxic effects to normal, healthy cells. Several basic and groundbreaking discoveries paved the way for the development of mAbs, the theoretical magic bullets. Abs are produced by terminally differentiated B cells known as plasma cells as part of the healthy immune response. Each single B cell and its plasma cell clonal progeny produce only one Ab with a single specificity, that is, a mAb. In a normal immune response, when many B cells are stimulated by Ag, multiple B cell clones develop, leading to multiple plasma cells, and the resultant Abs in the serum have diverse specificity for the Ag (polyclonal Abs). In 1971, Norman Klinman (4) developed an in vivo/in vitro B cell cloning method that could produce limited quantities of Abs in tissue culture from single cells, the first mAbs (Fig. 1). In 1975, Köhler and Milstein (5) made the critical breakthrough when they immortalized B cells by fusing them together with myeloma cells, thereby allowing the production of unlimited quantities of mAbs. This was a momentous step in the history of immunology and it led to their receipt of the Nobel Prize. However, even Köhler and Milstein may not have initially realized that their discovery would lead to drugs that would change the way we treat cancer. At about the same time, Stevenson and Stevenson (6) discovered that each malignant B cell, being derived from an antecedent mature B cell, expressed a unique Ig on its surface that could serve as a tumor-specific target. Therefore, in B cell lymphoma, all of the patient’s malignant cells can be specifically recognized by their unique cell surface (idiotypic) Ig structure. Soon thereafter we made mAbs against these tumor-derived idiotypes (7). When we administered these custom-made mAbs to patients with B cell lymphoma, most responded to treatment and had durable remissions of their tumors (8–10). Despite its therapeutic efficacy, the need to produce a different Ab for each patient was impractical, although with newer technology it may one day be revisited.

Earlier, in 1980, Nadler et al. (11) had infused a lymphoma patient with an mAb of undetermined specificity, but it was blocked by circulating cell-free substances and produced transient and clinically insignificant effects. In that same year they discovered CD20, one of the first non-Ig B cell–specific Ags (12). CD20 is highly expressed on normal and malignant B cells (12), and thus it could act as a target for mAbs in B cell lymphoma.

Up to this point, all of the mAbs administered to patients had been entirely of mouse origin. Surprisingly, patients with B cell lymphoma did not make Ab responses against the foreign mouse proteins. However, for patients with T cell lymphoma (13, 14) and other types of malignancy, human anti-mouse Abs against the murine components did arise, reducing therapeutic efficacy and decreasing the survival of the thera-
peutic Ab (15). Surprisingly, no serious side effects from immune complexes of human anti-mouse Ab have been documented (16). Later, advances in recombinant DNA technology allowed the creation of chimeric mAbs, which carried murine and human portions in their variable and constant regions, respectively (17). These developments allowed the generation of C2B8, the first chimeric anti-CD20 mAb, also eventually known as rituximab (18). This Ab was able to deplete B cells in cynomolgus monkeys with no effects on other cell populations and with very little evidence of toxicity. In the early 1990s, we reported the first phase I clinical trial of rituximab (19). The Ab was administered as a single injection at various doses to 15 patients with relapsed low-grade B cell lymphoma. The mAb was well tolerated and 6 of 15 cases showed clinically significant tumor responses at all the doses and with no dose-limiting toxicity. These promising results led to a phase II multicenter study where 37 patients with low-grade B cell lymphoma were treated with four weekly infusions of rituximab at 375 mg/m² (20), a dose chosen according to the maximum that could be easily manufactured at the time. Seventeen patients showed tumor response to single-agent rituximab. Although normal B cells were depleted in the blood, the serum Ig levels did not fall and the patients did not show evidence of immunodeficiency. Considering that this population of patients had relapsed after multiple rounds of chemotherapy, it was extremely promising that any of them responded to a single mAb. After a larger confirmatory trial that showed a remarkably consistent response rate (21), rituximab became the first mAb to be approved for the treatment of cancer by the U.S. Food and Drug Administration. Of note, this approval was granted even though no formal phase III trial comparing rituximab to other lymphoma therapies had been conducted. In part, this was because of the relative freedom of rituximab therapy from serious side effects, in marked contrast with chemotherapy drugs. Since the approval of rituximab, we estimate that >4 million people have been treated with rituximab worldwide for lymphoma and other diseases such as rheumatoid arthritis.

