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Drugs Targeting B-Cells in Autoimmune Diseases

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Drugs Targeting B-Cells in Autoimmune Diseases

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Preface

Systemic autoimmune diseases are complex multisystemic illnesses whose management may involve any specialty, although the most closely involved physicians are normally rheumatologists and internists.

The majority of systemic autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, progressive systemic sclerosis, primary Sjögren syndrome, inflammatory myopathies, and ANCA-associated vasculitis, have been treated principally with cytotoxic agents and corticosteroids, which, although effective in improving disease manifestations and survival, produce severe adverse events and do not prevent relapses, while a varying proportion of patients are refractory to treatment. The need for safer, more effective drugs, together with increased knowledge of the pathogenesis of autoimmune diseases is reflected by the interest shown in biologicals, with clinical trials of the B-cell depleting agent rituximab arousing great hope.

Indeed, the emergence of B-cell-targeted therapies has opened a new era in the therapeutic approach to systemic autoimmune diseases. Four agents deserve specific mention: (1) rituximab, used since 2002 in nearly 2,000 reported patients (1,000 in uncontrolled studies). In 2011, the US Food and Drug Administration (FDA) approved rituximab plus glucocorticosteroids as a front-line therapy for adults with granulomatosis with polyangiitis (Wegener's granulomatosis) and microscopic polyangiitis. This new indication for rituximab represents the first ever FDA-approved therapy for these two diseases and the first alternative to cyclophosphamide for the treatment of severe disease in nearly four decades. Rituximab has also been successfully deployed in other autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, mixed cryoglobulinemia, primary Sjögren syndrome, inflammatory ocular disorders, and hematological autoimmune disorders and has been shown to be suitable for patients refractory to conventional immunosuppressant agents; (2) belimumab, which has been tested in more than 2,000 patients in controlled trials, was approved by the FDA and the European Medicines Agency (EMEA) in 2011 for the treatment of systemic lupus erythematosus; (3) epratuzumab, tested in trials including nearly 300 patients; and (4) ocrelizumab, trials of which have recently been halted due to an unexpectedly high rate of severe infections.

The use of B-cell-depleting agents in clinical practice, overwhelmingly restricted to rituximab, is principally centered on patients who do not respond or are intolerant to standard therapy and those with life-threatening presentations. Forthcoming studies of B-cell-directed strategies, particularly investigations of off-label rituximab use and post-marketing studies of belimumab, will provide new insights into the utility of these treatments in the routine management of patients with autoimmune diseases. Careful evaluations of the risk/benefit profiles of these biologic agents will be essential as their full role in the treatment becomes established.

The main objective of *Drugs targeting B-cells in autoimmune diseases* is to offer the reader the latest opinions of the leading international clinical experts on the practical use of biological agents directed against B cells in autoimmune disorders, both systemic and organ specific. Clinical guidelines for the correct use of these agents can only made by a restricted number of physicians with long clinical and research experience.

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Historic Outline of the Development of Drugs Targeting B Cells

Maria J. Leandro

Abstract In the 1980s and 1990s numerous groups worked on the development of monoclonal antibody (mAb) technology for cell identification and characterization and for therapeutic uses. The initial drive to specifically target B cells was part of the effort to develop more effective and safer therapeutic agents to treat B-cell malignancies. Anti-idiotype antibodies were effective but a different antibody needed to be developed for each patient, contributing to preventing these antibodies from being used in normal clinical practice. Monoclonal antibodies to B-cell lineage-specific antigens were more attractive but choice of antigen was difficult as, for example, shedding or modulation of the antigen could lead to diminished efficacy. Technological advances enabling the production of chimeric human–mouse and humanized or fully human mAb decreased their immunogenicity, increased their half-life, and improved mAb recruitment of host immune effector mechanisms that target cells expressing the specific antigen.

1 Introduction

B cells are targeted nonspecifically by many of the immunosuppressive drugs used in the treatment of autoimmune diseases. Effective, relatively well-tolerated, specific B-cell depletion has been made possible by the availability of rituximab, a chimeric monoclonal antibody (mAb) targeting the CD20 antigen developed for the treatment of B-cell non-Hodgkin's lymphoma (NHL). Rituximab is currently licensed for the treatment of NHL (low-grade, follicular, and large cell subtypes), chronic lymphocytic leukemia (CLL), rheumatoid arthritis (RA), and ANCA-

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associated vasculitis (MabThera® Summary of Product Characteristics 2013; Rituxan® Summary of Product Characteristics 2012; McLaughlin et al. 1998; Robak et al. 2010; Edwards et al. 2004; Stone et al. 2010). It is also widely used in the treatment of other B-cell malignancies, autoimmune diseases and to target B cells in the context of organ transplantation. The other currently licensed anti-CD20 mAb are mainly used in the context of cancer and include the unlabeled anti-CD20 mAb, ofatumumab, which is licensed for the treatment of CLL and murine anti-CD20 mAbs combined with radioactive isotypes, tositumomab-I131 and ibritumomabtiuxetan, licensed for NHL. A completely different mAb, belimumab, which targets the cytokine BLyS/BAFF, a cytokine with a very important role in B-cell survival and function, is licensed for the treatment of systemic lupus erythematosus (SLE) (Furie et al. 2011).

In the 1980s and 1990s numerous groups worked on the development of mAb technology for cell identification and characterization and for therapeutic uses. The initial drive to specifically target B cells was part of the effort to develop more effective and safer therapeutic agents to treat B-cell malignancies (Foon 1989; Foran 2002). Anti-idiotype antibodies were effective but a different antibody needed to be developed for each patient, contributing to preventing these antibodies from being used in normal clinical practice (Meeker et al. 1985). Monoclonal antibodies to B-cell lineage-specific antigens were more attractive but choice of antigen was difficult as, for example, shedding or modulation of the antigen could lead to diminished efficacy. Technological advances enabling the production of chimeric human–mouse and humanized or fully human mAb decreased their immunogenicity, increased their half-life, and improved mAb recruitment of host immune effector mechanisms (by the human Fc portion) that target cells expressing the specific antigen (Liu et al. 1987). Unlabeled mAb were developed together with mAb conjugated with toxins capable of inhibiting protein synthesis or with radioactive elements and bispecific antibodies directed against both to cellular surface antigens and toxins (immunotoxins) (Foon 1989; Foran 2002). Antigen characteristics are essential in determining the efficacy of mAb directed to cell surface antigens and the ideal characteristics change depending on whether unlabeled mAb or immunotoxins are being developed. With unlabeled mAb there are also differences depending on whether the main objective of antigen-binding is engaging agonistic functions or direct cell toxicity or effective recruitment of host immune mediator mechanisms capable of killing the cells, mainly antibody-dependent cellular cytotoxicity, antibody-induced phagocytosis, or complement-dependent cytotoxicity. If an immunotoxin is being developed, then modulating antigens like CD22 are usually preferred (Bonardi et al. 1993; Amlot et al. 1993). If the recruitment of host immune mechanisms is the main objective, then cell surface antigens that do not modulate upon mAb binding and do not shed are ideal. The ideal antigen should be specific for the cells being targeted but not expressed by stem cells, should be highly expressed on cell surface and not exist circulating in a soluble form that will consume mAb aimed for cell targeting. The CD20 antigenic molecule and the mAb rituximab eventually fulfilled the necessary requirements for

therapeutic use for specific B-cell depletion of malignant and normal B cells in humans (Gopal and Press 1999; McLaughlin 2000, 2001).

2 The CD20 Antigen and Rituximab

Following the identification of surface immunoglobulin, CD20 (previously named B1) was the first cell-surface differentiation antigen on human B cells to be identified by a monoclonal antibody (Stashenko et al. 1980). Previously, specific and associated B-cell antigens had been identified by heteroantisera. The CD20 antigen is expressed on the cell surface of the majority of normal B cells and in 95 % of B-cell neoplasms but not on the pluripotent stem cells or very early B-cell precursors in the bone marrow or on terminally differentiated plasma cells (Anderson et al. 1984). This allows repopulation with new B cells following rituximab clearance and also continued production of immunoglobulins by longer-lived plasma cells during the period of B-cell depletion contributing to its relative safety. In most studies the CD20 antigen is described as being exclusively expressed by cells of B-cell lineage (normal or malignant) allowing for specific targeting of these cells (Stashenko et al. 1980; Nadler et al. 1981). However, a small number of reports described the presence of low expression of the CD20 antigen in non-B lymphocytes (Hultin et al. 1993; Algino et al. 1996; Leandro et al. 2006). The CD20 antigen is highly expressed on the B-cell surface, it usually does not modulate to a great extent upon antibody binding, and it does not shed and exist in soluble form, which makes it an ideal antigen to be targeted by unlabeled therapeutic mAbs (Liu et al. 1987; Press et al. 1994). Earlier studies had stated that CD20 did not modulate upon mAb binding, but more recent work has now shown that binding of CD20 by some anti-CD20 mAb, including rituximab, can be followed by some antigen modulation and that this may influence therapeutic efficacy (Beers et al. 2010; Stevenson and Stevenson 2012). The CD20 molecule is a 35 kDa transmembrane protein with only a small part of the molecule being extracellular. The close proximity of the binding mAb to the cell surface is thought to lead to more effective recruitment of immune effector mechanisms and therefore to cytotoxicity. Amazingly, even in this context, small differences in epitope specificity between the different anti-CD20 mAb that have been developed over the years can result in significant differences in function in vitro and in vivo in terms of both efficacy and safety (Beers et al. 2010; Stevenson and Stevenson 2012; Klein et al. 2013). For example, 1F5, a mouse anti-CD20 antibody, which was the first anti-CD20 mAb to be used to treat four lymphoma patients activates a G0 to G1 cell cycle transition in resting B lymphocytes and was therefore not felt to be ideal for further development (Golay et al. 1985, Press et al. 1987). Antibodies that are more efficient at activating complement, such as ofatumumab, can be associated with an increased risk of infusion reactions.

Rituximab (IDEC-C2B8) is a genetically engineered, chimeric human–mouse mAb containing human IgG1 heavy-chain and kappa light-chain constant region

sequences and murine variable region sequences directed against the CD20 antigen (Reff et al. 1994). Rituximab was developed by IDEC Pharmaceuticals for the treatment of NHL (San Diego, CA, USA) and is currently commercialized by Genentech (Rituxan; San Francisco, CA, USA) and by Roche (MabThera; F. Hoffmann-La Roche, Basel, Switzerland). Rituximab is thought to kill B cells mainly by antibody-mediated cellular cytotoxicity and antibody-mediated phagocytosis with complement activation and complement-mediated cytotoxicity contributing (Reff et al. 1994; Grillo-Lopez et al. 1999; Gopal and Press 1999; Rezvani and Maloney 2011). In vitro, rituximab can induce cell cycle arrest and apoptosis of certain lymphoma cell lines but to what extent these mechanisms contribute to its effectiveness in vivo is not known. Initial primate and human studies showed that rituximab in large enough doses was very efficient at depleting B cells in the peripheral blood and in the bone marrow but that depletion in lymph nodes was more variable (Reff et al. 1994; Maloney et al. 1994). Results from several human and animal studies have suggested that this variability is related not only to dose administered and tissue penetration but also to individual B-cell characteristics, microenvironment and individual factors that influence efficiency of host immune recruited mechanisms (McLaughlin 2001; Rezvani and Maloney 2011; Stevenson and Stevenson 2012). Many attempts to improve mAb efficacy in vivo have included mechanisms to increase cellular antigen expression, affinity of Fc receptor binding, or activation of effector immune cells (McLaughlin 2001).

