Antibody-Based Therapies in Systemic Lupus Erythematosus

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Abstract: Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder that is characterized by pathologic manifestations in multiple organs and elevated morbidity. Traditional management of SLE has included the use of non-steroidal anti-inflammatory drugs, anti-malarials and immunosuppressive drugs such glucocorticoids, azathioprine, cyclophosphamide, and mycophenolate mofetil. Although many of these therapies have shown great efficacy, they often associate with adverse effects, due to their systemic activity. The development of safer therapies for SLE has led to recent emphasis on targeting selected pathways that can be important in the inflammatory response in SLE. In this context, the use of biological agents such as monoclonal antibodies has seen a rapidly increasing progress, and is poised to be some part of the clinical practice for SLE in a near future. This review provides an update on the ongoing clinical trials and the promise and obstacles in the use of biologics in SLE.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disorder characterized by dysregulated immunity and multi-system involvement. The disease is a challenging one for clinicians because the manifestations, disease activity, and disease severity differ greatly among individual patients, and the presentation of these manifestations range from rash and arthritis to severe, and often fatal, cardiovascular events, lupus nephritis, and neuropsychiatric lupus. During the past decade, the mortality and morbidity of SLE have significantly improved due to earlier diagnosis and expanded therapeutic options. However, the complexity of the disease and its considerable heterogeneity in clinical manifestations are still responsible for a more aggressive disease course and unresponsiveness to treatments in some SLE patients. Furthermore, immunosuppressive therapies have systemic effects which can lead to organ toxicity.

Immunologically, the pathogenesis of SLE includes the loss of immune tolerance, an increased antigenic load, excessive T cell help, cytokine imbalance, B cell hyperactivity with production of autoantibodies, formation and deposition of immune complexes in tissue, and complement activation which causes tissue injury. The advances in the understanding of the biology of the immune system have identified cellular and molecular targets that could potentially modulate the pathogenesis of SLE by allowing more effective and less toxic therapies in those SLE patients with refractory disease or in those patients that cannot tolerate conventional therapy.

ANTIBODY-BASED THERAPIES IN SLE

Biologic therapies such as monoclonal antibodies and fusion proteins have become promising treatments in the management of rheumatic diseases, and they have already been evaluated for safety and utilized in the clinic, mostly for oncology indications. These types of drugs are produced by means of biological processes involving recombinant DNA technology and typically target key immune cells, surface molecules, and cytokines with specific action, and thus, reduce generalized immunosuppression and toxicity.

One biologic therapy employs the use of monoclonal antibodies (mAbs), which specifically target a single molecule without affecting other molecules and can be produced in elevated doses through hybridoma techniques. The four types of mAbs used in clinical therapy are murine, chimeric, humanized, and human antibodies. Particularly, the last three types have replaced the murine analogues, as much as recombinant DNA technology, transgenic mice, and phage display have replaced hybridoma technology [1]. Another antibody-based therapy utilizes fusion proteins, also known as chimeric proteins, in which the receptor portion – which provides the specificity - is linked to an immunoglobulin structure. The newer therapeutic mAbs used in clinic are less immunogenic, exhibit longer half-lives, and efficiently affect immune functions in humans [2,3]. Even more advanced approaches to improve use and efficacy of mAb treatments include designing of mAb of specific subclasses and utilizing engineered Fc regions that can enhance or reduce antibody effector function.

The therapeutic effect of mAbs is achieved through various mechanisms, including direct induction of apoptosis (programmed cell death) or indirect effects including antibody-dependent cell-mediated cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). In ADCC, the Fc fragment of the mAb can bind the Fc receptors on monocytes, macrophages, and natural killer (NK) cells, and cell death is then induced by engulfment or cytokines. In CDC, the mAbs can bind to the receptor of the target cell and initiate the complement cascade that results in a membrane attack complex that causes the lysis of the target cell.

Currently, antibody-based therapies constitute the majority of recombinant protein therapy in clinical studies [4].

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Table 1. Antibody-Based Therapies in SLE

Therapeutic Target	Mechanism of Action	Therapeutic Agent	Trade Name	Monoclonal Antibody Type
B cells				
	Anti-CD20 Ab	rituximab	Rituxan	chimeric
		ocrelizumab		humanized
		SBI-087		humanized
	Anti-CD22 Ab	epratuzumab	LymphoCide	humanized
	Anti-BlyS/BAFF Ab	belimumab	LymphoStat-B	human
		AMG 623		human
	Anti-BAFF-R Ab	BR3-Fc		human
	TACI-Ig	atacicept		human
Cytokines				
	Anti-TNF Ab	infliximab	Remicade	chimeric
		etanercept	Enbrel	human
	Anti-IL-1 Ab	anakinra	Kineret	human
	Anti-IL-6R Ab	tocilizumab	Actemra	humanized
	Anti-IL-10 Ab	B-N10		murine
	Anti-IFN Ab	rhuMAb, IFN alpha		humanized
		MEDI-545		human
		AMG 811		human
Co-stimulatory molecules				
CD28/B7	CTLA4Ig	abatacept	Orencia	human
CD40L/CD40	Anti-CD40L Ab	IDEC-131		humanized
ICOS/B7RP-1	Anti-B7RP-1 Ab	AMG 557		human
LFA-1/ICAM	Anti-CD11a Ab	efalizumab	Raptiva	humanized
Complement				
	Anti-C5 Ab	eculizumab	Soliris	humanized
	Anti-C5aR Ab	C5aRAM		human
	Anti-CR1 Ab	ETI-201		chimeric

According to a recent report by IMS Health, the worldwide sales of biologic medications increased by 12.5% in 2007 to $75 billion, nearly double the 6.4% increase in sales of traditional pharmaceuticals. Moroever, new agents are constantly added to this evolving field.

SLE PATHOGENESIS

Immune homeostasis is a process maintained by several mechanisms of immune tolerance that police and regulate the responsiveness to self antigens, to prevent autoimmunity. When immune tolerance breaks down and homeostasis is disrupted, the discriminative ability to recognize 'self' from 'nonself' is lost, and autoimmune disease ensues. Autoimmune diseases such as SLE result from the failure of the tolerance mechanisms to prevent the expansion of autoreactive T cells, which in turn promotes B cells to produce autoantibodies, and overactive B cells produce an abundance of antibodies that play central role in the immune dysfunction of SLE.

Given these premises, the targets for antibody-based treatment in SLE include B-cell depletion, interference with the T-cell/B-cell interactions, cytokine-based therapies, the induction of tolerance, and the interference with complement activation (Fig. **1**).

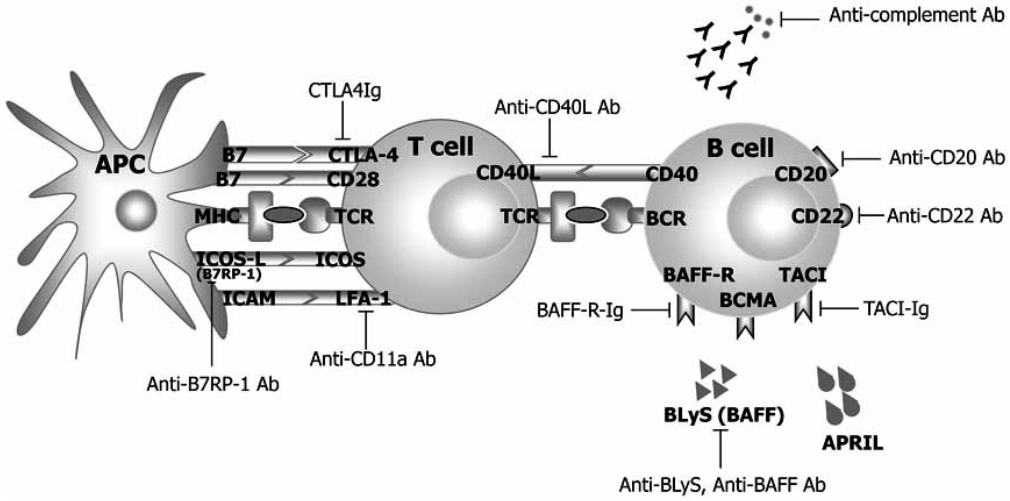

Fig. (1). Targets for antibody-based therapies. Normal T cell activation requires the interaction of MHC molecules presenting the peptide on APCs to the TCR, along with the interaction of co-stimulatory molecules. The negative co-stimulatory pathways are important mechanisms of peripheral tolerance. Two of the main negative pathways are the CTLA-4 and the programmed death-1 (PD-1) pathway. CTLA-4, expressed on T cells, binds to CD80 and CD86 of APCs and attenuates T cell activation. PD-1, expressed on both T and B cells, interacts with PD-L1 and PD-L2 of APCs to inhibit T cell proliferation and cytokine production [157]. These negative regulatory pathways are counterbalanced by several positive stimulatory pathways, the most prominent being the pathway between CD28 expressed on T cells and its ligands CD80/86 (same as the ligands of CTLA-4) found on the APCs. The binding of CD28 and CD80/86 lowers the threshold for TCR signaling that is required for T cell activation, intensifies cytokine responses, and promotes T cell proliferation, survival, and differentiation.

