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Editorial

Rheumatoid Arthritis Clinical Benefits from Abatacept, Cytokine Blockers, and Rituximab Are All Linked to Modulation of Memory B Cell Responses

Abatacept (ABA) is a biologic agent with great proven efficacy in patients with rheumatoid arthritis (RA)[1]. At a molecular level, ABA is composed of recombinant domains of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), fused to the constant region domains of human IgG1, which serves to increase *in vivo* half-life. This agent binds to CD80/CD86 with a much higher avidity than the CD28 on many T cells, and therefore can act as a physiologic competitive inhibitor that interrupts cell-cell co-stimulatory interactions. In earlier reports, ABA treatment of patients with RA was shown to have striking effects on the modulation of T cell subsets[2].

The recent report by Scarsi, *et al* highlights the bidirectional nature of the CD28-CD80/86 interaction and the profound effects that ABA can have on the B cell compartment of the immune system, especially for memory B cells[3]. With the proven efficacy of the B cell-targeted anti-CD20 agent rituximab (RTX), the central roles of B cells in RA have become well accepted, and B cells also commonly express the co-stimulatory molecules CD80/86[4]. Even at the earliest onset of clinical signs and symptoms, patients with RA display dysregulated immune-cell trafficking and maturation. Compared to healthy subjects, patients with RA also have abnormal levels of circulating memory B cells (identified by CD27 expression), and this may in part reflect their recruitment to the synovial compartment or secondary lymph nodes[5].

In a small study of 28 patients with RA, Scarsi, *et al* showed that after 6 months of ABA treatment, patients with clinical responses had significant decreases in levels of switched memory B cells, with persistent decreases in memory B cell subsets also found at 12 months[3]. ABA therapy also significantly reduced levels of serum total IgG, IgA, and IgM, reflecting a reversal of disease-associated hypergammaglobulinemia. There were also significant decreases in anticitrullinated protein antibody (ACPA) IgG and IgA levels, as well as rheumatoid factor (RF) autoantibodies[3]. These findings reiterate evidence from a small exploratory study of patients with ACPA-positive early RA and undifferentiated arthritis, in whom ABA treatment also reduced autoantibody levels[5]. These circulating disease-associated autoantibodies, a central hallmark of RA, are believed to primarily arise from autoreactive B cells in the hyperplastic synovia of affected joints[6].

Patients with ACPA-positive RA in fact may have better responses with ABA treatment as compared to ACPA-negative patients[7]. Scarsi, *et al* found that ABA treatment also normalized the RA-associated increases in levels of free light chains[3], a marker of dysregulated immunoglobulin production commonly seen in multiple myeloma, as well as in RA and systemic lupus erythematosus (SLE). Taken together, these new data suggest that ABA treatment restores regulation within the memory B cell compartment, and these treatment effects lead to a compensatory surge in levels of naive B cells at 6 months[3].

While ABA may also affect professional antigen-presenting cells (APC) of the myeloid series that are important drivers of RA pathogenesis, the earliest murine experimental models showed that CTLA-4 Ig had *in vivo* effects on both activated T cells and B cells[8]. These findings suggested that ABA treatment might dampen the co-stimulatory interaction between T and B lymphocytes, leading to amelioration of autoimmunity-driven inflammation. Memory B cells expressing CD80/86 may be especially efficient APC for the recruitment and maintenance of antigen-specific memory and effector T cells[9]. CD80/86 may also mediate pro-survival signals for APC. In an earlier synovial biopsy study, ABA treatment had the greatest effects on B cell representation in affected joints, because those cells rapidly disappeared from the RA synovium[10]. These findings contribute to an emerging perspective that biologic agents that act through very different primary

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targets to provide clinical benefits for patients with RA may display common immunoregulatory effects that normalize the B cell defects in RA[11,12].

RTX and B Cell Modulation

In many ways, the immunologic outcome of ABA treatment is highly reminiscent of the effect of B cell-targeted therapy with RTX, which initially causes marked peripheral blood B cell depletion. At 6 months, clinical response rates for RTX are very similar to those of ABA and for tumor necrosis factor (TNF) inhibitors. When serum levels of the RTX antibody wane after many months, there is a return of circulating blood B lymphocytes due to repopulation of the peripheral compartment. In patients with more prolonged clinical responses after single RTX treatments, levels of CD27+IgD+ (unswitched) B cells and CD27+IgD– (switched) B cells were higher in those who experienced an early relapse than in patients who experienced a late relapse[13,14]. During the earliest phase of peripheral reconstitution, there are heightened levels of transitional B cells that are presumably newly generated in the bone marrow. However, within weeks there is a shift to naive mature B cells that may reflect a resetting of the adaptive immune system that is associated with longer periods of treatment-induced clinical benefits[13,14]. Hence, akin to the findings with ABA, anti-CD20 therapy induces clinical responses linked to lower levels of activated B cells and memory B cells[13,15] as well as modest decreases in ACPA and RF autoantibody levels[13,16].

TNF Blockade and B Cell Modulation

TNF inhibitors have been approved for the treatment of RA since 1998, and clinical efficacy attained with these agents has become the yardstick by which we currently judge all other agents. While there are now 5 different approved TNF inhibitors, with nuances in their designs, modes of administration, and treatment regimens, these all have generally comparable clinical response rates and safety profiles.

