Is B-cell depletion still a good strategy for treating immune thrombocytopenia?

Bertrand Godeau1, Roberto Stasi2,3, This article is dedicated to the memory of Roberto Stasi

1. Assistance publique—Hôpitaux de Paris, CHU Henri-Mondor, service de médecine interne, centre de référence des cytopénies autoimmunes de l’adulte, UPEC, 94000 Créteil, France
2. St George’s Hospital, Haematology SpR, Blackshaw Road, Tooting, London SW17 0QT, UK

Correspondence: Bertrand Godeau, AP–HP, CHU Henri-Mondor, service de médecine interne, 51, avenue du Maréchal-de-Lattre-de-Tassigny, 94000 Créteil, France. bertrand.godeau@hmn.aphp.fr

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Summary

B cells play an important role in the pathophysiology of immune thrombocytopenia (ITP). Thus, a rational approach to ITP treatment involves B-cell depletion such as with rituximab. More than 10 years after the first reports of data suggesting that anti-CD20 MoAbs could be effective treatment for ITP, we have now a clear view of its efficacy, with an overall response in about 60% of patients. The report of fatal opportunistic infections was initially a matter of concern, but to date, reassuring data have been reported and rituximab appears well tolerated with an acceptable risk of infection. In view of these data, rituximab may always be a valid option for ITP. However, relapses are frequent, and the long-term response appears modest. Therefore, strategies to ameliorate the long-term efficacy of the treatment must be developed. Several options may be tested including giving rituximab upfront or early on after ITP diagnosis, maintenance treatment with repeated infusions, and combining rituximab with other treatments able to modulate T-cell compartment to achieve a synergistic effect. New generations of B-cell targeted treatment, including new-generations anti-CD20 MoAbs, may be also tested.

The pathophysiology of immune thrombocytopenic purpura (ITP) is complex and remains little understood. For many decades a widely accepted hypothesis was that ITP is primarily due to platelet autoantibodies that mediate phagocytosis and destruction of platelets by macrophages in the reticuloendothelial system within the spleen [1]. We now know that these autoantibodies can also inhibit megakaryocyte proliferation and maturation both in vitro and in vivo [2]. Additionally, T-cell–mediated peripheral platelet destruction and megakaryocyte inhibition or destruction in the bone marrow have been shown to lead to thrombocytopenia [3].

1 Auteur décedé.
B cells play an important role in the immune response. Their major role is to produce antibodies. However, B cells are also efficient antigen-presenting cells for T cells and secrete various cytokines such as interleukin 1 (IL-1), IL-10, IL-6, interferon γ, and tumor necrosis factor α (TNF-α), which activate macrophages, dendritic cells, and immunoregulatory cells. The role of B cells in the pathophysiology of ITP was reported more than 50 years ago, with the demonstration that platelet destruction was related to a plasma-derived factor [4]. Thus, a rational approach to ITP treatment could involve B-cell depletion such as with rituximab.

Rituximab was found a potential effective treatment for ITP in an open study more than 10 years ago [5]. It is now largely used as second- or third-line treatment by many groups. However, rituximab is not licensed in some countries and the availability of new effective treatments for ITP such as thrombopoietin receptor agonists could limit the interest in its further development. Although rituximab is still largely used, several unresolved questions remain: what is its long-term efficacy? Is it safe? Can we determine robust predictive factors of response? Should rituximab be associated with other treatments to increase its efficacy? Is there a place for the development of new generations of anti-CD20 antibodies or other options to modulate the B-cell compartment in ITP?

Rituximab for ITP: mode(s) of action

Human CD20 is a non-glycosylated phosphoprotein exclusively expressed in the B-cell hematopoietic lineage. Its exact function and natural ligands are unknown. It is not expressed in hematopoietic stem cells or plasma cells. Rituximab is a human-to-mouse chimeric anti-CD20 monoclonal antibody (MoAb) that induces rapid, profound and prolonged B-cell depletion. It was initially developed 20 years ago to treat lymphoma, but it is now used to treat various autoimmune diseases. The mechanisms of B-cell depletion after rituximab infusion are not completely understood, but antibody-dependent cellular cytotoxicity (ADCC) appears crucial [6]. Complement-dependent cytotoxicity (CDC) and apoptosis via a direct effect of MoAb binding to CD20 may be involved.