Another important counteracting positive stimulatory pathway is represented by CD40, which is expressed on APCs such as B cells, macrophages, and DC, and its ligand CD40L (CD154), which is expressed on the surface of activated T cells following TCR engagement. This interaction facilitates both humoral and cellular immune responses, driving B cell proliferation and enhancing T cell responses [158]. Other receptor:ligand co-stimulatory interactions are ICOS:ICOSL and OX40:OX40L.

In addition to co-stimulatory molecules, cellular regulators of peripheral immune tolerance are the Tregs. The fact that these cells have a role in autoimmunity is underscored by the finding that patients with a deficit of functional regulatory T cells with the X-linked immunodeficiency syndrome IPEX (immune deregulation, polyendocrinopathy, enteropathy, X-linked syndrome) have autoimmune disease in multiple organs, inflammatory bowel disease, atopic dermatitis, and fatal infection [159]. Different types of Tregs constitutively express CTLA-4 and can produce transforming growth factor (TGF) -β or IL-10 to suppress the differentiation and function of Th 1 and Th2 effector cells, in addition to antibody-producing B cells [160].

In the presence of antigen, B cells that display on their surface and secrete immunoglobulins with high affinity for that antigen are stimulated by the helper T cells. The positive co-stimulatory interaction of CD28-CD80/86 on the T cells and the APCs activates the T cell, and cytokines are released. These cytokines stimulate B cells to divide, promoting the switch of antibody production from IgM to IgG. Additionally, B-cell activators, such as the protein BLyS become up-regulated in lupus and further promote B-cell survival [54]. Autoantibodies, and the activation of complement, ultimately lead to tissue damage in SLE.

B-CELL-BASED THERAPIES

B cells play a central role in the pathogenesis of SLE. B cells secrete autoantibodies, present autoantigens, produce and respond to proinflammatory cytokines and chemokines, and regulate dendritic cell (DC) function [5-7]. Therefore, B cell activation has been considered a possible important therapeutic target in SLE, and the use of mAbs to target and selectively deplete B cells is the forefront of the new therapeutic approaches in SLE. A schematic representation of B cell development with related expression of specific markers targeted in therapy is shown in (Fig. **2**).

CD20 (Human B-lymphocyte-Restricted Differentiation Antigen; Bp35)

CD20, a hydrophobic transmembrane protein, has a molecular weight of approximately 35kDa. CD20 has no known natural ligand and its function is still unclear. CD20 has been suggested to play a role in Ca^{2+} influx across plasma membranes, maintaining intracellular Ca^{2+} concentration and allowing activation of B cells [8]. It is found on pre-B and mature B lymphocytes, but not on hematopoietic stem cells, pro-B cells, normal plasma cells or other normal tissues. Plasmablasts and stimulated plasma cells may also express CD20. Free CD20 is not found in the circulation, and it does not shed from the cell surface or internalize upon antibody binding [9].

Rituximab (Rituxan™, *Genentech*; MabThera™, *Hoffmann-La Roche*)

The chimeric monoclonal immunoglobulin (Ig) G1 antibody, rituximab, targets the pan-B-cell marker CD20. It was developed by IDEC Pharmaceuticals and was approved in 1997 by the FDA for low-grade non-Hodgkin B-cell lymphomas. This IgG1κ immunoglobulin contains murine light

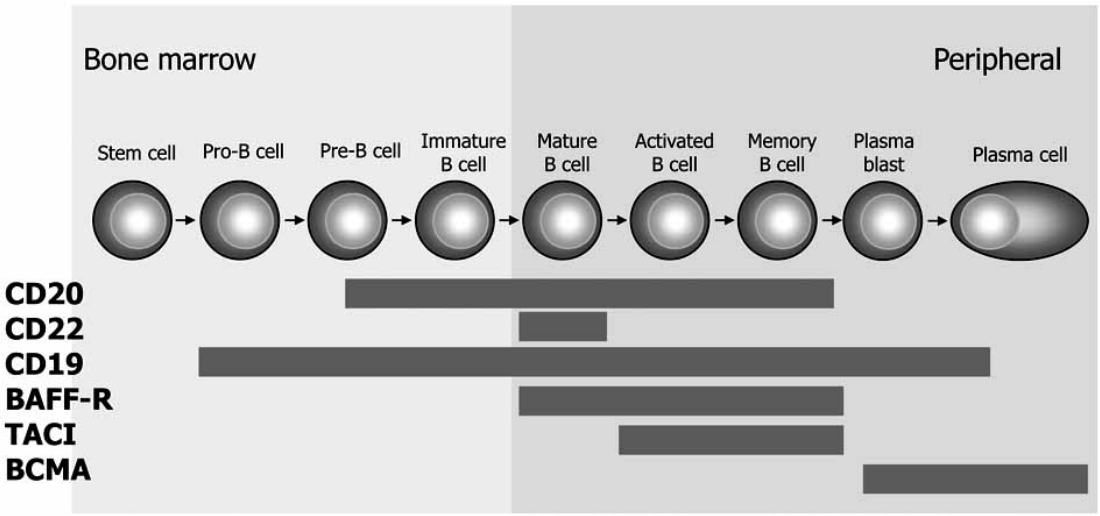

Fig. (2). Stages of B cell development and related expression of specific markers targeted in therapy.

and heavy-chain variable regions and a human constant region. The murine Fab domain of rituximab binds to the CD20 antigen of B cells, and the human Fc domain recruits immune effector functions which causes B cell lysis [10]. B cell lysis is mediated by induction of ADCC and CDC and initiation of apoptosis when cross-linked by Fc-γ receptor-bearing cells [10]. Immature, naïve, memory, and germinal center B cells are depleted while pro-B cells, early pre-B cells, and plasma cells are spared [11]. B cell receptors are down-regulated, and the ability of B cells to act as antigen presenting cells (APCs) to stimulate the immune system is reduced.

Successfully applied to the treatment of B cell lymphomas, the first autoimmune disease to be tested with rituximab was rheumatoid arthritis (RA) [12]. This study showed that when combined with methotrexate or cyclophosphamide, rituximab was effective in RA, whereas other studies suggested that rituximab could be considered before anti-tumor necrosis factor (TNF) therapy in RA [12,13]. In SLE, rituximab has shown efficacy in several case series and in large open studies [14-19]. A recent study of 32 patients with refractory SLE treated with rituximab and cyclophosphamide revealed clinical remission that lasted for a mean of three years in a third of the patients [18]. Similarly, in 16 SLE patients, improvement of disease state was observed in a majority of patients receiving rituximab and cyclophosphamide, and shorter depletion time was found to correlate with baseline CD19 absolute number [19]. In addition, a phase I/II prospective open-label study followed 24 patients with active lupus refractory to conventional immunosuppressant. The results were variable despite the majority being responsive to rituximab: 1/4 of the patients had B-cell return to baseline at 24 weeks, and 1/3 developed human anti-chimeric antibody, which correlated with poor B-cell depletion [20].

Mechanistically, rituximab successfully reduces circulating levels of self-reactive lupus antibodies and reduces levels of circulating memory B cell [15,21]. Rituximab normalizes previously raised levels of co-stimulatory molecules such as CD40 and CD80 on residual B cells, and a reduction of CD40 ligand (CD40L or CD154) on T cells has also been observed [22,23]. However, although symptomatic relief is observed, there is no overall change in serum levels of anti-double stranded (ds) DNA antibodies, and it does not seem to significantly reduce Ig (except for IgM) [16,24]. Despite the profound effects on B cells, rituximab has little effect on circulating T cells [25].

