Austin Publishing Group

B lymphocytes in Autoimmune Rheumatic Diseases: Pathogenesis to Treatment

Reem Hamdy A Mohammed

Department of Rheumatology and Rehabilitation, Kasr Alainy School of Medicine, Cairo University, Egypt

***Corresponding author:** Reem Hamdy A Mohammed, Department of Rheumatology and Rehabilitation, Kasr Alainy School of Medicine, Cairo University, Egypt, Email: rmhamdy@yahoo.com

Published Date: May 04, 2015

ABSTRACT

Autoimmune diseases represent a set of disorders of indefinite etiology. In such category of immune disorders, the immune system of a genetically susceptible individual encounters a potentially pathogenic external trigger that initiates the spark for breakdown of tolerance to self antigens provoking a self directed immune attack. The immune-pathogenic constructs in many of the recognized autoimmune diseases appear quite heterogeneous, certain diseases are predominantly B cell driven while others are primarily T cell driven and many undoubtedly represent a combination of both. The knowledge of the exact nature of the initial drive in these diseases is crucial for designing an effective therapeutic strategy. The role of B cells in adaptive immunity encompasses a vast array of immune-stimulatory as well as immune- regulatory responses passing from the secretion of autoantibodies to autoantigen presentation, reciprocal interactions with the T cells, secretion of pro-/anti-inflammatory cytokines and the generation of ectopic germinal centers with chronic inflammation. A hyperactive B cell status with defective regulatory functions can therefore facilitate break down of immune tolerance. A large body of experimental evidence validates the potential effects of B-cell depletion therapies in multiple autoimmune diseases. B cell depletion therapeutic strategy has been successfully employed in a number of autoimmune diseases. Many of these diseases are classified as typically of B-cell in

origin including systemic lupus erythematosus (SLE), idiopathic autoimmune thrombocytopenia, dermatomyositis and vasculitis, with others being classified as T cell driven diseases in which B lymphocytes are considered as prime movers like rheumatoid arthritis [1-7].

Contents: 1. Effector role of B lymphocytes; 2. The B lymphocytes and self tolerance; 3. B cell routes of immune-stimulation and autoimmunity; 4. B-cell depletion; 5. Regulatory Function of B lymphocytes; 6. B cell depletion biologic strategies.

Keywords: Autoimmune diseases; B lymphocytes; Immune tolerance; Antibody dependent cell mediated cytotoxicity; Antibody independent signaling pathway; Reciprocal T-B cell co-stimulation; B cell Targets-B cell depletion.

THE EFFECTOR ROLE OF B LYMPHOCYTES

The B lymphocytes possess a number of effector functions that have been proven to stand a pivotal role in the maintenance of immune competence and homeostasis. They feature the humoral arm of the immune response by being the initiator and moderator of antibody dependent cell mediated responses. At the same time the B cells are capable of playing an antibody independent role providing synergy to T cell mediated arm of the immune response.

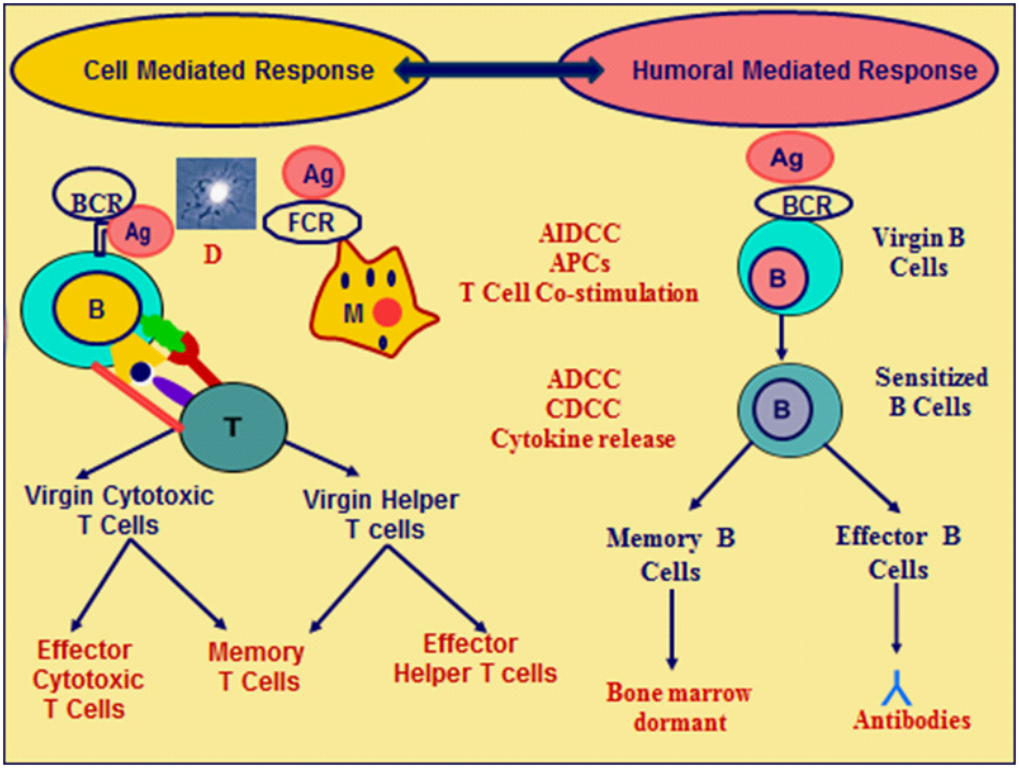

Figure 1: The effector role of B lymphocytes in the immune response.
BCR= B cell receptor, FCR= F C Gamma receptor, Ag= antigen, M= macrophage, D= dendritic cells, AIDCC= antibody independent cell mediated cytotoxicity, APCs= antigen presenting cells, ADCC= antibody dependent cellular cytotoxicity, CDCC= complement dependent cellular cytotoxicity.

Antibody Dependent Cell Mediated Cytotoxicity ADCC

The initiation of the humoral response involves distinct sequential phases of B cell differentiation and activation. Naive B cells bear millions of distinct surface antigen-specific receptors. The initial antigen recognition phase starts when clusters of the mature B lymphocytes expressing BCR (antigen specific IgM and IgD surface immunoglobulins) encounter their cognate antigen, this binding sparks signals that lead to further clustering (up-regulation) of antigen receptors with stimulation of B lymphocyte activation. B cell activation ends up by the subsequent formation of effector antibody secreting B cells and memory B cells. The antigen antibody binding activates the complement pathway with complement fixation, chemotaxis, phagocytosis and the formation of immune complex deposits in the tissues. In situations where the B cell surface receptor fails to encounter its cognate antigen, the B cell undergoes apoptosis. The majority of the antibody producing B lymphocytes stay in the circulation targeting the provoking antigen, whilst some of these antibody producing plasma cells migrate to the bone marrow and remain in-situ for several years providing a long lived source of antibodies following clearance of the provoking antigen. Bone marrow resident antibody producing B lymphocytes contribute to almost 50% of the immunoglobulins pooling into the circulation upon antigenic stimulation. Pre-activation of the circulating B lymphocytes via their respective cognate antigen results in up-regulation of the co-stimulatory molecules a prerequisite for facilitation of antigen uptake and successful antigen presentation for immune stimulation of the T cell responses [8,9].

The Antibody Independent Signaling Pathway

The antibody independent drive of B lymphocytes is another cornerstone in immune dysregulation in the territory of autoimmune diseases. The antibody independent role has been highlighted by some evidences in experimental and human studies: 1) In mouse models of lupus, the B cells were found critical to the development of disease even when they were unable to secrete autoantibodies. 2) The efficacy of B-cell depletion was found to be dissociated from changes in levels of autoantibodies in some autoimmune diseases; 3) The most compelling efficacy data for B-cell depletion occurs in diseases that were traditionally viewed as T-cell driven like Rheumatoid Arthritis and Multiple Sclerosis. The antibody independent signaling pathway encompasses a network of co- stimulatory events involved in programming of immune dysregulation passing from antigen presentation to cytokine production and T-B lymphocytes co-stimulation creating a positive feedback route [9-19].

Reciprocal T-B cell Co-Stimulation

The activation process of the T lymphocytes requires two signals:

A. An initial signal that involves engagement of the TCR on CD4 T helper cells to specific antigenic peptides presented in an MHC restriction response pattern on APCs. Engagement of the MHC-peptide complex to TCR on CD4 T-helper cells stimulates cytokine release with the resultant reciprocal stimulation of B cell proliferation and differentiation into plasma cells.

B. Second, synergistic co-stimulatory signals that sustain and integrate TCR signaling arranging for optimal T cell proliferation and differentiation.

Reciprocal T-B Co-stimulation: The definition of co-stimulation encompasses a series of immune events starting by an initial ligand-receptor interaction at the surfaces of a responder lymphocyte followed by the action of an "accessory" cell which is the antigen presenting cell APC represented by the dendritic cells, the activated macrophages acting as the first line APC with the activated B lymphocytes featuring the more specific activation signal for the T cells. Following the binding of the antigenic epitopes to the Ig receptors on the surface of B cells, the antigenic proteins gets internalized into the endosomal vesicles where they get processed and their peptides get presented to helper T cells at the surface in corporation with MHC II molecules. Thus activation of B lymphocytes sparks more antigen specific co-stimulatory signals to the primed T lymphocytes for their further activation. The memory B cells (previously described as poor APC) recently described as CD19-CD27-CD80. B cells are currently regarded as an effective rapid responder being capable of initiating immediate and robust memory effector responses potentially capable of T cell stimulation [20].

Such reciprocal interactions require the presence of the so-called co-stimulatory molecules. These are surface molecules that modulate cell to cell signaling and they have the capacity to either up or down-regulate immune responses by stimulating release of a number of pro-inflammatory or regulatory cytokines. The CD28 receptor is one of the best characterized T cell co-stimulatory molecules, which binds to two costimulatory molecules, B7-1 (CD80) and B7-2 (CD86). CD28 is constitutively expressed on 95% of CD4+ T cells and 50% of CD8+ T cells in humans. B7-1 and B7-2 are expressed mainly on APCs, including dendritic cells, macrophages, and B cells. The expression of B7-1 and B7-2 on APCs is enhanced by the presence of microbes and by cytokines that are produced in response to microbes. This regulated expression of B7 co-stimulators ensures that T cells respond best only when necessary - that is, when faced with pathogens [21].

THE B LYMPHOCYTES AND SELF TOLERANCE

Immune Tolerance refers to reduction or absolute inhibition of the individuals' ability to develop a reactive immune response to an antigen (self/foreign). Immune tolerance can be natural or acquired. Natural Immune Self-tolerance aims to abort any attempt at immune attack of self-antigens [22].

Tolerance starts in the bone marrow and is perfected by the peripheral lymphoid tissue. The currently identified mechanisms involved in the induction and maintenance of this tolerance include: clonal deletion/anergy, receptor editing and receptor down-modulation [23,24].

Clonal Deletion describes the process of maturational arrest and follicular exclusion of auto-reactive developing B cell population leading to premature death.

Along their course of development, the B lymphocytes encounter a number of checkpoints at

which the auto-reactive ones are effectively eliminated to preserve natural immune tolerance. Following the elimination of auto-reactive B cells by the bone marrow, the immature B cells migrate to the spleen, where they may encounter autoantigen not present in the bone marrow. B cells with high avidity to autoantigen are deleted (clonal deletion), while those having low to very-low avidity interactions progress to clonal anergy or ignorance, respectively. Anergy is defined as the inability of chronically stimulated auto-reactive cells to respond to further antigenic stimulation.

In contrast to clonal deletion and anergy there comes the clonal selection. In such case an encounter with a foreign antigen triggers the migration of the B lymphocytes to the T-cell zone of the germinal centers where they get activated by antigen-specific CD4+ T cells. During the ensuing rapid proliferation phase B cells undergo somatic hyper-mutation predominantly of the variable regions of their immunoglobulins. Only those B cells that express antibodies with increased affinity are selected (clonal selection) to survive and exit the germinal centers as antibody producing plasma cells or memory cells [25-33].