The physiology of B cell proliferation is known to involve the tightly regulated expression of TNF, and hence dysregulated TNF production has been implicated as a cause of the B cell abnormalities commonly found in patients with RA[17,18]. This may explain why treatment with the anti-TNF antibody infliximab also results in decreases in blood pre-switched memory B cell numbers with compensatory increases in peripheral blood transitional and naive B cells[17,19].

Etanercept (ETN) represents a recombinant fusion protein of a human TNF receptor (TNFR II p75) with an IgG constant region to increase *in vivo* half-life, and *in vivo* ETN acts as a decoy receptor that blocks both TNF-α and lymphotoxin β, a factor linked to ectopic lymphoid tissue formation. Anolik and colleagues have reported that ETN treatment results in normalized memory B cell levels and an increase in the proportion of transitional B cells and naive B cells due to effects on lymphoid germinal centers, in which immune complexes arrayed on follicular dendritic cells are involved in B cell clonal selection[20]. Therefore, during RA pathogenesis, the defects in B cell tolerance may be reversed by TNF inhibitors, which suggests these are linked to inflammation.

Interleukin 6 (IL-6) Blockade and B Cell Dysregulation

The anti-IL-6 receptor agent tocilizumab (TCZ) has also become a major resource for the treatment of RA. IL-6 has diverse and protean effects on many cell types, involved in both homeostatic and inflammatory pathways. This cytokine was first identified as a B cell growth factor that contributes to differentiation of B cells into plasma cells, the antibody-producing factories of the body[21]. In patients with RA, TCZ is another agent that has a dramatic effect on B lineage cells, with decreases in levels of circulating switched and unswitched memory B cells and with overall decreases in measured levels of antibody gene hypermutation in blood B cells[22,23]. However, it has little or no effect on IgG levels. In a study of 15 patients with SLE, similar effects of TCZ treatment have been documented in circulating B cell subsets after 12 weeks of IL-6 receptor blockade, as the frequency of $CD27^{HIGH}$ $CD38^{HIGH}$ IgD-plasmablasts/plasma cells and IgD-CD27+ post-switched memory B cells, as well as IgG+ memory B cells, declined[24]. Conversely, TCZ treatment also increased the frequency of IgD+CD27– antigen-inexperienced B cells. The levels of $CD38_{LOW}$ mature naive B cells increased significantly. These changes were concurrent with decreases in activated lymphocytes, and the frequency of CD4+CD45RA+CCR7+ naive T cells also increased[24].

B Cell Activation Factor (BAFF) Blockade Mechanism of Action Is Different

Not all agents that target B cell pathways bring about the same immunomodulatory effects and clinical benefits for patients with RA. The TNF family member (TNFSF13) BAFF, also called B lymphocyte stimulator (BLyS), serves essential functions in B cell survival and maturation during early antigen-independent phases in the bone marrow or after transitional B cells traffic and differentiate into mature antigen-naive B cells in the periphery. BAFF also supports the end-differentiation of plasma blasts and plasma cells[25]. Notably, BAFF blockade has very different effects on the distribution of human B cell subsets in the bloodstream than other biologic agents, as described above, and these same patterns have been seen with the anti-BAFF antibody belimumab, as well as the more recently developed anti-BAFF antibody tabalumab, and the decoy receptor TACI-Ig (atacicept). Indeed, in contrast to the seemingly consistent immunomodulatory effects described above with RTX, TNF inhibitors, IL-6 R blockade, and now described with CTLA4-Ig (ABA), BAFF blockade instead causes increases

in blood levels of memory B cells, which after several months return to pretreatment levels[26,27]. Most importantly, BAFF blockade attained with agents of various designs[28,29] has not provided the level of clinical benefits in RA seen with other currently approved biologic agents. Nonetheless, BAFF blockade has proven clinical benefits in SLE[30], which on the other hand suggests that B cells play very different roles in the immunopathogenesis of these diseases.

There is now extensive evidence that diverse biologic agents can provide clinical benefits associated with shifts in the B cell compartment of the adaptive immune system in patients with RA. Intriguingly, these shared features of effective biologic agents for normalization of peripheral blood memory B cell levels are commonly concordant with increases in naive B cells and reductions in disease-associated serum autoantibodies. It remains controversial whether these changes are directly responsible for clinical benefits, and whether the known alterations or deficiencies in B cell clonal selection and trafficking are main drivers of the disease. Yet not all approaches to target B cells are equally effective in RA; there are now results from clinical trials showing that several different anti-BAFF/BLyS agents all demonstrated a lack of efficacy in RA, a finding that in part further highlights the differences between RA and SLE immunopathogenesis.

As for the mechanism of action of ABA, mounting evidence now suggests that beneficial therapeutic effects are most probably related to interference with the bidirectional interactions involving T cell-mediated stimulation of pathogenic B cells. More importantly, there is much data to suggest that the inflammatory environment of RA may be a major contributor to activation and even inappropriate pro-survival effects on post-germinal center B cells. This influence may stoke the self-perpetuating immune response in RA and be part of the defect in immune tolerance that contributes to inflammatory synovitis, which in turn contributes to further B cell defects. While defects in peripheral immune tolerance checkpoints have been described in patients with RA[19], it remains controversial whether these defects are primary to the disease or in fact represent a byproduct induced by cytokine imbalances[18]. Plasma cell populations can become expanded in certain disease states because of an increase in the survival niches in affected inflamed tissues. The central lesson may be that efficacy in RA appears to require treatment-induced normalization of disease-associated B cell defects.

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