For SLE, the maximum therapeutic effect seems to be at or after 12 weeks, although B-cell depletion from peripheral blood can be rapid and variable from 2 to 18 months [16,26]. The pharmacodynamics of rituximab still remain uncertain. Repopulation of peripheral B cells following rituximab administration occurs when serum rituximab is no longer detectable and with a delayed recovery of $CD27^+$ memory cells – however, with variability among patients [16,26]. In terms of relapse, clinical manifestations are often preceded by the reappearance of B cells and/or autoantibodies [27]. An incomplete B cell depletion seems to be associated with certain FcR genotypes, African-American ancestry, human anti-chimeric antibodies, and lower serum rituximab [16,28].

Rituximab also provides benefits for SLE patients with glomerulonephritis, vasculitis, critical peripheral ischemia, arthritis, refractory skin disease, neuropsychiatric symptoms, hemolytic anemia, thrombocytopenia, pulmonary hypertension, anti-phospholipid antibody syndrome and juvenile-onset SLE (although more extensive randomized controlled trials are required to establish its long-term safety and efficacy) [16,29-32]. Overall, rituximab seems to be effective in severe SLE refractory to conventional treatment, as well as with the addition of it to current medications.

Caution must be taken for the risk of severe acute infections and reactivation of latent infections, such as JC virus reactivation leading to progressive multi-focal leukoencephalopathy (PML) [33]. Deaths of two SLE patients with treated with rituximab from PML and 23 cases of PML following rituximab therapy in patients with non-Hodgkin's lymphoma

Table 2. Current Clinical Trials for SLE (Information Obtained from ClinicalTrials.gov)

Drug	Phase	Clinical Trials.gov ID	Date of Completion	Sponsors/Collaborators
rituximab	Phase II	NCT00556192	December 2009	Chinese University of Hong Kong
	Phase II/III	NCT00137969		Genentech
	Phase II/III	NCT00381810		Genentech
	Phase II	NCT00726518		National Cancer Institute (NCI)
	Phase I	NCT00278538	August 2011	Northwestern University
	Phase III	NCT00282347		Genentech
ocrelizumab	Phase III	NCT00626197		Genentech, Hoffmann-La Roche
SBI-087	Phase I	NCT00714116	September 2010	Wyeth, Trubion Pharmaceuticals
epratuzumab	Phase III	NCT00383513	November 2012	UCB
	Phase II	NCT00660881	January 2014	UCB
	Phase II	NCT00624351	April 2009	UCB
belimumab	Phase II	NCT00583362	September 2010	Human Genome Sciences
	Phase III	NCT00724867	December 2010	Human Genome Sciences, GlaxoSmithKline
	Phase III	NCT00712933	December 2010	Human Genome Sciences, GlaxoSmithKline
	Phase II	NCT00732940	August 2012	Human Genome Sciences, GlaxoSmithKline
	Phase III	NCT00410384	February 2010	Human Genome Sciences, GlaxoSmithKline
	Phase III	NCT00424476	December 2009	Human Genome Sciences, GlaxoSmithKline
atacicept	Phase II/III	NCT00624338	February 2011	EMD Serono
infliximab	Phase II/III	NCT00368264		Medical University of Vienna and others
etanercept	Phase II	NCT00447265	February 2009	National Institute of Allergy and Infectious Disease (NIAID)
rhuMAb	Phase I	NCT00541749		Genentech
MEDI-545	Phase I	NCT00482989	June 2010	MedImmune LLC
	Phase II	NCT00657189	December 2009	MedImmune LLC
AMG 811	Phase I	NCT00818948	February 2011	Amgen
abatacept	Phase II	NCT00774852	October 2014	National Institute of Allergy and Infectious Disease (NIAID)
	Phase II/III	NCT00430677	May 2011	Bristol-Myers Squibb
AMG 557	Phase I	NCT00774943	Amgen	

and other hematologic malignancies, most of whom had received multiple immunosuppressive regimens (e.g., other chemotherapy, stem cell transplant) have been reported [33,34].

Currently, there are several ongoing clinical trials with rituximab. A phase II/III multicenter study is evaluating the efficacy and safety of rituximab in combination with an immunosuppressive drug in SLE subjects (EXPLORER and VOYAGER) (Study ID Numbers: U2971g; U3389g; ClinicalTrials.gov Identifier: NCT00137969; NCT00381810). Other studies evaluate the safety and efficacy of rituximab when combined with cyclophosphamide or mycophenolate mofetil (MMF) for lupus nephritis (Study ID Numbers: SLE-2005-006; U2970g; ClinicalTrials.gov Identifier: NCT00556192; NCT00282347). Two other studies are overseeing the effects of hematopoietic stem cell transplant post rituximab treatment in SLE patients (Study ID Numbers: DI SLE.Auto2003; CDR0000600160, NCI-04-C-0095; ClinicalTrials.gov Identifier: NCT00278538; NCT00726518).

Ocrelizumab (*Genetech*, *Hoffman*-La Roche)

Ocrelizumab is a humanized (90%) anti-CD20 mAb in phase III clinical trial for patients with lupus nephritis (BE-

LONG Phase III) (Study ID Numbers: ACT4072g, WA20500; ClinicalTrials.gov Identifier: NCT00626197).

Ofatumumab (HuMax-CD20™, *Genmab*, *GlaxoSmith-Kline*)

Ofatumumab is a human (100%) anti-CD20 mAb which inhibits early-stage B lymphocyte activation. Currently it is in clinical trials for RA, multiple sclerosis, lymphoma, and leukemia (phases I~III).

Veltuzumab (IMMU-106, hA20, HCD20, *Nycomed*, *Immunomedics*)

This is a humanized (90-95%) mAb with antigen-binding determinant similar to rituximab, but with some chemical differences. Veltuzumab is similar to rituximab in that it induces ADCC, apoptosis, and growth inhibition; however, veltuzumab has a significantly lower off-rate (increased residence time on lymphoma cells) and demonstrates a significantly higher CDC on certain human lymphoma cells *in vitro* [35]. Veltuzumab comes in a subcutaneous formulation, and preclinical trials for RA will be launched. The drug has shown efficacy, safety, and tolerability in phase II clinical trials for non-Hodgkin lymphoma and phase I/II trials for idiopathic thrombocytopenic purpura.

TRU-015 (*Wyeth*, *Trubion Pharmaceuticals*)

TRU-015 is a CD20-targeted small modular immunopharmaceutical (SMIP) designed to target and deplete B cells using a balance of effector functions optimized for autoimmune and inflammatory diseases. SMIP drugs have potent ADCC activity and attenuated CDC activity and are engineered for full binding and activity function of a mAb. They are smaller in size compared to conventional therapeutic mAbs but they maintain the same *in vivo* half-life and expression levels (Trubion Pharmaceuti-cals, http://www.trubion.com).

Currently, a phase II clinical trial is ongoing with active RA patients. A phase I clinical trial for SLE patients was scheduled in 2007; however, it was terminated due to its similar nature to the SBI-087 clinical trial (see next section).

SBI-087 (*Wyeth*, *Trubion Pharmaceuticals*)

This is a fully humanized anti-CD20 SMIP. Phase I clinical trial to determine the safety, pharmacokinetics and pharmacodynamics of SBI-087 with SLE patients is set to be completed in 2010 (Study ID Numbers: 3227K2-1002; ClinicalTrials.gov Identifier: NCT00714116).

CD22

CD22 is a 135-kDa, B-cell adhesion molecule and a co-receptor of the B cell receptor (BCR). It is a type I transmembrane sialoglycoprotein of the Ig superfamily that seems to have both positive and negative regulatory actions [36]. The main function of CD22 is to regulate B cell responses through recruitment of key signaling molecules to the antigen/receptor complex. It attenuates BCR-mediated signaling by recruiting the inhibitory tyrosine phosphatase, Src-homology-2-domain-containing tyrosine phosphatase (SHP) 1 or interacting with Src-homology-2-domain-containing inositol polyphosphate-5'-phosphatase (SHIP) [37]. The multiple ligands of CD22 bind to α2-6-linked silica acid residues present on glycoproteins expressed by activated T and B cells, monocytes, neutrophils, erythrocytes, and activated endothelial cells [38].

In contrast to CD20 which is expressed from pre-B cells to early plasmablasts, CD22, which is present in the cytoplasm of pro- and pre-B cells, is only expressed on the cell surface of IgM^+Ig^+ mature B cells and disappears with B cell differentiation into plasma cells and after B cell activation [39]. CD22 also plays a key role in B cell development, survival, and function. Preclinical studies shows that when CD22 is blocked, there is a selective cytotoxic effect; however, with the same CD22 block, primary B cell proliferation can also be observed, suggesting that modulation of CD22 may differ depending on the B stage of differentiation [39,40].