3 Rituximab in the Treatment of B-Cell Malignancies

Rituximab was licensed for the treatment of NHL of B-cell origin in 1997 in the USA and in 1998 in Europe. In the first lymphoma phase II trial using standard dose (4×375 mg/m^2 weekly infusions) normal B cells were depleted from the peripheral blood during a period lasting usually 6–9 months (Maloney et al. 1997a). Rituximab has a long half-life and can be found circulating and bound to cells for a long period after its administration (up to more than 6 months) (Berinstein et al. 1998). The overall response to rituximab monotherapy in relapsed or refractory follicular or low-grade NHL in the pivotal phase II/III trial was around 50 % (McLaughlin et al. 1998). Response rates were significantly higher (more than 90 %) when it was used in combination with chemotherapy (Czuczman et al. 1999). In vitro, rituximab sensitizes lymphoma cell lines to the cytotoxic and apoptotic effects of different chemotherapeutic drugs (Demidem et al. 1997; Alas et al. 2001). Response rates to rituximab monotherapy in previously untreated patients with lymphoma are higher than in relapsed or refractory cases (70–75 % compared to around 50 %) and progression-free survival is approximately 18 months (compared with around 12 months in relapsed or refractory cases) (Ghielmini 2005).

The good results obtained with rituximab monotherapy and its relatively good safety profile led to trials using higher doses and increased dose frequency (extended and maintenance dose schedules). Various alternative dosing schedules

have been used, including same single dose weekly infusions (375 mg/m^2) for 8 weeks, repeat of the standard dose regimen every 6 months (up to a maximum of 4), further single doses every 2 months for a total of four additional infusions and increase in infusion weekly frequency with or without increase in single dose particularly in B-cell malignancies that are associated with lower surface densities of the CD20 antigen (small lymphocytic lymphoma and CLL). In general these studies have reported better results, when compared with the standard 4 weekly course, with no increased toxicity. Studies that have used maintenance regimens (scheduled, intermittent re-treatment) have reported significantly longer duration of response to treatment with median progression-free survivals varying between 22 and 37 months (Hainsworth et al. 2002, 2005). When maintenance therapy was compared with re-treatment with the standard 4-week dose at the time of lymphoma progression the ultimate duration of rituximab benefit was similar but the final overall and complete responses were higher in the maintenance group (Hainsworth et al. 2005). Studies using higher dose and higher frequency schedules have improved rates of response in malignancies with lower expression of CD20, in particular CLL (O'Brien et al. 2001).

4 Rituximab in the Treatment of Autoimmune Diseases

From very early on there was a huge interest from some groups in trying rituximab for specific B-cell depletion in autoimmune diseases, in particular those associated with autoantibodies, despite initial arguments that it would probably not lead to positive responses due to the absence of documented significant decreases in serum immunoglobulin levels in the lymphoma trials. The possibility that the kinetics of autoantibody production were more dependent on continuous formation of new plasma cells from their B-cell precursors and therefore more susceptible to depletion of the pathogenic B-cell clones than total serum immunoglobulin levels and protective antibodies was considered very early on. Autoimmune syndromes associated with pathogenic monoclonal antibodies presumably produced by a monoclonal benign or malignant B-cell clone were also targeted.

The first publications of the use of rituximab to treat autoimmune diseases involved patients with cold agglutinin disease and IgM-associated polyneuropathy (Lee and Kueck 1998; Levine and Pestronk 1999). In both these situations, clinical manifestations are frequently a consequence of the pathogenicity of an IgM paraprotein and are therefore associated with clonal proliferation of B cells. In cold agglutinin disease, the pathogenic autoantibodies are typically monoclonal IgM kappa immunoglobulins against carbohydrate antigen1 on red blood cells (Berentsen et al. 2001). Frequently, a lymphoplasmacytic clone is identified in the bone marrow. These cells typically express CD20 and have low rates of proliferation. Conventional immunosuppressive or cytotoxic treatment is often unable to control the disease. In IgM-associated polyneuropathies, monoclonal antibodies to GM1 ganglioside or to myelin-associated glycoprotein (MAG) have

been identified (Levine and Pestronk 1999). These patients frequently respond to intravenous immunoglobulin (IVIG) and to a combination of plasmapheresis and cyclophosphamide. Reduction in serum autoantibody titers is usually associated with amelioration of the neuropathy.

Also in 1999, Zaja et al. reported a case of a patient with type II mixed cryoglobulinemia (with IgM monoclonal component) associated with hepatitis C that responded to rituximab treatment (Zaja et al. 1999). Around the same time, several reports appeared in the literature of patients with autoimmune manifestations associated with the presence of autoantibodies many in the context of lymphoproliferative diseases or graft versus host disease that improved following treatment with rituximab. Most of these patients had either hematological (cytopenias) or neurological manifestations.

5 Rituximab in the Treatment of Rheumatoid Arthritis

In RA, rituximab was first used based on a hypothesis developed by Edwards and Cambridge, that particular auto-reactive B-cell clones and particular subspecies of autoantibodies were engaged in a vicious cycle of self-perpetuation and induction of inflammation by macrophage activation (Edwards and Cambridge 1998; Edwards et al. 1999). Involvement of pathogenic B-cell clones both in the afferent and efferent arms of the abnormal immune reactions involved in disease pathogenesis was suggested. If the pathogenic B-cell clones were depleted and if the depletion of B cells lasted long enough to allow plasma cells to die and serum levels of pathogenic autoantibodies to decrease significantly or even disappear, then long-term improvement of rheumatoid arthritis should be achieved.

An initial open label study in five patients with active RA who were refractory to standard therapy suggested that treatment with a B-cell depletion protocol based on rituximab could lead to significant improvement in disease manifestations with a good safety profile (Edwards and Cambridge 2001). The five patients were treated with a protocol similar to the standard rituximab dose and schedule (as licensed for lymphoma) combined with cyclophosphamide and oral prednisolone. As mentioned above, studies in lymphoma had showed that combination of rituximab with CHOP increased the rate of responses from 50 % to more than 90 % (McLaughlin et al. 1998; Czuczman et al. 1999). The combined protocol was used in RA to try to achieve as profound B-cell depletion as possible using drugs that rheumatologists were familiar with in combination with rituximab. All five patients achieved major improvements in symptoms and signs of active disease following treatment. In two of the five patients clinical relapse coincided with the return of B cells to the peripheral blood at 7 months while in the other three clinical response continued beyond B-cell repopulation.

This open label study was then extended to include a total of 22 patients with active, refractory RA. In this extended trial, the effect of different doses of rituximab with or without cyclophosphamide or oral prednisolone was investigated based on the oncology concept of rolling mini-phase I studies (Leandro et al. 2002).

Results from these studies led to the randomized phase II proof of concept trial that proved the efficacy and safety of rituximab in the treatment of RA (Edwards et al. 2004). Phase IIb and Phase III trials followed and led to the licensing of rituximab for the treatment of RA in combination with methotrexate (Emery et al. 2006; Cohen et al. 2006).

6 Rituximab Doses and Protocols in Autoimmune Diseases

The standard lymphoma dose of 375 mg/m^2 for four weekly doses used in the first open label phase II trial in patients with relapsed or refractory follicular or low-grade NHL was selected based on the results from the phase I single dose and phase I multiple dose trials (Maloney et al. 1994, 1997a, b). What is now called the rituximab "autoimmune" dose and schedule, i.e. two 1,000 mg doses given 2 weeks apart, was initially developed by Professor Jo Edwards and his team based on a small group of patients with RA treated with different protocols based on the oncology concept of rolling mini-phase I studies as discussed above (Edwards and Cambridge 2001; Leandro et al. 2002; Edwards et al. 2004). The two 1,000 mg doses 2 weeks apart is the licensed dose for RA (MabThera® Summary of Product Characteristics 2013). The dose licensed for ANCA-associated vasculitis [granulomatosis with polyangiitis (Wegener's granulomatosis) and microscopic polyangiitis] is the standard lymphoma dose (Rituxan® Summary of Product Characteristics 2012). The initial combination with cyclophosphamide and higher doses of corticosteroids developed for RA in 1998 is still used in variously adapted forms by different teams, for example, in patients with severe ANCA-associated vasculitis or with severe, refractory systemic lupus erythematosus.

7 Conclusion

Specific, relatively well-tolerated B-cell depletion was made possible by the development of rituximab, a chimeric anti-CD20 mAb, for the treatment of NHL of B-cell origin. The initial use of rituximab in autoimmune diseases was aimed at decreasing the production of pathogenic autoantibodies, which were frequently associated with proliferation of monoclonal B-cell clones expressing CD20 such as in cold agglutinin disease and IgM-associated polyneuropathies and mixed cryoglobulinemia. In RA, rituximab was first used in 1998 based on a hypothesis that particular auto-reactive B-cell clones and certain species of autoantibodies were responsible for the initiation and perpetuation of inflammation in patients with RA. Interestingly, in RA, its use remained controversial until the publication of the proof of concept phase II trial in 2004 mainly due to resistance to accept a primary role for B cells in disease persistence. Rituximab initial success in diseases like RA where controversy exists regarding the pathogenic role of disease-associated

autoantibodies, together with animal studies in different autoimmune disease models suggesting a pathogenic role for B cells independent of secretory antibody, led to other hypothesis of the possible role of B-cell depletion that may underlie its clinical efficacy in autoimmune diseases and that takes into account other known B-cell functions, such as antigen-presentation, cytokine production and influence on other immune cell differentiation and function.

In many of the autoimmune diseases currently treated with B-cell depletion based on rituximab, clinical improvement is followed by relapse and need for re-treatment. Rituximab preclinical and clinical studies in lymphoma provide important knowledge to understand currently used protocols and to inform further optimization of drug and schedules of administration to improve responses in autoimmune diseases.

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Understanding B Cell Biology

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Abstract Humoral autoimmunity reflects failures in B cell tolerance and regulation. Accordingly, B cells have long been proposed as targets for treating autoimmune disease. The last decade has witnessed substantial growth in the number of therapeutic agents that target B cells themselves, or molecules key to B cell survival or function. In order to understand, develop, and eventually predict the outcomes of B cell targeted therapies, a thorough understating of the mechanisms underlying B cell development, activation, and regulation is necessary. Here we summarize B cell genesis, differentiation, and tolerance, and illustrate how an understanding of basic B cell biology can afford insight into the design and action of therapeutic agents.