The mechanisms of action of rituximab in ITP are not fully understood. Rituximab has direct effects on antibody production but also indirect effects on cellular immunity [7]. Rituximab can induce a marked decrease of titers of platelet autoantibodies, particularly in ITP patients in whom rituximab is effective. However, this pattern of response is not consistent even in responders, suggesting that other mechanisms are involved. Stasi et al. [8,9] showed that the rituximab response is related to changes in the T-cell compartment with a resolution of skewed distribution of human T-cell receptors, changes in T-helper subset distribution and improved regulatory T-cell function. In ITP patients showing no response to rituximab, Audia et al. [10,11] found a preferential Th1 and Tc1 T-lymphocyte polarization, associated with an increase in frequency of splenic effector memory CD81 T cells and a restricted pattern of the CD81 T-cell repertoire. These results demonstrate an activation of splenic CD81 T cells in ITP patients without response to RTX and suggest their involvement in platelet destruction in these patients. The interesting findings by Mahévas et al. [12] in patients with failure to respond to rituximab and who underwent splenectomy may explain one of the mechanisms of resistance to rituximab in ITP. The authors identified antibody-secreting cells as the major splenic B-cell population resistant to rituximab. Antiplatelet-specific plasma cells were detected in spleens up to 6 months after rituximab treatment, and the plasma cell population showed a long-lived program, thus explaining, for most of these patients, the absence of response to rituximab and the response to splenectomy. Plasma cells showed a gene expression profile characteristic of both long-lived plasma cells and proliferating plasmablasts. Therefore, the milieu generated by B-cell depletion may promote the differentiation and settlement of long-lived plasma cells in the spleen.

These data suggest several complex mechanisms responsible for failure of rituximab in ITP.

Efficacy of rituximab in ITP

More than 10 years ago, Stasi et al. [5], in an open prospective study, gave rituximab to 25 adults with chronic ITP according to the infusion regimen used for lymphoma (i.e., 4 weekly infusions of 375 mg/m² rituximab). The authors reported an overall response rate of 52%. In view of these encouraging results, other groups conducted uncontrolled studies. Six years later, Arnold et al. [13] conducted an extensive review of the literature involving 313 patients and confirmed the good short-term efficacy of rituximab for ITP, with an overall response rate of 62.5% that lasted from 2 to 48 months. The same group conducted a new systematic review and meta-analysis including results of more recently published controlled studies [14]. Platelet count > 50 g/L at 6 months was more frequent for patients receiving rituximab than placebo (relative risk 1.46, 95% confidence interval 1.18–1.80). However, rituximab was not associated with reduced risk of bleeding or increased risk of infection. An open prospective study was then conducted in France to assess the efficacy and safety of rituximab as an alternative to splenectomy in 60 adults with chronic ITP [15]. After a follow-up of 2 years, 40% of patients achieved response. A recent meta-analysis of published studies of 368 non-splenectomized ITP patients receiving rituximab confirmed these results, with an overall response rate of 57% [16]. Gahnima et al. [17], in a prospective double-blind randomized study, compared rituximab with placebo and standard of care as second-line treatment for ITP without splenectomy. The rate of complete response at 24 weeks was greater with rituximab than placebo, with a trend toward a lower rate of splenectomy...
in the rituximab arm. However, rituximab did not reduce the rate of treatment failure, defined as a composite of splenectomy or meeting the predefined criteria for splenectomy if splenectomy was not performed.

The long-term response of rituximab is less well known because the duration of follow-up has been limited in most reported studies. A retrospective study of 72 adults and 65 children found 5-year estimates of persistent response of 21% and 26%, respectively [18]. Unlike the multiple bias due to the retrospective design of this multicentric study, the high frequency of relapse appears not questionable. Unfortunately, we can define no clear predictive factors of sustained response. Better prolonged response could be associated with young age and short duration of ITP before treatment with rituximab [16]. However, these predictive factors appear not robust enough to be used for individuals.

Safety of rituximab in ITP

Assessing the safety of rituximab in ITP is a crucial question. Rituximab was initially developed to treat lymphoma, and tolerance in this setting has been considered excellent but may be overestimated. In lymphoma, rituximab is associated with chemotherapy and with adverse events, assessing the respective responsibility of rituximab and chemotherapy is difficult. Physicians could accept severe treatment-related side effects when a treatment is used for onco-haematologic indications with spontaneous life-threatening prognosis, but such side effects would be not acceptable for non-malignant autoimmune diseases.

