The immunological basis of B-cell therapy in systemic lupus erythematosus

Mo Yin MOK

Division of Rheumatology & Immunology, Department of Medicine, Queen Mary Hospital, The University of Hong Kong, Hong Kong

Abstract
Loss of B-cell tolerance is a hallmark feature of the pathogenesis in systemic lupus erythematosus (SLE), an autoimmune disease that is characterized by hypergammaglobulinemia and autoantibody production. These autoantibodies lead to formation of immune-complex deposition in internal organs causing inflammation and damage. Autoreactive B-cells are believed to be central in the pathophysiology of SLE. Other than its role in the production of antibodies that mediate humoral immune response, B-cells also function as antigen-presenting cells and are capable of activating T-cells. Activated B-cells may also produce pro-inflammatory cytokines that aggravate local inflammation. Abnormal B-cell homeostasis has been described in SLE patients. This may occur as a result of intrinsic B-cell defect or from aberrant regulation by maturation and survival signals. B-cell-based therapy is the current mainstream of research and development of novel therapies in SLE patients with severe and refractory disease. Potential cellular and molecular targets for B-cell therapies include cell surface molecules such as CD20 (rituximab) and CD22 (epratuzumab); co-stimulatory molecules involved in B-cell–T-cell interaction such as CTLA4 and B7 molecules (abatacept); maturation and growth factors such as B-cell activating factor and a proliferation-inducing ligand (belimumab, briobacept, atacicept) and B-cell tolerogen (abetimus). This article provides an overview on normal B-cell physiology and abnormal B-cell biology in SLE that form the immunological basis of B-cell-targeted therapy in the treatment of these patients with refractory diseases.

Key words: B lymphocytes, biologic treatment, pathogenesis, systemic lupus erythematosus.

INTRODUCTION
Systemic lupus erythematosus (SLE) is an autoimmune disease that is characterized by hypergammaglobulinemia and a phlethora of autoantibodies. This disease is associated with significant morbidity and mortality. Corticosteroid and immunosuppressive agents are the mainstay of treatment but there remains a proportion of patients who have refractory disease or have complications with treatment-related toxicity. Thus, there is a need for novel therapies with higher efficacy and favorable side-effect profiles. A number of potential cellular or molecular targets for new therapeutic strategies have been revealed on the basis of better understanding of pathophysiology of the disease. B-cell targeted therapy is the current mainstream of research and clinical trials in the treatment of patients with SLE.

B-lymphocytes are key players in the immune system. Plasma cells, the terminally differentiated cells of B-cell lineage, are effector cells that produce immunoglobulins involved in humoral response of adaptive immunity. The important role of B-cells as antigen-presenting cells in innate immunity has also been increasingly recognized in autoimmunity. Indeed, B-cell-based therapy now provides alternative
treatment options to patients who have moderate to severe active rheumatoid arthritis (RA) refractory to conventional disease-modifying agents and biologic therapy that targets tumor necrosis factor (TNF)-α. Anti-B-cell therapy has also been increasingly used off-label to treat SLE patients with refractory diseases. A number of B-cell-targeted biologic therapies is down the pipeline undergoing phase II or III clinical trials in the treatment of SLE with moderate to severe disease. This article provides an overview of normal B-cell biology and abnormal B-cell phenotype in SLE that form the immunological basis of B-cell therapy in these patients.