1 Introduction and Overview

During the last decade, therapeutics targeting B cells have emerged as attractive candidates for treating autoimmune diseases. In addition to making antibodies, B cells perform several other roles critical to normal immune system function, including antigen presentation and regulatory cytokine production. Further, the extent and nature of each function varies based on the B cell subset involved, the anatomic context, and the nature of inducing stimuli. Thus, unraveling—and eventually predicting—the basis for B cell targeted therapeutic activity requires understanding the developmental, selective, and homeostatic mechanisms governing naïve, activated, and antigen experienced B cell pools. Accordingly, this chapter focuses on current understanding of these processes in both mouse models and humans. First, an overview of B lineage commitment, subsets and primary B cell development is provided, followed by considerations of the selective

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and homeostatic processes active in establishing and maintaining pre-immune B cell pools. In subsequent sections, we discuss alternative routes of B cell activation, as well as the generation of effector and memory B cell subsets. Finally, we briefly discuss the relevance of these considerations to current thought and practice in B cell targeted therapies.

2 B Cell Commitment, Lineages, and Development

B cells are produced continuously throughout life, initially arising from the fetal liver and then from hematopoietic stem cells in the bone marrow (BM) (reviewed in Busslinger 2004; Dorshkind 2002; Georgopoulos 2002). As with all eukaryotic cells, B lineage commitment is based on transcription factor competition and cross-regulation (Warren and Rothenberg 2003). Accordingly, acquiring B cell identity involves both the onset of a B cell transcriptional program and the loss of other immune cell potentials (Rothenberg and Pant 2004). Key features of B lineage commitment are tied to the initiation of gene rearrangements at the immunoglobulin (Ig) heavy and light chain loci and the expression of several "master" transcription factors, notably Pax5 (Nutt and Kee 2007; Cobaleda et al. 2007). Once common lymphoid progenitors commit to the B lineage, the transcription factor E2A modifies chromatin marks to activate EBF and Pax5, which in turn activate a cascade of B cell-specific genes (Allman et al. 1999; Li et al. 1996; Nutt and Kee 2007; Medvedovic et al. 2011; Singh et al. 2007; Johnson et al. 2009). More recently, several studies have highlighted regulatory aspects of microRNAs (MiRs) in B cell development, particularly MiR-150 (Xiao et al. 2007; Li et al. 2013). While epigenetic regulation of B cell development is beyond the scope of this chapter, details of this topic and its link to lymphoma are discussed elsewhere (Xiao and Rajewsky 2009; Fernando et al. 2012).

B cells can be separated into two lineages: B-1 and B-2. Debate remains as to whether B-1 and B-2 cells derive from a common progenitor and diverge based on antigen-driven selection, or instead reflect the products of distinct, lineage-restricted progenitors (Ghosn et al. 2007; Berland and Wortis 2002; Montecino-Rodriguez and Dorshkind 2012). Regardless of their exact origins, each lineage plays distinct yet overlapping roles in humoral immunity, reflecting differences in their generation, antigen receptor diversity, and anatomic niche (Table 1). The phenotypic and functional characteristics of B-1 cells are well established in mice, but their likely human counterpart was only recently revealed (Griffin et al. 2011). In contrast, the characterization of B-2 cells is well advanced in both human and mice, affording more extensive comparisons.

Murine B-1 cells are derived primarily from the fetal liver and are sustained largely by self-renewal in the periphery (Hardy 2006; Berland and Wortis 2002; Ghosn et al. 2007). In contrast, B-2 cells arise mostly from the bone marrow and are produced throughout life, albeit at reduced output rates with advanced age. Thus, despite the early production and brief predominance of B-1 cells in fetal and

Table 1 Overview of B-1 and B-2 B cells

Ontogeny and function	B-1	B-2
Major roles	Immune barrier; rapid, early immune responses; natural Abs; TI responses	Surveillance; adaptive immune responses; memory; produce Ab targeted to pathogens; secondary immune responses; TI and TD responses
Anatomic locations	Coelomic cavities Mucosal interfaces Spleen	Secondary lymphoid organs Lymphatics blood
Development	Fetal liver; adult bone marrow; self-renewal in periphery	Continuous generation from bone marrow HSC pool
Major subsets	B-1a, B-1b	Transitional (TR), follicular (FO), marginal zone (MZ), germinal center (GC), memory B (MBC)
Pool size	Small	Large overall; FO B cells comprise the majority in young adult life
Primary antibody isotype(s) secreted	IgM, IgA	IgM, IgG
BCR/repertoire	Generated by somatic recombination J-proximal V_H segments Lack junctional diversity	Generated by somatic recombination; random use of entire V_H cluster; high junctional diversity Somatic mutation in GC, memory B

Key differences between B-1 and B-2 B cells in the context of this chapter are shown. These are extensively reviewed in Montecino-Rodriguez and Dorshkind (2012) and Herzenberg (2000)

neonatal life (Haughton et al. 1993), continuous B-2 cell production yields a much larger steady-state B-2 pool in secondary lymphoid organs (Krop et al. 1996).

2.1 BCR Expression and Early B Cell Differentiation

The expression of a functional B cell antigen receptor (BCR) is fundamental to B cell identity. Further, because BCRs are clonally distributed—each mature B cell expresses only one combining site specificity—a large repertoire of BCRs must be established in the pre-immune B cell compartment to afford the selectivity and specificity associated with adaptive immune responses. In mammals, this diverse array of BCRs is established through the rearrangement of V, D, and J gene segments at the Ig heavy and light chain loci (Tonegawa 1983; Alt et al. 1984). In addition to the considerable permutations provided by the random splicing of multiple gene segments and independent Ig heavy-light chain pairing, nucleotide insertion mechanisms at gene segment junctions further amplify the breadth of BCR diversity (Komori et al. 1993). Notably, while both B-1 and B-2 lineages undergo VDJ rearrangement, the B-1 repertoire is comparatively restricted in terms of heavy chain V segment use, and most B-1 cells lack junctional insertions

(Pennell et al. 1989a, b; Pennell 1995; Seidl et al. 1999; Gu et al. 1990; Kantor et al. 1997; Griffin et al. 2011; Alugupalli et al. 2004; Stoel et al. 2005).

The discrete, sequential steps of VDJ recombination provide the basis for current nomenclatures describing the developmental stages of BM-derived B-2 cells (Melchers 1997; Melchers et al. 1989; Hardy 1989). The three most commonly used nomenclatures are outlined and compared in Table 2. The pro-B cell (Hardy fractions A–C) is the earliest of these developmental stages, where recombinase activating genes 1 and 2 (RAG1/2) join a D and J_H segment at the Ig heavy chain (*IgH*) locus, followed by a V_H to DJ_H rearrangement (Oettinger et al. 1990; Schatz et al. 1989). After a successful V_HDJ_H recombination event at *IgH*, the resulting heavy chain gene product pairs with surrogate light chain (λ5-Vpre-B) to form the pre-BCR. Reflecting the order of Ig heavy chain constant region genes, this initially expressed heavy chain utilizes the J_H-proximal μ constant region. The pre-BCR complex, which includes the signaling components Ig-α and Ig-β, is trafficked to the cell surface (Pillai and Baltimore 1987; Karasuyama et al. 1994). Signaling through the pre-BCR is critical for continued B cell differentiation, presumably as a checkpoint for successful Ig heavy chain expression (Kitamura et al. 1992). Pre-BCR signals lead to reduced RAG1/2 protein levels (Jung et al. 2006), as well as a proliferative burst in these so-called large pre-B cells (Hardy fraction C′). The RAG1/2 proteins are then re-expressed, commencing light chain rearrangement and marking the small pre-B cell stage (Hardy fraction D). Productive light chain rearrangement at either the Ig kappa or lambda light chain locus yields expression of a complete BCR, demarcating the immature (IMM) BM B cell stage (Hardy fraction E).

While most details of B-2 cell differentiation and Ig gene rearrangement were established from studies in mice, human B cell development is strikingly similar. A decade after Cooper and colleagues suggested that different lymphoid lineages mediate antibody production versus delayed-type hypersensitivity in animal models, B cell precursors were described in human fetal liver (Cooper et al. 1965, 1966; Gathings et al. 1977). While these studies were largely geared towards diagnosing and characterizing leukemia (Preud'homme and Seligmann 1972; Vogler et al. 1978), they initiated work leading to an understanding of human B cell development. As in mice, human B cells arise in the fetal liver or bone marrow and are continuously generated throughout life (Nunez et al. 1996). Furthermore, the molecular mechanisms and temporal sequence of events underlying human BCR expression mirror the processes described in mice (LeBien 2000). One apparent difference between mouse and human B cell development is the contribution of the common γ chain cytokine interleukin 7 (IL-7). While murine pro- and pre-B cells rely on IL-7 for survival and differentiation, human B cell progenitors are IL-7 independent (Namen et al. 1988; Prieyl and LeBien 1996; Puel et al. 1998; Noguchi et al. 1993).

Table 2 B-2 developmental stages in the bone marrow

Developmental stages			
Osmond	Melchers and Rolink	Hardy	Status of Ig loci
Pro-B	Pre-pro B	A	Germline
	Pro-B	B	$D–J_H$ rearrangement
		C	$V_H–DJ_H$ rearrangement
Pre-B	Large pre B	C′	V_H DJ_H pairs with λ5-Vpre-B Pre-BCR surface expression
	Small pre B	D	$V_κ–J_κ$ or $V_λ–J_λ$ rearrangement
Immature B	Immature B	E	Complete BCR (receptor editing can occur)

Comparison of the nomenclatures used to identify developmental B cell subsets and how they relate to key VDJ recombination events (comprehensively reviewed in Osmond et al. 1998; Hardy et al. 2000)

2.2 *Peripheral B-2 Cell Maturation and Homeostasis in Pre-Immune Pools*

Once developing B cells reach the IMM stage, they will exit the BM within several days, entering the circulation as transitional (TR) B cells. The TR B cell pool can be further divided into numbered subsets, T1, T2, and T3, according to surface marker and functional criteria (Allman et al. 2001; Carsetti et al. 1995; Loder et al. 1999). TR cells are found in the blood and spleen, but rarely enter the lymphatics. Moreover, they are the last stage before developing cells enter one of the two mature pre-immune B-2 pools: the follicular (FO) or marginal zone (MZ) B subsets (Pillai and Cariappa 2009). Whereas FO B cells are recirculating and thus found in the blood and secondary lymphoid organs, MZ B cells—at least in mice—are sessile and instead home to and reside within the marginal zone of the splenic white pulp (Gray et al. 1982; Pillai et al. 2005; Lu and Cyster 2002). Besides occupying different physical niches, FO and MZ B cells display different BCR signaling characteristics and serve distinct functions (Martin and Kearney 2002; Pillai and Cariappa 2009; MacLennan et al. 1982; Oliver et al. 1997). While the mechanisms dictating which mature subset TR B cells will enter are not fully understood, BCR specificity, cytokine availability, and competition with preexisting mature B cells are all contributors (Martin and Kearney 2002; Thien et al. 2004; Allman and Pillai 2008). For example, MZ B cells express a skewed repertoire of BCR specificities, sharing some features with the B-1 repertoire. Further, under normal homeostatic conditions most TR B cells enter the FO pool, but under B lymphopenic conditions the MZ fate is favored (Agenes and Freitas 1999; Srivastava et al. 2005).