Receptor editing is a process of ongoing gene rearrangement in a BCR that already has a productive heavy or light chain gene rearrangement, successful editing converts the specificity of a self-reactive antibody into a non-self-reactive antibody. This step takes place within the bone marrow during early stages of B cell development [34].

Receptor Down-regulation involves down-regulation of the surface Ig D and Ig M antibody on the surface of developing B lymphocytes decreasing their avidity to autoantigens. Receptor dilution is another mechanism that aims at decreasing surface antibody expression either partially or totally via intracellular sequestration of the auto-reactive receptors and co-expression of two light chains [28,35].

The altered expression and/or down regulation of certain B cell surface receptor molecules have been associated with breakdown of immune tolerance like FcϒRIIb and complement receptor (CR) 1 (CD35) which is a powerful inhibitor of both the classical and the alternative pathway C3- and C5-convertases, due to its decay accelerating capacity and co-factor activity for C3b and C4b cleavage [36].

B CELL ROUTES OF IMMUNE-STIMULATION AND AUTOIMMUNITY

The initiation and perpetuation of an autoimmune response proved to be clearly multifactorial, while the breakdown of tolerance initiates the spark, the intensity of the response depends upon other variables including, the number of antigen presenting cells, the number and activity of regulatory T cells and regulatory B cells, the nature and amount of antigenic peptides generated, the intensity of the immune complex deposits and the presence of co-stimulatory signals.

The exact trigger of B cell hyperactive immune status phenomenon remains undefined. Theories include: (a) intrinsic hyper-reactivity leading to polyclonal B-cell activation with disturbed activation thresholds and ineffective negative selection, (b) lack of immune-regulatory

functions, (c) secondary effects of an overactive inflammatory environment, such as overactive germinal center and ectopic follicular activity, and/or (d) disturbed cytokine production by non-B immune cells.

B Lymphocytes and the Breakdown of Self Tolerance

Breakdown of tolerance represents the principal drive towards self-immune attack. Evidences of defective tolerance have been illustrated in a variety of ARDs like rheumatoid arthritis, systemic lupus erythematosus, Sjogren's syndrome and vasculitis.

The etiology appears to be multifactorial with a number of potential triggers mostly genetic alterations (mutations) being proposed, some of these mutations had been identified in experimental models only while others displayed similar findings in human disease [37-39]. Identified forms of some genetic mutations include:

(1) Impaired negative selection at the immature B cells stage: Experimental studies have shown that mutations located within the Sle1 locus in mice impaired negative selection of auto-reactive B cells at the immature B-cell stage with subsequent breakdown of tolerance [40].

(2) Increased B-cell signaling by overexpression of BCR signal-enhancing molecules or down-regulation/deficiency of molecules inhibiting BCR signaling: CD19 is a B-cell surface molecule that decreases the threshold for BCR stimulation. Experimental studies have demonstrated an association between the overexpression of CD19 and the increase in the levels of serum antibodies and B-cell activation in mice. A process that is reversed after loss of CD19 [41,42].

Deficiency of molecules that inhibit BCR-signaling, such as SHP-1, Lyn, or FcRIIB, enhances B-cell signaling and provokes autoimmune diseases in experimental animals. Similar findings have been illustrated in humans where the B cells from patients with lupus were found to express lower levels of FcRIIB on their surface due to polymorphisms in their FcRIIB promoter or the receptor itself [43-48].

(3) Somatic Hyper-mutations: Under normal circumstances the autoimmune B cells may either not receive necessary survival signals or be eliminated. During affinity maturation the massive somatic hyper-mutations can also cause the inadvertent development of auto-reactive immunoglobulins and accumulation of autoreactive B cells as reported in ARDs [49].

(4) Impaired Apoptosis of auto-reactive B cells: B-cell activation factor (BAFF) and its' homologue APRIL are B-cell survival factors. Experimental studies demonstrated that overexpression of BAFF was associated by expansion of peripheral B cell compartments with higher autoantibody levels and the development of a lupus-like disease in the animals [28]. Elevated serum levels of BAFF have been found in patients with rheumatoid arthritis, systemic lupus erythematosus, and primary Sjörgren's syndrome. These observations make BAFF a potential target for therapy. Neutralization of BAFF was shown to be associated with loss of mature B cells and reduced symptoms of autoimmune diseases in animal models [50-53].

BCR on B Cells

The BCR plays a pivotal role in maintenance as well as breakdown of tolerance. The antigen specific BCR-mediated uptake is 10-100 folds more efficient than pinocytosis which enables the B lymphocytes to function efficiently as APC even at low antigen concentrations. This Ag specific receptor molecule has the potential to boost or shield certain protein determinants from the proteolytic attack in endocytic compartments to modulate antigen processing and thereby the nature of MHC-displayed T-cell determinants. The binding of certain autoantigens to the BCR on immature B cells might induce apoptosis, receptor editing, or developmental arrest on one hand. On the other hand, the binding of BCRs to self-antigens can result in ineffective negative selection with positive selection of auto-reactive B cell population promoting their MHC restricted presentation to T cells facilitating breakdown of immune tolerance and provoking autoimmunity. Interestingly, experimental studies have illustrated that conditional ablation of the BCR might result in rapid B cell death. Incorporation of BCRs with auto-reactive potential into the memory compartment represents another route by which the BCR predisposes to autoimmune disease. Such memory B cells are high affinity, long-lived B cells that can rapidly differentiate into Ab-forming cells (AFC). They produce Abs of the stable IgG isotypes, they are not easily tolerated and recently proven to play a principal role in self-directed immune responses [54-57].

Formation of Ectopic Germinal Centers

B cells aid in the de novo generation of ectopic germinal centers (GCs) within inflamed tissues that can be observed during periods of chronic inflammation. These ectopic structures are probably not a unique disease-specific occurrence, but a consequence of chronic inflammation. Activated T and B cells that infiltrate the site of chronic inflammation express membrane-bound lymphotoxin $\alpha\alpha 1\beta\beta 2$ ($LT\alpha\alpha 1\beta\beta 2$). High levels of $LT\alpha\alpha 1\beta\beta 2$ eventually promote the differentiation of resident stromal cells into follicular dendritic cells (FDCs) and the development of ectopic GCs. These structures are similar to the GCs of secondary lymphoid organs and have been described in systemic lupus erythematosus, Hashimoto's thyroiditis, Graves' disease, rheumatoid arthritis, Sjögren'ssyndrome. The function and potential pathogenic role of ectopically formed lymphoid structures within inflamed tissues remains unclear. However, plasma cells residing within the ectopic GCs secrete autoantibodies making it plausible that ectopic GCs have a role in the maintenance of immunepathology [58-61].

B-CELL DEPLETION

The B lymphocytes pass into multiple developmental (transitional) stages with variable potentials ending up by the formation of mature B cells and antibody producing plasma cells. During their maturation B cells undergo two key processes: the generation of functional Ag-specific receptors and the selection of lymphocytes that express useful Ag receptors.

B-lymphocyte development, which takes 2 to 3 days, requires the concerted action of a network of cytokines and transcription factors that positively and negatively regulate gene expression.

Each transitional stage is marked by specific surface molecules, some of these molecules stand a promising interventional target in the B cell depletion strategy, for example the germinal Center B cells are denoted as IgD-, CD38++, CD10+ and CD27++ whereas, the splenic marginal zone B lymphocytes are typically CD27+, CD21++, CD23+/-, CD1c+ and IgD- [62-63].

Table 1: The different stages of B cell maturation and their surface markers.

Marker	Immature B cells	Transitional Stages			Naive B cells	CD27+ Memory B cells		
		T1	T2	T3		Un-switched	Switched	Plasma Cells
CD27	-	-	-	-	-	+	+	High
CD19	+	+	+	+	+	+	+	Low
CD10	+	+	+/-	-/+	-	-	-	+/-
CD24	+++	+++	++	+	+	++	++	-
IgD	-	+	++	++	++	+	-	-
IgM	++	+++	++	+	+	+	-	-
R123	+	+	+	Int	-	+	+	+
CD38	+++	+++	++	+	+	low	low	high

B cell depletion is a process directed at either elimination of B cell by arresting its' development, suppression of survival. The B cell depletion theories established a novel therapeutic strategy not only in B cell mediated ARDs but also in T cell mediated diseases in which the B cells act as prime movers [62-63].

Potential Targets for B Cell Depletion Strategy

The B-cell targeted therapy can be categorized into four main classes: a- neutralization of survival factors BAFF and APRI, b- killing of B cells using monoclonal antibodies directed to CD19, CD20, and CD22, c- induction of apoptosis using reagents targeting the BCR itself or BCR associated transmembrane signaling proteins such as CD79, d- ablation of the formation of ectopic GCs by antibodies against lymphotoxin- receptor (LTR) [64-69]. In the territory of autoimmune diseases, neutralization of survival factors BAFF /APRIL or B cell depletion using monoclonal antibodies directed to CD19, CD20, and CD22 are the most commonly evaluated in majority of researches and trials.

B lymphocyte survival factor

BAFF (B lymphocyte survival Factor, BLyS) is a member of the tumor necrosis factor (TNF) family and an essential component of the innate immune response. BAFF is induced in myeloid DC by type I interferons (IFNs), it is expressed on the surface of monocytes, dendritic cells (DC), neutrophils, stromal cells, activated T cells, malignant B cells and epithelial cells.

BAFF binds to three different B cell surface and transmembrane receptors, BAFF-R, TACI (transmembrane activator and calcium modulator and cyclophilin ligand interactor) and BCMA (B cell maturation protein). These receptors are expressed differentially during the various stages of B cell development and maturation.

Role of BAFF in humoral and cell mediated immune responses

- BAFF enhances long-term B cell survival primarily by up-regulating anti-apoptotic proteins provoking a prompt response of B cells to BCR activation.

- BAFF and its homologue APRIL (A proliferation-inducing ligand) additionally enhance the survival of plasma cells that express TACI and/or BCMA.

- BAFF up-regulates Toll-like receptor (TLR) expression which promotes B cell survival and, together with IL-6, promotes Ig class-switching and plasma cell differentiation.

- In the territory of cell mediated immunity, T cell-independent type II responses require the interaction of BAFF 60-mer or membrane BAFF with TAC. This interaction is vital for T cell-dependent immunoglobulin (Ig) M responses.

In contrast, survival and reactivation of the antibody producing memory B cells are BAFF-independent.

Soluble BAFF and APRIL are expressed at high levels in the sera and the target organs of patients with antibody dependent autoimmune diseases [69-73].

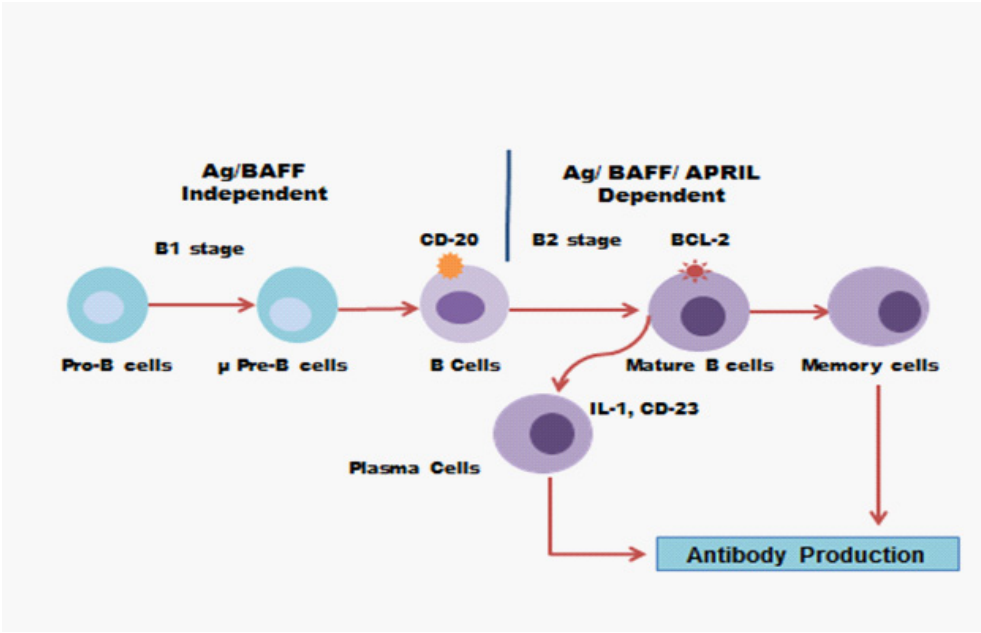

Figure 2: BAFF and the development of B lymphocytes [73].