**B-CELL DEVELOPMENT**

B-cell development involves sequential rearrangement of heavy- and light-chain immunoglobulin genes and takes place in the bone marrow. Pro-B-cells are the earliest B-lineage cells derived from pluripotential hematopoietic cells. Rearrangement of heavy-chain immunoglobulin genes in pro-B-cells leads to pre-B-cells, the next stage of B-cell development. In pre-B-cells, signals for cessation of heavy-chain rearrangement occur such that each cell ends up with only a single rearrangement heavy-chain gene. Subsequent assembly of light-chain genes and heavy-chain genes leads to expression of immunoglobulin M (IgM) molecules on cell surfaces which marks the stage of immature B-cells. At this stage, the immature B-cells are being censored for autoreactivity. B-cells that react strongly with self-antigens are induced to undergo apoptosis, clonal deletion or anergy, a state of non-reactivity. The remaining B-cells that do not recognize self-antigens survive, leave the bone marrow and emerge into the periphery where they undergo further differentiation into mature B-cells that express both surface IgD and IgM. These mature B-lymphocytes become competent for immunologic function by acquiring expression of class II major histocompatibility complex (MHC) molecules, which enable them to present antigens to helper T-cells as well as surface CD40, a protein involved in receiving T-cell help. Naïve B-cells recirculate through secondary lymphoid tissues and return to the blood via the lymphatic system.

**ACTIVATION OF B CELLS**

Initial encounter of antigen by B-cells occurs in peripheral lymphoid organ where free antigens gain access via lymphatics or are carried by homing dendritic cells, professional antigen-presenting cells from peripheral tissues. The B-cell receptor on B-lymphocytes efficiently captures antigen which is then internalized, processed and returned to the cell surface as peptides bound to Class II MHC molecules. Antigen-activated B-cells then migrate toward the T-cell zones of the lymphoid tissue. Humoral response to most protein antigens requires help from CD4+ T-cells. B-cell–T-cell interaction leads to activation, proliferation and further differentiation of B-cells into plasma cells. Some B-cells migrate from the T-cell zone into a nearby lymphoid follicle where they proliferate and differentiate and establish secondary germinal centers. The rapid proliferation of cells in the germinal centre greatly increases the number of B-cells specific for the pathogen that initiated the antibody response. Furthermore, in the germinal center, somatic hypermutation of immunoglobulin-variable domain genes and affinity maturation occur such that there is a switch from IgM to other isotypes of antibodies and increase in the affinity of antibodies for the inducing antigen. These antigen-activated B-cells then come into contact with specialized stromal cells called follicular dendritic cells that bear unprocessed antigens trapped within the lymphoid follicles. These cells provide survival signals for mature B-lymphocytes that bind cognate antigen on their surface with high affinity. Those B-cells that fail to bind die by apoptosis. Thus, those B-cells that have high-affinity binding to antigens survive the selection process, leave the germinal center to become either memory B-cells or antibody-secreting plasma cells. Plasma cells migrate to the bone marrow and produce the majority of circulating immunoglobins. B-cells that become memory B-cells reside in the lymphoid organ and can be rapidly activated upon subsequent challenge with the same antigen.

**B-CELL–T-CELL INTERACTION**

Helper T-cells which recognize antigen on the surface of B-cells become activated and synthesize both cell-bound and secreted effector molecules that synergize in B-cell activation (Fig. 1). CD40 ligand (CD40L) is expressed on activated helper T-cells, that binds to CD40 on B-cell surface. Antigen binding and CD40–CD40L interaction provide signals that drive B-cell activation, proliferation and differentiation into plasma cells. Activated B-cells also express other co-stimulatory molecules such as surface B7.1 and B7.2 proteins that bind to CD28 on the surface of
T-cells to enhance cognate interaction as well as driving T-cell activation. The B7 molecules are members of the immunoglobulin superfamily that bind to CD28 on naïve T-cells and an additional receptor, CTLA-4 that is expressed on activated T-cells. CTLA-4 binds B7 molecules with higher avidity than CD28 and transduces a negative signal to the activated T-cells in order to limit excessive proliferative response of these activated T-cells. Soluble factors like cytokines are also important inducers of B-cell activation. Interleukin (IL)-4 preferentially induce switching of immunoglobulin isotype to IgG1 and IgE, whereas tissue growth factor (TGF)-β induces switching to IgG2b and IgA. Interferon (IFN)-γ induces IgG2a and IgG3 production by activated B-lymphocytes.