Czuczman et al. (22) first reported that rituximab could be safely and efficaciously administered with chemotherapy in patients with low-grade lymphoma. The lack of toxicity on hematopoietic cells has also allowed rituximab to be safely combined with chemotherapy, even in elderly patients (23). In groundbreaking phase III clinical trials in diffuse large B cell lymphoma, rituximab was added to standard CHOP chemotherapy and it improved every measure of clinical outcome, including overall survival of the patients (23–26). In low-grade lymphoma, rituximab shows clinical benefit even when used as a single agent. The Swiss Group for Clinical Cancer Research demonstrated that single-agent rituximab, administered weekly for four doses, then twice monthly for a further four doses, in newly diagnosed or relapsed/refractory FL could establish long-term disease control (27). This has allowed low-grade lymphoma patients to defer chemotherapy, or autologous transplantation, treatments with considerable morbidity and mortality risks. In low-grade lymphomas, when rituximab was combined with chemotherapy as initial treatment (28, 29) or with salvage therapy (30), improvements in responses and survival are also achieved. Furthermore, the use of rituximab as a maintenance dose every 2 mo, for up to 2 y, after consolidation chemotherapy was both well tolerated and delayed disease progression in responders, with as many as 60% of patients free of disease progression 6 y after treatment (31, 32). Longer follow-up of these patients will be required to know whether this strategy of maintenance rituximab will result in longer overall survival of the patients.

Frequently, new therapies come forward to the clinic before they are fully understood. Despite the success of rituximab in the clinic, the mechanistic intricacies of the mAb still remain a matter of discussion and are extensively reviewed elsewhere (33, 34). We need to understand how rituximab works against lymphoma and the mechanisms of tumor resistance if we are to be able to make more potent Abs and to improve on the present results. Preclinical and clinical evidence both suggest that after anti-CD20 mAb binds to its target, CD20 on B cells, the Ab’s constant Fc domain binds to the host immune effector cells such as NK cells and macrophages. These immune effector cells either phagocytose the target cells directly or they release cytotoxic molecules such as perforin and granzyme to lyse the target cells. These processes are known as Ab-directed cellular cytotoxicity (ADCC) and phagocytosis, respectively. In line with this hypothesis, individuals who bear Fc receptors with higher affinity to the Fc domain of Igs (FcRs) also have better responses to rituximab (35–37). Alternatively, anti-CD20 mAbs might kill by activating the host complement pathway or directly inducing death signals in the cell upon binding to CD20 (reviewed in Ref. 34). Earlier work suggested that anti-CD20 mAbs were capable of in-
Toxic cell signaling and a "classical" form of cell death called apoptosis and hence sensitization to cytotoxic chemotherapy. However, most of these earlier studies were performed in cell lines and not primary tumor cells, or the mAb required artificial cross-linking (38-40). Recently published data show that cell death induced by anti-CD20 mAbs is in fact non-apoptotic and is dependent on the type of anti-CD20 used (41). Finally, there is some evidence that anti-CD20 mAbs induce T cell-mediated immunity (42, 43). Consistent with this latter idea is the observation that responses to rituximab in patients can be delayed by as long as several months after administration (20), a period of time that would allow induction of T cell immunity. Furthermore, lymphoma patients who respond to rituximab the first time can have more durable responses on retreatment (20), a curious phenomenon that might be accounted for by the presence of memory T cells.

From the work of Cragg, Glennie, and colleagues (44-47) we know that not all anti-CD20 mAbs are the same. They can be classified into either type I or II, depending on their in vitro activity in various assays. This distinction is based on the ability of the mAb to redistribute CD20 in Triton X-100-insoluble lipid rafts, induce homotypic adhesion, elicit complement-dependent cytotoxicity (CDC), and/or direct cell death, as discussed earlier. Type I mAbs such as rituximab are more potent at translocating CD20 into lipid rafts and inducing CDC, whereas type II mAbs such as tositumomab and obinutuzumab are more effective at inducing homotypic adhesion and direct cell death induction through an actin-dependent, lysosome-mediated pathway (41, 48).

Evidently, understanding why anti-CD20s fail is as important as knowing how they work. Approximately 30% of cases of B cell non-Hodgkin lymphoma either do not respond to rituximab or relapse after initially responding, and they eventually become refractory to rituximab (reviewed in Ref. 49). We found that the level of the complement defense molecules CD46, CD55, and CD59 on lymphoma cells did not correlate, either negatively or positively, with responses to rituximab (50). A series of comprehensive studies from Taylor and colleagues (51–53) showed trogocytosis as a potential mechanism of rituximab resistance. Trogocytosis is a process whereby phagocytic cells selectively remove bound Ag/IgG complexes (CD20/rituximab, in this case) from the surface of target cells rather than phagocytose them (51, 52).