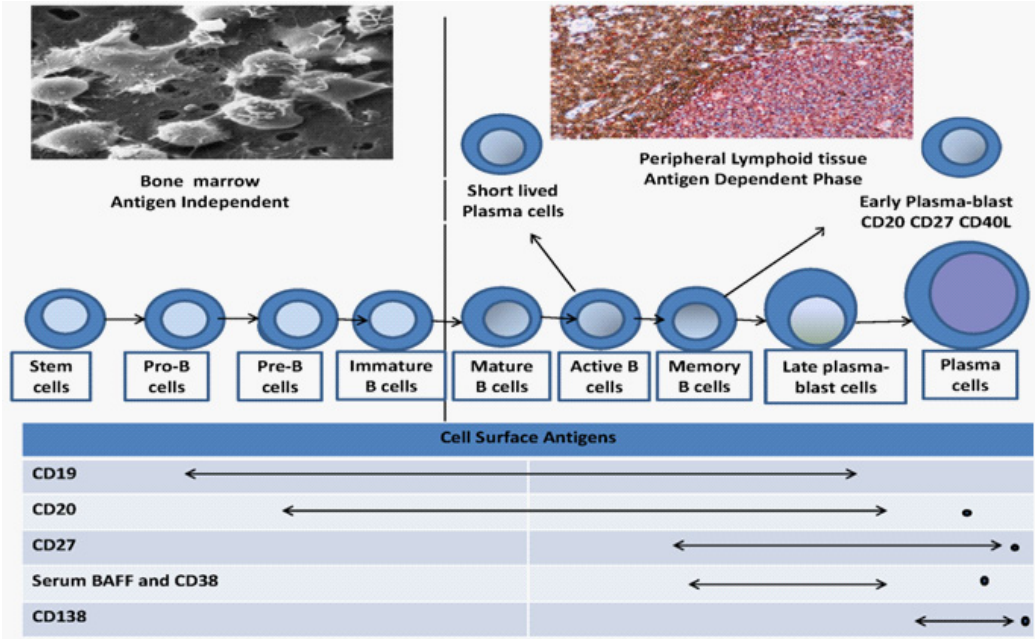

Figure 3: The different developmental stages of B lymphocytes with potential cell surface target molecules [73].

B cell surface molecules

CD-20 cell surface molecule

A 297-amino acid activated glycosylated trans-membrane phosphoprotein specifically expressed on the surface of B cells, starting at the early pre-B cell stage and persists until the differentiation of B cells into plasma cells. CD-20 is not expressed on hematopoietic stem cells, pro-B cells, or normal plasma cells. Plasma-blasts and stimulated plasma cells may express CD20. CD20 is co-expressed on B cells with CD19, another B cell differentiation marker. CD20 appears to play a crucial role in B cell development, differentiation, proliferation and cell-cycle regulation events. The CD-20 density on B cells appears to be important and highly correlates with complement dependent cytotoxicity (CDC). CD20 may act as a signaling molecule to trigger apoptosis and complement fixation when engaged with CD20 mAb. Overexpression of the CD-20 antigen has been illustrated in B cell mediated disorders with clonal B cell expansion including lymphomas, leukemias and ARDs in variable densities [73-77].

CD-22 cell surface molecule

CD22 is a 135-kDa trans-membrane sialoglycoprotein, a member of the immunoglobulin superfamily. Its expression is restricted to lymphocytes of the B cell lineage. CD22 is present in the cytoplasm of pro- and pre-B cells and becomes detectable on the cell surface only at mature

stages of B cell differentiation and is lost during terminal differentiation into plasma cell and after B cell activation.

The CD22 molecule has multiple ligands because it binds to α2–6-linked sialic acid residues present on glycoproteins expressed by activated T and B cells, monocytes, neutrophils, erythrocytes, and activated endothelial cells.

The exact role of this surface molecule remains unrevealed. In vitro studies demonstrated some positive and negative roles for CD-22 in the regulation of B cell activation through BCR signaling and cell adhesion. In vivo experimental studies on genetic disruption of CD22 revealed some important biological functions suggesting a key role for CD22 in B cell development, survival, and function. CD22-deficieny was associated with a shorter life span, a reduced number of mature B cells in the bone marrow and in the circulation, and a chronic exaggerated antibody response to various auto-antigens [78-80].

CD-19 cell surface molecule

CD19 is a B-cell specific membrane protein that is broadly expressed during B-cell development, from the pro-B cell to the early plasma cell stage. Although CD19 and CD20 mAb share common effector mechanisms, therapies targeting CD19 might offer several unique advantages for the treatment of RA compared with currently available CD20-directed immunotherapies [44].

REGULATORY FUNCTION OF B LYMPHOCYTES

B lymphocytes might additionally exhibit the potential of suppressing immune stimulation with inflammation. They are capable of down regulating the immune response via the release of regulatory/ anti-inflammatory cytokines. Experimental studies on arthritis models have illustrated that certain B cell subsets particularly the ones derived from the gut lymphoid tissue have the potential to secrete the IL-10 and up-regulate the expression of CD1d with down-regulation of IL-1 associated inflammatory cascades and signal transducer and activator of transcription 3 (STAT3) activation.

The B lymphocytes can induce the differentiation of tolerogenic CD41 T cells via antigen presentation. They also mimic their sister T cells in their patterns of effector immune function. By revealing their immune regulatory potential the B cells can be defined as B effector 1 and 2 cells: the B effector 1 cells produce Th1-associated pro-inflammatory cytokines, including tumor-necrosis factor (TNF)-a, IFN-c and IL-12, whereas B effector 2 cells produce Th2-associated cytokines, including IL-4, IL-13, IL-10 or TGF-b that possess inhibitory functions in autoimmune diseases. Another possible hypothesis suggests that the removal of apoptotic tissues and circulating self-antigens by the B cell derived autoantibodies facilitates the rapid clearance of such autoantigens which may reduce the risk of autoimmune diseases in some situations [81].

B CELL DEPLETION BIOLOGIC STRATEGIES

B cell depletion strategies are currently achieved either via antibodies targeting the surface molecule CD20 (e.g., Rituximab and Ofatumumab) or antibodies targeting the B cell survival factors. Treatment with these antibodies depletes B cells by a combination of antibody-mediated cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and antibody-triggered apoptosis.

Anti-CD-20 Biologic Drugs

Rituximab

Rituximab, a chimeric human/mouse IgG1 antibody directed at human CD20, which is found on only pre-B and mature B cells. The principal B-cell-depleting activity involves antibody-dependent cellular cytotoxicity and, to a lesser extent, complement-dependent cytotoxicity. The drug is specifically directed to CD-20 surface molecule. It effectively and completely depletes B lymphocytes, yet this effect is transient. B cell depletion can be observed as early as 6-12 weeks after initiation of therapy with complete depletion by 6 months. B cell repopulation recovery time is variable and full recovery of the B cells might get delayed up to two years from initiation of therapy.

The drug has been approved by the food and drug administration in the USA and by the Committee for Medicinal Products for Human Use of the European Agency for the Evaluation of Medicinal Products in Europe, in 2006, for the treatment of refractory rheumatoid arthritis (particularly seropositive disease). A number of trials demonstrating potential success of different strategies of rituximab in RA have been published. RTX has shown efficacy in the treatment of biologic naïve patients with RA who failed to respond to conventional DMARDs therapy, in RA patients who were previously exposed to anti-TNF- therapy, and more recently in methotrexate and biologic naïve early RA (the drug is given as intravenous infusion of 375mg/m^2 x 2 over 2 weeks to be repeated every 6- 9 months). Follow up of B cell repopulation along the course of treatment provides an important investigational step prior to repetition of rituximab therapy [82-89].

Table 2: Randomized clinical trials with rituximab in patients with rheumatoid arthritis [82-89].

Author	No. Patients	Arms	RA	Primary Endpoint/ Follow-up	Clinical Outcomes	Remarks
Edwards et al., 2004	161	MTX (*n* = 40) or RTX (*n* = 40) or RTX + MTX (*n* = 40), or RTX + CYC (*n* = 41)	Anti-TNFα naive	ACR50 response/24 weeks	ACR50: 13 vs. 33 vs. 43 vs. 41	Only RF patients were included. MTX arm used very low doses (10 mg/week)
Cohen et al., 2006REFLEX trial	520	MTX + PBO (*n* = 209) vs. RTX + MTX (*n* = 308)	Anti-TNFα failure	ACR20 response/24 weeks and post-treatment 2 years	At week 24: ACR20: 18 vs. 51 ACR50: 5 vs. 27 ACR70: 1 vs. 12 EULAR moderate or good: 22 vs. 65	Most patients positive for RF (79%); non-significant differences at 6 months in radiographic progression. Significant reduction at 1 year
Emery et al., 2006 DANCER trial	465	PBO + MTX (*n* = 149) or RTX 2× 500 mg + MTX (*n* = 124) or RTX 2× 1000 mg + MTX (*n* = 192)	DMARD or anti-TNFα failure	Proportion of RF-positive patients who met the ACR 20%/24 weeks	ACR20: 28, 55 and 55, respectively. ACR70: 5,13, 20 EULAR good response: 4, 14, 28	No radiological outcomes measured. Better responses in patients not previously exposed to anti-TNFα
Finckh et al., 2007	116	RTX (*n* = 50) vs. alternative anti-TNFα (*n* = 66)	Anti-TNFα failure	Change from baseline of DAS-28 score/at least 6 months	Mean decrease in DAS-28: −1.61 in RTX vs. −0.98 in anti-TNFα	Treatment with RTX was more effective than a 2nd or 3rd anti-TNFα
Tak et al., 2009 IMAGE study	715	PBO + MTX (*n* = 232), RTX + MTX (2× 500 mg) (*n* = 239) or RTX + MTX (2× 1000 mg) (*n* = 244)	Early RA MTX naive	Change from screening in the mTSS at week 52/52 weeks	Mean change in mTSS at 52 weeks 1.08 vs. 0.65 vs. 0.36 ACR50: 41 vs. 59 vs. 64	RTX (2× 1000 mg) + MTX significantly improved clinical outcomes and inhibited joint damage compared with MTX alone. Published only in abstract form
Mease et al., 2010 SUNRISE trial	475	One open-label course of RTX. From week 24 RTX (retreatment group) vs. PBO	Anti-TNFα failure	ACR20 response at 48 weeks/48 weeks	ACR20: RTX vs. PBO 54 vs. 45%, mean change in DAS 28: −1.9 vs. −1.5	Patients with very high disease activity at baseline (mean DAS-28, 6.7). No differences in ACR50, ACR70, and EULAR responses in both groups

In Systemic Lupus Erythematosus (SLE)

In ARDs like SLE where the B lymphocytes master the scenarios of immune events, the concept to ameliorate the autoimmune phenomena by suppression of autoantibody production and thereby nullifying autoantibody-dependent effector mechanisms has served as the main rationale for the off label use of B-cell-directed therapies. The use of such therapy additionally supported the inhibition of B-cell-mediated processes such as antigen presentation, cytokine production and reciprocal activation of T cells.

Rituximab is an off-label biologic that can be used in patients with refractory SLE. The off-label use was first reported in 2002, after which the drug has been progressively used in SLE [89].