Epratuzumab (hLL2, LymphoCide™, *UCB*, *Immunomedics*)

Epratuzumab is a fully humanized antibody against CD22. Rather than being a B cell depleting antibody, epratuzumab is an immunomodulatory agent which negatively regulates hyperactive B cells through its inhibitory effect on the BCR, and it does not bind to the ligand-binding domain but induces rapid CD22 internalization and phosphorylation [41]. Because of the limited surface expression of CD22, epratuzumab is unable to cause full B-cell depletion (which is probably via ADCC); only a B-cell population reduction about 30-45% is achieved [42,43]. Epratuzumab is also associated with little immunogenicity, and no significant changes in circulating autoantibodies, immunoglobulin or T cell levels are observed [42,44].

In vitro, epratuzumab preferentially inhibits the exaggerated B cell proliferation in SLE patients. Clinical efficacy of epratuzumab was also shown in an open-label study with SLE patients. Currently, there are ongoing clinical trial studies assessing long-term use (phase III; Study ID Numbers: SL0006 IMMU-103-04X, Eudra CT Number: 2006-004496-36; ClinicalTrials.gov Identifier: NCT00383513), dose response and dose frequency (phase II; Study ID Numbers: SL0007, Eudra CT Number: 2007-002566-35; ClinicalTrials.gov Identifier: NCT00624351), and safety of the epratuzumab in SLE patients (phase IIb; Study ID Numbers: SL0008, Eudra CT Number: 2007-002589-37; ClinicalTrials.gov Identifier: NCT00660881).

Refractory and recurrent non-Hodgkin lymphoma clinical trials have shown that a combination of anti-CD20 and anti-CD22 mAbs is effective than either one of them alone. A recent study using a recombinant, fused, bispecific anti-CD20/22 combination antibody demonstrated a more potent inhibition of lymphoma cell proliferation *in vitro* with higher ADCC activity and other distinct properties from the parental mAbs, suggesting a possible new candidate therapeutic molecule [45].

SOLUBLE MEDIATORS

B-lymphocytic Stimulator (BLyS) (B-Cell-Activating Factor, BAFF; TALL-1; THANK; zTNF4)

B-lymphocyte stimulator (BLyS) protein is a 285-amino-acid member of the TNF family and plays a key role in B

lymphocyte differentiation into plasma cells, survival, and activation. It is expressed as transmembrane protein on various cell types including monocytes, DC and activated T cells and some malignant B cells, or it is released in a soluble form. Interferon (IFN)-γ, IFN-α, interleukin (IL)-10, granulocyte-colony stimulating factor (G-CSF), and CD40L stimulate BLyS production in certain cell types [46,47].

BLyS binds to surface receptors on activated B cells. There are three membrane receptors involved: B-cell-activating factor receptor (BAFF-R, BR3); transmembrane activator and calcium modulator and cyclophylin ligand interactor (TACI); B cell maturation antigen (BCMA). These receptors are not present in early B cell precursors or in pre-B cells when CD20 receptors appear, but are present mainly in primary mature B cells and mature B cells, the latter being the time when CD20 receptors have disappeared. Additionally, TACI is predominantly expressed on $CD27^+$ memory B cells and found on a subset of T cells, and BCMA on plasmablasts, plasma cells, and tonsillar germinal center B cells. BAFF-R is present on all peripheral B cells and it is the predominant receptor on circulating B and T cells, and induces B cell survival. TACI is generally thought of as a negative regulator of B cells [48-50]. Signaling through BAFF-R and BCMA increases levels of Bcl-2 and stimulates B lymphocytes to undergo proliferation and inhibit apoptosis. Additionally, stimulation of all three receptors increases intranuclear levels of nuclear factor-kappa B (NF-κB), which activates differentiation and proliferation. During various stages of B cell and plasma cell development, the three known receptors for BLyS are differentially expressed; therefore, BLyS blockade may differ from B-cell depletion by anti-CD20 treatment [51].

BLyS/BAFF-R is the main axis transducing B-cell survival signals, and interference of this pathway (anti-BLyS antibody, BR3-, TACI-, BACMA-Fc fusion proteins) results in B cell reduction in mice, non-human primates, and humans [52]. Excessive BLyS production has been shown to trigger severe autoimmune disease resembling SLE in mouse [52]. Furthermore, serum levels of BLyS are elevated and correspond with autoantibody titers in lupus patients, and therefore, neutralization of this protein has been suggested as a therapeutic approach [53,54].

Interestingly, serum BLyS increases after B cell depletion with rituximab in RA, SLE, and Sjögren's syndrome patients [55-57]. Perhaps increased serum BLyS can provide additional survival signals for cells that were not removed with rituximab and for new B cells, further suggesting the importance of therapeutic targeting of BLyS.

A PROLIFERATION-INDUCING LIGAND (APRIL; TALL-2; TRDL-1; TNFSF13A)

APRIL is also a member of the TNF-ligand superfamily expressed in monocytes, DC, T cells, and malignant B cells. Unlike BLyS, APRIL is processed inside the cell before secretion and only functions as a soluble factor. APRIL binds to two of the BLyS receptors, BCMA and TACI, but not to BAFF-R. APRIL alone has little or no effect on B-cell biology, but APRIL and BLyS can form BLyS/APRIL heterotrimers (BAHTS) which exert BLyS-like activities *in vitro* and circulate *in vivo*. It has also been suggested that BAHTS may bind to TACI [58].

Belimumab (LymphoStat-B™, *Human Genome Sciences*, *GlaxoSmithKline*)

Belimumab is a fully human IgG1λ mAb that binds to BLyS, inhibits BLyS's stimulation of B cell development, and restores the potential for autoantibody-producing B cells to undergo the normal process of apoptosis. In phase I, tolerability, immunogenicity, and pharmacology of belimumab was studied in mild to moderate SLE patients, and a 12-47% reduction in circulating $CD20^+$ cells was observed [59]. Phase II study failed to show efficacy in reducing symptoms of SLE although it demonstrated improvement in SLE disease activity measures in serologically active patients [59]. However, a subsequent extension phase of the study showed significant decrease in disease activity and serological improvement (normalized IgG, reduced autoantibody and immunoglobulin isotype, while increasing complement) [59]. Peripheral B cell depletion seems to occur later with belimumab and at a less magnitude with decrease in circulating autoantibody, IgG, IgE, and IgM levels. There are currently phase III studies for human anti-BLyS antibody treatment for SLE evaluating the efficacy, safety, tolerability, and impact on quality of life (Study ID Numbers: HGS1006-C1057, BLISS-52; HGS1006-C1056; ClinicalTrials.gov Identifier: NCT00424476; NCT00410384). Other phase III studies address the long term efficacy and safety of belimumab in SLE patients (Study ID Numbers: HGS1006-C1074; HGS1006-C1066; ClinicalTrials.gov Identifier: NCT00712933; NCT00724867).

AMG 623 (*Amgen*, *Anthera Pharmaceuticals*)

Phase Ib study in SLE patients was recently completed, and the initiation of phase II is scheduled for AMG 623, an Fc-conjugated peptide fusion protein that binds to BLyS (http://www.anthera.com).

BR3-Fc (Briobacept, *Biogen, Genentech*)

This is a homodimeric recombinant fusion glycoprotein built from the extracellular ligand-binding portion of BAFF-R and the Fc portion of an IgG1 molecule. It blocks BLyS from binding to BAFF-R and inhibits B cell activation, resulting in apoptosis. A phase I single dose, safety study in RA patients showed no toxicity and 55% reduction in peripheral B cells[1].

There are also preclinical studies using anti-BR3 antibodies which directly act as an antagonist on the receptor. These anti-BR3 antibodies are effective in blocking BLyS-dependent B-cell survival both *in vitro* and *in vivo* and in reducing B-cell population *in vivo* [60]. The reduction of B cells is superior to anti-CD20 and any of the other previously reported anti-BAFF reagents (anti-BLyS antibodies, BR3-Fc, TACI-Fc) [61]. Although results may be preliminary, these mAbs are promising therapeutic means for SLE.

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[1] Shaw, M.; Trapp, R.; Del Giudice, J.; Burnette, M.; Beckman, E.; Anand, B.; Cheu, M.; Maciuca, R.; McLean, L. The effects of repeated doses of briobacept (BR3-FC) in patients with rheumatoid arthritis, [Abstract] *from* European League Against Rheumatism Congress, Paris, 2008.

