Prospects for B-cell-targeted therapy in autoimmune disease

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Reasons for targeting B cells in autoimmune disease date back to the discovery of autoantibodies over 50 yr ago [1]. The idea became of practical interest when anti-B cell monoclonal antibodies were developed in the early 1990s [2, 3]. Fortuitously, at about the same time it became clear that B cells are not simply the subordinate foot soldiers of an immune response but may be as important as T cells in its genesis and regulation. Moreover, it seemed possible that B cells might actually be the driving force behind human autoimmunity. The concept of therapeutic B-lymphocyte depletion (BLyD) emerged subsequently in the pages of this journal [4]. Concept was transformed into reality as soon as B cells return. Thus, chronic depletion would seem to be required. Practically, this would make BLyD a not very attractive proposition in the long-term.

The development of B-lymphocyte depletion

BLyD therapy as designed for RA is quite different in approach from that for agents such as methotrexate or etanercept, which suppress inflammation only for as long as they are administered. BLyD was designed to induce sustained remission [4], and is perhaps closer to a traditional 500 mg course of intramuscular gold or high-dose cytotoxic therapy with stem cell rescue. This concept of ‘remission induction’ is familiar in the context of intravenous cyclophosphamide in systemic lupus erythematosus (SLE) or Wegener’s granulomatosis. It has important implications for the use of the agent, and in particular dosage and timing.

Trials of BLyD in RA have shown that it can induce major clinical responses in a significant proportion of patients [5–10]. A short course can reduce a C-reactive protein level of 90 to 1.5 and not infrequently leaves the patient with minimal joint swelling or tenderness. Initial studies in other autoantibody-associated diseases, such as immune thrombocytopenia and SLE, have suggested similar efficacy [11–14]. Severe cytopenias and progressive glomerular disease have in some cases resolved at the timing.

Clinical pathology is antibody-mediated

This is the most obvious reason, as removing B cells should remove the precursors of pathogenic antibody-producing plasma cells and therefore ameliorate disease. The first problem with this idea is that BLyD using anti-CD20 does not remove these plasma cells. On the positive side, although some lymphoma studies showed that anti-CD20 therapy was not associated with a major fall in immunoglobulin levels, in patients with autoimmune disease, our and other studies have found that autoantibody levels often do fall significantly. The conclusion is therefore that some autoantibody-producing plasma cells are relatively short-lived and BLyD prevents replenishment from B cells [20]. The positive practical implications are therefore obvious but we are left with an even more urgent need to understand what controls the production of the pathogenic antibodies. The more serious problem with this rationale, therefore, is that if antibody is simply an effector mechanism for tissue damage, with its production controlled by T cells, then autoantibodies will return as soon as B cells return. Thus, chronic depletion would seem to be required. Practically, this would make BLyD a not very attractive proposition in the long-term.
Removal of B cells might starve T cells of autoantigen-presenting cells

B cells have a powerful and highly selective antigen-presenting capacity. However, other powerful antigen-presenting cells, such as dendritic cells, are readily available in most tissues. Even if B cells were to have a dominant role in antigen presentation, this suggestion suffers from the same problem as the first suggestion. Disease would be expected to reappear as soon as B cells return, so chronic depletion will be necessary. In theory, removal of the antigen-presenting capacity of B cells might induce putative autoreactive T cells to die off and allow ‘restoration of tolerance’. Unfortunately, this is difficult to verify experimentally because of the paucity of reproducible tests for autoreactive T cells [21, 22].

Removal of self-perpetuating B cells responsible for the vicious cycle of antibody production

We embarked on BLyD treatment in RA on the basis of a third rationale, which suggested that response might be long-lasting [23]. The concept is that autoantibodies and their parent B cells are involved in a self-perpetuating vicious cycle which, if broken by removal of specific pathogenic B-cell clones, may collapse permanently [4, 23]. Although the idea of a vicious cycle as the basis of autoimmunity is not new, it has received relatively little attention. We have therefore included a brief outline of the basic concepts involved.