Multiple evidences from human and experimental researches have indicated that B lymphocytes

play a central role in the pathogenesis of SLE. Moreover, the efficacy of B-cell depletion usinganti-CD20 monoclonal antibodies in murine models of SLE has been demonstrated. In human studies, RTX successfully loweredCD20 B-cell levels in peripheral blood within days to weeks (an effect sustained for up to 6 months), reduced anti-dsDNA and anti-nucleosome antibodies, and reversed B-cell homeostasis abnormalities. Although RTX should not be used as first-line treatment in SLE or in patients with a predominantly mild form of the disease, the results of its off-label use in patients with severe, refractory SLE seems to be sufficiently positive to warrant its use in this subgroup of patients. Uncontrolled trials and case studies illustrated encouraging results except for two recent trials the EXPLORER and the LUNAR studies. The two RCTs addressed the hypothesis that the addition of rituximab to the standard of care, corticosteroids and immune-suppressants was superior to addition of placebo for the control of SLE activity, with insignificant differences [90-110].

Table 3: Reported efficacy of rituximab in some of the Non-randomised trials of systemic lupus erythematosus.

Study	Rituximab regimen	Organ-specific disease	Number of patients/follow-up (months)	Method of assessment (mean disease activity score before/after B-cell depletion)
Anolik and colleagues	Variable	No (7 LN)	17/12	SLAM improved in patients achieving effective B-cell depletion (6.8/5.2)
Cambridge and colleagues	2-dose	No (12/15 LN)	15/6	BILAG
Tokunaga and colleagues	Variable	Yes, NPSLE	10/7 to 45	Neurological parameters (GCS)
Reynolds and colleagues	Variable	No	11/10	BILAG (median reduction of 7.5)
Li and colleagues	2-dose	Yes, LN	19/12	SLEDAI (9.2/2.5)
Pepper and colleagues	2-dose + MMF maintenance	Yes, LN	20/12	Renal parameters improved in 14/18 at 12 months
Catapano and colleagues	4-dose (15) or2-dose + CYC (16)	No (11 LN)	31/30	BILAG (14.5/3.5 at 24 months)
Smith and colleagues	4-dose, retreated with 2-dose	No	11/24	BILAG (14/2)
Gunnarsson and colleagues	4-dose	Yes, LN	7/6	SLEDAI (15/3)
Galarza and colleagues	4-dose	No	43/12	SLEDAI (12.5/4.5)
Boletis and colleagues	4-dose	Yes, LN	10/38	Renal parameters
Melander and colleagues	4-dose regimen (10 retreated)	Yes, LN	20/22	12/20 improved

BILAG, British Isles Lupus Assessment Group; CYC, cyclophosphamide; GCS, Glasgow Coma Scale; MMF, mycophenolatemofetil; SLAM, systemic lupus activity measure; LN, lupus nephritis; NPSLE, neuropsychiatric systemic lupus erythematosus; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index. [a]Randomised controlled trial. [b]Same cohort in these studies.

Table 4: Comparing Randomized Controlled Trials [94,96,100].

Study	Rituximab regimen	Concomitant therapy	Endpoints	Results
LUNAR	Randomised 1:1 to receive either rituximab or placebo on days 1, 15, 168, and 182	MMF and corticosteroids	Primary: (i) % patients with complete or partial renal responses at week 52. Secondary: (ii) patients with BL UPCR >3 to UPCR <1; (iii) % change from BL in anti-dsDNA; and (iv) mean change from BL in C3 (mg/dl)	(i) and (ii) no significant difference; (iii) placebo (50%) and rituximab (69%) (P <0.01); and (iv) placebo (25.9%) and rituximab (37.5%) (P <0.03). % patients requiring a new immunosuppressive agent placebo (11.1%) and rituximab (1.4%)
EXPLORER	Randomised 1:2 to receive placebo or rituximab, methyl prednisolone 100 mg and acetaminophen and diphenhydramine or placebo on days 1, 15, 168, and 182	Usual dose prednisolone and either azathioprine 100 to 250 mg/day, MMF 1 to 4 g/day or MTX 7.5 to 27.5 mg/week, and additional prednisolone (0.5 mg/kg, 0.75 mg/kg, or 1.0 mg/kg), tapered beginning on day 16 to a dosage of 10 mg/day over 10 weeks and 5 mg/day by week 52	Primary: effect of placebo or rituximab in achieving and maintaining a major, partial or no response at week 52 in each of the eight BILAG index organ system scores. Secondary: described earlier	Primary EP: major clinical response 15.9% vs. 12.4% and PCR 12.5% vs. 17.2% for placebo and rituximab, respectively. In the African American/Hispanic group: major clinical response 9.4% vs. 13.8% and PCR 6.3% vs. 20.0% for placebo and rituximab, respectively
Li and colleagues	Randomised to receive either rituximab or a combination of rituximab and cyclophosphamide 750 mg on day 1 and day 15, followed by intravenous methylprednisolone 250 mg and oral prednisolone 30 mg from day 2 to day 5, then 0.5 mg/kg for 4 weeks and then reducing the dose by 5 mg every 2 weeks to 5 mg/day	Other medications were stopped except for hydroxychloroquine, oral prednisolone and statins. All patients also received angiotensin-converting enzymes inhibitors	Primary: in each of the groups, % patients with complete response at week 48. Secondary: % patients with partial response; and duration of complete $CD19^+$ B-lymphocyte depletion, histological assessment, adverse effects or death at week 48	Primary EP: no significant difference between the two groups. Overall, at week 48, 21% had a complete response, 58% achieved partial response, 11% remained the same and 11% worsened. Secondary EP: 42% patients achieved a complete response; 95% achieved effective depletion; no significant difference in the proportion of patients achieving a complete depletion at weeks 4, 8, 24 and 48 between the two groups except at week 2; a significant improvement in mean serum albumin levels (28.1 to 39.4), changes in the concentration of serum C3 (0.55 to 0.85), dsDNA antibody (693 to 8) and immunoglobulins. At week 48, the urinary protein excretion improved and there was an improvement in the ESR (62.1 to 30) and SLEDAI (9.2 to 2.5)

BL, baseline; EP, endpoint; ESR, erythrocyte sedimentation rate; MMF, mycophenolatemofetil; MTX, methotrexate; PCR, partial clinical response; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; UPCR, urine protein creatinine ratio.

Table 5: Possible Explanations for the apparent discrepancy between Real life-case studies DBRCTs [94].

	Clinical experience	Randomised controlled trials
Disease activity	Refractory to conventional immunosuppressants	Rituximab was used as an add-on therapy to background immunosuppressants
	Favourable response reported in life-threatening cases, often including a range of organ-system involvement such as CNS manifestations, cytopenias and others	Life-threatening cases and those with CNS manifestations were not evaluated in controlled trials. This setting warrants a dedicated study
Clinical response	No defined pretreatment, therefore complete and partial responders might not be clearly distinguished	Predefined endpoints were stringent, perhaps driven by the impressive responses seen in clinical experience in an uncontrolled setting
	Improvement in one system alone might qualify for response, regardless of a flare or lack of response in another organ system	Predefined and usually stringent. For example, despite clinical response and steroid-sparing effect, a reduction in proteinuria that does not meet the predefined threshold would not qualify as complete/ partial response
Background immunosuppressants	Flexibility in changes to background immunosuppressants including the dose of corticosteroids	Changes to or deviation with predefined background therapy would qualify as nonresponder
	Concomitant use of large dose of steroids is uncommon	Concomitant use of large dose of corticosteroids might have limited any beneficial effects of rituximab, the extent of which may be more restricted in such a setting than previously assumed
Rituximab dosing-regimen	Variable between reports	Predefined dosing regimen
Steroid tapering	Steroid-sparing effect is not a requirement to define response and therefore favourable response might be overestimated	Steroid dosing effect was included in the definition of clinical response
Adverse events	No standardised reporting of adverse events. Therefore, the true incidence of serious adverse events in clinical practice is not comparable with that reported in other uncontrolled studies or controlled clinical trials	Rituximab therapy appears to be safe as no there were no significant differences in serious adverse events when compared with standard-of-care treatment
Follow-up period	Not defined, therefore it is not known how many responders had sustained response in the long term	Predefined, therefore, unless long-term studies are undertaken, it would be difficult to detect the importance of effects seen at relatively short-term follow-up
CNS, central nervous system.		

In vasculitis

Rituximab (anti-CD-20 therapy) showed considerable efficacy in different subtypes of vasculitis especially cryoglobulinemic vasculitis, ANCA associated vasculitis and cutaneous vasculitis with connective tissue diseases including rheumatoid arthritis, systemic lupus erythematosus and Sjogren's syndrome. Rituximab can be considered as an effective alternative line of therapy in newly diagnosed as well as refractory ANCA-associated vasculitis. Wegener's Granulomatosis (granulomatosis with polyangiitis) patients with retro-orbital granulomas tend to be less responsive to rituximab therapy. A number of case reports and studies reported success of off label use of Rituximab in refractory ANCA associated vasculitis. The two major trials of rituximab in ANCA associated vasculitis the RAVE and RITUXVAS concluded that rituximab was non-inferior to other conventional immunosuppressive regimens in refractory as well as newly diagnosed cases.

The great limitation of rituximab use in some cases with GPA or microscopic polyangiitis might be the need for retreatment in the absence of well-defined consensus on the off label use of the drug in such cases to be retreated following initial treatment [111-118].

Rituximab showed efficacy in cases with HCV-mixed cryoglobulinemia. The rationale behind anti-B-cell therapy in mixed cryoglobulinemic vasculitis relies on the concept that the chronic stimulation by hepatitis C virus as a lymphotropic virus induces polyclonal B cell proliferation with the subsequent production of mixed cryoglobulinsby the infected B cells with subsequent formation of immune complex deposits. Rituximab was successfully used in combination with antiviral agents as well as mono-therapy in HCV cryoglobulinemic vasculitis. Rituximab combined with Peg-IFN-α/ribavirin delete both virus-dependent and -independent B-cell clones. Antiviral therapy alone decreased the memory B cells, whereas in association with rituximab, naive B cells are the main depleted population [111,119,120].

In sjogren syndrome

The use of B cell targeted therapy in Sjogren syndrome is restricted to a number of case studies and case reports with the drug used as an off label alternative to conventional therapy showing promising results. Several uncontrolled studies have reported successful off-label use of RTX in small series of patients (20 patients) with primary SS. The first open-label study was reported in 2005 and included 15 patients (including 7 patients with B-cell lymphoma) who received four weekly infusions of 375 mg/m^2 of RTX with a significant improvement in subjectivesymptoms and increased salivary gland function in patients with residual glandular function [120]. Another study by Devauchelle-Pensec and colleagues studied 15 patients with primary SS who received two weekly doses of RTX (375 mg/m^2). Depletion of peripheral B cells was complete in all patients after 12 weeks from the infusion therapy, without significant changes in the levels of natural killer, T helper, and cytotoxic T cells [121]. In a retrospective study of 16 patients with primary SS and systemic features, treatment with RTX was associated with decreased serum levels of RF, globulins, and 2-microglobulin [122]. B-cell depletion showed an inverse relationship with serum BLyS levels that was sustained up to 18 months in 3 out of 15 patients with primary SS treated with rituximab in the BIOGEAS register (multicenter Spanish register) [123]. Rituximab was found to improve sicca features, salivary flow, ocular tests, fatigue and quality of life in another two recent RCTs [125-126].

In inflammatory myopathies

Various small open-label studies have used RTX to treat severe, refractory inflammatory myopathies. RTX induced partial remission of muscular involvement in some cases. RTX stabilized and/or improved pulmonary involvement in 7 of 11 (64%) patients with anti-synthetase syndrome (presenting severe, progressive interstitial lung disease refractory to immunosuppressive agents) 6 months after treatment. The BIOGEAS Multicenter Study Group has included the largest series of patients so far including, 20 patients with inflammatory myopathies (11 with dermatomyositis, 4

with polymyositis, and 5 with antisynthetase syndrome), of whom 11 (55%) achieved a complete response, 6 (30%) achieved a partial response, and 3 (15%) were classified as non-responders. The therapeutic response was excellent for muscular (94%), cutaneous (80%), and pulmonary involvement (75%) with 40% relapsing after a mean follow-up of 19 months. RTX increased risk of serious infections in some of the studied patients with one death in the BIOGEAS from serious infection [90,124,127].