Atacicept (*ZymoGenetics*, *EMD Serono*)

Atacicept is a recombinant fusion protein built with extracellular ligand binding portion of TACI fused to the Fc portion of human IgG. It blocks activation of TACI by APRIL and BLyS. Phase IIb dose-escalating study conducted on RA patients showed that atacicept reduced 40-50% peripheral B cells and significantly reduced IgM, IgG, rheumatoid factor activity, and anti-cyclic citrullinated peptide antibodies[2]. Currently, phase II/III is in progress to evaluate the most effective dose of atacicept in SLE patients (Study ID Numbers: 27646; ClinicalTrials.gov Identifier: NCT00624338).

CYTOKINE-TARGETED THERAPIES

Defective regulation of both helper T cell (Th) 1 and Th2 cytokines promote and modulate SLE. The cytokine profile in SLE patients differs from both healthy control and patients with other autoimmune diseases such as RA. Even among SLE patients, cytokines differ according to disease stage and phenotypes. Because the imbalance in the levels of cytokines and their receptors in SLE are crucial to the development of the pathology of the disease, cytokines are seen as important therapeutic targets in SLE.

Tumor Necrosis Factor-α (TNF-α)

The role of TNF-α in SLE is somewhat ambiguous. TNF-α is both a proinflammatory and an immunoregulatory cytokine with differential effects on B cells, T cells and DC, as well as on the process of programmed cell death. In lupus-prone mice, levels of TNF-α differ among strains and disease stage, and yet, with renal inflammation, there is usually increased renal TNF expression. Several studies in lupus-prone mice have shown that either TNF-α expression is low or high, and that TNF administration could be beneficial or TNF blockade could be therapeutic [62-65].

As with mice, there are both variability and contradicting results for TNF-α levels in SLE patients. While some studies have shown that serum TNF-α levels are relatively low in SLE patients and TNF-α seem protective, other studies have shown elevated concentration of TNF-α in both active and inactive disease, or no significant differences of TNF-α levels between healthy individuals and SLE patients [66-68]. Moreover, concentrations of TNF-α may correlate with clinical disease activity and appear elevated in lupus nephritis, correlating with renal activity [69]. Additionally, soluble TNF receptors were also found to be high, correlating with disease activity (even though these receptors are TNF inhibitors and should reduce the biologic activity of TNF-α) [67,70].

Infliximab (Remicade™, *Centocor*), Etanercept (Enbrel™, *Amgen*, *Wyeth*), Adalimumab (Humira™, *Abbott*)

TNF blockers have been very successful for autoimmune disease such as RA, Crohn's disease, and psoriasis. Attempts to use TNF blockers with SLE must be cautiously carried out due to the induction of autoantibodies and lupus-like syndromes during anti-TNF therapy [71-73]. However, the vast majority of those induced autoantibodies (anti-dsDNA) belong to the IgM isotype and most probably are non-pathogenic, and with the cessation of the drug, the lupus-like symptoms disappear. Yet, a few cases of nephritis in RA patients after use of TNF blockers have been described [74,75].

To explain the formation of autoantibodies after the use of TNF blockers, some hypotheses have been suggested. The first is that TNF down-regulates IFN-α, and by reducing TNF, IFN-α is released [76]. The second hypothesis is that TNF blockade diminishes IFN-γ, which in turn down-regulates cytotoxic T cell activity to eliminate autoimmune B cells [77]. Another possibility is that chronic TNF exposure impairs T cell activation through the down-regulation of T cell receptor (TCR) components, and, when TNF is blocked, T cells become activated [78]. A fourth hypothesis is that, as a result of chronic TNF exposure, there is increased apoptosis, which allows for more target antigen available for autoantibody formation [79].

In lupus patients, anti-TNF treatment has been successful in improving proteinuria, polyarthritis, subacute cutaneous lupus, and disease activity, and lupus nephritis improvement could last for several years [80,81]. However, a decline in efficacy and severe infusion reaction were observed when TNF blockade was used as a monotherapy [81].

Currently, a phase II/III multi-center trial in Europe is looking at the use of infliximab combined with azathioprine in lupus membranous nephritis (Study ID Numbers: TRIAL V, Eudract-Nr. 2005-004067-30, Protocol EU-116, EK Nr:110/2006; ClinicalTrials.gov Identifier: NCT00368264). In addition, a randomized, double-blind, multi-center phase II study evaluating the safety and tolerability of etanercept with patients with active lupus nephritis started in February 2008 and is scheduled to complete in 2009 (Study ID Numbers: DAIT ALN01; ClinicalTrials.gov Identifier: NCT00447265).

Interleukin-1 (IL-1)

IL-1 is a proinflammatory cytokine produced by macrophages, monocytes and DC, and is up-regulated by TNF-α. The IL-1 superfamily includes the pro-inflammatory cytokines IL-1α and IL-1β. IL-1 binds to IL-1 receptor (IL-1R), and the IL-1 receptor antagonist (IL-1Ra) competes for receptor binding with IL-1α and IL-1β, blocking their role in immune activation.

IL-1 is overexpressed in inflamed kidneys of MRL/lpr and NZB/W lupus mice, and low-dose administration of IL-1 accelerates renal disease in the latter strain of mice [62,63]. Also, IL-1R deficiency causes arthritis in mice [82].

In human SLE, both IL-1α and IL-1β are detected in glomerulonephritis and low serum levels of IL-1Ra coincide with kidney involvement, while IL-1β concentrations appear increased in the serum and cerebrospinal fluid [83,84]. However, there are also reports of increased IL-1Ra during active disease and decrease during flares [84,85]. Additionally, in

[2] Tak, P.; Thurlings, R.; Dimic, A.; Mircetic, V.; Rischmueller, M.; Nasanov, E.; Shmidt, E.; Emery, P.; Rossier, C.; Nesterov, I.; Hill, J.; Munafo, A. A phase Ib study to investigate atacicept (TACI-Ig) in patients with rheumatoid arthritis. [Abstract] *from* The 70th Annual Scientific Meeting of the American College of Rheumatology, Washington, DC, 2006.

MRL/lpr mice, IL-1 administration accelerated renal disease, but IL-1Ra did not improve nephritis [62,86].

Anakinra (Kineret™, *Amgen*)

Approved for the treatment of RA in 2001, anakinra is a non-glycolated version of human IL-1Ra that exerts its action in the same manner as the endogenous antagonist. It neutralizes the biological activity of IL-1 by competitively inhibiting IL-1 binding to IL-1 receptor 1 and has been shown to have both safety and efficacy when used in SLE patients [87].

Interleukin-6 (IL-6) and Interleukin-6 Receptor (IL-6R)

IL-6 is induced by TNF-α and IL-1, and in turn, IL-6 induces B-cell differentiation to plasma cells, T cell proliferation, cytotoxic T cell differentiation, and local inflammation. It plays a critical role in B cell hyperactivity, secretion of antibodies, and SLE immunopathology, possibly directly mediating tissue damage. The signaling of IL-6 is mediated through two membrane molecules: a ligand-binding membrane bound IL-6R (mIL-6R) and a non-ligand-binding signal transducer gp130, and a soluble IL-6R (sIL-6R) which lacks transmembrane and cytoplasmic domains.

Serum IL-6 is significantly elevated in active SLE patients and correlates with disease activity and anti-dsDNA antibody [88]. IL-6 is highly up-regulated in SLE kidneys and is detected in the urine of lupus nephritis patients [83,89]. High levels of IL-6 are also associated with the development of cardiopulmonary complications of SLE (pericarditis, pneumonitis, pulmonary hypertension, interstitial pneumonitis), and IL-6 is elevated in the cerebrospinal fluid of SLE patients with neuropsychiatric symptoms [90,91]. Finally, sIL-6R is elevated in SLE patients and in experimental lupus nephritis, and blockade of IL-6 improves renal disease [92].

Tocilizumab (Actemra™, MRA, *Roche, Chugai*)

Tocilizumab is a humanized mAb directed against the IL-6 receptor. Tocilizumab binds both mIL-6R and the sIL-6R to inhibit IL-6 signaling [93].

IL-6 receptor blockade decreases lymphocyte activation and alters B and T cell homeostasis by blocking differentiation and trafficking. It induces significant improvement of disease activity in RA, Crohn's disease, Castleman's disease, and systemic-onset juvenile idiopathic arthritis, and is actively studied for the treatment of SLE [94-97].