The risk of infection has been initially a matter of concern. Fatal progressive multiple leuкоencephalopathy (PML) following rituximab therapy has been reported [19]. These cases occurred mainly in patients receiving treatment for lymphoma or severe autoimmune diseases with rituximab associated with immunosuppressive agents, including high-dose steroids. To our knowledge, only 2 cases of PML in ITP patients have been reported [19,20]. In an extensive review of the literature, Arnold et al. reported a poor tolerance of rituximab in ITP, with death in 2.9% of cases [13]. The authors noted that 4 patients died of infection, but assessing the real responsibility of rituximab is difficult. Moreover, they attributed some deaths to rituximab, which could have been due to ITP rather than the treatment itself. Rituximab may have been initially indicated as treatment for severe refractory ITP in severe immuno-compromised patients with several lines of immunosuppressive drugs and prolonged steroid treatment. Better tolerance was observed in patients without heavy pre-treatment as suggested by the results of a French study of patients without splenectomy [15]. In this study, 60 patients showed no severe side effects at 2-year follow-up.

The safety of rituximab has been better studied in other autoimmune diseases. A large prospective registry of patients receiving treatment in daily practice for rheumatoid arthritis in France included 1303 patients monitored for a mean of 1.2 ± 0.8 years. The rate of severe infections was 5 per 100 patient-years and was similar to that for patients with rheumatoid arthritis receiving other biotherapies [21]. However, no such study existed for ITP. Moreover, in real life, the side effects of rituximab, particularly after a long delay, could be underestimated. To answer this important question, a prospective registry of ITP patients receiving rituximab was established in France 3 years ago. Preliminary results for 209 patients at a mean follow-up of 18 months gave reassuring data, with absence of opportunistic infection [22]. Analysis with more patients and prolonged follow-up is in progress.

Several other risks of rituximab treatment are well known and some can be easily prevented. [23]. Rare, fatal, infusion-related reactions have been reported in patients with rheumatoid arthritis, and health authorities recommend systemic premedication with 100 mg methylprednisolone. Rituximab can be associated with virus reactivation, and cases of fatal fulminating B hepatitis (HBV) have been reported. Patients should be screened for HBV infection and carriers should receive prophylaxis with lamivudine and monitored for the development of hepatitis during and several months after the therapy. In the presence of markers of active HBV infection, other therapeutic options should be considered.

Transient late-onset neutropenia occurring usually several months after the administration of rituximab has been observed mainly in patients with chronic lymphocytic leukemia. This risk for patients receiving rituximab for ITP is rare.

In our experience, reduced low level of immunoglobulins is occasionally observed a long time after rituximab infusions [24]. Ensuring that this complication is not the first manifestation of common variable immunodeficiency (CVID) syndrome is difficult. In this case, substitution with intravenous immunoglobulins should be discussed. Even if the incidence of this complication remains unknown and may be probably low, IgG levels should be monitored over the long term [23].

B-cell depletion may impair vaccine responses. Recently, Nazi et al. [25] investigated the effects of rituximab on antibody and cellular responses to Streptococcus pneumoniae polysaccharide and Haemophilus influenzae type b (Hib) vaccines in ITP patients. Response to these 2 vaccines was lower for patients previously treated by rituximab as compared with ITP controls. Antibody responses were impaired for at least 6 months after rituximab. Cellular immunity was reduced in parallel with depleted B-cell pools. In view of these results, anti-pneumococcal, -haemophilus, and -meningococcus-C vaccinations appear crucial for patients without splenectomy who receive rituximab. Patients should receive these vaccinations ideally at least 2 weeks before the first rituximab infusion.
Optimal use of rituximab?

Historically, rituximab was tested in ITP with the “standard” regimen used to treat lymphoma (i.e., four weekly infusions of 375 mg/m²). Several groups proposed a fixed dose of 4 weekly injections of 100 mg [26–28]. With this regimen, a prospective open study of 48 adults found an overall response and time to response similar to that with the standard regimen [28]. However, the duration of the response appeared shorter. A retrospective study compared the standard dose and the regimen of 2 fixed doses of 1000 mg on days 1 and 15 as used in other autoimmune diseases such as lupus, anti-neutrophil cytoplasmic antibody-related necrotizing vasculitis and rheumatoid arthritis [29]. The overall response rate and tolerance were similar with both regimens, so this schema may replace the standard regimen as a more convenient schedule for patients.