While not absolutely congruent with the analogously named subsets in mice, four B cell subsets are defined among human peripheral blood B cells, based on the differential expression of CD19, CD38, CD27, CD24, and IgD (Table 3). These include TR, FO, MZ-like, and memory B cell populations. A more detailed discussion of subset demarcation, and comparisons with the corresponding mouse

Table 3 Comparison of mouse and human peripheral B cell subset phenotypes

Subsets	Mouse	Human
Transitional	$B220^+AA4.1^+CD24^{hi}IgM^+$ $BR3^+TACI^+$	$CD20^+CD27^-CD38^{hi}IgM^+CD24^{hi}$ $BR3^+$
Mature pre-immune	$CD23^+CD21/35^+$ $IgD^{hi}IgM^{lo}$ (FO^+) $CD23^-CD21/35^{hi}$ $IgD^{lo}IgM^{hi}CD1d^+$ (MZ^+) $BR3^+TACI^+$	$CD20^+CD27^-CD38^+$ IgM^+IgD^+ (Naïve$^+$) $CD20^+CD23^-CD21^{hi}$ $IgD^{lo}IgM^{hi}CD1d^+$ (MZ^+-like) $BR3^+TACI^+$
Germinal center	$B220^+GL7^+Fas^+PNA^+$ IgD^-IgM^- $BR3^+$	$CD20^+CD38^+IgD^-$ $BR3^+$
Plasma cell	IgD^-B220^{lo}, $CD138^{hi}$ $TACI^+$ and/or $BCMA^+$	$CD20^-CD38^{hi}CD27^{hi}CD138^+$ $TACI^+$ and/or $BCMA^+$
Memory B cell	$B220^+CD80^+CD73^+PD\text{-}L2^+$	$CD20^+CD38^-CD27^+$

Major surface marker differences between pre-immune and antigen experienced B cell subsets including BLyS receptor expression are shown. Memory B cell BLyS receptor profiles remain poorly defined (Tangye et al. 2006; Scholz et al. 2011; Tomayko et al. 2010)

subsets, can be found elsewhere (Scholz et al. 2011). Recent studies of human B cell reconstitution after B cell depletion indicate that these peripheral subsets and their differentiative order largely recapitulate murine B cell ontogeny; BM émigrés initially seed the TR B cell pool, followed by appearance of the more mature FO and MZ-like subsets (Anolik et al. 2007; Roll et al. 2006; Leandro et al. 2006; Palanichamy et al. 2009; Suryani et al. 2010).

Once established, the maintenance of mature pre-immune B cell pools relies on signals from survival cytokines, primarily those in the BLyS family of ligands and receptors. This subfamily of the tumor necrosis factor (TNF) superfamily consists of two cytokines, BLyS (B Lymphocyte Stimulator a.k.a. BAFF) and A proliferation-inducing ligand (APRIL); and three receptors, BLyS receptor 3 (BR3, a.k.a. BAFF-R), transmembrane activator and cyclophilin ligand interactor (TACI), and B cell maturation antigen (BCMA) (Hahne et al. 1998; Kelly et al. 2000; Madry et al. 1998; Moore et al. 1999; von Bulow and Bram 1997). BLyS binds with the greatest affinity to BR3, less strongly to TACI, and with low affinity to BCMA (Bossen and Schneider 2006; Day et al. 2005). In contrast, APRIL binds with high affinity to both TACI and BCMA, but negligibly to BR3.

Within the pre-immune B-2 cell pools, TR, FO, and MZ B cells express BR3 (Stadanlick et al. 2008; Hsu et al. 2002) and require signals via this receptor for their survival. Accordingly, both BLyS and BR3 deficiencies independently yield profound reductions in TR and mature B cell numbers (Harless et al. 2001; Lentz et al. 1996, 1998; Miller and Hayes 1991; Miller et al. 1992; Yan et al. 2001). Conversely, BLyS transgenics or mice given exogenous BLyS show increased FO and MZ B cell numbers (Mackay et al. 1999; Thien et al. 2004). The current models for peripheral B cell homeostasis posit that B cells fill the mature pre-immune pools until most of the available BLyS is bound to cell surface BR3 and TACI; and at that point B cell

capacity is maximal so the pool size remains constant unless BLyS levels change substantially.

Far less is understood about the homeostatic mechanisms operating in B-1 B cells. However, B-1 cell homeostasis differs fundamentally from B-2 cells in two ways. First, unlike B-2 cells, the B-1 compartment is maintained largely by self-renewal, rather than by the continuous influx of new cells generated from HSC-derived progenitors. Second, B-1 B cells are largely independent of BLyS, since BLyS depletion in mice has little or no effect on B-1 pools, despite the profound depletion of B-2 cells (Scholz et al. 2008).

Though human B cell homeostasis is less extensively characterized, evidence suggests mechanisms parallel to those in mice. For example, human B cells also bind BLyS and express BR3 in both TR and naïve pools (Darce et al. 2007; Palanichamy et al. 2009; Ng et al. 2004; Sims et al. 2005; Carter et al. 2005). Furthermore, homozygous BR3 deletion results in a B cell developmental block at the TR stage, severely reducing numbers of mature pools—as has long been appreciated in BR3- or BLyS-deficient mice (Warnatz et al. 2009; Thompson et al. 2001; Schiemann et al. 2001). These observations imply an inverse relationship between total B cells and BLyS levels, conceptually consistent with the notion that BLyS signals via BR3 are key homeostatic regulators of the pre-immune B cell pools. Indeed, BR3 deficiency, B cell lymphopenia, or B cell depletion therapy leads to elevated serum BLyS levels (Cambridge et al. 2006; Kreuzaler et al. 2012). Nevertheless, there is also evidence that human and nonhuman primate B cells are somewhat less sensitive to BLyS depletion than murine B cells. In contrast to murine FO B cells, a higher percentage of human B cells survive in culture without BLyS and show only small improvements in survival with added BLyS (Avery et al. 2003; Sims et al. 2005; Tangye et al. 2006). Furthermore, antibody-mediated BLyS depletion partially ablates late TR and mature naïve B cell subsets in humans and nonhuman primates, but to a lesser degree than in mice (Scholz et al. 2008; Calero et al. 2010; Halpern et al. 2006; Vugmeyster et al. 2006; Baker et al. 2003). Differences in B cell sensitivity to BLyS may reflect differences in BLyS receptor expression levels and/or BLyS availability within different anatomic locales: for example, splenic MZ B cells of both mice and nonhuman primates are highly sensitive to BLyS (Scholz et al. 2008; Vugmeyster et al. 2006). In toto, these studies indicate a critical role for BLyS ligands and receptors in the size and content of the primary human B cell repertoire.

3 Immune Tolerance and the Selection of Pre-Immune B Cell Pools

Early demonstrations of acquired tolerance led to the clonal selection paradigm, which posits the selective elimination of clones bearing autoreactive antigen receptors (Billingham et al. 1953; Owen 1945; Burnet 1976). Indeed, the random recombination and nucleotide insertion mechanisms underlying Ig gene expression unavoidably

yield self-reactive BCRs, necessitating mechanisms to eliminate or silence potential autoreactivity. In accord with this idea, multiple checkpoints are imposed during B cell development that reduce the likelihood that self-reactive B cells will enter the mature FO and MZ pools.

3.1 Deletion and Receptor Editing in the Bone Marrow

While some losses occur among developing B cells at the pre-B stage, the first point at which a complete BCR specificity can be leveraged for selection is at the IMM BM stage. Several powerful transgenic mouse models have identified two general mechanisms through which autoreactive specificities are eliminated or altered at this stage. Following the seminal findings of Nossal and Pike, compelling evidence has accumulated for the selective elimination of IMM B cells bearing self-reactive BCRs, driven by strong BCR ligation (Nossal and Pike 1975; Goodnow 2007; Nemazee and Weigert 2000). In addition, avid BCR signaling at the IMM B cell stage can lead to continued RAG expression and successive light chain gene rearrangements, thus altering BCR specificity via a process dubbed receptor editing (Tiegs et al. 1993; Gay et al. 1993; Luning Prak et al. 2011). This specificity-based central tolerance checkpoint is stringent, as only about 10 % of IMM B cells proceed through this checkpoint and exit the BM (Allman et al. 1993; Forster and Rajewsky 1990).

Evidence for similar processes in humans was established through single cloning and re-expression of Igs from human B cell subsets. In these studies, Nussenzweig and colleagues showed that nearly 75 % of BM precursors express autoreactive or polyreactive BCRs, and that these are purged from the repertoire as cells transit successive maturation stages (Wardemann et al. 2003). Interestingly, in some autoimmune patients these checkpoints were faulty (Meffre and Wardemann 2008; Yurasov and Nussenzweig 2007; Yurasov et al. 2005).

3.2 Transitional B Cell Selection

Despite the ~ 90 % losses due to negative selection in the BM, autoreactive and polyreactive clones nonetheless enter TR pools. While no longer capable of RAG reactivation and editing, TR cells remain subject to deletional tolerance mechanisms (Allman et al. 2001; Fulcher and Basten 1994; Goodnow et al. 1988; Rolink et al. 1998; Carsetti et al. 1995). Moreover, in addition to negative selection mediated by avid BCR signals, cells at the TR checkpoint also undergo a form of positive selection, whereby a minimal level of so-called tonic BCR signaling is required for survival and ultimate maturation (Monroe 2006). Thus, under normal physiological conditions, only about 30 % of TR B cells—and thus about 3 % of the original IMM B cell cohort—successfully continue to the mature FO or MZ pools (Allman et al. 1993). Importantly, and in contrast to BM selection, the stringency of peripheral tolerance is flexible and determined through interclonal competition

based on BCR signal strength and the ability to acquire BLyS (Cyster et al. 1994; Thien et al. 2004; Hondowicz et al. 2007). Thus, excess BLyS relaxes peripheral selection, allows autoreactive clones to enter otherwise forbidden mature pre-immune pools, and is associated with development of humoral autoimmunity in mice (Groom et al. 2002; Khare et al. 2000; Mackay et al. 1999).