The vicious cycle of antibody production

All antibody production is a vicious cycle. B lymphocytes proliferate because they receive growth signals derived from the interaction of their own antibodies with antigen. One of these signals comes via T cells, to which both the B cell and other cells, such as dendritic cells, present antigen that has been picked up by cell surface antibody (attached to an immunoglobulin receptor for non-B cells). The other signal comes from an interaction between an immune complex, carrying the complement fragment C3d, and the B cell, via both B-cell surface antibody and complement receptor 2 [23]. Encounter of antibody expressed on the B cell with antigen therefore stimulates antibody production. It is designed to be an explosive chain reaction which only stops when antigen has been removed from the body. In autoimmunity the antigen cannot be removed [23].

Thus, the idea of autoimmunity as a vicious cycle of B-cell proliferation and antibody production is no more than standard dogma. The critical question is how the cycle can engage for an autoreactive cell when the system is supposed to be designed so that damaging autoreactivity cannot occur.

Prior to about 1995, it was widely believed that as T cells drive (most) antibody production, autoantibody production must arise because autoreactive B cells are under the control of specific autoreactive helper T cells. (Either Th1 or Th2 cells will do, the main difference being that Th2 cells support IgE production.) However, for the major rheumatological autoantigens, including IgG Fc, DNA, Ro/La and topoisomerase-I, T-cell responses are hard to detect. Rheumatoid factor (RF) production is not, as far as is known, supported by anti-IgG T cells but by T cells recognizing foreign antigens [22]. T-cell responses to La do not appear to determine anti-La production [24] and twins discordant for scleroderma and anti-topoisomerase antibodies had the same anti-topoisomerase T-cell responses [25]. In myasthenia, T-cell autoreactivity may occur in the thymus but there appear to be no autoreactive T cells capable of getting into the muscle [26]. There is also the unanswered question: how could functionally significant numbers of autoreactive T cells arise in the first place? No convincing arguments have been given.

Aberrant cycle engagement: autoantibodies as immunomodulators

Such immunomodulatory interactions could only work for a few autoantigens, which would explain why most autoimmunity is directed at no more than about 40 of 40 000 or so potential self antigens. The most obvious candidate for this is the interaction between IgG RF and itself, in which the rules of antibody–antigen interaction break down. The precise signalling aberrations that arise are complex, but effectively the tail and the dog are wagging each other. Once an ‘unusual’ RF has been generated (e.g. by class switching or hypermutation), normal safeguards controlling its production and the survival of parent RF producing B cells are able to be bypassed [23]. Anti-C1q antibodies in lupus can similarly disturb immune complex-based signalling by activating complement in an inappropriate way [27]. The overproduction of antibody product from daughter plasma cells of these self-perpetuating clones is ultimately responsible for the pathology. Another interesting example of aberrant immune complex-based signalling is the binding of DNA-containing immune complexes to Toll-like receptor 9 on RF-specific B cells, providing an explanation for RF production as a by-product of anti-DNA antibody production [28]. Anti-acetylcholine receptor antibodies may modulate the thymic environment by binding to myoid cells [26]. Mechanisms for other autoantibodies have been suggested but are as yet more difficult to define [23].

The crucial implication of such immunomodulatory signals is that they provide a means whereby autoreactive B cells can engage a vicious cycle of expansion in the context of completely normal T cell responses. Abnormal T-cell responses may help initiate the autoreactive response and still contribute to the action of the immunomodulatory antibodies, but as yet the evidence of their being the major driving force behind the chronicity of the response is sparse.

What happens to autoimmune responses after BLyD?

So what do clinical and immunological changes during BLyD tell us about the forces underlying autoantibody production? Is there evidence of an aberrant cycle that might be breakable, or is BLyD therapy of limited potential because of some unknown underlying T-cell-driven response? The essential practical question is why patients relapse, in half of cases only after up to 4 yr of more or less complete remission.
Before addressing this question it is necessary to take a step back and reconsider the relationship between antibody and clinical disease in RA, because this has in the past been less obvious than in most other autoantibody-associated disorders.

**Relationship between antibodies and clinical response**

Initial scepticism about B-cell-targeted therapy in RA may have related to the fact that it invokes not one but two paradigm shifts. Perhaps surprisingly, the idea that B cells might play a role in immunoregulatory drive was in some ways the less controversial. The importance of both afferent feedback signals from antibody (feeding a vicious cycle) and of B cells in directing T-cell responses had been emphasized by Carson for some years [29], and more recently by Shlomchik [30], even if the concept of immunomodulatory autoantibody had not been enunciated as such. The more radical shift appeared to be the suggestion, based on our studies of immunoglobulin Fc receptors, that inflammation in RA was antibody-mediated after all [31–33].