In systemic sclerosis

Rituximab had been evaluated in systemic sclerosis in three open label in addition to standard treatment. Rituximab administration in these trials showed improvements in Rodnan score, histological analysis of skin biopsies revealed a significant reduction in the myofibroblast score with elimination of B cells skin infiltrates with stabilization of pulmonary function tests after treatment. The potential efficacy of RTX in SSc associated pulmonary arterial hypertension has not yet been evaluated [128-130].

Ocrelizumab

Ocrelizumab is a humanized anti-CD20 mAb. The drug has been tried in patients with rheumatoid arthritis RA in combination with methotrexate (two regimens used: 200 mg and 500 mg ×2 every 6 months) and was effective in reducing signs and symptoms of uncontrolled inflammation and joint damage. The use of Ocrelizumab was associated by a significant increase in serious infections in four double blind randomized controlled trials DBRCTs raising concerns regarding safety versus efficacy in RA.

Ocrelizumab was investigated in two trials: the BEGIN study for non-renal SLE (cancelled early) and the BELONG study for proliferative lupus nephritis. The drug was administered using different regimen from those in RA at either 400 or 1,000 mg intravenously ×2 at trial entry with repeat, single dosing every 4 months. In the BELONG study greater treatment effects of ocrelizumab were observed when combined with the EUROLUPUS cyclophosphamideregime (renal response of 65.7% for ocrelizumab vs.42.9% for EUROLUPUS alone) than with mycophenolatemofetil (renal response of 67.9% for ocrelizumab vs. 61.7% for mycophenolate alone) with results showing adverse events when ocrelizumab was given with mycophenolate combination [94,131,132].

Table 6: Safety and efficacy of ocrelizumab in lupus nephritis: design and results of the BELONG study [94].

Patients and methods	Concomitant therapy	Endpoints	Results
A total of 381 patients with class III or class IV (80%) LN were randomised equally to receive either: placebo, OCR 400 mg or OCR 1,000 mg on days 1, 15 and every 16 weeks thereafter, >74% received three infusions and >50% received four infusions	In addition, either: MMF up to 3 g/day (63%); or EL (cyclophosphamide 500 mg ×6/2 weeks) followed by azathioprine 2 mg/kg up to 200 mg/day; and a steroid taper regimen - intravenous steroids: allowed up to 3 g by day 15, given in divided pulses), oral steroids: 0.5 to 0.75 mg/kg (≤60 mg/day) with taper to ≤10 mg over 10 weeks	Complete renal response: normal serum creatinine and ≤25% higher than baseline; urinary protein to creatinine ratio <0.5; inactive urinary sediment	In all modified intention-to-treat populations, there was a treatment difference of 12.2% with 54.7% vs. 66.9% for placebo (n = 75) and OCR (n = 148) groups, respectively
		Partial renal response: serum creatinine ≤25% above baseline value; and 50% improvement in the urine protein to creatinine ratio, and if baseline ratio >3.0 then a urine protein to creatinine ratio <3.0	ORR higher in OCR (400 mg) + EL (65.6%) and OCR (1,000 mg) + EL (74.2%) groups vs. placebo + EL (42.9%), ORR was similar in OCR+ MMF (67.9%) vs. placebo + MMF (61.7%)
		Nonresponse: not achieving either a complete or partial renal response. Patients who died or discontinued the study prior to week 48 (and had no renal data within 12 weeks of week 48) were considered nonresponders	≥50% reduction in urine protein-to-creatinine ratio occurred in 69.6% vs. 58.7 % for OCR and placebo groups, respectively
			Urine protein-to-creatinine ratio <0.5 was achieved in 39.9% vs. 37.3% for OCR and placebo, respectively
			Serious adverse effects imbalance appeared to be driven by the combination with MMF: OCR 400 mg (41.8%) compared with 1,000 mg OCR + MMF (24.1%) and placebo + MMF (21.3%). Serious adverse event rates in EL groups were not reported as higher in the OCR arms
			Serious infection imbalance appeared to be driven by the OCR combination with MMF. MMF groups: OCR 400 mg (32.9%) compared with 1,000 mg OCR (19%) and placebo + MMF (16.3%). EL groups: OCR 400 mg (12.8%) compared with 1,000 mg OCR (10.4%) and placebo + MMF (11.1%)

EL, EUROLUPUS regimen (cyclophosphamide followed by azathioprine); LN, lupus nephritis; MMF, mycophenolatemofetil; OCR, ocrelizumab; ORR, overall renal response

Ofatumumab

Ofatumumab is a human immunoglobulin (Ig) G1κ monoclonal antibody (mAb) that specifically binds to the human CD20 antigen inducing potent B cell lysis. Ofatumumab recognizes a membrane-proximal epitope on the human CD20 molecule, distinct from the epitope recognized by rituximab and other anti-CD20 mAb. In addition, Ofatumumab has a slower rate of dissociation from its' CD20 target epitope than RTX, which results in greater complement-dependent cytotoxicity with efficient lysis of RTX refractory B-cell lines. The drug is approved for the treatment of chronic lymphocytic leukemi is fractory to fludarabine and alemtuzumab. Clinical trials (phase/II) revealed that ofatumumab doses of 300 mg, 700 mg, and 1000mgadministered intravenously as 2 infusions 2 weeks apart demonstrated significant clinical benefit compared with placebo in patients with active rheumatoid arthritis (RA) who had an inadequate response to disease-modifying anti-rheumatic drugs (DMARD) [133].

In another single-blind, phase I/II study that aimed to investigate the safety and tolerability of a single subcutaneous (SC) dose of ofatumumab,35 patients with RA were randomized in 5 cohorts to receive a single subcutaneous (SC) ofatumumab dose ranging from 0.3 to 100 mg, or placebo, following premedication with oral acetaminophen and antihistamine. Patients were followed for 24weeks with extended follow up to monitor B cell and immunoglobulin recovery for up to 2 years if required. Treatment of RA patients with SC ofatumumab doses of 30 mg or higher resulted in profound and prolonged B cell depletion in blood. Single doses up to 60 mg were tolerated without glucocorticoid premedication. Infusion reactions resulting from rapid B cell depletion and cytokine release are commonly observed with IV administration of anti-CD-20 biologic drugs and may be severe. Approaches including increased volume of infusion, increased infusion time, and use of IV glucocorticoid premedication successfully reduced the incidence and severity of infusion reactions [134].

Anti-CD-22 Epratuzumab

Epratuzumab is a humanized monoclonal antibody targeting the CD22 receptors on the B lymphocytes. Two multicenter, placebo-controlled, randomized, double-blind studies are available (EMBODY™ 1 and EMBODY™ 2), designed to evaluate the efficacy, safety, tolerability, and immunogenicity of Epratuzumab in patients with moderate to severe SLE, each including 780 subjects over 54 weeks. Ongoing experimental studies addressing the role of anti-CD22 in ANCA vasculitis are being run. The two EMBODY™ Phase 3 clinical studies for epratuzumab in Systemic Lupus Erythematosus (SLE) did not meet their primary clinical efficacy endpoints in either dose in both studies. Treatment response in patients who received epratuzumab in addition to standard therapy was not statistically significantly higher than those who received placebo in addition to standard therapy [135-139].

Anti-CD19-Directed Therapies

The MDX-1342 (Medarex, Princeton, NJ) is a fully humanized antibody that selectively to CD19 and induces the depletion and elimination of B cells expressing CD-19 (other than stem cells or fully differentiated plasma cells, which lack CD19 expression).

Preliminary data available from an ongoing phase I study of MDX-1342 in subjects with active RA (despite treatment with MTX)has demonstrated potent B-cell depletion effects with a single-dose (10 or 30 mg) administration of MDX-1342 [44,78].

Targeting B cell Survival Factors

Therapeutic antagonism of BAFF and its homologue APRIL (a proliferation-inducing ligand) targets an important homeostatic signal for B cell survival and selection.

Belimumab

B-lymphocyte stimulator (BLyS), also called B-cell activating factor (BAFF) is a growth factor required for B-cell survival, maturation, and activation, germinal-center formation, the development of B cells into plasma cells and immunoglobulin production. Many of the subsets of maturing B cells are completely dependent on the binding of BAFF receptors by BLyS to survive and mature. Mature, activated B cells differentiate into plasmablasts or memory B cells, memory cells lack BAFF receptors.

Belimumab is a fully humanized IgG1-λmonoclonal antibody that binds to soluble BLyS abrogating its binding to its receptors and thus suppressing its activity. Belimumab depletes activated and naive B cells as well as plasma cells but not memory B cells. Belimumab is an anti-BLyS monoclonal antibody (LymphoStat-B) that binds specifically and with high affinity to soluble BLyS and inhibits its binding to TACI, BCMA, and BR3. The persistence of memory B cells in patients with SLE could be a limitation of belimumab therapy, because these cells can give rise to progeny that can secrete the entire undesirable autoantibody repertoire, it is also an advantage, because protective antibodies against influenza, pneumococcus, and tetanus are maintained and can be successfully induced with revaccination [71,140-142].

In rheumatoid arthritis

Belimumab is not an approved therapy in RA it has been investigated in RA and results were rather modest. In one randomized, double blind, multicenter placebo-controlled phase II clinical trial (DB-PCT) by McKay and colleagues in 2005, belimumab was investigated in a total of 283 RA patients with moderate-to-severe disease activity. Belimumab at doses of 1mg/kg, 4mg/kg, 10 mg/kg intravenously administered on days 0, 14, and 28, and then every 4 weeks through week 24, in addition to standard-of-care therapy (concurrent DMARD) showed modest improvement in ACR 20 response criteria with significantly higher gains compared to placebo. In another phase II

clinical DB-PCT, belimumab therapy wasn't associated by significant increase in the incidence of adverse or serious adverse events. The most frequent adverse events observed were arthralgia, upper respiratory tract infections, urinary tract infections, diarrhea, joint swelling, headache, fatigue, peripheral edema, pain in extremities, cough, pruritus, and sinusitis [144,145].

In systemic lupus erythematosus

Elevated plasma levels of soluble BLyS has been found in 50% of patients with active SLE suggesting a key role for this molecule in the pathogenesis of the disease.

The safety and efficacy of belimumab in SLE has been evaluated in several RCTs. Two large, phase 3, multicenter, prospective, randomized, controlled trials have compared belimumab with placebo in patients with SLE who were receiving standard therapies. The trials were designated according to their duration in weeks as BLISS-52 (865 patients from Latin American, Asian-Pacific and Eastern Europe) and BLISS-76 (819 patients from North America, Central America, and Europe). The trials included patients with active disease, who were seropositive to ANA titer ≥1:80 or anti-dsDNA antibody titer ≥30 IU per milliliter at entry. In both trials patients were randomly assigned to belimumab at a dose of 1 mg per kilogram of bodyweight, belimumab at a dose of 10 mg per kilogram, or placebo. The study drugs were administered by intravenous infusion on days 0, 14, and 28 then every 28 days. The primary outcome measure in both studies was the Systemic Lupus Erythematosus Responder Index (SRI) at week 52. Both studies showed significant improvement in the SRI with 10 mg of belimumab per kilogram as compared with Placebo.

Belimumab was approved by the FDA in 2011 for use in patients with active SLE. The FDA approval specifies that this agent is indicated for patients with active uncontrolled autoantibody-positive SLE who are receiving standard therapy, including glucocorticoids, antimalarial agents, immunosuppressive agents, and nonsteroidal antiinflammatory drugs. Belimumab is given intravenously at a dose of10 mg per kilogram on days 0, 14, and 28 and then every 28 days. The infusions, especially the first two infusions, should be administered at an infusion center, because reactions to the first two infusions are not unusual. Preinfusion treatment with acetaminophen, diphenhydramine, intravenous glucocorticoids, or a combination of these agents can be used to mitigate such reactions. Belimumab has no known drug interactions, and no dose adjustments are required for renal or hepatic dysfunction [140,146].