CELL SURFACE MOLECULES ON B-CELLS

Each of the stages of B-cell development is characterized by cell-surface protein expression of the B-cell lineage (Fig. 2). CD19 is expressed throughout B-cell development. CD20 is present on pre-B and mature B-cells. It is not found on stem cells, pro-B-cells and normal plasma cells. CD22 is expressed in low levels in immature B-cells, strongly expressed on mature B-cells and is absent in plasma cells. CD138 is expressed on plasma cells but not on mature circulating B-lymphocytes.

SURVIVAL FACTORS FOR B CELLS

Cytokines like IL-7 produced by bone marrow stromal cells, induce proliferation of pro-B-cells and have little effect on subsequent stages of B-cell development. B-cell activating factor (BAFF), also known as B-lymphocyte stimulator (BlyS), is a TNF family ligand that is important in B-cell immunity. BAFF is a fundamental maturation and survival factor for transitional and mature B-cells via inhibition of apoptosis. BAFF is present as a transmembrane protein on myeloid cells including macrophages, monocytes, dendritic cells and is also expressed by non-myeloid cells including bone marrow stromal cells. BAFF forms homotrimers and also exists in a soluble form that is biologically active.
and transduces signaling on B-cells through three different receptors, namely BAFF-receptor (BAFF-R), transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI) and the B-cell maturation protein (BCMA). BAFF mediates its effect specifically via the BAFF-R and shares TACI and BCMA and with another ligand, a proliferation-inducing ligand (APRIL). APRIL has close sequence homology with BAFF and influence B1 cell activity, humoral responses and class-switching of immunoglobulins. Information in regard to the distinct function of these receptors for BAFF is still lacking.4

ABNORMAL B-CELL BIOLOGY IN SLE

Although the pathogenesis of SLE is not fully known, B-cells are believed to play a central role as loss of B-cell tolerance with emergence of autoreactive B-cells is a hallmark feature of lupus pathogenesis. More circulating B-cells with spontaneous expression of high levels of co-stimulatory molecules such as B7 molecules have been reported in SLE patients compared to controls.5 Altered B-cell subset in peripheral blood has also been shown in SLE patients, with increased immature transitional B-cells, memory B-cells, plasma blasts6 and circulating plasma cells that spontaneously produce autoantibodies.7,8 Autoantibodies produced by these autoreactive cells lead to immune-complex formation that deposit in internal organs causing inflammation and organ damage. Indeed, B-cells producing anti-double stranded (ds)DNA antibodies have been postulated to have a direct pathogenic role in lupus nephritis.9 The abnormal phenotypes of B-cells in SLE may also disturb immune homeostasis including autoantigen presentation, cytokine production, modulation of T-cell repertoire and memory T-cells. Indeed, the mere presence of B-cells that are non-productive of autoantibodies has been shown to contribute to disease in the murine lupus model10,11 suggesting broader immune effects of B-cells and the central role of B-cells in the pathogenesis of SLE.

Dysregulated BAFF was first linked to lupus pathogenesis from the lupus-like phenotype manifested by BAFF transgenic mice. These mice had hypergammaglobulinemia, raised levels of anti-dsDNA antibodies and increased immunoglobulin deposition in the kidneys.12 There were also increased circulating B-cells, hepatosplenomegaly, increased germinal center reaction and expansion of marginal zone B-cells in the lymphoid nodes. Marginal zone B-cells are able to activate naive T-cells and have a lower stimulating threshold than recirculating immature B-cells for activation, proliferation and differentiation into plasma cells. These cells are involved very early in immune responses and are involved in T-independent immune responses.13 Indeed, elevated serum BAFF levels compared to controls have been reported in 20–40% of SLE patients across different cross-sectional studies.14–16 Patients who had elevated serum BAFF were also more likely to have higher serum immunoglobulin and anti-dsDNA antibody levels.15 BAFF has been shown to correlate with SLE disease activity in cross-sectional analysis and predicted active disease in a longitudinal study16 supporting its therapeutic implication as a potential target in biologic therapy.