As indicated above, it is theoretically possible for BLyD to benefit a condition with a T-cell effector response by removal of antigen-presenting cells. It was therefore important to see whether improvement with BLyD correlated with the absence of B cells themselves (in the role of antigen presenters) or the relative absence of antibody. Kinetic studies are still very much in progress. We addressed this question in our studies of BLyD in 22 patients with RA [20]. Two important observations emerged.

First, clinical improvement followed the decline in autoantibody levels more closely than the fall in B-cell numbers; on many occasions the correlation with autoantibody levels was very close [20]. B cells disappear within days but clinical improvement and autoantibody decline may progress over as long as 9 months. Substantial clinical improvement was seen immediately in patients treated with protocols involving high doses of steroid and cyclophosphamide, but this is less evident with lower levels of steroid and no cyclophosphamide.

Secondly, B-cell return was only associated with clinical relapse in half of the cases. The other half relapsed anything up to 2yr later. Whether or not relapse was temporally associated with B-cell return, it was invariably associated with a rise in autoantibodies to levels comparable with those present before treatment [20]. As previously observed following high-dose chemotherapy with bone marrow rescue, IgM RF levels appear to be the best predictors of clinical relapse.

The same patterns of response have recently been confirmed in a further 15 patients with RA. Another key observation is that, of seven patients known to the authors with seronegative arthritis (RF-negative but often positive for another autoantibody) considered clinically to be RA who have received BLyD, none has shown a clinical response [6, 7, 9]. Moreover, evidence to date is that autoantibody-negative disorders, such as psoriasis and Crohn's disease, show no response [10, 34]. It is looking increasingly as if B cells are only important in a human inflammatory disease if the clinical condition is associated with autoantibodies. The most obvious conclusion is that is because the antibodies are pathogenic.

In summary, although conclusive proof of pathogenicity of autoantibodies in RA may always be elusive, the evidence from BLyD strongly supports a pathogenic role for a subset of autoantibodies, and very possibly the IgG or IgA RF known to form the small immune complexes predicted to be the cause of TNF-α production [31]. (IgM RF is likely to have a secondary, if more universal role.) This conclusion has the attraction that it seems anomalous for autoantibodies to be considered pathogenic in almost all diseases in which they occur, except RA! Moreover, acceptance of a role for pathogenic autoantibodies in RA makes the development of B-cell-targeted therapy much easier, both because there is a practical rationale and because antibody levels provide a means of monitoring pharmacodynamics.

**Reasons for relapse**

If, as suggested, antibodies are pathogenic in RA, as assumed in other autoimmune disorders treated, then the reason for clinical relapse following BLyD in all these conditions would appear to be autoantibody return. It then becomes vital to discover why the autoantibodies return when they do. In fact there are two questions: why do autoantibodies return at the time of B-cell return in some patients and why is return delayed for many months in others?

On the basis of the vicious cycle mechanism proposed for autoantibody production, autoantibodies might return for one of two reasons [20]. First, B-cell clones committed to production of autoantibodies capable of self-perpetuation via afferent signals may not have been cleared and may re-expand. Secondly, these B-cell clones may have been deleted but their daughter plasma cells may be able to produce antibody capable, via the same afferent survival signals, of 'educating' new B-cell clones to take part in a self-perpetuating cycle.

The reappearance of autoantibodies in some patients at the time of B-cell return may not help distinguish these possibilities. There is something odd about B-cell return following anti-CD20 treatment, in that it does not occur for about 8 months, much longer than the drug's half-life. This makes interpretation complex. What the pharmacodynamics seem to be telling us is that the reappearance of sufficient autoantibody to induce clinical relapse requires the generation of new B cells. What it does not tell us is whether the expansion of these new B cells requires the survival of the original B-cell clones. The problem is that the vicious cycle hypothesis predicts not only that pathogenic autoantibody-producing cells will expand, but so will irrelevant clones producing antibodies of the same specificity, whether or not they are in themselves pathogenic—what might be called the 'karaoke' effect, with irrelevant antibodies 'singing along' with the pathogenic ones. The prediction is that a good proportion of autoantibodies will be epiphenomenal and pathogenic species may be hard to identify. This fits with the fact that autoantibodies often occur in the absence of clinical disease.