A phase I study on lupus patients treated with tocilizumab showed a decrease in complement, IgG, and anti-dsDNA antibody levels, as well as improvement of disease activity scores (Study ID Numbers: 020272, 02-AR-0272; ClinicalTrials.gov Identifier: NCT00046774).

Interleukin-10 (IL-10)

Produced by monocytes and lymphocytes, IL-10 is generally considered a regulatory cytokine which inhibits synthesis of pro-inflammatory cytokines such as IFN-γ, TNF-α, GM-CSF, IL-2, and IL-3. It also suppresses the antigen presentation capacity of APCs. However, in B-cells, IL-10 causes activation, differentiation, and antibody production and stimulates the expression of human leukocyte antigen (HLA) class II molecules on resting B cells.

When NZB/W lupus mice were given anti-IL-10 antibody, anti-DNA antibody serum levels were significantly inhibited, and the onset of proteinuria and glomerulonephritis were delayed; while in contrast, when these mice were given IL-10, the disease was accelerated [98]. However, results seem to vary according to the strain of the mice [99].

IL-10 levels are consistently high in the serum of SLE patients and correlate with clinical disease activity and serological disease activity (anti-dsDNA antibody) [100]. The variation in IL-10 production seems predisposed to genetic regulation and seems to be controlled at the transcription level [101]. When anti-human IL-10 antibody was added to lupus patient cell cultures, total Ig and IgG productions were inhibited [102].

B-N10

B-N10 is a murine anti-IL-10 mAb that neutralizes human IL-10. A small study with B-N10 treatment showed disease activity improvement in SLE patients, and disease inactivity was observed at 6 months [103].

Interferon-α (IFN-α)

IFN-α plays a role in the activation, differentiation, and survival of B cells, T cells, and DC. It stimulates antibody production in B cells. IFN-α is mainly produced in plasmacytoid DC (pDC), which were originally termed natural IFN-α-producing cells and they are key effector cells in the innate immune system because of their ability to produce large amounts of IFN-α in response to microbial and viral infections [104].

Several studies have shown interferon dysregulation in SLE patients, and DNA-containing immune complexes within SLE serum stimulate pDCs to produce IFN-α [105,106]. IFN-α levels also correlate with anti-ds DNA antibody production, complement activation, and IL-10 production [107].

MEDI-545 (*MedImmune LLC*)

MEDI-545 is a fully human antibody that blocks multiple type 1 IFN-α subtypes. Phase Ib and IIa trials evaluating the safety and tolerability of multiple intravenous and subcutaneous doses of MEDI-545 in lupus patients are ongoing (Study ID Numbers: MI-CP152, MI-CP179; ClinicalTrials.gov Identifier: NCT00482989, NCT00657189).

rhuMAb IFNalpha (*Genentech*)

rhuMAb IFNalpha is a recombinant humanized monoclonal antibody against IFN-α. A phase I dose-escalation of single and repeat doses of rhuMAb IFNalpha in SLE patients is in progress (Study ID Numbers: IFN3958g; ClinicalTrials.gov Identifier: NCT00541749).

Interferon-γ (IFN-γ)

IFN-γ is a 20kDa Type 2 IFN that is secreted by Th1 cells, cytotoxic T cell, DC, and NK cells. It signals through a multimeric receptor-complex made of two different transmembrane chains: IFN-γ receptor 1 (IFN-γR1), which is a

high-affinity receptor-binding subunit, and IFN-γ receptor 2 (IFN-γR2), which is also called the species-specific accessory factor.

The role of IFN-γ in lupus still seems to not have reached consensus, although studies do indicate the importance of it and the effect it has on the disease. Levels of IFN-γ are high in NZB/W lupus mice, correlating with disease stage, and IFN-γ administration accelerates disease while anti-IFN-γ antibody treatment significantly delays the onset of the disease [108,109]. The use of soluble IFN-γ receptor or IFN-γ receptor-Ig also showed efficacy with therapeutic benefits and improved renal disease in these animal lupus models [110]. However, in MRL/lpr mice, early prophylactic treatment exhibited favorable effects, while a late institution of treatment accelerated SLE development [111].

In SLE patients, serum IFN-γ is high and correlates with disease activity and renal disease [84,112]. However, like in lupus models, not all studies show increased IFN-γ in SLE patients, and some studies even show a decrease of IFN-γ with lupus nephritis [113].

AMG 811 (*Amgen*)

This is a fully human mAb that binds to IFN-γ. A phase Ib, randomized, multicenter study that will assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of multiple dose for SLE patients with and without glomerulonephritis will start in 2009. Stdy ID number: 20070283; Clinical Trials.gov identifier: NCT (0818948).

CO-STIMULATORY MOLECULES AND T CELL-TARGETED THERAPY

In the pathogenesis of lupus, the hyperproduction of high-affinity IgG autoantibodies is due to the stimulation by antigen and T helper cells. To stimulate the T-cell receptor on T cells, antigen is presented within the major histocompatibility complex (MHC) class II on the APC, with a second step represented by T-cell co-stimulation of receptor-ligand interactions between the APCs and T cells.

There are several co-stimulatory molecule pairs on T cells and APCs: CD28 and CD80 (B7-1) and CD86 (B7-2); CD40L (CD154) and CD40; inducible T cell co-stimulator (ICOS, CD278) and B7-related protein-1 (B7RP-1, CD275); 4-1BB (CD137) and 4-1BBL; and lymphocyte function-associated antigen-1 (LFA-1, CD11a/CD18) and intercellular adhesion molecule 1 (ICAM-1, CD54).

CD28 and Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4; CD152) and CD80/86

The CD28 molecule expressed on T lymphocytes binds CD80 (B7.1) and CD86 (B7.2) on the APCs. CD28 is expressed on almost all $CD4^+$ T cells and about half $CD8^+$ T cells and plays a role in activation of the immune response. CD28 stimulation augments T-cell immune responses by amplifying signals through the TCR and stabilizing the immunological synapse, which lowers the threshold for naïve T-cell activation, facilitating entry into the cell cycle, and inducing expression and secretion of IL-2, TNF-α, lymphotoxin, IFN-γ, granulocyte-macrophage colony-stimulating factor (GM-CSF) in normal human T cells. Without this co-stimulatory interaction, T cells become either apoptotic or anergic [114].

Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is a CD28-family receptor found on activated T cells. It binds to the same ligands as CD28, CD80 and CD86, but has a 20-100 fold higher binding avidity for the B7 ligands than CD28, and through this binding, CTLA-4 inhibits T cell function and blunts the immune response. CTLA-4 inhibits T cell responses directly via SHP2 and protein phosphatase 2A (PP2A) dephosphorylation of TCR-proximal signaling proteins [115,116]. Unlike CD28, CTLA-4 is not constitutively expressed; it is rapidly up-regulated after CD28 ligation and T cell activation, approximately 48 hours after activation [117].

The two B7 ligands are both type I proteins and like their receptors, belong to the Ig superfamily. B7.1 is expressed on activated B cells, activated T cells, and macrophages [118]. B7.2 is expressed on DC, Langerhans cells, memory and germinal center B cells, and monocytes and is up-regulated through IFN-γ stimulation. Both B7 ligands are capable of binding to CD28 and CTLA-4, and in general, B7-2 is the first of the two encountered. Although contradictory reports exist, a majority of the studies support a model where B7.1 preferentially binds CTLA-4 and inhibits T cell function, while B7.2 is favored by CD28 and can effectively promote T cell responses [119].

Abatacept (Orencia™, BMS-188667, *Bristol-Myers-Squibb*)

The CTLA4Ig, abatacept is a receptor fusion protein linking the extracellular domain of CTLA-4 and the Fc portion of IgG1, blocks the CD28-B7 co-stimulatory pathway. Like CTLA-4, but with an even higher avidity, abatacept competes with CD28 for CD80 and CD86 binding, and by doing so it has negative regulatory effects [120]. Rather than acting directly towards B cells, CTLA4Ig impairs T cell help for B cells, and therefore, activated and memory T cells become less dependent on this pathway [121]. CTLA4Ig may also alter the expression of adhesion molecules and chemokine receptors, which inhibit inflammatory cells to attack, target organs, and prevent up-regulation of ICOS [122]. Combining CTLA4Ig with another agent (cyclophosphamide, LFA-1 blockade, ICOS-Ig, TACI-Ig) provides marked synergistic benefit and improved survival in mice compared with either agent alone [123].