Consequently, the target cells are no longer opsonized and are able to escape detection by immune effector cells. This process is thought to be mediated by localized deposition of Ag/IgG deposits, such as may occur owing to translocation of rituximab into lipid rafts, as well as concomitant complement deposition, which aids FcR binding (54). In a proof-of-principle study to support the evidence for trogocytosis, 12 chronic lymphocytic leukemia (CLL) patients were treated with lower doses of rituximab (20 or 60 mg/m² three times a week for 4 initial weeks, as compared with conventional 375 mg/m² weekly for 4 wk). The study demonstrated that even at doses as low as 20 mg/m², rituximab was able to effectively clear as much as 75% of the circulating CLL cells, and less trogocytosis was seen in this group compared with the group given 60 mg/m² (55). These results, however, are at odds with another previous study in CLL, which showed a dose–response relationship between rituximab and overall response rate (56). It is also not known whether trogocytosis would similarly apply to solid B cell malignancies where the tumor cells are less accessible to the peripheral vasculature than are circulating CLL cells.

Another possible mechanism of rituximab resistance is the loss of surface CD20 expression after rituximab therapy, which might be due to transcriptional downregulation or selection of a CD20*-clone (57-59). A further means by which target cells can evade anti-CD20-mediated killing is by internalization (59, 60) of the Ag/Ab complex into the target cell, thereby preventing its from recruiting immune effector cells. Normal and malignant B cells express the inhibitory Fc receptor FcγRIIB, which binds to the Fc domain of rituximab. Together the CD20/rituximab/FcγRIIB complex is then internalized into the target cell and degraded. Furthermore, unlike normal B cells, which express a consistent level of FcγRIIIB on the cell surface, malignant B cells have a heterogeneous expression of FcγRIIIB, with the level of FcγRIIIB on the B cells correlating with the extent of rituximab internalization. Thus, it is possible that FcγRIIIB expression on malignant B cells might serve as a biomarker for response to rituximab. Alternatively, internalization might be overcome by employing type II anti-CD20s, which seem to internalize far less than do type II anti-CD20s, or by combination of rituximab with an anti-FcγRIIB inhibitor (60).

Recently, new anti-CD20s have entered the clinic. Ofatumumab (type I) and obinutuzumab (type II) were both approved by the U.S. Food and Drug Administration in 2009 and 2013, respectively, for the treatment of CLL. Unlike chimeric rituximab, both Abs are humanized, meaning they contain only the minimum critical murine sequences, that is, the CDR grafted into a human framework, with the theoretical benefit being to reduce immunogenicity of mouse sequences. Ofatumumab is a type I mAb and it recognizes a distinct epitope, and it has a slower dissociation rate from CD20 compared with rituximab, thus displaying greater CDC activity than rituximab (47). As a consequence, ofatumumab is able to lyse human lymphoma cell lines more effectively than does rituximab (47, 61). In contrast, obinutuzumab is a type II mAb with a sugar residue removed from its Fc domain to enhance Fc receptor binding (62, 63). Similar to other type II anti-CD20s, obinutuzumab is also superior in inducing lysosomal-mediated cell death, without the need for mAb cross-linking (48). The two new anti-CD20 mAbs have different effects on immune effector cells (64). Obinutuzumab was superior at enhancing NK cell activation and ADCC whereas ofatumumab was better at inducing Ab-directed cellular phagocytosis by macrophages. These differences may in part be due to a modified Fc domain of obinutuzumab that increases its binding affinity to FcγRIIIa on NK cells, but has less effect on FcγRIIa, the predominant activatory FcγR present on macrophages (65).

The hope is that the new anti-CD20 Abs will be more efficacious than rituximab, but the clinical data so far are mixed. One study of a head-to-head comparison between rituximab and obinutuzumab in relapsed indolent lymphoma showed no difference in progression-free survival (66). This phase II study compared obinutuzumab at a dose of 1000 mg to rituximab at the standard approved dose of 375 mg/m². These differing doses were argued to be comparable in serum levels achieved because of differing pharmacokinetics. Com-
parable overall responses of 32 of 74 and 29 of 75 patients were reported. In a recent head-to-head comparison in patients with CLL (67), obinutuzumab was superior to rituximab when combined with the drug chlorambucil, but again the doses and even the schedules of the two Abs were not matched. In short, although in vitro and xenograft studies support the potential of the new anti-CD20 mAbs over rituximab, the present trials are unclear as to whether these new mAbs are indeed an advance. The dosing and schedule differences between the mAbs being compared make it difficult to conclude whether any observed clinical benefits are due to differences in their mechanisms of action.