Atacicept

Atacicept is a human recombinant fusion protein that comprises the binding portion of a receptor for both BLyS (B-Lymphocyte Stimulator) and APRIL (A PRoliferation-Inducing Ligand), Atacicept has shown selective depletion of the mature B cells and the late stages of B cell development with blocking of plasma cells, while sparing B-cell progenitors and memory cells. Experimental studies demonstrated the efficacy of atacicept in animal models of autoimmune disease and the biological activity of atacicept in patients with systemic lupus erythematosus

(SLE) and rheumatoid arthritis (RA) has been demonstrated with reduction of the Ig M and Ig G levels. The use of atacicept as an alternative B cells targeted therapy in refractory vasculitis remains to be investigated [147-149].

Other anti BLyS strategies

Blisimod (A623) soluble and membrane bound BLyS and Tabalumab (LY2127399) soluble and membrane BLyS that are currently being investigated [150].

B-lymphocyte tolerogens

Abetimus (LJP-394) is a B-cell tolerogen. It consists of four double-stranded DNA (dsDNA) epitopes on a polyethylene glycol platform. It cross-links anti-dsDNA surface immunoglobulin receptors on B-cells, leading to anergy or apoptosis. It also reduces titers of anti-dsDNA antibodies. Abetimus was the first B-cell tolerogen developed for SLE and was studied in human trials for the treatment of nonrenal lupus and lupus nephritis. Initial trials suggested a reduction in renal flares in patients who have high-affinity antibodies to the DNA epitope contained within the abetimus molecule. After an analysis of a phase III Abetimus Sodium in patients with a history of lupus nephritis (ASPEN) trial, the trial was terminated when interim efficacy analysis indicated no benefit to continue. TV-4710 (Edratide) another tolargen peptide composed of 19 amino acids based on the complementarily determining regions (CDR1) of a human anti-dsDNA antibody, was tested in a phase II trial. This study has been concluded but there are yet no results released [151-153].

The novel strategy of the B-cell-targeted biologic therapy is an encouraging field for research. The proven effectiveness of this strategy in a variety of ARDs is increasing the demand for more research. In depth studies to address the potential role of regulatory B cells and the desire of a novel interventional approach targeting the memory B cell compartment represent unmet needs that might add to the effectiveness of this strategy.

References

1. Edwards JCW, Szczepanski L, Szechinski J, Filipowicz-Sosnowska A, Emery P, et al. Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. N Engl J Med. 2004; 350: 2572–2581.
2. Looney RJ, Anolik JH, Campbell D, Felgar RE, Young F. B cell depletion as a novel treatment for systemic lupus erythematosus: a phase I/II dose-escalation trial of rituximab. Arthritis Rheum. 2004; 50: 2580-2589.
3. Hasegawa M, Hamaguchi Y, Yanaba K, Bouaziz JD, Uchida J. B-lymphocyte depletion reduces skin fibrosis and autoimmunity in the tight-skin mouse model for systemic sclerosis. Am J Pathol. 2006; 169: 954-966.
4. Ahuja A, Shupe J, Dunn R, Kashgarian M, Kehry MR. Depletion of B cells in murine lupus: efficacy and resistance. J Immunol. 2007; 179: 3351-3361.
5. Sanz I, Anolik JH, Looney RJ. B cell depletion therapy in autoimmune diseases. Front Biosci. 2007; 12: 2546-2567.
6. Xiu Y, Wong CP, Bouaziz J-D, Hamaguchi Y, Wang Y, et al. B lymphocyte depletion by CD20 monoclonal antibody prevents diabetes in non-obese diabetic mice despite isotype-specific differences in Fc{gamma}R effector functions. J Immunol. 2008; 180: 2863–2875.
7. Lanzavecchia A. Receptor-mediated antigen uptake and its effect on antigen presentation to class II-restricted T lymphocytes.

Annu Rev Immunol. 1990; 8: 773-793.

8. Hampe CS. B Cells in Autoimmune Diseases. Scientifica. 2012.

9. Berzofsky JA. T-B reciprocity. An Ia-restricted epitope-specific circuit regulating T cell-B cell interaction and antibody specificity. Surv Immunol Res. 1983; 2: 223-229.

10. Simitsek PD, Campbell DG, Lanzavecchia A, Fairweather A, Watts C. Modulation of antigen processing by bound antibodies can boost or suppress class II major histocompatibility complex presentation of different T cell determinants. Journal of Experimental Medicine. 1995; 181: 1957–1963.

11. Davidson HW, Watts C. Epitope-directed processing of specific antigen by B lymphocytes. J Cell Biol. 1989; 109: 85-92.

12. Martin F, Chan AC. B cell immunobiology in disease: evolving concepts from the clinic. Annu Rev Immunol. 2006; 24: 467-496.

13. Duddy ME, Alter A, Bar-Or A. Distinct profiles of human B cell effector cytokines: a role in immune regulation? J Immunol. 2004; 172: 3422-3427.

14. Mamula MJ. Epitope spreading: the role of self peptides and autoantigen processing by B lymphocytes. Immunol Rev. 1998; 164: 231-239.

15. Manca F, Fenoglio D, Kunkl A, Cambiaggi A, Sasso M, et al. Differential activation of T cell clones stimulated by macrophages exposed to antigen complexed with monoclonal antibodies. A possible influence of paratopespecifcity on the mode of antigen processing. Journal of Immunology. 1988; 140: 2893–2898.

16. Amigorena S, Bonnerot C. Role of B-cell and Fc receptors in the selection of T-cell epitopes. Curr Opin Immunol. 1998; 10: 88-92.

17. Lanzavecchia A. Antigen-specific interaction between T and B cells. Nature. 1985; 314: 537-539.

18. Garside P, Ingulli E, Merica RR, Johnson JG, Noelle RJ. Visualization of specific B and T lymphocyte interactions in the lymph node. Science. 1998; 281: 96-99.

19. Cambier JC, Gauld SB, Merrell KT, Vilen BJ. B-cell anergy: from transgenic models to naturally occurring anergic B cells? Nat Rev Immunol. 2007; 7: 633-643.

20. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nat Rev Immunol. 2013; 13: 227-242.

21. Sharpe AH, Abbas AK. T-cell costimulation--biology, therapeutic potential, and challenges. N Engl J Med. 2006; 355: 973-975.

22. Manjarrez-Orduño N, Quách TD, Sanz I. B Cells and Immunological Tolerance. J of Invest Derm. 2009; 129: 278–288.

23. Duddy M, Niino M, Adatia F, Hebert S, Freedman M. Distinct effector cytokine profiles of memory and naive human B cell subsets and implication in multiple sclerosis. J Immunol. 2007; 178: 6092-6099.

24. Anolik JH, Barnard J, Owen T, Zheng B, Kemshetti S. Delayed memory B cell recovery in peripheral blood and lymphoid tissue in systemic lupus erythematosus after B cell depletion therapy. Arthritis Rheum. 2007; 56: 3044-3056.

25. Goodnow CC, Sprent J, Fazekas de St Groth B, Vinuesa CG. Cellular and genetic mechanisms of self tolerance and autoimmunity. Nature. 2005; 435: 590-597.

26. Mandik-Nayak L, Bui A, Noorchashm H, Eaton A, Erikson J. Regulation of anti-double-stranded DNA B cells in nonautoimmune mice: localization to the T-B interface of the splenic follicle. J Exp Med. 1997; 186: 1257-1267.

27. Noorchashm H, Bui A, Li HL, Eaton A, Mandik-Nayak L. Characterization of anergic anti-DNA B cells: B cell anergy is a T cell-independent and potentially reversible process. Int Immunol. 1999; 11: 765-776.

28. Liu X, Manser T. Antinuclear antigen B cells that down-regulate surface B cell receptor during development to mature, follicular phenotype do not display features of anergy in vitro. J Immunol. 2005; 174: 4505–4515.

29. Rui L, Vinuesa CG, Blasioli J, Goodnow CC. Resistance to CpG DNA-induced autoimmunity through tolerogenic B cell antigen receptor ERK signaling. Nat Immunol. 2003; 4: 594-600.

30. Shlomchik MJ. Sites and stages of autoreactive B cell activation and regulation. Immunity. 2008; 28: 18-28.

31. Cambier JC, Gauld SB, Merrell KT, Vilen BJ. B-cell anergy: from transgenic models to naturally occurring anergic B cells? Nat Rev Immunol. 2007; 7: 633-643.

32. Sanz I, Anolik JH, Looney RJ. B cell depletion therapy in autoimmune diseases. Front Biosci. 2007; 12: 2546-2567.

33. Sanz I, Wei C, Lee FE, Anolik J. Phenotypic and functional heterogeneity of human memory B cells. Semin Immunol. 2008; 20: 67-82.

34. Luning Prak ET, Monestier M, Eisenberg RA. B cell receptor editing in tolerance and autoimmunity. Ann N Y Acad Sci. 2011;

1217: 96-121.

35. Gay D, Saunders T, Camper S, Weigert M. Receptor editing: an approach by autoreactive B cells to escape tolerance. J Exp Med. 1993; 177: 999–1008.

36. Kajsa E Prokopec, Mia Rhodiner, Peter Matt, Ulla Lindqvist, Sandra. Down regulation of Fc and complement receptors.

37. Wang H, Shlomchik MJ. Autoantigen-specific B cell activation in Fas-deficient rheumatoid factor immunoglobulin transgenic mice. J Exp Med. 1999; 190: 639-649.

38. Sharif MN, Tassiulas I, Hu Y, Mecklenbräuker I, Tarakhovsky A. IFN-alpha priming results in a gain of proinflammatory function by IL-10: implications for systemic lupus erythematosus pathogenesis. J Immunol. 2004; 172: 6476-6481.

39. Llorente L, Richaud-Patin Y, Garcia-Padilla C, Claret E, Jakez-Ocampo J, et al. Clinical and biologic effects of anti-interleukin-10 monoclonal antibody administration in systemic lupus erythematosus. Arthritis & Rheumatism. 2000; 43: 1790–1800.

40. Medina F, Segundo C, Campos-Caro A, Gonzalez-Garcia I, Brieva JA. The heterogeneity shown by human plasma cells from tonsil, blood, and bone marrow reveals graded stages of increasing maturity, but local profiles of adhesion molecule expression. Blood. 2002; 99: 2154–2161.

41. Sato S, Steeber DA, Jansen PJ, Tedder TF. CD19 expression levels regulate B lymphocyte development: human CD19 restores normal function in mice lacking endogenous CD19. J Immunol. 1997; 158: 4662-4669.

42. Tedder TF, Poe JC, Fujimoto M, Haas KM, Sato S. CD19-CD21 signal transduction complex of B lymphocytes regulates the balance between health and autoimmune disease: systemic sclerosis as a model system. Current directions in autoimmunity. 2005; 8: 55–90.

43. Cappione A 3rd, Anolik JH, Pugh-Bernard A, Barnard J, Dutcher P. Germinal center exclusion of autoreactive B cells is defective in human systemic lupus erythematosus. J Clin Invest. 2005; 115: 3205-3216.

44. Tedder TF. CD19: a promising B cell target for rheumatoid arthritis. Nat Rev Rheumatol. 2009; 5: 572-577.

45. Tedder TF, Engel P. CD20: a regulator of cell-cycle progression of B lymphocytes. Immunol Today. 1994; 15: 450-454.

46. Shultz LD, Rajan TV, Greiner DL. Severe defects in immunity and hematopoiesis caused by SHP-1 protein-tyrosine-phosphatase deficiency. Trends Biotechnol. 1997; 15: 302-307.

47. Hibbs ML, Tarlinton DM, Armes J, Grail D, Hodgson G. Multiple defects in the immune system of Lyn-deficient mice, culminating in autoimmune disease. Cell. 1995; 83: 301-311.

48. Bolland S, Ravetch JV. Spontaneous autoimmune disease in Fc(gamma)RIIB-deficient mice results from strain-specific epistasis. Immunity. 2000; 13: 277-285.

49. Vinuesa CG, Tangye SG, Moser B, Mackay CR. Follicular B helper T cells in antibody responses and autoimmunity. Nat Rev Immunol. 2005; 5: 853-865.

50. Vincent FB, Morand EF, Mackay F. BAFF and innate immunity: new therapeutic targets for systemic lupus erythematosus. Immunol Cell Biol. 2012; 90: 293-303.