In accordance with this relationship, BLyS levels correlate with serum autoantibody titers in Sjogren's syndrome and other systemic rheumatic diseases (Mariette et al. 2003; Cheema et al. 2001; Stohl et al. 2003). Despite the effectiveness of negative and peripheral selection, autoreactive B cells are found in mature pools in a quiescent state, suggesting the presence of additional poorly understood regulatory mechanisms (Wardemann et al. 2003). Finally, studies in humans receiving B cell ablation therapies such as rituximab or stem cell transplantation have provided not only detailed kinetics of human TR maturation but also novel surface markers to identify these immature B cell subsets (Palanichamy et al. 2009; Suryani et al. 2010; Anolik et al. 2007; Roll et al. 2006; Leandro et al. 2006). Further phenotypic marker studies, as well as functional and gene expression analyses, should help to further discriminate human B cell subsets (Anolik et al. 2009). Lastly, studies of human BCRs at the IMM and TR stages of development suggest that selection is based on specificity, and that either or both of these tolerogenic checkpoints are defective in humoral autoimmune diseases (von Boehmer and Melchers 2010; Meffre and Wardemann 2008; Wardemann et al. 2003; Yurasov et al. 2005; Wardemann and Nussenzweig 2007).

4 B Cell Activation and Humoral Immune Responses

BCR ligation initiates downstream signaling systems that foster activation. Characteristics of the subsequent humoral immune response are dictated by the type of antigen, the B cell differentiative subset(s) involved, the avidity of BCR cross linking, and intercellular interactions. In general, B cell responses follow the two-signal paradigm (Bretscher and Cohn 1970), whereby BCR ligation (signal 1) must be followed by additional activation and differentiation cues (signal 2) that are delivered via other cells or molecules. Two broad categories of humoral responses are defined based on the source of the second signal. The thymus-dependent (TD) response involves second signals that are delivered when B cells internalize, process, and present protein antigens to CD4 helper T cells. In contrast, the second signal in thymus-independent (TI) responses is delivered through innate immune receptors such as Toll like receptors (TLRs) expressed by the B cells themselves (TI-1), or through exceptionally intense BCR cross linking alone (TI-2).

Important differences between TD and TI responses include the pre-immune B cell populations that participate, the antibody isotypes generated, the response duration, the ultimate antibody affinity, and the extent of immunological memory established. In general, B-2 cells, particularly those in the FO compartment, are the major contributors to TD responses. In contrast, TI responses arise primarily from

either B-1 cells or the B-2 lineage MZ subset. These differences likely reflect the BCR signaling characteristics and differentiative potential of these pools, as well as the nature of inducing signals. TI responses are of short duration and skewed towards IgM production, whereas TD responses are more protracted and usually culminate in substantial class switched antibody of the IgG isotypes. Moreover, TD responses display a gradual but profound increase in average antibody affinity—a process termed affinity maturation. Finally, while both types of response can generate relatively long-lived antibody forming cells and memory B cells, TD responses are substantially more robust in this regard.

4.1 T-Independent Responses and Natural Antibodies

Within days after TI antigen challenge, substantial numbers of antibody secreting plasma cells (PCs) appear in splenic extrafollicular regions (Tarlinton 2008; Gourley et al. 2004). The antibodies made by this extrafollicular response are largely IgM and display comparatively low affinity for antigen. Within 2–3 weeks, the vast majority of these PCs die, although recent evidence indicates some long-term PC persistence and memory B cell (MBC) formation (Bortnick et al. 2012; Obukhanych and Nussenzweig 2006).

In addition to participating in responses to overt TI antigenic stimuli, some B-1 B cells are apparently constitutively activated and produce so-called natural antibodies (Bos et al. 1989; Baumgarth 2011). These polyreactive antibodies of the IgM and IgA isotypes bind epitopes on pathogens and commensals, as well as self-components such as cellular debris and phospholipids (Haas et al. 2005; Binder and Silverman 2005; Griffin et al. 2011). In conjunction with their use of a restricted set of IgH and IgL variable regions that do not include junctional insertions, these features suggest that B-1 B cells are "innate-like," serving both barrier and housekeeping functions with a limited and relatively invariable set of ligand receptors (Herzenberg 2000).

Several autoimmune prone mice highlight a role for TI activation of autoreactive B cells; particularly from the standpoint of antigens containing TLR7, 8, and 9 ligands (Pisitkun et al. 2006; Leadbetter et al. 2002; Herlands et al. 2008). How these activation cues lead to sustained autoantibody production nonetheless remains unclear and is an active area of investigation.

4.2 T-Dependent Responses, Germinal Centers, and Affinity Maturation

As with TI responses, within days of TD antigen challenge, substantial numbers of PCs that generate low-affinity IgM appear in splenic extrafollicular regions.

However, a few days later clusters of proliferating B cells appear at the borders of B cell follicles and T cell zones in the lymph nodes and spleen (Nieuwenhuis and Opstelten 1984; Jacob et al. 1991). These are germinal centers (GCs); transient structures wherein the unique functional features of TD responses emerge, including affinity maturation as well as efficient memory B cell (MBC) and long-lived plasma cell generation.

GC formation requires a series of cognate, bi-directional interactions between activated CD4 T cells and activated, antigen-presenting B cells. Detailed discussions of these interactions are found elsewhere (Victora and Nussenzweig 2012), but they include MHCII-restricted presentation by the B cell, costimulation via CD40-CD40L, and key cytokines such as IL-21. Together, these interactions result in the adoption of a GC B cell transcriptional program driven largely by Bcl-6 (Allman et al. 1996; Dent et al. 1997; Shaffer et al. 2000; Basso and Dalla-Favera 2010). A key gene upregulated in GC B cells is activation-induced deaminase (AID), which creates point mutations in Ig V regions (Muramatsu et al. 2000; Pavri et al. 2010). This so-called somatic hypermutation (SHM) mechanism results in clonal variants of GC B cells with altered antigen affinity and specificity (Pavri and Nussenzweig 2011). Through selective competition and survival, clonal variants with higher affinity for antigen are selectively preserved, whereas those with lower affinity are at a selective disadvantage and die (Zotos and Tarlinton 2012). The details surrounding preferential survival remain an area of intense investigation, but clearly involve competition for antigen as well as survival signals. Currently popular models posit that the anatomically defined GC light zones are where competition for antigen and T helper cell survival factors occurs; whereas proliferation and AID-mediated SHM occur in GC dark zones (MacLennan 1994). AID also mediates class switch recombination (Muramatsu et al. 2000). Regulation of GC formation and resolution, light and dark zone designations and functions, and outcomes are broadly similar between mice and humans (Victora et al. 2012; Schmidlin et al. 2009; Diehl et al. 2012; Durandy et al. 2007; Peron et al. 2007).

Since GC B cells undergo a random BCR diversification process, the formation of autoreactive specificities is an unavoidable consequence (Diamond and Scharff 1984; Alabyev et al. 2007). Accordingly, active selection against incipient autoreactive GC B cell clones must also occur, although the mechanisms remain debated (Zou and Diamond 2013). Current models include direct death signaling through Fas–FasL interactions, as well as an inability to access survival cytokines due to loss of cognate antigen-presenting ability. Nonetheless, there is clear evidence for GC and/or post-GC selective checkpoints in both mice and humans (Wong et al. 2012; Yan et al. 2012; Tiller et al. 2007). Moreover, there is evidence for defects in this tolerance checkpoint in some SLE patients (Cappione et al. 2005).

4.3 Long-Lived Plasma Cells and Memory B Cells

Humoral responses, particularly TD responses, culminate in the establishment of long-lived PCs and MBC. Long-lived PCs can persist for the life of the organism; however, the basis for their longevity and precise differentiative origin remain an

area of intense investigation. Commitment to the PC fate involves the expression of B lymphocyte induced maturation protein 1 (Blimp1), which extinguishes the mature B cell gene expression program (Shaffer et al. 2002). Blimp1 initiates the PC transcriptional program in part through repression of both Bcl6 and Pax5 (Angelin-Duclos et al. 2000; Martins and Calame 2008). In addition, a plethora of stress response genes, presumably to cope with sustained antibody secretion (Oracki et al. 2010), are upregulated via the transcription factor Xbp1 (Reimold et al. 2001). Long-lived PCs home to and reside in the BM, affording stable and high antibody titers for the lifetime of the host (Schittek and Rajewsky 1990; Manz et al. 1997). For example, TD responses from vaccines or pathogens confer protection for years or decades in humans (Pinna et al. 2009; Plotkin 2008; Amanna et al. 2007). Accordingly, given their robust nature and remarkable lifespan, long-lived BM PCs are of particular concern in the context of autoimmunity. Indeed, among patients where long-lived PCs are the source of pathogenic autoantibodies, ablative therapies targeting pre-immune and MBC pools may have little impact (Slifka et al. 1998). Thus, specifically targeting PCs is an important yet comparatively unexplored area in therapeutics for humoral autoimmune disorders.

MBCs are the result of antigen-driven clonal expansion long after an immunological challenge (Crotty et al. 2003). They remain in the host at elevated frequencies and are less dependent on T cell help for their reactivation (Maruyama et al. 2000). Whether antigen persistence plays a role in their maintenance remains debated, but at least some MBCs endure in the apparent absence of antigen (Vieira and Rajewsky 1990). Furthermore, MBC have a lower BCR signaling threshold, enabling more rapid entry into cell cycle compared to pre-immune pools (Gagro et al. 2003; Good et al. 2009; Yefenof et al. 1986). Moreover, the Ig genes of MBCs can be highly mutated or not, and MBCs can express either switched or unswitched BCRs (Gourley et al. 2004; Anderson et al. 2007). Lastly, MBCs are generally derived from GCs; however, evidence also exists for GC-independent MBC generation (Shlomchik and Weisel 2012).

In contrast to the relationship between BLyS and pre-immune B cell homeostasis, survival requisites for memory and plasma cells are not yet resolved. Alternative members of the BLyS ligand and receptor family may play a role, but are likely redundant with other survival promoting mechanisms. For example, B cells stimulated with TLR-4, 7, and 9 ligands upregulate TACI expression, suggesting APRIL or BLyS may be important for the differentiation of short-lived PCs and/or their persistence (Treml et al. 2007; Groom et al. 2007). Similarly, long-lived PCs express both TACI and BCMA, suggesting APRIL may be an important cytokine for long-lived plasma cell homeostasis. Indeed, reductions—but not complete elimination—of plasma cells were noted when BLyS and APRIL were simultaneously blocked in vivo (Benson et al. 2008). Other cytokines, interleukins, and chemokine receptors are clearly involved in PC survival, suggesting considerable redundancy (Oracki et al. 2010). Whether these can be targeted individually or en masse to achieve therapeutic benefit is not yet clear, but may raise considerable off-target hurdles, inasmuch as eliminating long-lived memory and PC pools could significantly impact preexisting immunity to pathogens or vaccine antigens.

5 Overview of B Cells as Therapeutic Targets

Therapeutics targeting different B cell subsets and activation points are likely to differ in their activity and efficacies both within and between various autoimmune diseases. Indeed, several therapeutic agents that target B lineage cells are in clinical use or development for treating autoimmune diseases (Chugh 2012). The basic strategies involve targeting B cell-specific surface markers, depleting key survival factors, or disrupting critical intercellular or intracellular functions. Key examples of each of these approaches include rituximab (anti-CD20), belimumab (anti-BLyS), CD40 blockade, and bortezomib (proteasome inhibitor), respectively. Here, we briefly consider the effects and implications of each therapy, in order to illustrate how an understanding of B cell biology may provide insight into predicted outcomes, mechanism(s) of action, and potential drawbacks.