The role of APRIL is less clear in lupus pathogenesis. APRIL transgenic mice showed increased T-cell survival and T-independent antibody responses.17 Increased APRIL level in SLE patients has been reported whereas inverse correlation between APRIL level and disease activity was reported in another study.19 This discrepancy has been postulated to be related to over-expression of heterotrimers between APRIL and BAFF, that were reported in many systemic autoimmune diseases, thus interfering with the detection of individual protein in patient sera.20 However, the function of these biologically active heterotrimers remains unknown.

B-CELL BASED THERAPIES AND THEIR MECHANISMS OF ACTION

The central role of B-cells in the pathogenesis of SLE has led to the development of a number of novel therapies targeting these cells. The overview on B-cell biology provides a brief background on the potential cellular or molecular targets for these B-cell targeted biologic agents. A variety of B-cell-based therapies has been under research and on-going phase II and III clinical trials. These include antibodies to B-cell surface antigens, B-cell tolerogens, blockers of co-stimulatory molecules and inhibition of cytokines with direct B-cell effects (Fig. 3).

B-CELL-DEPLETING THERAPIES

Anti-CD20

Rituximab, a mouse–human chimeric monoclonal anti-CD20 antibody, was initially been approved by the Food and Drug Administration (FDA) of the US for treatment of refractory or relapsed B-cell lymphoma in 1997. This drug was subsequently been approved by
the FDA in the treatment of RA in 2006. It has recently been widely used off-label in the treatment of SLE patients with refractory disease with or without concomitant use of cyclophosphamide (CTX) with promising results. Rituximab causes depletion of B-cells from peripheral blood more rapidly and efficiently than those in the lymphoid tissue.\(^{21}\) The mechanisms of action of rituximab involve complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity and stimulation of the apoptotic pathway.\(^{22}\)

A systematic review of off-label use involving 188 SLE patients revealed an overall clinical response of 91% in rituximab-treated patients.\(^{23}\) The largest single-centre cohort of rituximab use in SLE involved over 50 patients using two infusions of 1000 mg 2 weeks apart, in combination with high-dose glucocorticoids with or without CTX.\(^{24}\) All treated subjects had pronounced B-cell depletion, improved British Isles Lupus Assessment Group (BILAG) disease activity score, decreased anti-dsDNA antibody and increased C3 level. At 6 months, 42% and 47% of patients had major and partial remission, respectively. Only 11% of patients did not respond to treatment. B-cell repopulation occurred at a median of 6 months. Sustained depletion of circulating CD19+ B-cells beyond 12 months post-treatment was observed in approximately one-third of patients. Protective antibodies including tetanus and pneumococcal antibodies from previous vaccinations were not affected by rituximab.\(^{25}\)

Apart from depletion of peripheral B-cells, rituximab has also been shown to deplete CD20+ cells in the kidneys in patients with active refractory lupus nephritis, accompanied by improvement in various clinical parameters.\(^{26,27}\) Rituximab has also been suggested to restore normal peripheral B-cell homeostasis\(^{28}\) and extend its immune effect to other immune cells. B-cell depletion by rituximab is accompanied by decrease in activated peripheral T-cell populations\(^{29}\) and increase in FoxP3 expression of regulatory T-cells.\(^{30}\) However, two phase III clinical trials involving patients with severe lupus (EXPLORER) and patients with Class III or IV lupus nephritis (LUNAR) did not meet the primary endpoints of significantly reducing disease activity at 52 weeks.\(^{31}\) The phase III clinical trial on ocrelizumab, a humanized anti-CD20 monoclonal antibody for patients with active lupus nephritis\(^{32}\) has been stopped prematurely because of an increased rate of serious infections reported in the ocrelizumab arm compared to placebo.