51. Mackay F, Schneider P. Cracking the BAFF code. Nat Rev Immunol. 2009; 9: 491-502.

52. Hsu H, Khare SD, Lee F, Miner K, Hu YL, et al. A novel modality of BAFF specific inhibitor AMG623 peptibody reduces B-cell number and improves outcomes in murine models of autoimmune disease. Clinical and Experimental Rheumatology. 2012; 30: 197–201.

53. Jagessar SA, Heijmans N, Oh L, Bauer J, Blezer EL. Antibodies against human BLyS and APRIL attenuate EAE development in marmoset monkeys. J Neuroimmune Pharmacol. 2012; 7: 557-570.

54. Lanzavecchia A. Antigen-specific interaction between T and B cells. Nature. 1985; 314: 537-539.

55. Celis E, Zurawski VR Jr, Chang TW. Regulation of T-cell function by antibodies: enhancement of the response of human T-cell clones to hepatitis B surface antigen by antigen specific monoclonal antibodies. Proceedings of the National Academy of Sciences of the United States of America. 1984; 81: 6846–6850.

56. Takai T. Fc receptors and their role in immune regulation and autoimmunity. J Clin Immunol. 2005; 25: 1-18.

57. Takai T. Roles of Fc receptors in autoimmunity. Nat Rev Immunol. 2002; 2: 580-592.

58. Drayton DL, Ying X, Lee J, Lesslauer W, Ruddle NH. Ectopic LT alpha beta directs lymphoid organ neogenesis with concomitant expression of peripheral node addressin and a HEV-restricted sulfotransferase. J Exp Med. 2003; 197: 1153-1163.

59. Hutloff A, Büchner K, Reiter K, Baelde HJ, Odendahl M. Involvement of inducible costimulator in the exaggerated memory B cell and plasma cell generation in systemic lupus erythematosus. Arthritis Rheum. 2004; 50: 3211-3220.

60. Drayton DL, Liao S, Mounzer RH, Ruddle NH. Lymphoid organ development: from ontogeny to neogenesis. Nat Immunol. 2006; 7: 344-353.

61. Aloisi F, Pujol-Borrell R. Lymphoid neogenesis in chronic inflammatory diseases. Nat Rev Immunol. 2006; 6: 205-217.

62. Cain D, Kondo M, Chen H, Kelsoe G. Effects of acute and chronic inflammation on B-cell development and differentiation. J Invest Dermatol. 2009; 129: 266-277.

63. Melchers F. The pre-B-cell receptor: selector of fitting immunoglobulin heavy chains for the B-cell repertoire. Nat Rev Immunol. 2005; 5: 578-584.

64. Dörner T, Burmester GR. New approaches of B-cell-directed therapy: beyond rituximab. Curr Opin Rheumatol. 2008; 20: 263-268.

65. Looney RJ, Anolik J, Sanz I. B cells as therapeutic targets for rheumatic diseases. Curr Opin Rheumatol. 2004; 16: 180-185.

66. Browning JL. B cells move to centre stage: novel opportunities for autoimmune disease treatment. Nat Rev Drug Discov. 2006; 5: 564-576.

67. Li Y, Chen F, Putt M, Koo YK, Madaio M. B cell depletion with anti-CD79 mAbs ameliorates autoimmune disease in MRL/lpr mice. J Immunol. 2008; 181: 2961-2972.

68. Gatumu MK, Skarstein K, Papandile A, Browning JL, Fava RA. Blockade of lymphotoxin-beta receptor signaling reduces aspects of Sjögren's syndrome in salivary glands of non-obese diabetic mice. Arthritis Res Ther. 2009; 11: R24.

69. Cancro MP. The BLyS/BAFF family of ligands and receptors: key targets in the therapy and understanding of autoimmunity. Ann Rheum Dis. 2006; 65 Suppl 3: iii34-36.

70. Thien M, Phan TG, Gardam S, Amesbury M, Basten A. Excess BAFF rescues self-reactive B cells from peripheral deletion and allows them to enter forbidden follicular and marginal zone niches. Immunity. 2004; 20: 785-798.

71. Davidson A. Targeting BAFF in autoimmunity. Curr Opin Immunol. 2010; 22: 732-739.

72. Pers JO, Daridon C, Devauchelle V, Jousse S, Saraux A. BAFF overexpression is associated with autoantibody production in autoimmune diseases. Ann N Y Acad Sci. 2005; 1050: 34-39.

73. Mohammed RHA. Recent Advances in the Management of Refractory Vasculitis. In: Lazaros I Sakkas, Christina Katsiari, editors. Updates in the Diagnosis and Treatment of Vasculitis. Croatia: InTech. 2013.

74. Glennie MJ, French RR, Cragg MS, Taylor RP. Mechanisms of killing by anti-CD20 monoclonal antibodies. Mol Immunol. 2007; 44: 3823-3837.

75. Cragg MS, Walshe CA, Ivanov AO, Glennie MJ. The biology of CD20 and its potential as a target for mAb therapy. Curr Dir Autoimmun. 2005; 8: 140-174.

76. Townsend M, Monroe JG, Chan AC. B-cell targeted therapies in human autoimmune diseases: an updated perspective. Immunological Reviews. 2010; 237: 264–283.

77. Tony HP, Burmester G, Schulze-Koops H, Grunke M, Henes J, et al. Safety and clinical outcomes of rituximab therapy in patients with different autoimmune diseases: experience from a national registry (GRAID). Arthritis Research and therapy. 2011; 13: R75.

78. Engel P, Nojima Y, Rothstein D, Zhou LJ, Wilson GL. The same epitope on CD22 of B lymphocytes mediates the adhesion of erythrocytes, T and B lymphocytes, neutrophils, and monocytes. J Immunol. 1993; 150: 4719-4732.

79. Cyster JG, Goodnow CC. Tuning antigen receptor signaling by CD22: integrating cues from antigens and the microenvironment. Immunity. 1997; 6: 509-517.

80. Otipoby KL, Andersson KB, Draves KE, Klaus SJ, Farr AG. CD22 regulates thymus-independent responses and the lifespan of B cells. Nature. 1996; 384: 634-637.

81. Yang M, Rui K, Wang S, Lu L. Regulatory B cells in autoimmune diseases. Cell Mol Immunol. 2013; 10: 122-132.

82. Engel P, Gómez-Puerta JA, Ramos-Casals M, Lozano F, Bosch X. Therapeutic targeting of B cells for rheumatic autoimmune diseases. Pharmacol Rev. 2011; 63: 127-156.

83. Cohen SB, Emery P, Greenwald MW, Dougados M, Furie RA, et al. Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: Results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. Arthritis Rheum. 2006 ; 54: 2793-2806.

84. Emery P, Fleischmann R, Filipowicz-Sosnowska A, Schechtman J, Szczepanski L, et al. The efficacy and safety of rituximab in patients with active rheumatoid arthritis despite methotrexate treatment: results of a phase IIB randomized, double-blind, placebo-controlled, dose-ranging trial. Arthritis Rheum. 2006; 54: 1390–1400.

85. Finckh A, Ciurea A, Brulhart L, Mo¨ller B, Walker UA, et al. Which subgroup of patients with rheumatoid arthritis benefits from

switching to rituximab versus alternative anti-tumour necrosis factor (TNF) agents after previous failure of an anti-TNF agent? Ann Rheum Dis. 2010; 69: 387–393.

86. Tak P, Rigby WFC, Rubbert-Roth A, Peterfy CG, van Vollenhoven RF, et al. Rituximab in combination with Methotrexate (MTX) significantly inhibits joint damage and improves clinical outcomes in patients with early active RA who are naïve to MTX: a randomized active comparator placebo-controlled trial (IMAGE). Arthritis Rheum. 2009; 60: S238.

87. Mease PJ, Cohen S, Gaylis NB, Chubick A, Kaell AT, et al. Efficacy and safety of retreatment in patients with rheumatoid arthritis with previous inadequate response to tumor necrosis factor inhibitors: results from the SUNRISE trial. J Rheumatol. 2010; 37: 917–927.

88. Leandro MJ, Cambridge G, Edwards JC, Ehrenstein MR, Isenberg DA. B-cell depletion in the treatment of patients with systemic lupus erythematosus: a longitudinal analysis of 24 patients. Rheumatology (Oxford). 2005; 44: 1542-1545.

89. Perrotta S, Locatelli F, La Manna A, Cennamo L, De Stefano P, et al. Anti-CD20 monoclonal antibody (Rituximab) for life-threatening autoimmune haemolyticanaemia in a patient with systemic lupus erythematosus. Br J Haematol. 2002; 116: 465–467.

90. Looney RJ, Anolik JH, Campbell D, Felgar RE, Young F. B cell depletion as a novel treatment for systemic lupus erythematosus: a phase I/II dose-escalation trial of rituximab. Arthritis Rheum. 2004; 50: 2580-2589.

91. Driver CB, Ishimori M, Weisman MH. The B cell in systemic lupus erythaematosus: a rational target for more effective therapy. Ann Rheum Dis. 2008; 67: 1374-1381.

92. Ahuja A, Shupe J, Dunn R, Kashgarian M, Kehry MR. Depletion of B cells in murine lupus: efficacy and resistance. J Immunol. 2007; 179: 3351-3361.

93. Ng KP, Cambridge G, Leandro MJ, Edwards JC, Ehrenstein M. B cell depletion therapy in systemic lupus erythematosus: long-term follow-up and predictors of response. Ann Rheum Dis. 2007; 66: 1259-1262.

94. Reddy V, Jayne D, Close D, Isenberg D. B-cell depletion in SLE: clinical and trial experience with rituximab and ocrelizumab and implications for study design. Arthritis Res Ther. 2013; 15 Suppl 1: S2.

95. Cambridge G, Leandro MJ, Teodorescu M, Manson J, Rahman A, et al. B cell depletion therapy in systemic lupus erythematosus: effect on autoantibody and antimicrobial antibody profiles. Arthritis Rheum. 2006; 54: 3612-3622.

96. Ramos-Casals M, Díaz-Lagares C, Khamashta MA. Rituximab and lupus: good in real life, bad in controlled trials. Comment on the article by Lu et al. Arthritis Rheum. 2009; 61: 1281-1282.

97. Anolik JH, Barnard J, Cappione A, Pugh-Bernard AE, Felgar RE. Rituximab improves peripheral B cell abnormalities in human systemic lupus erythematosus. Arthritis Rheum. 2004; 50: 3580-3590.

98. Tokunaga M, Saito K, Kawabata D, Imura Y, Fujii T. Efficacy of rituximab (anti-CD20) for refractory systemic lupus erythematosus involving the central nervous system. Ann Rheum Dis. 2007; 66: 470-475.

99. Reynolds JA, Toescu V, Yee CS, Prabu A, Situnayake D. Effects of rituximab on resistant SLE disease including lung involvement. Lupus. 2009; 18: 67-73.

100. Li EK, Tam LS, Zhu TY, Li M, Kwok CL. Is combination rituximab with cyclophosphamide better than rituximab alone in the treatment of lupus nephritis? Rheumatology (Oxford). 2009; 48: 892-898.

101. Pepper R, Griffith M, Kirwan C, Levy J, Taube D. Rituximab is an effective treatment for lupus nephritis and allows a reduction in maintenance steroids. Nephrol Dial Transplant. 2009; 24: 3717-3723.

102. Catapano F, Chaudhry AN, Jones RB, Smith KG, Jayne DW. Long-term efficacy and safety of rituximab in refractory and relapsing systemic lupus erythematosus. Nephrol Dial Transplant. 2010; 25: 3586-3592.

103. Smith KG, Jones RB, Burns SM, Jayne DR. Long-term comparison of rituximab treatment for refractory systemic lupus erythematosus and vasculitis: Remission, relapse, and re-treatment. Arthritis Rheum. 2006; 54: 2970-2982.

104. Lu TY, Ng KP, Cambridge G, Leandro MJ, Edwards JC, et al. A retrospective seven-year analysis of the use of B cell depletion therapy in systemic lupus erythematosus at University College London Hospital: the fi rst fi fty patients. Arthritis Rheum. 2009; 61: 482-487.