For relapse after an initial response, Hasan et al. [30] tested the value of retreatment with rituximab with the same schedule as the initial course or a doubling dose with and without cyclophosphamide. The authors found a similar response rate for 75% of patients with the tested options. Repeated courses of rituximab for other autoimmune diseases such as RA may be well tolerated, but we do not have any data concerning the tolerance of such strategy in ITP. This therapeutic strategy should be avoided and reserved for selected patients with disease refractory to other therapies.

The optimal time to administer rituximab is a difficult and unresolved question [31]. After failure of first-line treatment (mainly steroids and intravenous immunoglobulins), the respective place of the available ITP second-line treatments, particularly splenectomy, rituximab and thrombopoietin mimes, is debated. We lack randomized trials to help the decision. Ideally, we should have controlled study “face-to-face” comparing these options, such studies are unlikely to be conducted in the near future. A international consensus of the opinion of a panel of experts gave a recommendation grade of B for treatment with rituximab, without distinguishing between patients with and without splenectomy [32]. The American Society of Hematology (ASH) recently published guidelines [33] that gave a weak recommendation for rituximab (Level C). Experts from the ASH based their recommendations on the relatively poor durable response rate and the relatively high frequency of adverse effects. They recommended splenectomy as second-line treatment for adults showing failure of steroids. However, most physicians are so far reluctant to recommend splenectomy, and patients are often hesitant to accept this invasive and irreversible treatment with its possible postoperative complications and definite risk of overwhelming sepsis. Because each approach has advantages and disadvantages, treatment needs to be individualized, and patient participation in decision making is paramount, as indicated by the international consensus and the ASH guideline.

The main limitation of rituximab in ITP is the high rate of relapse. As we previously indicated, the mechanisms of relapse are not well understood and probably complex and non-univocal. Ideally, we should have robust clinical predictive factors of response, but the literature gives conflicting results, and such factors for individuals are lacking. Several studies focused on genetic polymorphisms (Fcy and KIR receptors), and studies of B- and T-cell compartments are in progress and may help in better understanding response failure or relapse in some patients. However, we doubt that the studies will allow for guiding individual decisions for treatment.

An important objective for the future could be to test associations to decrease the risk of relapse. Two randomized studies of patients with newly diagnosed ITP compared rituximab as first-line therapy to dexamethasone alone and dexamethasone plus 4 weekly injections of rituximab at a “standard dose” of 375 mg/m² [34,35]. The 2 studies report the superiority of rituximab with dexamethasone over dexamethasone alone, with better initial and long-term responses. However, a comparison of rituximab alone and rituximab with dexamethasone is not available. Also, there is still uncertainty regarding the relative efficacy of high dexamethasone and standard dose prednisolone as initial treatment for ITP. Accordingly, the use of this combination upfront should be pondered carefully.

Bussel’s group retrospectively assessed the response to rituximab combined with 3 cycles of 3 to 4 days of high-dose dexamethasone (28 mg/m², maximal dose 40 mg) at 2-week intervals in 67 adults and pediatric patients [36]. The authors found an overall initial response of 75%. When this treatment was administered to a subgroup of patients with ITP duration < 2 years, the estimated long-term response rate was 59%. From historical data, the long-term response could be comparable to that obtained with splenectomy. The treatment was associated with risk of hypogammaglobulinemia. Other interesting results were recently reported by a Chinese group, who compared low-dose rituximab alone (4 weekly injections of 100 mg) with or without 2 weeks of recombinant thrombopoetin (rhTPO) in 114 patients [37]. Complete initial responses were more frequent in the RTX-rhTPO group (43% vs 29%), and significantly longer time to relapse in the combination-therapy group.

New anti CD20 antibodys

New-generation CD20-targeted therapy is in development, mainly for B-cell malignancies [38]. Some are potent CDC inducers; others strongly promote cell death by apoptosis. Their activities have not been tested in autoimmune disease and whether high CDC activity could be useful for ITP remains to be determined. Also, high CDC activity may be associated with increased risk of infection.