Two biologicals that have been applied to humoral autoimmunity are largely targeted towards eliminating members of pre-immune B cell subsets: rituximab and belimumab. Rituximab directly depletes B cells by targeting the CD20 surface molecule. Although effective for treatment of RA, rituximab has yielded perplexing results in off-label use for SLE (Looney et al. 2004; Sanz et al. 2011; Stohl et al. 2011). The basis for such confounding outcomes is unclear, but might reflect unwanted effects on B cell selection, or the lack of activity on relevant subsets in some subjects. For example, since BLyS levels are inversely related to mature B cell numbers, serum BLyS levels increase when B cells are ablated (Cambridge et al. 2006; Kreuzaler et al. 2012). Thus, depletion of mature pre-immune B cells pools without comcomitantly limiting BLyS availability could lead to temporarily relaxed TR selection, affording entry of autoreactive clonotypes to mature naïve pool (Cambridge et al. 2006). Alternatively, because long-lived PCs lack CD20 surface expression, rituximab may not target the cells responsible for pathogenic antibody production in some subjects (Pescovitz 2006).

Belimumab—an anti-BLyS monoclonal antibody that neutralizes soluble BLyS—is one of several therapeutic agents designed to target BLyS family members (Cancro et al. 2009; Vincent et al. 2013). This approach ablates pre-immune B cell pools, albeit through survival cytokine blockade rather than direct B cell targeting. Clinical trial results demonstrated reductions in serum BLyS levels following treatment, as expected; along with significant and sustained reductions in mature pre-immune and activated B cells (Wallace et al. 2009; Furie et al. 2011). Nonetheless, the degree of depletion was less than might have been predicted by mouse studies, possibly reflecting the comparatively lower BLyS reliance of human FO B cells (Tangye et al. 2006). Therefore, reducing BLyS levels concomitant with B cell ablation may "normalize" TR selection, although this has not yet been directly assessed.

Other agents have been developed to target activated and antigen experienced B cell subsets. Some impede interactions of activated B cells with elements of T cell help, possibly influencing ongoing or emerging GC reactions, while others are aimed primarily at antibody secreting plasma cells per se. Thus, in SLE patients,

administration of CD40L blocking antibody results in decreased PCs, lowered anti-double-stranded DNA antibody levels, and reduced proteinuria (Grammer et al. 2003). Whether this reflects disruption of ongoing GC responses where negative selection has failed is unclear, but warrants further investigation. Indeed, there is ample evidence that some autoimmune disorders require T cell help (Jiang et al. 2007; Diamond et al. 1992; Shlomchik et al. 1990; Mohan et al. 1995), so blocking T-B interactions may be a highly attractive therapeutic approach. Accordingly, further understanding of B cell selection and tolerance checkpoints in TD responses may suggest future therapies.

How memory or plasma cell subsets are impacted by current ablative approaches remains unclear, probably reflecting heterogeneity and insufficient phenotypic delineation of memory B cell subsets (Anolik et al. 2009). For example, despite results from both mouse studies and clinical trials indicating that MBC and PC pools are not substantially affected by anti-BLyS treatment, initial increases in circulating MBC, followed by a gradual return to baseline levels, were observed in belimumab clinical trials (Wallace et al. 2009; Furie et al. 2011). Nevertheless, there were sustained and significant decreases in a plasma cell subset implicated in SLE pathogenesis, along with IgG anti-dsDNA Ab and ANA titers, while Ab titers to previous immunizations were maintained (Jacobi et al. 2003; Chatham et al. 2012; Furie et al. 2011; Navarra et al. 2011). These results raise the possibility of targeting pathogenic MBCs or PCs while sparing others.

Bortezomib is a proteasome inhibitor originally developed for multiple myeloma. Because PCs synthesize massive amounts of antibody, inhibiting proteasome function induces apoptosis through the unfolded protein response (Obeng et al. 2006). Therefore, inhibiting the proteasome has become an attractive novel therapy. Treatment of lupus prone mice with bortezomib protects from nephritis (Neubert et al. 2008). Similar results were also produced in an experimental model of autoimmune myasthenia gravis (Gomez et al. 2011). Interestingly, bortezomib selectively targets TD generated PCs but spares early TI type 2 responses (Lang et al. 2010). Unfortunately, because molecular inhibitors are global, the therapy lacks the specificity that antibody-based therapeutics provide. A more detailed discussion about bortezomib's role in treating humoral autoimmune disorders can be found elsewhere (Fierabracci 2012).

6 Perspective

Over the past two decades, research in basic B cell biology has cleared the path for the development of therapeutic agents for treating autoimmune disease. With increasing understanding of development, tolerance checkpoints, and function, the coming years promise to yield increasingly targeted agents to allow manipulation of specific B cell types as improved therapies are designed.

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Pharmacological Effects and Mechanisms of Action of Agents Blocking B Cells

Ignacio Sanz

Abstract Spearheaded by the development of anti-CD20 monoclonal antibodies capable of effectively killing B cells, the FDA approval in 1999 of the anti-CD20 monoclonal antibody rituximab for the treatment of B cell malignancies and reassurance about the overall safety of B cell depletion, the field of anti-B cell therapies has expanded over the last decade to the treatment of multiple autoimmune diseases. Moreover, growing knowledge of the biology of B cells and their functional and phenotypic heterogeneity combined with advances in biotechnology have enabled investigators and pharmaceutical companies to develop new agents that target different types of B cells through multiple mechanisms of action. Together with improved understanding of the heterogeneous nature of autoimmune diseases and better approaches to their segmentation, these developments should provide in the near future multiple choices for the rationale design of clinical trials and eventually, multiple choices for safer and more effective modalities that allow clinicians to use different agents, either combined or sequentially, to induce and maintain disease remission. In this chapter, we shall discuss the rationale for B cell therapies, the different strategies that can be used to target B cells and the mechanisms of action of drugs currently used for the treatment of autoimmune diseases.

1 Introduction

Spearheaded by the development of anti-CD20 monoclonal antibodies capable of effectively killing B cells, the FDA approval in 1999 of the anti-CD20 monoclonal antibody rituximab for the treatment of B cell malignancies and reassurance about the overall safety of B cell depletion, the field of anti-B cell therapies has expanded over the last decade to the treatment of multiple autoimmune diseases (Sanz 2009;

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Tony 2010). Moreover, growing knowledge of the biology of B cells and their functional and phenotypic heterogeneity combined with advances in biotechnology have enabled investigators and pharmaceutical companies to develop new agents that target different types of B cells through multiple mechanisms of action (Martin and Chan 2004; Looney et al. 2010; Calero et al. 2010; Townsend et al. 2010; Dörner et al. 2010). Together with improved understanding of the heterogeneous nature of autoimmune diseases and better approaches to their segmentation, these developments should provide in the near future multiple choices for the rationale design of clinical trials and eventually, multiple choices for safer and more effective modalities that allow clinicians to use different agents, either combined or sequentially, to induce and maintain disease remission.

In this chapter, we shall discuss the rationale for B cell therapies, the different strategies that can be used to target B cells and the mechanisms of action of drugs currently used for the treatment of autoimmune diseases.

2 Rationale for B Cell Targeting

B cells have central contributions to the pathogenesis of autoimmune diseases through both antibody-dependent and antibody-independent functions with the latter including antigen-presentation, cytokine production, and organization of secondary and tertiary ectopic lymphoid tissue (Manjarrez-Orduno et al. 2009; Townsend et al. 2010). Through these activities, B cells produce pathogenic autoantibodies and immune complexes and pro-inflammatory cytokines (Townsend et al. 2010). Similarly, B cells may regulate CD4 T helper cells, CD8 T cells, iNKT cells, and T regulatory cells as well as dendritic cells and therefore exert a profound influence on the overall homeostasis and function of the immune response. It is important to bear in mind, however, that the final functional outcome induced by B cells and therefore by their depletion, is rather complex as B cells may exert opposing activities (Matsushita et al. 2008; Lund 2008; Yanaba et al. 2008; Manjarrez-Orduno et al. 2009; Calero et al. 2010). This Janus-like nature of B cells is illustrated by their ability to either induce or suppress T regulatory cells and to produce either protective natural IgM autoantibodies (Stoehr et al. 2011; Notley et al. 2011) or pathogenic IgG autoantibodies. Also, of significant importance, subsets of B cells with regulatory properties mediated at least in part by the production of IL-10 have been reported in humans over the last few years (Blair et al. 2010; Iwata et al. 2011). The cellular and molecular basis of this complex behavior remain to be fully understood but is likely to depend at least in part on functional differences between separate B cell populations and to functional modifications induced by the pro-inflammatory milieu of autoimmune diseases. These limitations in knowledge notwithstanding, the recognition of the rather complex behavior of B cells should provide a better foundation for the understanding of the consequences of universal B cell depletion and of the relative advantages of more targeted B cell therapies.

Table 1 Strategies for therapeutic targeting of B cells and plasma cells

		Effect on cellular target		
Mechanism	Molecular target	B cells	Plasma cells	Drug
Direct depletion	CD20	Killing by CDC, ADCC and apoptosis	Deplete precursors, early CD20+ PB	Rituximab, others
	CD19	Killing	Deplete precursors, PB, CD19+ PC	Antibodies
	CD38, CD138, CD27	??	Deplete PC	ATG
	Proteasome	Killing of GC and activated B cells	Deplete PC	Inhibitors
Depletion and inhibition	CD79α/β	Deplete and inhibit proliferation	Deplete precursors	Antibodies
Survival and/or differentiation	BAFF	↓ N and Tr cell survival, promote tolerance; ↓ GC survival	Deplete some precursors	Belimumab
	BAFF + APRIL	↓ N and Tr cell survival, promote tolerance; ↓ GC survival	Deplete SLPC, spleen LLPC; ↓ BM PC	Atacicept
	Type 1 IFN	↓ N cell activation; ↓ M cell differentiation ↓ MZ B cell precursors/migration	↓ Generation	Antibodies to IFN, IFN-R
Interruption of abnormal germinal center reactions	IL-21	↓ N, inhibit GC, M cell differentiation, survival	↓ Generation/ survival	Antibodies
	IL-6	↓ M cell differentiation	↓ Generation/ survival	Tocilizumab
	IL-17	↓ GC reactions; ↓ B cell proliferation and switching	↓ Antibody production	Antibodies
	IFNγ	↓ GC reactions; ↓ Th1/T_{FH} cell-dependent autoantibodies	↓ Generation?	Antibodies
BCR inhibition	Syk, Btk, PKCδ, PI3K/AKT/ mTOR	↓ Activation and survival	↓ Generation?	Inhibitors
Inhibitory co-receptors	CD22	↓ N and Tr cell	None direct	Antibodies
	FcRγIIb	Inhibit B cell activation	↓ PC survival	IVIG; antibodies
Co-stimulation	CD40L	Inhibition of early B cell activation/GC formation	↓ Generation?	Antibodies[a]

