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Translational Mini-Review Series on B Cell-Directed Therapies: The pathogenic role of B cells in autoantibody-associated autoimmune diseases – lessons from B cell-depletion therapy

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Summary

B cell depletion therapy with rituximab (BCDT) is a licensed treatment for rheumatoid arthritis and has shown promising results in the treatment of severe, refractory patients with other autoantibody-associated autoimmune diseases (AAID). The exact role that B cells play in the pathogenesis of AAID and consequently the mechanisms by which BCDT is effective are not known. The two more widely discussed hypotheses are that BCDT is effective because it removes the precursors of plasma cells producing pathogenic autoantibody species, or because it depletes a critical mass of autoreactive B cell clones that present antigen to pathogenic autoreactive T cells. This review will focus on the effects of BCDT and whether the response of patients with AAID to BCDT could be due ultimately to its effects on autoantibodies. A better knowledge of the main role that B cells play in the pathogenesis of the different diseases and a better understanding of the most likely mechanism of relapse following an earlier response to BCDT would help to guide further developments of B cell targeting therapies and potentially increase the chance of designing a protocol that could induce a long-term remission.

Keywords: autoantibodies, autoimmune diseases, B cell, B cell depletion, rituximab

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Introduction

A primary role for B cells in the pathogenesis of several autoimmune diseases has long been suggested by the presence of autoantibodies in the patients' sera. Direct pathogenic mechanisms based on autoantibodies were among the earliest proposed to explain disease manifestations in autoantibody-associated autoimmune diseases (AAID) and are accepted widely in many situations, including autoimmune peripheral cytopenias, thyroid dysfunction in Graves'disease and pemphigus [1–3]. However, in several AAID, the lack of a close correlation between autoantibody levels detectable in the peripheral blood and clinical manifestations of disease and the absence of a clear pathogenic mechanism as a direct consequence of the known autoantibodies has contributed in the past to the loss of interest.

B cells are the precursors of antibody-producing cells (plasma cells) [4]. In the process of undergoing activation and maturation into memory B cells and plasma cells they are very efficient antigen-presenting cells (APCs) to T cells of soluble antigens that are bound specifically by the B cell antigen receptor (surface immunoglobulin) and secrete a variety of soluble factors that can influence the function of other cells, such as follicular dendritic cells [4–9]. B cells also seem to play an important role in promoting normal follicular dendritic cell formation and normal lymphoid architecture in the development of secondary lymphoid tissues [10–12].

B cell depletion therapy based on rituximab (BCDT) was first used in the treatment of AAID with the objective of depleting pathogenic B cell clones and decreasing production of pathogenic species of autoantibodies [13–17]. In the

last 10 years, promising results in improving patients with severe, refractory forms of different AAIDs has contributed to a renewed interest on B cells and their exact role or roles in the pathogenesis of these diseases. Is BCDT effective because it removes the precursors of plasma cells producing pathogenic autoantibody species, because it depletes a critical mass of autoreactive B cell clones that present antigen to autoreactive T cells, because it eliminates an important cellular source of cytokines and chemokines, or because it disrupts the architecture and function of secondary lymphoid tissue?

To try to understand the effects of B cell targeting therapies, it is important to consider both normal B cell and plasma cell homeostasis and how they possibly change in AAID, and what is known about the pharmacokinetics and pharmacodynamics of the therapeutic agents used. Good recent reviews on B and plasma cell biology can be found in the literature [18,19]. It is likely that B cells play different pathogenic roles in different AAID, and even in different manifestations of the same disease. It is also possible that what we classify as a particular disease is a syndrome with different primary pathogenic processes. Furthermore, during a disease natural history secondary pathogenic mechanisms may develop and become established and B cells may play different roles at different disease stages. Also, when discussing the pathogenic role of B cells in AAID in the context of BCDT it is useful to think of how the autoreactive B cell clones have escaped normal tolerance mechanisms. Was it because T cell tolerance had been lost previously? Were there abnormalities in the B cell tolerance mechanisms that predisposed to the emergence of autoreactive B cell clones? Are specific characteristics of their B cell receptors or secreted autoantibody species responsible for their perpetuation by generating signals that allow these cells to escape normal tolerance mechanisms? These questions are particularly important when considering why patients relapse following an early response to BCDT. The degree of response observed in many patients treated with BCDT indicates that the disease, at least in these patients, is B cell-dependent. This review will focus on the effects of BCDT and whether the response of patients with AAID to BCDT could be due ultimately to its effects on autoantibodies.

What do we know about the effects on B cell lineage cells of BCDT?

