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Anti-CD20 antibody is an efficient therapeutic tool for the selective removal of autoreactive T cells

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Rituximab, a chimeric IgG_1 monoclonal antibody that specifically binds to CD20 on B cells, has demonstrated surprising efficacy in the treatment of autoimmune diseases that are predominantly mediated by T helper (T_H) cells, such as rheumatoid arthritis, multiple sclerosis (MS), lupus nephritis and idiopathic thrombocytopenic purpura,[1–5] as well as in mouse models of autoimmune diabetes.[6] These findings have led investigators to speculate that B cells might have a primary and initiating role in these autoimmune diseases. In this Viewpoint I argue, however, that the action of anti-CD20 therapy is in fact specific for autoreactive T cells.

The roles of B cells in the action of rituximab are generally thought to relate to autoantigen presentation, excessive costimulation, and inflammatory cytokine and chemokine production, which all lead to the attraction and expansion of autoreactive inflammatory T cells at the expense of regulatory T (T_{REG}) cells. Other antigen presenting cells (APCs) such as dendritic cells (DCs) and macrophages, however, are activated and prevalent in lesions of T_H-cell-mediated autoimmune diseases. These cells are also quite capable of carrying out the pathogenic functions usually attributed to B cells. Autoreactive B cells could be the most efficient APC for autoreactive T cells previously primed by autoantigen, which would be an important factor in explaining the efficacy of rituximab in T_H-cell-mediated autoimmune diseases if the amount of autoantigen was limited; however, this is not the case in these autoimmune diseases, where the entire target organ—such as the brain in multiple sclerosis or all the nucleated cells undergoing apoptosis in the case of lupus—provides abundant supply of autoantigens. Moreover, B cells might go on to produce proinflammatory cytokines to induce expansion of T_H17 cells, but autoreactive T_H1 cells are the initiators of autoimmunity in all of the above mentioned diseases, as well as in murine lupus,[5] and also have a major role in disease pathogenesis. B cells cannot, however, present autoantigens to induce T_H1 cell differentiation because they do not produce interleukin 12.

Although anti-CD20 therapy might operate by several concurrent mechanisms, one possibility has, so far, been overlooked in all discussions and editorial commentaries regarding this issue. In my opinion, phagocytes and other inflammatory cells not only remove anti-CD20-opsonized B cells, but at the same time remove autoreactive T cells that are interacting with the autoantigen-presenting B cells in a conjugating immunological synapse in peripheral lymphoid organs. These ectopic lymphoid-follicle-like structures resemble germinal centers and are found in the rheumatoid synovium, in the brain of patients with MS, or at the site of

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inflammatory infiltrates in the kidneys in mouse models of lupus and in human lupus nephritis.[7–11]

According to the linked recognition mechanism of cognate interaction between T_H cells and B cells, B cells that are specific for a particular antigen are 1,000-fold more efficient in interacting with and soliciting help from T cells that have receptors for determinants of the same antigen than T cells that do not have appropriate receptors.[12–14] It is reasonable, therefore, to believe that the T cells that interact with auto-antigen-presenting B cells at the sites of inflammation or autoantibody production in the above mentioned autoimmune diseases would be selectively enriched for autoreactive T cells. Autoantigen-specific and activated T cells hyper-expressing CD40 ligand, HLA-DR and CD69,[15] are reduced rapidly after anti-CD20 therapy—within a matter of weeks in the periphery.[2,3] This T-cell depletion might occur at even earlier time points; however, the rate of T-cell depletion in the organized foci of lymphoid neogenesis at sites of inflammation has yet to be explored.

The mechanisms of action of anti-CD20 antibodies *in vivo* are not completely known,[16] but antibody-dependent cell-mediated cyto-toxicity (ADCC), phagocytosis and complement-mediated cytotoxicity have been implicated.[17–19] Anti-CD20 antibodies bind to target B cells to form immune complexes, which then activate complement components and lead to the formation of a membrane attack complex that directly kills the B cells.[16] Alternatively, the immune complexes attract and activate phagocytes bearing complement receptors, which then engulf the B cells opsonized by anti-CD20 antibodies.[19] Moreover, the aggregated Fc portion of anti-CD20 antibodies that is bound to B cells activates macrophages and natural killer cells that bear the Fcγ receptor (FcγR), which kill the opsonized B cells by discharging cytotoxic mediators and granules (the ADCC mechanism) or by engulfing them (phagocytotic route).[16–19] ADCC and phagocytosis are probably the critical mechanisms in the action of anti-CD20 antibodies, because the response rate to rituximab is better in patients who have high-affinity polymorphisms in the gene encoding the FcγR.[17,20] The activity of ADCC also involves the internalization of anti-CD20-coated cells.[18] Moreover, complement activation by anti-CD20-opsonized B cells leads to the recruitment of neutrophils, which produce inflammatory mediators.[19] Thus, the action of anti-CD20 agents cannot be restricted solely to B cells because other cells interacting with the B cells in the autoimmune response are probably also affected. Moreover, as mentioned above, B-cell-bound anti-CD20 antibodies cross-link FcγRs on phagocytes in order to activate them; such phagocytes could then efficiently engulf not only B cells, but entire cellular aggregates made up of B cells, T cells and other APCs.

If these interpretations are correct, then the beneficial therapeutic effects of anti-CD20 antibodies should be most evident in patients with advanced or refractory diseases, in which chronic ongoing autoimmune responses occur in organized foci of stable interactions between T cells, B cells, DCs and other APCs. Indeed, these organized lymphoid-follicle-like structures —which resemble germinal centers and are found in the rheumatoid synovium, the brain of patients with MS and the kidneys of patients with severe lupus nephritis, as mentioned above[7–9,11]—might be disrupted by anti-CD20-antibody-mediated attacks on B cells and other interacting cells. Depletion of autoreactive T cells and other inflammatory APCs, along with autoreactive B cells from such foci of interactions, removes a source of inflammatory cytokines that counteract T_{REG} cell function,[3–5] as well as enable tolerogenic APCs, such as plasmacytoid DCs, to generate more T_{REG} cells.[10]

The hypothesis that the efficacy of rituximab in the treatment of predominantly T_H-cell-mediated autoimmune diseases is due to the simultaneous removal of autoreactive inflammatory T cells and anti-CD20-opsonized B cells could be experimentally tested, as antigen-specific interactions between T cells and B cells can be visualized. For example, ovalbumin-specific transgenic T_H cells bearing T-cell receptors and hen-egg-lysozyme-

specific transgenic B cells bearing B cell receptors could be adoptively transferred to mice, and the recipient mice could then be repeatedly immunized with a conjugate of ovalbumin and henegg lysozyme,[21] Such animals could subsequently be treated with mouse antibody to CD20[15] to determine whether concurrent depletion of both T cells and transgenic B cells occurs.

Thus, anti-CD20 therapy, although not specific for autoreactive B cells, could, ironically, be specific for autoreactive T cells because of the association of these T cells with autoreactive B cells. Consequently, it is not necessary to know what autoantigenic determinants or epitopes disease-relevant T cells recognize in order to selectively deplete them via B-cell-directed antibody therapies. This mechanism would be a major advantage of such therapy, because the autoantigen epitopes recognized by autoreactive T cells in most human autoimmune diseases are not well defined or are heterogeneous. In addition, the specificities of autoreactive T cells keep shifting with disease progression due to epitope spreading as a result of recognition of new determinants exposed by autoimmune inflammatory damage.[22]

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