Despite these difficulties in interpretation, the following scenarios for the basis of relapse are suggested by the observations in patients and by the vicious cycle hypothesis.

Those patients in whom relapse occurs as soon as circulating B-cell numbers normalize may have immediate reactivation of disease because of the persistence of pathogenic B-cell clones.

Those in whom relapse is delayed may have been cleared of original clones, and re-engagement of a vicious cycle may require the chance appearance of new clones secreting autoantibodies with appropriate immunomodulatory capacity.

Studies of residual B cells in solid tissues are now in progress and will hopefully shed light on these questions.

**Practical problems with BLyD with anti-CD20**

The exploration of BLyD in RA would not have gone ahead were it not for the evidence of few problems with infection with use of anti-CD20 in lymphoma. The intuitive assumption would have been that BLyD would be impractical because efficacy would be associated with hypogammaglobulinemia. Even with data available from lymphoma, there was a feeling that BLyD needed to be a one-off remission induction therapy to be practical. Certainly this seemed to be the case in the context of RA if, as was suspected, combination therapy with an agent such
as cyclophosphamide would be needed to achieve adequate depletion.

In the event, early results with BLyD in RA suggested not only that there was a selective effect on autoantibodies, with preservation of antibody levels to tetanus toxoid and pneumococcal polysaccharide, but also that total immunoglobulin levels tended to remain normal. However, recent experience indicates that over a period of 4–5 yr the repeated use of BLyD, with three or more courses, is in some cases associated with significant falls in immunoglobulin levels. IgG levels have not fallen to an extent considered worrying in the context of genetic immunodeficiency. However, in three cases IgM levels have become undetectable.

The clinical significance of this is uncertain. However, in the oncology context low IgM levels have also been seen and have been thought to be associated with increased risk of chest infection. There has certainly been a suggestion in the cases of RA treated at University College London that chest infection is more common than expected, but not obviously in relation to low immunoglobulin levels [14]. A significant proportion of respiratory episodes have occurred within days of rituximab administration, suggesting that they may not be truly infective but may be some form of delayed sensitivity reaction. The problem has resolved in all RA cases but protracted respiratory problems have been seen in one case of temporal arteritis. Severe respiratory complications are almost certainly rare. One case of interstitial pneumonitis in the literature appears to be exceptional [35]. The evidence so far certainly suggests that, whatever the cause, respiratory problems after BLyD are no more of an issue than the infective problems encountered with anti-TNF-α agents (they are probably less of an issue), but careful monitoring will be essential.

The implication of these findings would seem to be that BLyD as currently used may be of considerable value in achieving disease control in refractory patients in the medium term, but there is a major question mark over the long-term repeated use of BLyD, much as originally assumed. This raises the question of what alternative approaches to B-cell targeting might achieve the desired long-term remission without long-term compromise of humoral immunity.

Alternative strategies

The first alternative to the current BLyD protocol to consider is another anti-B-cell antibody that might have greater potency and perhaps achieve sustained responses in those cases currently responding for a year or less. New monoclonal reagents will now be either humanized or from mice carrying human antibody genes. Increased potency may well be achievable since different anti-CD20 antibodies have widely different activities in cell-killing assays. It is not certain which is the most relevant mechanism in vivo; complement-mediated lysis, antibody-dependent cellular cytotoxicity (ADCC) or induction of apoptosis through other means. Murine studies suggest that rituximab may kill chiefly through complement [36]. At least one other anti-CD20 antibody is being developed that appears to have greater cytolytic potency [37].

Rituximab itself may have greater potential at higher dosages, which so far have not been explored in detail in autoimmune disease. It may also be fruitful to look for more acceptable adjuvant agents to cyclophosphamide. Combination with methotrexate probably does not enhance the effect on autoantibody levels [8]. Cyclophosphamide does [8], but is too toxic for use in RA. Mycophenylate is a possibility, but may not be sufficiently selective and a rapidly cytolytic agent that could be combined in a short course of treatment would be preferable.

