Autoimmune disease

Treating human autoimmune disease by depleting B cells

R J Looney

Rituximab treatment is safe and justifies continued study

The development of rituximab has raised the hope of a new therapeutic approach for autoimmune diseases. In the United States rituximab is approved for indolent CD20+ B cell lymphomas, and it is also being evaluated in many different types of lymphomas as well as other B cell malignancies such as Waldenström’s macroglobulinemia and chronic lymphocytic leukemia. World wide more than 300,000 patients with B cell malignancies have been treated with rituximab. Because rituximab is generally well tolerated and because it selectively and profoundly depletes B cells, its role in immune mediated diseases, especially autoimmunity, is now also being explored. Two articles in this issue of the Annals of the Rheumatic Diseases report the clinical use of rituximab in patients with autoimmune diseases.1,2 The first article consists of three case reports using rituximab in three different autoimmune diseases, and the second reports a series of 22 patients with rheumatoid arthritis (RA) treated with rituximab with various combinations of glucocorticoids and cyclophosphamide. Both articles found rituximab was well tolerated, and both concluded that there might have been some therapeutic benefit.

These articles are not the first reports on the use of rituximab in autoimmunity. Indeed, the same group reporting treatment of 22 patients with RA in this issue has previously reported on five patients with RA who had prolonged remission of RA with a three week course of treatment combining high dose, daily glucocorticoids, three weekly doses of rituximab, and two doses of cyclophosphamide.3 Other groups have reported the use of rituximab in idiopathic thrombocytopenic purpura, autoimmune haemolytic anaemia, cold agglutinin disease, and neuropathy associated with monoclonal IgM.4-8 None of these reports was adequately controlled. Thus, additional randomised clinical trials are needed for definitive evaluation of the effectiveness of rituximab in autoimmune disorders. As these trials are being planned, it is a good time to reflect on the roles of B cells in autoimmune disease, on the effects of rituximab on the immune system, and on the possible effects of rituximab on autoimmune disease.

WHAT ARE THE ROLES OF B CELLS IN AUTOIMMUNE DISEASE?

B cells are by definition the source of all immunoglobulins, and in this capacity they obviously have a critical role in antibody mediated autoimmunity. B cells should not, however, be viewed as passive makers of immunoglobulins, with other cells, especially T helper cells, making all the important decisions. B cells also have a key role in determining the responses to antigen both directly as antigen presenting cells and indirectly by influencing other antigen presenting cells such as dendritic cells.

“Development of autoimmune disease may be blocked in animals deficient in B cells”

B cell deficient mice were first produced by administration of anti-µ antibody bodies beginning at birth.11-15 These animals had a severe defect in T cell priming, as assayed by proliferative responses and delayed-type hypersensitivity when antigen was given locally in complete Freund’s adjuvant. These B cell deficient mice were also resistant to certain autoimmune diseases such as experimental autoimmune encephalitis and spontaneous insulin dependent diabetes (in non-obese diabetic mice).16-20 Interestingly, immunisation with peptide rather than intact protein circumvented the need for B cells.21 Mice genetically deficient in B cells have now been produced by knocking out either the transmembrane (TM) domain of IgM (µTM) or the Jµ segment. These B cell deficient mice have generated controversy about the role of B cells in T cell responses, because in some (C57 Black/6) but not other backgrounds T cell responses were relatively well preserved. A recent publication has resolved some of these inconsistencies.22 In all backgrounds, including C57 Black/6, T cell responses were severely defective with low doses of protein antigens.

Moreover, responses to even high doses of protein were severely defective if mice with a C57 Black/6 background were allowed to develop normally (B cells are required for normal lymphoid node development) and then were irradiated and reconstituted with bone marrow from B cell deficient animals (µTM B6). Adoptive transfer of polyclonal B cells into µTM B6 reconstituted animals corrected the defect in T cell responses, but adoptive transfer of monoclonal B cells from an immunogenetic transgenic animal did not. These results suggest but do not prove the importance of antigen-specific B cells in T cell responses to soluble proteins.

The development of autoimmune disease may also be blocked in animals genetically deficient in B cells. For example, in B cell deficient Jµ− MRL lpr+ mice not only were autoantibodies completely absent but also the massive increase of T cells in lymphoid organs was prevented.23-25 When Jµ− MRL lpr+ mice were bred to express an IgM transgene that can only be expressed as a membrane and not as a secreted protein, autoantibodies were again absent but the massive increase of T cells in lymphoid organs was reconstituted, indicating that B cells play a role in the T cell activation in this model.26 Interestingly, nephritis with cellular infiltrates in the renal interstitium and vessels was seen in these animals despite the absence of autoantibodies.27 Thus, even apart from their production of autoantibodies, B cells may have an important role in autoimmunity by activating T cells in certain models of SLE. Experimentally induced autoimmune diseases may also fail to develop in B cell deficient mice—for example, myasthenia gravis in mice immunised with acetylcholine receptor, and collagen arthritis in mice immunised with type II collagen.28-29 In both of these models T cells from the immunised, B cell deficient animals respond to the immunising autoantigen, despite the absence of disease, suggesting that in these models the autoantibodies themselves may be critical. Thus, there is considerable evidence of a critical role for B cells both in normal T cell responses and in the induction of autoimmune disease.