Clinically, CTLA4Ig is effective and well-tolerated in RA patients with inadequate anti-TNF therapy response, and since 2006 has been approved for the treatment of this disease [124,125].

As for use in SLE, this agent decreases the production of autoantibodies, reduces nephritis, and prolongs life in murine SLE [126]. A phase III clinical trial evaluating the safety and efficacy of abatacept in the treatment of lupus nephritis was recently completed in China (Study ID Numbers: IM101-217; ClincialTrials.gov Identifier: NCT00705367). Currently recruiting participants is a phase II/III multicenter randomized clinical study which will evaluate abatacept in patients with active lupus nephritis who are also taking MMF and glucocorticoids (Study ID Numbers: IM101-075; Clinical-

Trials.gov Identifier: NCT00430677), and another phase II study that will see the effectiveness of adding abatacept to standard cyclophosphamide therapy (Study ID Numbers: ITN034AI; ClinicalTrials.gov Identifier: NCT00774852).

Belatacept (LEA29Y, *Bristol-Myers-Squibb*)

This second generation CTLA4Ig, which differs from abatacept by two amino acids, has been clinically studied for patients undergoing renal transplantation and has given promising results [127]. It has been shown to have superior binding to CD80 and CD86 compared with abatacept [127]. A phase I study of belatacept, to see the safety, preliminary efficacy, and immunogenicity, was completed late 2007 on RA patients.

CD40 Ligand (CD40L; CD154; gp39) and CD40

CD40L is regulated by CD28 and by ICOS and is a member of the TNF receptor superfamily expressed on APCs such as B cells, macrophages, DC, and endothelial cells. The binding of CD40L on T cells to CD40 activates APCs by increased expression of MHC and B7 molecules and secretion of IL-12, and induces a variety of downstream effects. The interaction leads to release of cytokines such as IL-10, and contributes to T cell activation by up-regulating the B7 ligands CD80 and CD86 on the APCs [128]. The interaction between CD40 and CD40L has been linked to the production of pathogenic autoantibodies and tissue injury in lupus nephritis in both animal models and SLE patients, and glomerular and tubular CD40 expression is markedly up-regulated in proliferative human lupus nephritis [129-131]. Also, inhibition of B-T cell interaction through the inhibition of the CD40-CD40L pathway is effective therapeutically in lupus-prone mice [132].

Ruplizumab (Antova™, BG9588, *Biogen*)

Ruplizumab, a 151-kD humanized anti-CD40L antibody, reduces autoreactive anti-dsDNA-producing B cells, abnormal B cell populations, proteinuria, and improves SLE disease activity index (SLEDAI) in lupus patients [128,133]. BG9588 blocks early, local proliferation of antigen-specific B and T cells by interrupting their cognate interaction and inhibition of germinal center formation [128,133]. However, studies conducted on SLE patients showed failed clinical efficacy and adverse thromboembolic events, perhaps due to the CD40 and CD40L interactions between endothelial cells and platelets [134-136].

In mouse models, when anti-CD40L is combined with CTLA4Ig, a striking therapeutic effect is observed [137]. There was prolonged effect on disease when the combination was given early in the course of disease, and B-cell tolerance to autoantigen was maintained without preventing immune responses to exogenous antigens [132]. A combined administration of CD40 or CD40L blockade with CTLA4Ig to SLE-prone mice was synergistic in preventing onset of SLE for many months after a short course of treatment, due to long-lasting blockade of T-cell help for autoreactive B cells [132].

IDEC-131 (*IDEC, Eisai*)

This humanized mAb against CD40L demonstrated in a phase II study for SLE that it was safe and well-tolerated by patients, but there was no significant efficacy compared to placebo [130,135]. No thromboembolic event has been noted with IDEC-131 in SLE.

Inducible T-cell Co-Stimulator (ICOS; CD278; AILIM) and B7-related protein-1 (B7RP-1; CD275; B7-H2; GL50, ICOSL)

ICOS is a CD28-family molecule, but unlike CD28, it is not expressed on naïve T cells. Instead, it is up-regulated rapidly after TCR ligation and is expressed on activated and memory T cells. It shares approximately 39% amino acid similarity with CD28 and CTLA-4 and is up-regulated by CD28 ligation, induces IL-4 and IL-10 production, and up-regulates CD40L on T cells [138,139]. Additionally, ICOS is important for germinal center formation, clonal expansion of T cells, antibody production, and class switching in response to various antigens. Finally, it is important in regulating cytokine production in effector T cells and recently activated T cells [138].

ICOS binds to B7RP-1, which is a B7-like molecule constitutively expressed on B cells and monocytes in inflamed tissues. Like CD28-mediated co-stimulation, ICOS-mediated co-stimulation can be down-modulated by CTLA-4. The expression of ICOS is increased on T cells in lupus mice and SLE patients and is overexpressed in peripheral blood $CD4^+$ T cells from active SLE patients, in which it induces the production of IFN-γ [138,140].

The blockade of ICOS ligand using anti-ICOS mAb results in reduced anti-dsDNA antibody and improved renal pathology in animal lupus models [141].

AMG 557 (*Amgen*)

This fully human mAb that binds to B7RP-1 can prevent functional interaction with ICOS on activated T cells. A phase I dose-escalation study of repeat subcutaneous doses with SLE patients is on-going (Study ID Numbers: 20060169; ClinicalTrials.gov Identifier: NCT00774943).

4-1BB (CD137) and 4-1BB Ligand (4-1BBL)

4-1BB is a member of the TNF receptor family expressed on activated $CD4^+$ and $CD8^+$ T cells, NK cells, natural killer T cells, $CD4^+CD25^+$ regulatory T cells (Tregs), monocytes, and DC. Its ligand, 4-1BBL, is expressed on activated APCs, IFN-γ-activated macrophages, activated and primary B cells, monocytes, splenic DC, and can also be induced on T lymphocytes. Signaling through CD137 by its ligand leads to increased TCR-induced T cell proliferation, cytokine production and functional maturation, and prolonged $CD8^+$ T cell survival. The importance of CD137 co-stimulation in the generation of a fully competent T cell response has been established in mice [142]. However, CD137 co-stimulation can also suppress T cell-dependent humoral immune response and autoantibody production, and CD137 agonist mAbs can suppress T-dependent humoral immunity and ameliorate SLE in lupus models [143]. When mice were given anti-CD137 mAb at an early age, they were protected from disease initiation, and those that were given the antibody at a later age had significantly reduced proteinuria and anti-dsDNA antibody production and prolonged survival. In MRL/lpr lupus mice, short-term anti-CD137 treatment blocked lymphadenopathy and spontaneous autoimmune

disease and increased their survival [143]. The suggested mechanisms of anti-CD137 mAbs are 1) they target $CD4^+$ T cells and markedly diminish their cytokine secretion, and 2) anti-CD137 mAbs generate Tregs [144-146]. In any case, CD137 signaling can either increase or inhibit immune responses depending on the stage of T cell responses and the types of inflammatory environment involved.

CD11a

CD11a is one of the two components, along with CD18, of LFA-1. CD11a is expressed on all leukocytes including B and T lymphocytes, particularly memory T cells, monocytes, macrophages, neutrophils, basophils and eosinophils, and on lower levels on polymorphonuclear cells.

CD11a/18 binds to CD54 (ICAM-1), CD102 (ICAM-2), and CD50 (ICAM-3). CD11a plays a central role in leukocyte intercellular cell-cell adhesion through heterophilic interactions with its ligands (ICAM-1-3) and functions in lymphocyte co-stimulatory signaling as an important role in T-cell activation, T cell adhesion to endothelial cells in the blood vessel, and T-cell extravasation and trafficking from the circulation into the skin [147].

Efalizumab (Raptiva™, *Genentech*, *EMD Serono*)

Efalizumab is a recombinant humanized mAb that binds to CD11a and acts as an immunosuppressant. It prevents T cell activation and re-activation, and migration to the skin. The drug has been approved and successfully used for the treatment of psoriasis[3].

Studies of efalizumab for the treatment of discoid lupus and subacute cutaneous lupus erythematosus have shown dramatic efficacy on cutaneous lesions [147,148].