Rituximab is a mouse–human chimaeric monoclonal antibody IgG1k directed against CD20 [20]. The surface antigen CD20 is expressed almost exclusively by B cells at different stages of differentiation, but it is not expressed by stem cells or the earlier precursors, known as pro-B cells, or by terminally differentiated plasma cells [21]. This allows for a period of depletion induced by rituximab to be followed by repopulation with naive B cells starting usually 6–9 months after and for sustained production of immunoglobulin by surviving plasma cells during the period of depletion [22].

Rituximab is very effective at depleting normal and malignant B cells. With the doses used currently, depletion of normal B cells is major but not complete. The mechanisms of depletion include antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity and apoptosis [20,23]. The relative importance of each of the mechanisms is still not completely clear and may vary depending on whether normal or tumour cells are involved, their location and possibly intrinsic B cell factors, including activation and differentiation status. *In vitro*, the sensitivity of different lymphoma cell lines to the different killing mechanisms can vary [24]. Studies in CD20-transgenic mice showed that in the spleen, marginal zone B cells and particularly germinal centre B cells were more resistant to rituximab-induced killing than follicular B cells [25]. Killing of peritoneal B cells correlated with their recirculation patterns, suggesting that these cells were killed mainly in peripheral blood, where they were susceptible to effector mechanisms [25]. However, a different mouse model using a mouse anti-mouse CD20 antibody showed efficient depletion of all B cell populations [26]. It is unknown if any of these models reflects the effects of rituximab in humans.

Depletion in peripheral blood, bone marrow, lymph nodes and synovial tissue varies [20,27–31]. In primate and early lymphoma studies there was a clear dose effect, with smaller doses inducing less depletion and a need for an increasing dose to deplete peripheral blood, bone marrow and solid lymphoid tissues in this order [20,27]. However, depletion can vary between individuals treated with the same dose and it has been noted that patients who receive lower doses (possibly above a certain threshold) can show unexpectedly high or prolonged depletion [20,29,32–34]. Interestingly, primate studies showed that, within each individual, the degree of depletion in different tissues seemed to be similar [20]. When smaller doses of rituximab are used, genotypic differences that translate into different affinities of FcgammaRcIIIa to IgG and potentially differences in ADCC efficacy seem to influence the degree of depletion [32]. Whether this is true when larger doses are used is not clear. Certain AAID such as systemic lupus erythematosus (SLE) and idiopathic inflammatory myopathy seem to be associated with an increased incidence of less pronounced or shorter depletion in the peripheral blood [33–36]. Both pharmacokinetics and pharmacodynamic differences probably contribute to these heterogeneities.

Depletion in the peripheral blood occurs usually very quickly [27,35,37,38]. A few studies suggest that the majority of B cell depletion in solid lymphoid tissues and other solid tissues occurs within the first few weeks following rituximab infusion [20,30]. Nevertheless, rituximab can be detected in circulation for months following its administration, and it is not clear whether the initial depletion is followed by a period of continued depletion [39]. A bone marrow study suggested that there is continuing depletion of B cell precursors probably until rituximab is cleared [28]. It is also not known what

effects rituximab has (if any) when it is bound to cells but the cells are not depleted; in particular, whether it renders them non-functional. There is little good information on the effect of BCDT on *in vivo* humoral responsiveness. The published immunization studies are heterogeneous and most include patients at different time-points following treatment with BCDT. Several of these studies showed decreased antibody responses following immunization to neo- or recall antigens [33,40,41]. In one study using influenza immunization, response to one of the specific antigens was decreased, but not to two others [42].

Peripheral blood B cell reconstitution after rituximab therapy occurs mainly with naive B cells and seems to follow ontogeny similarly to B cell reconstitution after bone marrow transplantation with an increased frequency of detectable transitional B cells and CD5 positive B cells [43–45]. It is possible that the time of B cell return to the peripheral blood depends mainly on clearance of rituximab and the regeneration capacity of the individual bone marrow [28]. The rate of B cell repopulation of the peripheral blood varies between patients [35,43,46]. Reconstitution of peripheral blood memory B cells can be delayed for several years [44,47]. At the start of repopulation an increased number of circulating plasmablasts/plasma cells can be seen in several patients [44]. The frequency and pattern of mutations in these cells seem to suggest that early B cell activation and differentiation when repopulation starts favours B cell clones, for which specific T cell help is available [48].