### Anti-CD22

Epratuzumab is a humanized anti-CD22 monoclonal antibody that was originally developed for the treatment of non-Hodgkin’s lymphoma and was found to have a very good safety profile. Unlike rituximab,
Epratuzumab induces B-cell modulation instead of causing significant B-cell depletion. The mode of action of epratuzumab involves antibody-dependent cell cytotoxicity as well as induction of signal transduction via the inhibitory CD22 membrane molecule, causing down-modulation of B-cell receptor signaling. This agent has been shown to partially deplete B-cells and to target preferentially naïve and transitional B-cells. In an open-label trial, epratuzumab has been shown to reduce peripheral B-cell levels by approximately 30%–50% and to improve British Isles Lupus Assessment Group (BILAG) disease activity score. Epratuzumab is currently undergoing a phase III trial in the treatment of patients with active SLE.

ANTI-CYTOKINE THERAPY
Anti-BAFF therapy
Targeting B-cells by depriving important differentiation and survival factors such as BAFF and APRIL offers alternative B-cell targeted therapy. BAFF can be targeted by anti-BAFF monoclonal antibody or BAFF-R fusion protein. Belimumab, a monoclonal antibody that targets BAFF, has been shown to decrease CD20+ B-cells by 63%–71% and reduce anti-dsDNA levels by 29% in a phase II clinical trial. Reduction of SLE disease activity after treatment by belimumab was more significant among serologically active SLE patients. In a recently reported phase III trial, belimumab was demonstrated to improve disease activity measured by Safety of Estrogens in Lupus Erythematosus National Assessment – Systemic Lupus Erythematosus Disease Activity Index (SELENA–SLEDAI), reduce disease flares and stabilize SLE among these patients with serologically active lupus.

Briobacept (BR3-Fc) is a recombinant fusion protein with two BAFF receptors linked to the Fc domain of human IgG1. BR3-Fc attenuated the lupus-like disease with decreased anti-dsDNA antibodies, diminished proteinuria and ameliorated glomerular changes in a lupus mouse model. Data on clinical trials using BR-3Fc has not yet been published.

TACI-Ig that inhibited both BAFF and APRIL has been demonstrated to result in improvement in proteinuria and prolong survival in murine lupus. Atacicept (TACI:Fc5), a recombinant fusion protein of the TACI receptor and human IgG1, has been used to treat patients with mild to moderate SLE in a phase I trial. Treatment with atacicept resulted in reduction in peripheral mature B-cells by 45%–60% and was well tolerated. It remains to be elucidated whether the broader effect of atacicept against both BAFF and APRIL would be translated into better clinical efficacy in subsequent phase II/III clinical trials.

B-CELL TOLEROGENS
The LJP394 compound (abetimus sodium, Riquent) has been evaluated as a B-cell tolerogen in SLE patients with histories of lupus nephritis, aiming to prevent renal or other major SLE flares. It is a synthetic agent made up of four double-stranded oligonucleotides attached to a polyethylene glycol platform that binds avidly to anti-dsDNA antibodies and cross-links B-cell receptors specifically on B-cells recognizing dsDNA, thus causing anergy or deletion of these anti-dsDNA antibody-producing B-cells. Early trials showed impressive safety data and potential efficacy in a subset of patients who had elevated anti-dsDNA antibodies. There was dose-dependent decrease in anti-dsDNA antibody levels in SLE patients treated with abetimus. However, its phase III trial was terminated abruptly after an interim analysis failed to reveal clinical benefit overall in SLE patients.

INHIBITION OF CO-STIMULATORY MOLECULES
Direct inhibition of B-cell–T-cell interaction via the CD40–CD40L pathway has been shown to reduce inflammation, vasculitis and fibrosis in lupus nephritis and prolong survival in the murine lupus model. Humanized anti-CD40L (BG9588) monoclonal antibodies have also been shown to decrease IgG anti-dsDNA antibody, increase C3 and decrease hematuria. However, this phase II open-label study was terminated prematurely because of thromboembolic events occurring in patients receiving the monoclonal antibodies.