105. Sfikakis PP, Boletis JN, Lionaki S, Vigklis V, Fragiadaki KG, et al. Remission of proliferative lupus nephritis following B cell depletion therapy is preceded by down-regulation of the T cell costimulatory molecule CD40 ligand: an open-label trial. Arthritis Rheum. 2005; 52: 501-513.

106. Gottenberg JE, Guillevin L, Lambotte O, CombeB ,Allanore Y, et al. Tolerance and short term efficacy of rituximab in 43 patients with systemic autoimmune diseases. Ann Rheum Dis. 2005, 64: 913-920.

107. Gunnarsson I, Sundelin B, Jónsdóttir T, Jacobson SH, Henriksson EW, et al. Histopathologic and clinical outcome of rituximab treatment in patients with cyclophosphamide-resistant proliferative lupus nephritis. Arthritis Rheum. 2007; 56: 1263-1272.

108. Galarza C, Valencia D, Tobon GJ, Zurita L, Mantilla RD, et al. Should rituximab be considered as the first-choice treatment for severe autoimmune rheumatic diseases? Clin Rev Allergy Immunol. 2008, 34: 124-128.

109. Boletis JN, Marinaki S, Skalioti C, Lionaki SS, Iniotaki A. Rituximab and mycophenolate mofetil for relapsing proliferative lupus nephritis: a long-term prospective study. Nephrol Dial Transplant. 2009; 24: 2157-2160.

110. Melander C, Sallée M, Trolliet P, Candon S, Belenfant X. Rituximab in severe lupus nephritis: early B-cell depletion affects long-term renal outcome. Clin J Am Soc Nephrol. 2009; 4: 579-587.

111. Mohammed RH, El Makhzangy HI. Hepatitis C related Vasculitides. In: Luis M Amezcua-Guerra, editor. Advances in the etiology, pathogenesis and pathology of vasculitis. Croatia: InTech. 2011.

112. Keogh KA, Wylam ME, Stone JH, Specks U. Induction of remission by B lymphocyte depletion in eleven patients with refractory antineutrophil cytoplasmic antibody- associated vasculitis. Arthritis Rheum. 2005; 52: 262-268.

113. Smith KG, Jones RB, Burns SM, Jayne DR. Long-term comparison of rituximab treatment for refractory systemic lupus erythematosus and vasculitis: Remission, relapse, and re-treatment. Arthritis Rheum. 2006; 54: 2970-2982.

114. Keogh KA, Ytterberg SR, Fervenza FC, Carlson KA, Schroeder DR. Rituximab for refractory Wegener's granulomatosis: report of a prospective, open-label pilot trial. Am J Respir Crit Care Med. 2006; 173: 180-187.

115. Jones RB, Ferraro AJ, Chaudhry AN, Brogan P, Salama AD, et al. A multicenter survey of rituximab therapy for refractory antineutrophil cytoplasmic antibody-associated vasculitis. Arthritis Rheum. 2009; 60: 2156-2168.

116. Stone JH, Merkel PA, Spiera R, Seo P, Langford CA. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. N Engl J Med. 2010; 363: 221-232.

117. Jones RB, Tervaert JW, Hauser T, Luqmani R, Morgan MD. Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. N Engl J Med. 2010; 363: 211-220.

118. Wong CF. Rituximab in refractory antineutrophil cytoplasmic antibody-associated vasculitis: what is the current evidence? Nephrol Dial Transplant. 2007; 22: 32-36.

119. Saadoun D, Rosenzwajg M, Landau D, Piette JC, Klatzmann D. Restoration of peripheral immune homeostasis after rituximab in mixed cryoglobulinemia vasculitis. Blood. 2008; 111: 5334-5341.

120. Saadoun D, Resche Rigon M, Sene D, Terrier B, Karras A. Rituximab plus Peg-interferon-alpha/ribavirin compared with Peg-interferon-alpha/ribavirin in hepatitis C-related mixed cryoglobulinemia. Blood. 2010; 116: 326-334.

121. Pijpe J, Meijer JM, Bootsma H, van der Wal JE, Spijkervet FK. Clinical and histologic evidence of salivary gland restoration supports the efficacy of rituximab treatment in Sjögren's syndrome. Arthritis Rheum. 2009; 60: 3251-3256.

122. Devauchelle-Pensec V, Pennec Y, Morvan J, Pers JO, Daridon C. Improvement of Sjögren's syndrome after two infusions of rituximab (anti-CD20). Arthritis Rheum. 2007; 57: 310-317.

123. Seror R, Sordet C, Guillevin L, Hachulla E, Masson C. Tolerance and efficacy of rituximab and changes in serum B cell biomarkers in patients with systemic complications of primary Sjögren's syndrome. Ann Rheum Dis. 2007; 66: 351-357.

124. Ramos-Casals M, García-Hernández FJ, de Ramón E, Callejas JL, Martínez-Berriotxoa A, et al. Off-label use of rituximab in 196 patients with severe, refractory systemic autoimmune diseases. Clin Exp Rheumatol. 2010; 28: 468–476.

125. Dass S, Bowman SJ, Vital EM, Ikeda K, Pease CT, et al. Reduction of fatigue in Sjo¨gren syndrome with rituximab results of a randomised, double-blind, placebo-controlled pilot study. Ann Rheum Dis. 2008; 67: 1541–1544.

126. Meijer JM, Meiners PM, Vissink A, Spijkervet FK, Abdulahad W. Effectiveness of rituximab treatment in primary Sjögren's syndrome: a randomized, double-blind, placebo-controlled trial. Arthritis Rheum. 2010; 62: 960-968.

127. Sem M, Molberg O, Lund MB, Gran JT. Rituximab treatment of the anti-synthetase syndrome: a retrospective case series. Rheumatology (Oxford). 2009; 48: 968-971.

128. Daoussis D, Liossis SN, Tsamandas AC, Kalogeropoulou C, Kazantzi A. Experience with rituximab in scleroderma: results from a 1-year, proof-of-principle study. Rheumatology (Oxford). 2010; 49: 271-280.

129. Lanzavecchia A, Sallusto F. Human B cell memory. Curr Opin Immunol. 2009; 21: 298-304.

130. Smith V, Van Praet JT, Vandooren B, Van der Cruyssen B, Naeyaert JM. Rituximab in diffuse cutaneous systemic sclerosis: an open-label clinical and histopathological study. Ann Rheum Dis. 2010; 69: 193-197.

131. Genovese MC, Kaine JL, Lowenstein MB, Baldassare A, Schechtman J, et al. Ocrelizumab, a humanized anti-CD20 monoclonal antibody, in the treatment of patients with rheumatoid arthritis: A phase I/II randomized, blinded, placebo-controlled, dose-ranging study. Arthritis Rheum. 2008; 58: 2652-2661.

132. Teeling JL, Mackus WJ, Wiegman LJ, van den Brakel JH, Beers SA. The biological activity of human CD20 monoclonal antibodies

is linked to unique epitopes on CD20. J Immunol. 2006; 177: 362-371.

133. Østergaard M, Baslund B, Rigby W, Rojkovich B, Jorgensen C. Ofatumumab, a human anti-CD20 monoclonal antibody, for treatment of rheumatoid arthritis with an inadequate response to one or more disease-modifying antirheumatic drugs: results of a randomized, double-blind, placebo-controlled, phase I/II study. Arthritis Rheum. 2010; 62: 2227-2238.

134. Kurrasch R, Brown JC, Chu M, Craigen J, Overend P, et al. Subcutaneously Administered Ofatumumab in Rheumatoid Arthritis: A Phase I/II Study of Safety, Tolerability, pharmacokinetics, and Pharmacodynamics. J Rheumatol. 2013; 40; 1089-1096.

135. Kalunian KC, Wallace DJ, Petri MA, Houssiau FA, Pike MC, et al. BILAG-measured improvement in moderately and severely affected body systems in patients with systemic lupus erythematosus (SLE) by Epratuzumab: Results from EMBLEM™, a phase IIb study. Ann Rheum Dis. 2010.

136. Wallace DJ, Kalunian KC, Petri MA, Strand V, Kilgallen B, et al. Epratuzumab demonstrates clinically meaningful improvements in patients with moderate to severe systemic lupus erythematosus (SLE): Results from EMBLEM™, a phase IIb study. Ann Rheum Dis. 2010.

137. Daridon C, Blassfeld D, Reiter K, Mei HE, Giesecke C, et al. Epratuzumab targeting of CD22 affects adhesion molecule expression and migration of B-cells in systemic lupus erythematosus. Arthritis Res Ther. 2010; 12: R204.

138. Jacobi AM, Goldenberg DM, Hiepe F, Radbruch A, Burmester GR. Differential effects of epratuzumab on peripheral blood B cells of patients with systemic lupus erythematosus versus normal controls. Ann Rheum Dis. 2008; 67: 450-457.

139. Dörner T, Kaufmann J, Wegener WA, Teoh N, Goldenberg DM. Initial clinical trial of epratuzumab (humanized anti-CD22 antibody) for immunotherapy of systemic lupus erythematosus. Arthritis Res Ther. 2006; 8: R74.

140. Hahn BH1. Belimumab for systemic lupus erythematosus. N Engl J Med. 2013; 368: 1528-1535.

141. Petri M, Stohl W, Chatham W, McCune WJ, Chevrier M. Association of plasma B lymphocyte stimulator levels and disease activity in systemic lupus erythematosus. Arthritis Rheum. 2008; 58: 2453-2459.

142. Jacobi AM, Huang W, Wang T, Freimuth W, Sanz I, et al. Effect of long-term belimumab treatment on B cells in systemic lupus erythematosus: extension of a phase II, double-blind, placebo-controlled, dose-ranging study. Arthritis Rheum. 2010; 62: 201-210.

143. Chatham WW, Wallace DJ, Stohl W, Latinis KM, Manzi S, et al. Effect of belimumab on vaccine antigen antibodies to influenza, pneumococcal, and tetanus vaccines in patients with systemic lupus erythematosus in the BLISS-76 trial. J Rheumatol. 2012; 39: 1632-1640.

144. McKay J, Chwalinska-Sadowska H, Boling E, Valente R, Limanni A, et al. Belimumab (BmAb), a fully human monoclonal antibody to B-lymphocyte stimulator (BLyS), combined with standard of care therapy reduces the signs and symptoms of rheumatoid arthritis in a heterogenous subject population. Arthritis Rheum. 2005; 52: 710–711.

145. Ding C, Jones G. Belimumab Human Genome Sciences/Cambridge Antibody Technology/GlaxoSmithKline. Curr Opin Investig Drugs. 2006; 7: 464–472.

146. Furie R, Stohl W, Ginzler EM, Becker M, Mishra N, et al. Biologic activity and safety of belimumab, a neutralizing anti-B-lymphocyte stimulator (BLyS) monoclonal antibody: a phase I trial in patients with systemic lupus erythematosus. Arthritis Res Ther. 2008; 10: R109.

147. Furie RA, Petri MA, Wallace DJ, Ginzler EM, Merrill JT. Novel evidence-based systemic lupus erythematosus responder index. Arthritis Rheum. 2009; 61: 1143-1151.

148. Hartung HP, Kieseier BC. Atacicept: targeting B cells in multiple sclerosis. Ther Adv Neurol Disord. 2010; 3: 205-216.

149. Dall'Era M, Chakravarty E, Wallace D, Genovese M, Weisman M, et al. Reduced B lymphocyte and immunoglobulin levels after atacicept treatment in patients with systemic lupus erythematosus: results of a multicenter, phase Ib, double-blind, placebo-controlled, dose-escalating trial. Arthritis and Rheumatism. 2007; 56: 4142–4150.

150. Yildirim-Toruner C, Diamond B. Current and novel therapeutics in the treatment of systemic lupus erythematosus. J Allergy Clin Immunol. 2011; 127: 303-312.

151. Postal M, Costallat LT, Appenzeller S. Biological Therapy in Systemic Lupus Erythematosus. International Journal of Rheumatology. 2012.