COMPLEMENT-TARGETED THERAPY

Low complement concentrations and activation of the complement system are characteristic of active SLE. Complements are involved in the pathogenesis of lupus and contribute to organ damage, consumed via the classical pathway during immune complex deposition. Accordingly, patients with active lupus characteristically have decreased C_3 and C_4. The concept that complement is involved in the pathogenesis of SLE emerged from observations of decreased levels of components with active disease and findings of complement and immunoglobulin deposits in affected organs, such as skin and kidneys. However, the paradox is that deficiency states within the classical pathway do not protect from SLE but strongly predispose to the disease.

Complement Component 5 (C5)

C5 is cleaved into two subunits: C5a, which plays an important role in chemotaxis, and C5b, which forms the first part of the complement membrane attack complex (MAC). C5a is an 11-kd peptide of the complement anaphylatoxin, and binds to two receptors: C5aR (CD88) and C5L2. C5aR is expressed on neutrophils, monocytes, eosinophils, and lymphocytes. It is through signaling of the C5aR that C5a seems to have biologic functions consisting of degranulation of mast cells, neutrophils, eosinophils, enhancement of smooth muscle contraction, increase of vascular permeability, and induction of leukocyte chemotaxis. The C5a/C5aR interaction also modifies the production of IL-12, thus regulating Th1 cell responses, and potentiates the production of cytokines such as Il-6, IL-8, and TNF-α. C5aR expression is also found in areas of acute inflammation of renal tissue from patients with lupus nephritis [149].

C5b recruits C6, C7, C8, and C9 to form the terminal complement complex MAC (C5b-9). At sublytic concentrations, MAC is a potent host cell activator and can cause the release of cytokines and other inflammatory mediators. Since both C5a and C5b-9 are associated with various complications of SLE, particularly lupus nephritis, blocking the C5 cleavage is a useful therapeutic target. When NZB/W lupus mice were given functionally blocking anti-C5 mAb for six months, it significantly ameliorated the course of renal disease with delayed onset of proteinuria and increased survival [150].

Eculizumab (Soliris™, *Alexion Pharamcetuicals*)

This humanized mAb directed against C5 has been approved for the treatment of paroxysmal nocturnal hemoglobinuria. It binds to the 2b subunit of the C5 convertase enzyme and reduces the esterase activity of this subunit preventing C5 convertase from hydrolyzing C5 into C5a and C5b.

In a SLE patient study, eculizumab was safe, well-tolerated and demonstrated improvement of disease[4]. The treatment period for this study was brief, and a higher dose over an extended time will require the therapeutic potential of this agent in SLE.

C5aRAM (CGS 27913)

This recombinant human C5a receptor antagonist that antagonizes the C5a receptor was found to alleviate nephritis in MRL/lpr mice [151].

ETI-201 (*Elusys*)

ETI-201 is a heteropolymer, de-immunized human chimeric antibody to complement receptor, CR1, cross-linked to dsDNA. The prototype ETI-104, a murine mAb, showed both safety and efficacy in SLE patients. It rapidly binds to erythrocytes, and in patients there is a rapid reduction in plasma dsDNA autoantibody levels 15 minutes after administration, with an antibody reduction of 56% [152]. Results of phase I/II trials demonstrated safety of ETI-201 in lupus patients and reduced circulating DNA antibody levels.

CONCLUSION

The recent past has seen a dramatic improvement in the management of various autoimmune diseases with the use of biologic therapies. This change has associated with increased efficacy and minimized side effects. Biologics have emerged as forerunners of therapy in RA, where many have become

[3] However, as of April 2009, Genentech has announced its decision to pull Raptiva of the U.S. market because of an increased risk of PML. (http://www.gene.com/products/information/immunological/raptiva/)

[4] Furie, R.; Matis, L.; Rollins, S.; Mojcik, C. A single dose, placebo-controlled, double blind, phase I study of the humanized anti-C5 hbG1.1 in patients with systemic lupus erythematosus.[Abstract] *from* The 64th Annual Scientific Meeting of the American College of Rheumatology, Philadelphia, 2000.

the mainstream drug choice for many patients. Biologics in the therapy for lupus lag behind RA, owing to different pathogenesis and in part to the complexity and diversity of clinical manifestations. However, it is this complexity and heterogeneity of the disease that makes it even more vital for novel therapies in SLE to aim at tailored individualized treatment. And it is also this complexity that provides multiple potential targets for biologic modifiers. Determining the mechanism of each drug and the best therapeutic choice for each individual patient will be a challenge. This is because the immune system is intricately structured and despite huge progress in the field, outcomes may not be fully predicted. Careful assessment needs to be made for the potential of immediate as well as delayed toxicities. An example is TGN1412 (CD28-SuperMAB; *TeGenero Immuno Therapeutics*), a humanized mAb that binds and is a strong agonist for CD28 receptor on T cells, originally intended for the treatment of B cell chronic lymphocytic leukemia and RA. In its first human clinical trial in March 2006, this agent caused massive cytokine storm and catastrophic systemic organ failure and hospitalization in the treated subjects [153, 154]. Studies after the incident showed that TGN1412 may not behave the same in nonhuman primate species as in humans, as human T cells express little to none of the CD33-related SIGLECs, which are inhibitory molecules that down-regulate cellular activation pathways in animal models [155]. Similarly, anti-CD3 antibodies were also found to induce acute polyclonal T cell activation with associated risk of cytokine release syndrome [156].

Drug resistance, which often is hard to predict, is also a problem. Resistance may be due to specific characteristics of the antibody (e.g., low affinity, poor penetration into tissue, or immunogenicity after use of human–mouse chimeric antibodies), the antigen (e.g., antigen specificity, low density on the target cell, biologic function, or mutation), and impaired immune effector mechanisms. The formation of anti-drug antibodies (human anti-murine antibodies, HAMA; human anti-chimeric antibodies, HACA; human anti-human antibodies, HAHA) is not common but is an issue that cannot be ignored.

Another hurdle for the use of novel therapeutics is an economic one. The emergence of biologic therapeutics has raised complex regulatory issues due to technical difficulties and safety issues of implementing generic versions of biologics. There are also pharmacoeconomic concerns of the high cost for biologic therapies due to use in the treatment of chronic disease.

The field is still in its exploratory phase for SLE, and more extensive studies are needed before possibly becoming common clinical practice, at least for some of the agents discussed here.

ACKNOWLEDGEMENTS

A.L.C. is supported by grants from the National Institutes of Health (AR53239) and the Southern California Chapter of the Arthritis Foundation.

ABBREVIATIONS

ADCC = Antibody-dependent cell-mediated cytotoxicity

APCs = Antigen presenting cells

APRIL = A proliferation-inducing ligand

BAFF = B-cell-activating factor receptor

BAHTS = BLyS/APRIL heterotrimers

BCMA = B cell maturation antigen

BCR = B cell receptor

Blys = B-lymphocyte stimulator

B7RP-1 = B7-related protein-1

CDC = Complement dependent cytotoxicity

CD40L = CD40 ligand

CTLA-4 = Cytotoxic T-lymphocyte antigen-4

DC = Dendritic cells

dsDNA = Double stranded DNA

G-CSF = Granulocyte-colony stimulating factor

HACA = Human anti-chimeric antibodies

HAHA = Human anti-human antibodies

HAMA = Human anti-murine antibodies

ICAM = Intercellular adhesion molecule

ICOS = Inducible T cell co-stimulator

IFN = Interferon

Ig = Immunoglobulins

IL = Interleukin

IPEX = Immune deregulation, polyendocrinopathy, enteropathy, X-linked syndrome

LFA-1 = Lymphocyte function-associated antigen-1

mAbs = Monoclonal antibodies

MAC = Membrane attack complex

MHC = Major histocompatibility

MMF = Mycophenolate mofetil

NF-κB = Nuclear factor-kappa B

NK = Natural killer cells

PD-1 = Programmed death-1

pDCs = Plasmacytoid dendritic cells

PML = Progressive multi-focal leukoencephalopathy

PP2A = Protein phosphatase 2A

RA = Rheumatoid arthritis

SHP = Src-homology-2-domain-containing tyrosine phosphatase

SHIP = Src-homology-2-domain-containing inositol polyphosphate-5'-phosphatase

SLE = systemic lupus erythematosus

SLEDAI = SLE disease activity index

TCR = T cell receptor

TGF = Transforming growth factor

Th = Helper T cell

TNF = Tumor necrosis factor

Tregs = Regulatory T cells

TACI = Transmembrane activator and calcium modulator and cyclophylin ligand interactor

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Received: 16 December, 2008 **Revised: 24 March, 2009** **Accepted: 26 March, 2009**

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