(continued)

Table 1 (continued)

Mechanism	Molecular target	Effect on cellular target: B cells	Plasma cells	Drug
	TLR	Inhibition of B cell activation Control of T cell autoimmunity	↓ Differentiation	Inhibitors
	ICOS	↓ CC differentiation, B cell activation	↓ Generation?	Antibodies
	BAFF/APRIL/IFN		As above	
	CD80/CD86/CD28	Inhibit B cell activation and APC function	↓ PC survival	Abatacept; antibodies
B cell homing and environment	CXCR4/CXCL12	Inhibit GC reaction	↓ BM homing, survival	Antagonists antibodies
	CXCR5/CXCL13	Inhibit GC, ectopic lymphogenesis	↓ Generation	Antagonists antibodies
	CXCR3/CXCL9-11	Inhibit/kill CXCR3+ effector B cells	Inhibit migration to target organs	Antagonists antibodies
	LTβR	Disrupt GC, ectopic lymphneoogenesis	↓ Generation?	Baminercept
Induction of regulatory B cells	CD40	Expand IL-10-producing T2 B cells	??	Agonistic CD40 antibody
	IL-15R	Expand IL-10-producing B cells	??	GM-CSF/IL-15 fusokine
	CD40 + IL-21R	Expand B10 cells	??	CD40L + IL-21

Partial description of anti-B cell/plasma cells mechanisms currently under investigation (ranging from preclinical studies to phase III clinical studies). The table includes the main known biological effects on B cells and plasma cells (on the basis chiefly of animal experiments but also human data when available). In many instances, impact on plasma cells was deduced from the effect of the intervention on antibody levels and not necessarily on direct cellular evidence

N naïve, *Tr* transitional, *M* memory, *BM* bone marrow, *GC* germinal center, *CC* centrocyte, *PC* plasma cells, *SLPC* short-lived PC, *LLPC* long-lived PC, *MZ* Marginal Zone

[a]A study of anti-CD40L antibodies in SLE was interrupted due to thromboembolic complications

?? Effect found in animal or in vitro studies but not proven clinically

3 Mechanisms of B Cell Targeting

Multiple strategies can be used to target B cells with the ultimate goal of generating a favorable, protective balance between pathogenic and protective functions (Calero et al. 2010; Sanz and Lee 2010). Different strategies including some not specifically discussed in this chapter are summarized in Table 1. In rather simplistic

terms, such balance requires either the selective elimination of pathogenic cells while preserving regulatory B cells; the direct expansion of regulatory B cells; or the universal depletion of B cells under conditions that promote repopulation with a predominance of protective cells. An extension of the latter concept is the possibility of using multiple agents (combined or sequentially) to try and restore B cell tolerance during the repopulation phase that ensues after B cell depletion.

The first and most commonly used form of B cell therapy is the direct killing of B cells using cytotoxic antibodies directed against B cell surface antigens. Alternatively, different degrees of B cell depletion can be obtained by inhibiting cytokines that promote the survival and/or differentiation of B cells. Other agents can be used to dampen the activation of B cells either by inhibiting BCR signaling or B cell co-stimulatory pathways or by engaging B cell inhibitory co-receptors. Anti-B cell activity could also be achieved with agents that inhibit abnormal germinal center reactions (Sweet et al. 2012), or agents that disrupt the B cell microenvironment in secondary and tertiary lymphoid tissues (Browning 2008). Finally, a critical B cell effector function, namely the generation of pathogenic autoantibodies could be targeted by agents that directly eliminate antibody-producing plasma cells or inhibit their differentiation and/or survival.

3.1 B Cell Elimination

3.1.1 Direct B Cell Depleting Agents

Anti-CD20 antibodies represent the paradigm of agents that target B cells by recognizing specific surface antigens and mediate B cell elimination through multiple mechanisms including: antibody-mediated cytotoxicity (ADCC); complement-dependent cytotoxicity (CDC); and direct B cell apoptosis. CD20 expression encompasses almost the whole B cell differentiation spectrum with initial upregulation that starts in pre-B cells and is extinguished in early plasmablasts. Hence, anti-CD20 antibodies eliminate a large swath of the B cell compartment but spare pro-B cells and antibody-secreting cells. Antibodies against other B cell surface markers have a different target profile depending on the relative expression of the marker in question. Thus, anti-CD19 antibodies would target a wider spectrum of B cells including pro-B cells and a large fraction of antibody-secreting cells. These antibodies could also play an important role in autoimmune diseases as indicated by their ability to decrease serum immunoglobulins in animal models (Yazawa et al. 2005). It should be noted, however, that they might be unable to target the most mature, terminally differentiated plasma cells which are devoid of CD19 expression (Sanz and Lee 2010; Mei et al. 2012). Antibodies directed against other B cell markers such as CD22 are also of significant interest and target a more limited fraction of B cells. However, given their different mechanism of action, they will be discussed in a separate section. Here we will focus on the mechanisms of action of rituximab and similar anti-CD20 antibodies since this agent has

contributed the vast majority of published studies, has been approved by the FDA for the treatment of anti-TNF resistant rheumatoid arthritis and ANCA-associated vasculitis and is frequently used in clinical practice for these indications and in multiple other autoimmune diseases (Sanz 2009).

Rituximab is a chimeric anti-CD20 monoclonal antibody with a mouse variable region that attaches to CD20 and a human IgG1 Fc region that mediates critical effector functions including CDC and ADCC, the central mechanisms of action of this agent. As discussed elsewhere in an excellent review, the relative contributions of these mechanisms to the in vivo activity of rituximab are largely deduced from in vitro studies (Weiner 2010). Some of these studies have clearly identified CDC as a major mechanism and this conclusion has also been supported in animal studies (Cang et al. 2012). However, Fc-mediated ADCC is undoubtedly important as well and this conclusion is perhaps best illustrated by the observation that variations in the elements that participate in this function help explain the limited activity of rituximab in some patients with lymphoma and autoimmune diseases alike (Weiner 2010). Thus, polymorphisms in the FcRγIIIA receptor that limit the efficiency of killing have been associated with decreased B cell depletion and clinical efficacy (Anolik et al. 2003). It is also of interest that NK cell activity, one of the main cell types involved in carrying out ADDC may be defective in SLE and possible other rheumatic diseases as well (Sibbitt and Bankhurst 1985), a factor that might contribute to decreased rituximab efficacy. At least in vitro, rituximab can also induce direct B cell apoptosis upon attachment to CD20, aggregation into lipid rafts and downstream inhibition of activation and anti-apoptotic pathways (Bonavida 2007). Finally, it is worth bearing in mind that rituximab might have synergistic effects with chemotherapeutic drugs, including cyclophosphamide, in the treatment of malignancies (Weiner 2010). Such synergy might be relevant to the rationale of combining rituximab and cyclophosphamide in the treatment of refractory SLE (Lu et al. 2009; Vital et al. 2012).

The timing of B cell depletion and repopulation following rituximab is reasonably well understood and also reasonably consistent in different diseases. Generally, maximal depletion is achieved within 4–8 weeks after treatment and most frequently, significant CD19+ B cell repopulation is observed 6–9 months thereafter. These times intervals are variable and selected patients have been reported in whom no significant B cell repopulation was observed for several years (Anolik et al. 2004; Anolik et al. 2007b) (Leandro et al. 2006; Lu et al. 2009; Leandro et al. 2005; Lu et al. 2008). The extent and quality of depletion and repopulation are critical for therapeutic efficacy (Looney et al. 2004; Anolik et al. 2007a; Albert et al. 2008; Lazarus et al. 2012). Thus, deep depletion (defined as reaching a level of $\leq$0.01 cells/μL rather than the level of 5 cells/μL unfortunately used in most studies to define "complete" depletion) appears to determine favorable and lasting responses (Vital et al. 2011b; Dass et al. 2008). This observation has two important corollaries. First, measurement of the actual level of depletion using high-sensitivity flow cytometry is imperative in order to discriminate between the failure of any given rituximab administration to induce complete B cell depletion and the failure of complete B cell depletion to induce disease response (Vital et al. 2011a).

Second, the correlation between deeper depletion and better clinical response would suggest the need for modified regimens aimed at improving the depth of depletion as indicated by the initial response or by patient segmentation approaches that might identify beforehand subjects more likely to be refractory to conventional administration. Potentially beneficial modifications could include the aforementioned co-administration of cyclophosphamide, administration of higher or more frequent doses of rituximab and combination therapies with other biological agents that might potentiate the depleting effects of rituximab and/or prevent the fast re-expansion of residual pathogenic B cells. Along these lines, we and other have reported that such residual cells largely include memory cells and plasma cells, the former possibly due to resistance to depletion and surging levels of BAFF after depletion (Cambridge et al. 2007), and the latter to lack of expression of CD20 (Anolik et al. 2004; Anolik et al. 2007b; Roll et al. 2007). Moreover, SLE patients with poor response to rituximab are characterized by quick re-expansion of memory cells (Anolik et al. 2007a) and SLE relapses after B cell depletion correlate either with expansion of plasmablasts in anti-DNA high patients or with IgD− CD27− B cells in patients with low anti-DNA titers (Lazarus et al. 2012) and poor therapeutic response in RA correlates with baseline levels of CD27+ memory cells and plasmablasts (Roll et al. 2007; Owczarczyk et al. 2011).

Multiple agents offer at least a theoretical rationale for combination therapy that might improve the depth and quality of B cell depletion. Such agents span from the FDA-approved anti-BAFF antibody belimumab to agents in preclinical development including type I interferons (Thurlings et al. 2010), and TLR inhibitors (Leadbetter et al. 2002) or cytokine inhibitors (Anolik and Aringer 2005), that might improve depletion and/or interfere with the survival, expansion, and pathogenic differentiation of residual memory cells. Similarly, combination therapy with agents aimed at plasma cells also represent an appealing alternative (Table 1). Of currently available agents, proteasome inhibitors might be especially advantageous due to their ability to target not only plasma cells but also activated germinal center and memory cells that might be more resistant to rituximab. Moreover, the very fast and short-lived effect of these drugs would allow to limit their impact to a predetermined time window thereby enhancing safety.

Different variables are known to impact the ability of rituximab to deplete B cells. These variables include the aforementioned FcRγIIIA polymorphisms, black race, at least in SLE and the generation of human anti-chimeric antibodies (HACA), which are more common and pronounced in SLE (Anolik et al. 2003; Looney et al. 2004; Albert et al. 2008). An interferon signature in blood mononuclear cells has also been correlated with poor response to rituximab in RA. While no direct analysis of correlation with B cell depletion, this study highlighted the incomplete tissue and bone marrow depletion as a possible explanation (Thurlings et al. 2010).