Among new-generation CD20-targeted therapy, only veltuzumab has been tested for ITP [39]. Veltuzumab is a second-generation
humanized MoAb. Low-dose veltuzumab was evaluated in 41 ITP patients. The patients received different doses initially by intravenous infusion (IV) or later by subcutaneous (SC) injections. Tolerance was excellent, with only one Grade 3 infusion reaction and no other safety issues. An objective response was observed in 55% of patients with IV or SC treatment. Responders who received veltuzumab during persistent ITP showed a longer median time to relapse (14.4 months) than those with chronic ITP (5.8 months). Three patients maintained a response for up to 4.3 years. These promising results appear similar to those obtained with rituximab.

**Other options to modulate B-cell compartment?**

A better knowledge of the cooperation between B- and T-cell compartments allowed for developing new therapeutic strategies in autoimmune diseases, particularly systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). CD40-ligand (CD154) is expressed on activated CD4+ T lymphocytes and is essential for the T-cell–dependent activation of B lymphocytes. Blockade of the CD40/CD154 signal has been tested with success by Kawana et al. [40], who reported increased platelet count in 3 patients receiving a humanized MoAb directed against CD154. However, previous studies of patients with SLE reported CD40 blockade associated with an high risk of thrombosis, which explains why the development of this strategy has been stopped in SLE [41].

T-cell activation requires several signals, particularly an interaction between the B7 family members and their ligand CD28, expressed on T cells. CTLA-4 is a second receptor for B7 family that is expressed on the membrane of activated T cells; it delivers a signal that inhibits T-cell activation. CTLA4Ig (abatacept) is a soluble version of CTLA-4 that down regulates subsequent immune-effector mechanisms. In vitro studies suggested that it could be an attractive strategy for ITP [42,43]. However, to our knowledge, it has been never tested with ITP.

CD22 is a cell-surface protein that is expressed by most mature B-cell lineages. It contributes to sensitive control of the B-cell response to antigens. Epratuzumab is a novel, fully human CD22-targeting MoAb that induces B-cell apoptosis. It is currently under investigation as treatment for SLE, with preliminary encouraging results [44].

Among the new strategies focused on B-cell compartment modulation, inhibition of B-cell activating factor (BAFF) is the most advanced. Belimumab is a human MoAb that inhibits BAFF. It has been demonstrated as effective treatment in SLE and was licensed in Europe and in the United States for this indication. It appears well tolerated and effective to treat rheumatologic SLE manifestations. Its activity against hematologic manifestations of SLE and particularly autoimmune cytopenia has not been specifically evaluated and appears modest [45]. Bortezomib is a proteasome inhibitor that has shown high activity in B cell malignancies such as multiple myeloma, mantle cell lymphoma, and Waldenstrom’s macroglobulinemia. It is an attractive agent, either alone or in combination, to treat refractory cases in which long-lived plasma cells are the source of pathogenic antibodies.

**Conclusions**

More than 10 years after the first reports of promising data suggesting that anti-CD20 MoAbs could be effective treatment for ITP, results of only a few randomized studies have been published, and evidence-based medical proof of the efficacy of rituximab in this setting are lacking. However, we have a clear view of its efficacy, with an overall response in about 60% of patients as reported by several prospective studies. The report of fatal opportunistic infections was initially a matter of concern, but to date, reassuring data have been reported and rituximab appears well tolerated with an acceptable risk of infection. In view of these data, rituximab may always be a valid option for ITP.

However, relapses are frequent, and the long-term response appears modest. Therefore, strategies to ameliorate the long-term efficacy of the treatment must be developed. One option could be to better select patients. Unfortunately, robust predictive factors of long-term response are lacking, and we doubt that a useful biological marker able to predict a sustained response can be found in the near future. Among the various tested clinical markers, one that appears as the best marker is duration of ITP, with better chance of sustained response when rituximab is administered to patients with persistent ITP. To use rituximab sooner as a second-line treatment is probably a good strategy. Another option could be to repeat several courses of rituximab with a maintenance treatment as proposed in other autoimmune diseases, but the tolerance of such strategy and notably the risk of infection and hypogammaglobulinemia should be evaluated. To combine rituximab with another treatment such as dexamethasone or thrombopoietin mimetics as suggested by pilot studies or with other immunosuppressive drugs specifically directed against the T-cell compartment with the hope of a synergistic effect could be another option, but encouraging preliminary results should be confirmed and the tolerance and notably the risk of infection should be examined carefully. Finally, the interest of a new generation of B-cell targeted treatment, including new-generation anti-CD20 MoAbs, may be tested.

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