Monoclonal antibodies directed at other B-cell surface molecules have been in development for some time. Anti-CD19 antibodies have been produced but because CD19 has a high rate of cycling in the membrane, killing through an anti-CD19 antibody is likely to be best achieved through internalization of a toxin or radioconjugate [38]. This may be acceptable in the treatment of malignant disease but is difficult to justify in autoimmune disorders. Anti-CD22 antibodies are also available [38], but their potential is uncertain. There is a view that CD20 just happens to be a good target for cytolytic antibodies and that it is relatively unlikely that a better B-cell target will be found. This does not rule out the possibility that combined therapy with antibodies to more than one surface molecule may not prove useful. There is also a possibility that the potency of cytolytic antibodies might be enhanced by concomitant blockade of complement inhibitors or other molecules that protect against cell death.

Another approach to BLyD is the blockade of growth factors such as B-lymphocyte stimulator (BlyS), alias BAFF and perhaps another molecule of the same co-ligand system; APRIL. Strategies targeting BlyS and APRIL are well into development, using either antibodies or receptor-based decoys [39, 40]. What is not yet clear is whether blockade of the BlyS/APRIL system can have as potent an effect as anti-CD20. Depletion is likely to be less profound. However, blockade of BlyS and or APRIL may have a selective effect on autoimmune B cells, at least in some conditions. This raises the issue of a more sophisticated strategy—selective B-cell depletion—which may have much greater potential than current BLyD with anti-CD20.

In many ways, current BLyD is using a sledgehammer to crack a nut. It would be preferable to induce the death of autoimmune B cells without affecting B cells recognizing foreign antigens. One strategy is to use ‘pseudoautoantigens’, which resemble autoantigens but instead of binding to antibody to form a complex capable of stimulating a parent B cell are designed to deliver a death signal to the autoreactive B cell [41]. This strategy has been under investigation but has yet to prove clinically effective. The main drawback of the approach is that it is necessary to know which autoantigens drive the pathogenic mechanism in each patient, which has proved to be a very complex issue.

What may be more realistic is to try to find a way of blocking the survival signals on which autoreactive B cells are selectively dependent. The existence of such signals is plausible. For instance, actively proliferating autoreactive B cells may be selectively sensitive to blockade of signalling through complement receptor 2, or T-cell help through cytokines or contact-mediated costimulatory pathways, or to manipulation of molecules that confer death sensitivity. The dependence on such signals may be greater for all autoimmune cells but it may be disease-specific. Thus, one could imagine that blockade of complement receptor 2 might induce the death of RF-specific B cells but might encourage the survival of B cells implicated in lupus-associated reactions. This might, for the sake of argument, explain why thiols such as penicillamine or gold thiomalate can reduce RF levels in RA but have a tendency to induce features reminiscent of lupus.

Looking far ahead, it may be that the ideal drug for each autoimmune disease will be a small molecule that discourages the survival of autoreactive B cells only of a type found in the target disease. At its best, gold thiomalate is the perfect drug for RA because it can induce complete and permanent remission. Its problem is that it achieves this so rarely, and more often produces toxicity. There is no reason to think that if we understood how drugs like gold, sulphasalazine and penicillamine really work it should not be possible to separate efficacy from toxicity. A reassessment of how these agents work might pay great dividends.

A few other approaches to B-cell targeting that may fit into the above scenario are worth mentioning. Inhibition of B-cell proliferation by blockade of intracellular mediators specific to B-cell activation and survival might be attractive. Cells of B lineage...
are dependent on specific chemokines at different stages of development and these or their receptors might be useful targets. There may be arguments for targeting plasma cells as well as B cells, with the aim of removing autoantibodies potentially capable of re-establishing a vicious cycle. Since it is relatively unlikely that autoreactive plasma cells can be targeted specifically, this strategy may require a major programme of immune restoration with vaccination. At present there are no safe effective agents for removing plasma cells selectively.

In more general terms, it may be that for some of the life-threatening autoimmune disorders a more aggressive immunosuppressive approach may still be justified even if it is less ideal. High-dose cytotoxic therapy with stem-cell rescue has produced very sustained remissions in SLE [42]. It might be, for instance, that a good risk–benefit profile could be achieved by combining a highly B-cell-selective agent with a moderately high-dose cytotoxic regimen, perhaps achieving the same benefit without the risk of mortality found with truly high-dose cytotoxic regimens.

In order to address all these questions there is an overwhelming need for an understanding of disease kinetics and pharmacodynamics. Only then can rational therapy be designed. Hopefully, the use of BLYD and related therapies will allow us to collect this information over the next few years and lead to the design of treatments capable of consistently inducing truly long-term remissions.

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References