Abatacept, a fusion protein of the extracellular domain of CTLA4 and immunoglobulin constant region, has been used to block co-stimulatory molecules including the CD28, CTLA4 receptors and B7 molecules on the B-cell surface. This drug has shown promising results in murine SLE by reducing proteinuria and prolonging survival. A phase III clinical trial in human SLE is currently underway.

CONCLUSIONS AND PERSPECTIVES
The preliminary results of a number of clinical trials involving B-cell-targeted therapy were negative and...
reported failure to meet the primary clinical endpoint of improvement in disease activity measured by various composite disease activity indexes. It is appreciated that the main impediment to clinical trials in SLE is the heterogeneity of the disease. This heterogeneity is observed over a wide spectrum of clinical manifestations, different genetic makeup of the affected individuals and a variety of pathophysiological mechanisms involved in different patients. Thus, clinical efficacy of rituximab is influenced by polymorphisms of FcγRIIa and FcγRIIIa which may account for incomplete B-cell depletion in some lupus patients. The elevated serum BAFF level found in 20–40% of SLE patients suggested that BAFF may not be the only pathogenic mechanism for all lupus patients.

Examination of B-cell phenotype in patients treated with B-cell-based therapy provides more information in regard to abnormal B-cell homeostasis in SLE patients. It is appreciated that some treatment agents demonstrated clinical efficacy in the absence of significant decrease in serum anti-dsDNA antibody levels, whereas other therapeutic agents lower anti-dsDNA antibody levels without accompanying clinical efficacy. Indeed, the pathophysiology involving B-cells in SLE extends beyond production of pathogenic autoantibodies and is likely related to their antigen-presenting function or cytokine production.

The persistence of high levels of anti-dsDNA antibodies in B-cell-depleted patients suggested that these pathogenic antibodies may also be produced by long-lived plasma cells in addition to short-lived plasma cells that are more readily eradicated by B-cell depletion therapy. It is possible that these clones may reside in the bone marrow as well as in other survival niches, thus are more resistant to B-cell depletion compared to peripheral B-cells. Indeed, B-cells from autoimmune mice appear to be more resistant to depletion with monoclonal antibodies than their non-autoimmune counterparts. SLE patients who had circulating B-cells which bear the phenotype of germinal center cells and plasmablasts were likely to have incomplete B-cell depletion. It remains to be elucidated whether resistance to treatment in these patients is contributed by ongoing exuberant germinal centre reactions, intrinsic resistance of these cells or anomalous co-stimulatory signals that favor B-cell survival.

Although clinical data on B-cell-based therapy are still limited, the findings reviewed point to a growing optimism for targeting B-cells in SLE. The accumulated evidence suggests that B-cell-based therapy with conventional immunosuppressive agents can be of therapeutic value in patients with severe disease. However, questions remain to be answered. First, anti-dsDNA antibody level is no longer a reliable biomarker for lupus disease activity in the setting of biologic clinical trials, posing a need for development of other biomarkers. Measurement of autoreactive VH4.34 B-cells has been postulated to be an additional and powerful biomarker of tolerance. Second, current data suggest that complete B-cell depletion is not necessary for clinical response, thus the level of B-cell depletion necessary for sustained clinical response with full restoration of B-cell tolerance and normalization of other immune compartments remains to be defined.

In conclusion, other than production of pathogenic autoantibodies, B-cells play an important role in lupus pathogenesis by presenting autoantigens to T-cells, expressing co-stimulatory molecules for other autoreactive cells, secreting chemokines and cytokines and regulating T-cell activation and functions of dendritic cells. Further research into the abnormal B-cell homeostasis in SLE and identification of patients presenting with distinct pathogenic mechanisms may provide more promising B-cell-based therapeutic strategies for treatment of these patients.

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