How do patients with AAID respond to BCDT: an autoantibody-producing role for B cells

In most AAID the main question has been centred on whether BCDT is effective because it ultimately decreases the levels of pathogenic autoantibodies or because it decreases the number of autoreactive B cells that can present autoantigen(s) to directly pathogenic autoreactive T cells. It is also important to remember that autoantibodies by forming immune complexes can increase antigen uptake and potentially antigen presentation by other antigen-presenting cells. One should not expect B cells to play the same role in different diseases, but it is interesting to note that there are many similarities in the way that patients with different AAID respond to BCDT.

Not all patients with a particular disease respond to BCDT. Non-response can be associated sometimes with evidence of insufficient depletion, but not in the majority of cases [29,34,35,43,49–51]. In RA, patients who are seronegative both for rheumatoid factor by agglutination assays and to immunoglobulin (Ig)G antibodies to citrullinated peptides by enzyme-linked immunosorbent assay (ELISA) are much less likely to respond to BCDT than patients who are seropositive and if they respond they usually respond less well [52,53]. It is not known whether seronegative rheumatoid arthritis patients who respond to BCDT have detectable autoantibodies by more sensitive methods.

Among the patients who respond, the degree of response varies with several patients in different AAID achieving clinical remission [35,37,53–55]. Interestingly, the kinetics of response, in particular the rate at which patients respond to BCDT, can vary; patients with rheumatoid arthritis usually take 2–3 months to respond to BCDT, while patients with autoimmune cytopenias or central nervous system (CNS) lupus can respond very quickly [56–58]. However, it is sometimes difficult to ascertain the kinetics of clinical response to B cell depletion *per se*, because many patients have been treated with rituximab in combination with other therapies that may contribute to a quicker response. Presumably a response within days would suggest a role dependent on the presence of B cells *per se* and would favour a role for B cells in antigen-presentation to autoreactive T cells or activation of other immune cells. A more slow and progressive response taking a few weeks to a few months would suggest a role mediated by autoantibodies with progressive decrease in autoantibody levels as a consequence of non-renewal of plasma cells. However, as far as the authors are aware, it is not known for how long activated effector T cells can survive and continue their activity and, on the other hand, if an autoantibody is produced only by short-lived plasma cells and cleared at a high rate, a major decrease may occur quickly. In a study on Graves' disease, a decrease in thyroid stimulating antibodies was seen at 21 days [59]. In SLE, antibodies to dsDNA can be seen to decrease within 1 month [35]. Also, in situations such as autoimmune cytopenias, direct competition from anti-CD20-coated B cells with autoantibody-coated red blood cells or platelets for effector mechanisms could contribute to an earlier increase in cell counts.

In many of the published studies, clinical improvement following BCDT is associated with a decrease in autoantibody serum levels. This includes patients with RA, SLE, anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis, pemphigus and others [37,38,51,55,60–64]. However, in most studies autoantibodies decrease both in patients who respond as well as in patients who do not respond to BCDT, and the levels do not necessarily correlate with the severity of manifestations. Frequently, the therapeutic effects of B cell depletion are disproportionately larger than the effects such treatment has on circulating autoantibody titres. Also, patients can achieve sustained remission despite persistence of detectable autoantibodies in the sera. Nevertheless, in a few studies, a higher decrease in autoantibody levels was reported in patients who responded when compared to patients who did not respond to BCDT [60,65]. A higher decrease in autoantibodies or lower baseline levels has also been associated with a more prolonged response [66,67]. In RA, synovial biopsy studies have shown that the clinical response was not associated with the degree of B cell depletion in the synovia *per se*, but with the decrease in synovial plasma cell numbers and immunoglobulin produc-

tion [30,31]. It is important to note that in several publications it is not so easy to interpret the antibody data. In many of them serial samples were not examined, and only values at baseline and 6 months are given. At 6 months, autoantibodies may already be increasing and an earlier decrease in response to therapy may be missed. Also, ideally, to avoid interassay variability, serial samples should be frozen and then thawed and tested simultaneously.