A somewhat different role for B cells is the induction of autoreactive T cells by antigens that cross react at the B cell level.30-32 For example, naïve mice immunised with mouse cytomegalovirus had no T cell response, but mice previously immunised with human cytomegalovirus and then immunised with mouse cytomegalovirus-specific T cell response. T cell response to mouse cytomegalovirus may be transferred from mice immunised with human cytomegalovirus not by T cells or serum but only by B cells. In these
experiments B cells from mice immunised with human cytochrome c expressed surface immunoglobulins that reacted with both human and mouse cytochrome c. Presentation of mouse cytochrome c by these self cross reactive B cells appears to break T cell tolerance. Once the autoreactive memory T cells are generated they recognise mouse cytochrome c presented by a variety of antigen presenting cells. Similar cross reactivity at the B cell level may be responsible for induction of an SLE-like illness in animals immunised with peptides from Sm.

B cell deficient animals may also have altered T cell responses; these changes in T function may be due to changes in dendritic cells. For example, B cell deficient animals have an enhanced cytotoxic response against transplantable tumours. The mechanism for this enhanced response to tumours was not investigated. However, T cells from B cell deficient animals have been found to be defective in their ability to provide B cell help or to produce interleukin (IL) 4. Dendritic cells from B cell deficient mice have enhanced production of IL12 and preferentially induce T helper 1 (Th1) cells. This phenotype is similar to the phenotype of IL10 knockout mice. Splenocytes from B cell deficient mice have low levels of IL10 mRNA. Moreover, IL10 treated dendritic cells from B cell deficient mice develop the ability to induce T cell production of IL4. Thus, B cells also appear to have important immunoregulatory effects that are mediated through dendritic cells, perhaps due to B cell production of IL10. Thus, B cells may have an immunoregulatory role.

**WHAT ARE THE EFFECTS OF RITUXIMAB ON THE IMMUNE SYSTEM?**

Rituximab is a humanised mouse monoclonal antibody (mouse variable regions and human IgG1 constant regions) directed at human CD20. Both mouse and human CD20 are B cell-specific membrane proteins with four transmembrane spanning domains, intracellular carboxyl and amino terminal...
domains, and a 50 amino acid loop that is extracellular. They are members of the M54A family that includes the FcγRIIB chain and in humans at least seven other expressed sequences. They can function at calcium channels but their physiological role is unknown. CD20 appears on B cells at the pre-B stage and disappears during differentiation to plasma cells. Thus, immature B cells and mature naive and memory B cells all express CD20. Rituximab can mediate both complement mediated cytotoxicity and antibody dependent cell mediated cytotoxicity. Moreover, cross linking CD20 on the surface of B cells using rituximab plus a secondary antibody or an FcR expressing cell can result in apoptosis. Experiments in mice transplanted with human B cell tumours suggest that FcγRIIB is critical for B cell depletion in vivo. The association of improved clinical response in patients with lymphoma with the high affinity allele of FcγRIIIa suggests the importance of FcγRIIIa for depletion of B cells by rituximab in humans. In patients with lymphoma remarkably effective and long term (6–12 months) depletion of peripheral blood B cells has been observed. In studies in non-human primates the effects of rituximab are relatively transient because of the development of an anti-rituximab immune response. Nevertheless, in these animals rituximab treatment immediately before immunisation completely prevented primary and secondary antibody responses, consistent with the expression of CD20 on naive and memory B cells. Immunoglobulin levels are generally well preserved after treatment with rituximab, presumably because of the lack of expression of CD20 on plasma cells.

Additional studies on the effects of rituximab on the normal immune system in humans are clearly needed. These clinical trials will need to be carefully designed to take advantage of the unique effects that B cell depletion may have on established disease. The clinical effects of rituximab and studies of the immune system of subjects in these trials should provide invaluable insight into the role of B cells in human autoimmune disease.


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Dr Looney has a grant from Genentech and IDEC to study the use of rituximab in systemic lupus erythematosus (SLE). He has also served as a consultant for these companies on the use of rituximab in autoimmune disease.

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