Many early phase, single-arm studies of rituximab and nonchemotherapeutic agents exist. As yet, none of these has replaced current conventional frontline rituximab-CVP or rituximab-CHOP chemotherapy in low-grade and high-grade lymphoma, respectively. Neither has any of these approaches been shown to be superior to standard salvage therapies. One of the strategies for enhancing the therapeutic effect of anti-CD20 mAbs is to administer a second Ab that stimulates NK cells responsible for ADCC. Preclinical studies show impressive therapeutic synergy between rituximab and an agonistic Ab against CD137, a target appearing on NK cells once they see an Ab-coated tumor cell (68). Clinical trials are now underway to test this concept in patients with lymphoma.

The initial use of rituximab drew concerns with regard to possible immunosuppressive activity on the host, being that it is a B cell–depleting mAb, and B cells are a vital part of our body’s defensive adaptive immune system. Surprisingly, to date, rituximab and the new anti-CD20 mAbs are generally well tolerated. The most common side effects encountered are related to the first mAb infusion, which is related to cytokine release and is manifested as fever and chills. Infusion-related events tend to be proportional to the patient’s tumor load and can be ameliorated by premedication with corticosteroids, slower infusion rates, and smaller initial mAb doses. The other notable side effect is late-onset neutropenia, the mechanism of which is poorly understood and tends to be transient and of significant consequence to the patient (reviewed in Ref. 69). The risk of infections attributable to anti-CD20 therapy per se is more difficult to assess. Lymphoma in itself causes host immunosuppression, and anti-CD20 is frequently used in combination with chemotherapy. In the Swiss Group for Clinical Cancer Research trial where patients with FL were treated only with rituximab, the infection rate was not significantly different, although persistent B cell depletion was associated with lower serum IgM levels (70).

Anti-CD20 mAb therapy has been associated with reactivation of viral diseases such as hepatitis B. This is likely to be due to the critical role played by B cells in Ab production and T cell–mediated immunity in the control of viral infections. Fortunately, early identification and antiviral prophylaxis and/ or treatment are generally sufficient to prevent or control viral reactivation during anti-CD20 therapy. Another less predictable and more severe viral complication is progressive multifocal leukoencephalopathy (PML). PML is a demyelinating condition of the CNS caused by reactivation of John Cunningham polyoma virus (71). It is most frequently associated with HIV infection where there is a reduction in the CD4+ T cell subset. Again, the precise pathology is unclear, as not all rituximab-treated patients who develop PML have reduced CD4+ T cells. As many as 52 cases of PML have been described in patients with lymphoma or CLL, and it carries a 90% mortality rate (71). However, it needs to be stressed that the absolute risk is of PML is very low, and there is little doubt that the clinical benefit of rituximab outweighs its risks.

Conclusions

Anti-CD20 mAb therapy has inaugurated a new era of cancer immunotherapy (Fig. 1). In 1957, Burnet and Thomas first suggested that the host immune system is capable of eliminating spontaneously occurring tumors (reviewed in Ref. 72), and scientists have attempted in a myriad of different ways to reawaken the host to fight cancer cells. There now exists a well-tolerated drug that has the ability to re-engage the host immune system and can be administered en masse to patients with B cell malignancies. Rituximab has indeed laid the foundation for mAb therapy, and it is no surprise that all attention is now focused on mAb therapy to many other different targets. The lessons learned from rituximab are important for the clinical use of these future mAbs. No one single drug or mAb is going to “cure cancer” but rather the future lies in new, scientifically rational combinations of drugs. Anticancer therapy is currently still dominated by chemotherapy. Zitvogel et al. (73) have demonstrated that chemotherapeutic agents are not merely cytotoxic but are also able to alter the immunogenicity of tumor cells, which may explain why rituximab is more effective in combination with chemotherapy. Perhaps as we learn to understand the requirements for effective anticancer therapy, newer mAbs will be able to extend the current ability to treat cancer while minimizing unwanted side effects.

Disclosures

The authors have no financial conflicts of interest.

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