Changes in autoantibody serum levels after treatment with BCDT have been studied in detail in some cohorts of patients with RA, SLE, ANCA-associated vasculitis and pemphigus. Effects on different autoantibody species have been compared and also between autoantibodies, total immunoglobulin levels and anti-microbial antibody levels. Decreases in some autoantibody levels were found to be higher than in anti-microbial or natural antibodies and for IgG and IgA autoantibodies higher than decreases in total levels of the respective immunoglobulin classes [38,45,51,60,64,66]. In SLE differences between the different autoantibodies have been described. Antibodies to dsDNA, to microsomes and to C1q were found to decrease, while autoantibodies to extractable nuclear antigens (ENA) were not [45,60]. SLE patients with antiENA antibodies before treatment were more likely to relapse early [47,68]. In an interesting study in Grave's disease, decreases in total levels of anti-thyrotropin receptor antibody levels were similar between patients treated with rituximab and methimazole when compared with patients treated with methimazole only, but at the stimulatory capacity of the TSH receptor autoantibodies, antibodies decreased only in patients treated with rituximab, suggesting that the susceptibility of different species of antibodies not differentiated in usual assays to rituximab may be different [59]. Interestingly, even in patients who achieve undetectable autoantibody levels and clinical remission, B cell repopulation is often followed by a detectable increase in autoantibody levels and clinical relapse [54].

In most AAID reported, patients relapse frequently following a response to an earlier course of therapy but in a few, such as thrombotic thrombocytopenic purpura (TTP) or a subset of idiopathic thrombocytopenic purpura, individuals can show sustained remission for several years after one course of BCDT [69,70]. This may be more frequent in situations that are more often monophasic, such as TTP. However, even in diseases where relapse is expected some patients can remain well for a prolonged period of time [53,54,68]. Clinical relapse has only rarely been reported to occur before B cells start repopulating the peripheral blood [54,71]. Interestingly, clinical relapse in AAID following an initial response to an earlier course of BCDT can occur either at the time of B cell return to the peripheral blood or at a variable time after [34,53,54]. Regardless of its relation to B cell repopulation, clinical relapse is usually preceded or associated with a rise in autoantibody titres [37,55,60]. In ANCA-associated vasculitis, pre-emptive retreatment at the time of rise in ANCA titres was associated with sustained clinical remission [37].

If the primary role of B cells in the pathogenesis of AAID is differentiation into plasma cells producing pathogenic species of autoantibodies, one would expect a close relationship between levels of autoantibodies measured and clinical disease. However, this is complicated by the fact that, as in a normal humoral response to a microbial agent, different species of autoantibodies are expected to be produced with potentially different pathogenic capacity. Differences in isotype, subclass and specificity (including fine specificity) are expected to underlie possible differences in pathogenicity. In many diseases, we do not know whether the autoantibodies detected in the peripheral blood are the more important species from the pathogenic viewpoint. Another factor that needs to be taken into consideration when looking at the effects of BCDT on antibody levels is that there are probably differences in the kinetics of production of different autoantibody species. Some may be produced mainly by short-lived plasma cells, while others may be produced by long-lived plasma cells or by a mixture of the two populations. To what extent formation of new plasma cells in an autoimmune humoral response depends upon the recruitment of naive or memory B cells may also differ. Autoantibodies produced at different stages in the natural history of the disease may have different roles and different production kinetics. Also, it is not known for how long plasma cells found in inflammatory sites live and to what extent they contribute to serum levels of autoantibodies or to the pathogenic autoantibody population. All these factors will influence the effects of BCDT on the serum levels of different autoantibodies.

Furthermore, there may be a mass effect needed to cause disease, or at least clinically detectable disease. It is possible that depending on the exact role played by the autoantibodies, this mass effect and/or threshold level may be different in different diseases and even in the same disease in different individuals. In many of the patients with AAID treated with BCDT who respond well to treatment, autoantibody levels decrease but are still detectable. This is observed both in diseases such as RA, where a direct pathogenic role for autoantibodies is more controversial, as in diseases such as pemphigus, where autoantibodies are known to be a direct cause of clinical manifestations. Interestingly, certain types of autoantibodies such as ANCA seem to be more prone to become undetectable than others such as rheumatoid factor or antibodies against citrullinated peptides.

Conclusion

The exact role that B cells play in the pathogenesis of AAID is not known. The two more widely discussed hypotheses are that B cells may drive the disease either through production of pathogenic autoantibodies, or by constituting a critical mass of antigen-presenting cells to pathogenic autoreactive

T cells. The extent of the response in many patients with different AAID shows that the disease, at least in these patients, is B cell-dependent. Importantly, following BCDT, individual patients with different AAID can show a sustained response for a variable period of time after the return of B cells to the peripheral blood. A better knowledge of the main role that B cells play in the pathogenesis of the different diseases and a better understanding of the most likely mechanism of relapse following an earlier response to BCDT would help to guide further developments of B cell targeting therapies and potentially increase the chance of designing a protocol that could induce response in an increased number of patients, and particularly a longer-term remission. Ideally, this should be achievable without a significant increase in the risk of side effects and without the need to keep patients B cell-depleted by the use of maintenance regimens.

Disclosure

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