In tune with the different roles described for B cells, the immunological consequences of B cell depletion and its impact in the course of the disease can be ascribed to the interruption of antibody-dependent and antibody-independent functions and to the potentiation of B cell regulatory functions. From an antibody standpoint, despite the lack of direct effect rituximab on most plasma cells, this

agent has been associated with significant decrease in serum levels of some autoantibodies but not others presumably reflecting the generation of the residual autoantibodies by long-lived plasma cells capable of surviving without replenishment from B cells (Martin and Chan 2006; Cambridge et al. 2006). Whether autoantibody decrease is the main reason for disease improvement remains a matter of debate. Nonetheless, both the clinical improvement observed in different diseases before substantial antibody decline is observed and the strong clinical response observed in patients that maintain their autoantibodies suggest the participation of other important mechanisms as well (Looney et al. 2004; Hauser et al. 2008; Stone et al. 2010). One such mechanism is the restoration of a protective B cell balance through B cell reconstitution dominated by transitional and naïve B cells. Whether this balance is created merely by the absence of pathogenic cells or by the expansion of regulatory B cells remains to be fully determined (Calero et al. 2010). However, tantalizing studies support the latter possibility as B cell depletion has been shown to restore the ability of naïve B cells to secrete regulatory IL-10 in Multiple Sclerosis (Duddy et al. 2007). More recently, the benefit of rituximab in SLE has been ascribed to the expansion of CD1d+ CD38hiCD24hi transitional B cells with restored functional ability to induce suppressive invariant NKT cells (Bosma et al. 2012). Interestingly, the same group has reported a deficiency of IL-10 mediated regulatory function of CD24hiCD38hi transitional cells in SLE (Blair et al. 2010), and of the ability of the same cells to induce T regulatory cells and suppress Th1 and TH17 development in RA (Flores-Borja et al. 2013). Thus, it would appear that repopulation dominated by transitional cells with restored regulatory functions may play a major role in the immunological and clinical benefit induced by rituximab. It will be of great interest to understand in future studies how the different functions ascribed to these cells in separate studies compare in side-by-side analysis and in different diseases and to correlate the different regulatory functions with disease improvement in different conditions.

Other studies in different diseases have also suggested that the benefit of rituximab may be mediated at least in part by inhibiting T cell activation (Iwata et al. 2010; Sfikakis et al. 2005) or the development of Th1 cells and Th17 cells (van de Veerdonk et al. 2011). The expansion of T regulatory cells following B cell depletion has also been described (Vigna-Perez et al. 2006; Audia et al. 2011; Stasi et al. 2008) but is worth noting that favorable effects on the balance of effector to regulatory T cells after rituximab has not been a consistent finding (Abdulahad et al. 2011) and that both favorable and unfavorable effects of B cell depletion on T cell differentiation may be demonstrated in different animal models (Weber et al. 2010)

Finally, it is possible that profound and sustained B cell depletion might afford the immune system a second chance to educate itself during the repopulation phase, thereby enforcing proper immunological tolerance. Restoration of tolerance might be promoted by the absence of danger signals in a non-inflammatory milieu, lack of exposure to offending antigens and/or lack of repetition of stochastic events that might generate specific autoreactive B cell clones (Sanz and Lee 2010; Edwards and Cambridge 2006).

The benefit of rituximab in multiple diseases has prompted the development of new generations of anti-CD20 antibodies. These antibodies include humanized and fully human antibodies including ocrelizumab (Maloney 2012) that elicit lesser immunogenic responses as well as antibodies engineered to acquire better ADCC or CDC and/or more favorable pharmacokinetics (Cang et al. 2012; Maloney 2012). The new antibodies can be differentiated on the basis of differential binding to CD20, modifications to the Fc region or introduction of different scaffolds including small modular immunopharmaceutical (Hayden-Ledbetter et al. 2009). In terms of CD20 binding properties, rituximab and other type I antibodies such as ofatumumab promote clustering of CD20 into membrane lipid rafts and possess strong CDC and ADCC relative to direct anti-B cell effects. In turn, ofatumumab has increased CDC activity due to enhanced C1q binding created by the recognition of a CD20 region that is more proximal to the transmembrane domain than the one recognized by rituximab (Wierda et al. 2010). In contrast, CD20 redistribution is not a consequence of type II antibodies (tositumomab and obinutuzumab) which are characterized by strong ADDC, weak CDC and strong anti-B cell tumor activity (Maloney 2012). Whether these new agents will translate into better and safer anti-CD20 B cell depleting agents remains to be determined.

3.1.2 Agents That Block B Cell Survival and/or Differentiation

BAFF and APRIL Blockade

Belimumab, an anti-BAFF/BLyS (B lymphocyte activation factor/BLyS), monoclonal antibody was recently approved by the FDA for the treatment of SLE and is now being tested in rheumatoid arthritis and Sjogren's syndrome (Sanz 2011; Jin and Ding 2013). These developments further substantiate the rationale of targeting B cells in patients with active SLE, a subset that benefits the most from this therapy (van Vollenhoven et al. 2012). The benefit of this approach also provides a different paradigm for more selective B cell targeting through a different mechanism of action, namely the inhibition of essential B cell survival factors such as BAFF and/or APRIL (a proliferation-inducing ligand) (reviewed in (Moisini and Davidson 2009). Human BAFF signals through three different receptors (BAFF-R, TACI and BCMA), the latter with low avidity, whereas APRIL only signals through TACI and BCMA. Accordingly, anti-BAFF antibodies (belimumab) or BAFF-R-Ig provide selective BAFF blockade. In contrast, a TACI decoy receptor (TACI-Ig fusion protein; Atacicept) binds both BAFF and APRIL, thereby inhibiting both signaling pathways. BAFF is essential for the survival of late transitional, follicular naïve and marginal zone B cells yet, it is not critical for the survival of memory cells. Nevertheless, it may contribute to the survival of germinal centers and plasmablasts generated from human memory cells (Avery et al. 2003). In contrast to B cells, murine short-lived and long-lived plasma cells survival is more dependent on TACI and BCMA, respectively, and accordingly, combined BAFF and APRIL inhibition with TACI-Ig induces more pronounced depletion of these cells than selective

BAFF blockade (Moisini and Davidson 2009). In most lupus models, TACI-Ig preferentially depletes short-lived IgM plasma cells while sparing long-lived IgG bone marrow plasma cells. The same agent, however, was able to deplete IgM and IgG plasma cells from the spleens of MRL/lpr mice (Liu et al. 2004).

The relative expression of the three BAFF family receptors in different human B cell subsets and the relative role of BAFF and APRIL in their homeostasis remain to be fully elucidated. Similarly, whether BAFF and/or APRIL blockade have similar B cell consequences in humans as in mice is also unclear. However, available data reviewed below indicate significant parallels between the two species in terms of the patterns of expression of the BAFF-R, TACI, and BCMA (Moir et al. 2004; Avery et al. 2003; Jacobi et al. 2010) and important information regarding B cells in patients treated with these agents is starting to emerge. Thus, in SLE patients treated with belimumab for up to 2.5 years (Jacobi et al. 2010), BAFF inhibition induced slow but substantial and sustained decreases in transitional cells and naïve cells with statistical significance reached after 3 months of treatment. Memory cells and plasma cells did not significantly decrease indicating that similar to their mouse counterpart, these human B cell subsets are independent of BAFF for survival. In contrast, a subset of isotype switched CD27-negative cells whose expansion correlates with active SLE (Wei et al. 2007), and with SLE flares after rituximab treatment (Lazarus et al. 2012), also underwent significant and sustained depletion. These findings have been largely confirmed and extended by the immunological analysis of the large number of patients treated in the phase III studies that led to the approval of belimumab (Stohl et al. 2012). This study demonstrated a global reduction of median numbers of total B cells by 50 % through 76 weeks of treatment with significant reductions demonstrated as early as 8 weeks of treatment. No T cell changes were appreciated but only global CD4 and CD8 T cell numbers were evaluated. B cell changes were largely explained by reduction in naïve cells (defined as CD20+ CD27−) that declined by a median of 40 % as early as 8 weeks and by 75 % at 76 weeks, without significant memory cell reductions. Significant changes were also observed in activated B cells (CD20+ CD69+) but only after 52 weeks of high-dose belimumab and after 76 weeks of low-dose therapy. Of great interest, decrease in naïve CD20+ CD27− B cells was the only cellular correlate of clinical improvement and lower risk of severe flare. Declines in different subsets of antibody-secreted cells were also observed but only 24 weeks of treatment, thereby raising the question of whether these changes may have been due to decline in disease activity or a consequence of pharmacological interference with plasma cell differentiation or survival. Unfortunately, more incisive understanding of the B cell consequences of BAFF inhibition in this unique study is limited by a phenotypic definition of B cells that precludes discrimination of naïve, transitional and CD27-negative memory cells and the use of imprecise and overlapping classifications of multiple plasmablasts and plasma cell subsets. Nonetheless, belimumab induced a significant and rather quick reduction in total serum IgG levels and anti-ds DNA antibody titers of 10 % and 20 %, respectively, after only 8 weeks of treatment. While total IgG remained relatively stable thereafter and anti-microbial antibody titers did not significantly change, anti-DNA titers continued to decline and were

40 % lower after 52 weeks. Combined, these observations are consistent with a preferential impact of BAFF inhibition on short-lived plasmablasts as compared to long-lived plasma cells. It should be noted, however, that approximately 30 % of patients treated with high-dose belimumab lost their serum titers of anti-Sm antibodies, a type of autoreactivity typically ascribed to long-lived plasma cells. However, the fact that about 20 % of placebo patients also lost these antibodies indicates that changes in disease activity may play a major role irrespective or in addition to BAFF blockade. Regrettably, no information was provided regarding other long-standing autoantibodies such as anti-Ro antibodies.

Combined, the two analysis of the immunological impact of BAFF blockade thus far performed in SLE, offer a picture that is both consistent with the known biology of this cytokine but that is also somewhat counterintuitive regarding prevailing notions of the pathogenesis of SLE. Indeed, the results discussed (Stohl et al. 2012; Jacobi et al. 2010) indicate that disease activity and early improvement after belimumab is largely dependent on CD27− cells (that include both naïve, transitional and isotype switched CD27− cells) rather than on more conventional CD27+ memory cells and plasmablasts. Given the regulatory potential of transitional and naïve cells and the substantial numerical contraction experienced by these compartments, it will be important to understand their functional activity and whether regulatory function is enhanced by BAFF blockade. It should be noted, however, that BAFF levels set the threshold for negative selection of early autoreactive B cells and that excess BAFF facilitates their selection into the mature compartment (Lesley et al. 2004). Therefore, it is tempting to postulate that the enforcement of tolerance presumably imposed by limiting BAFF stimulation of newly formed B cells may play a more important role than the number of B cells of presumed regulatory phenotype. Direct studies of regulatory function and autoreactivity will be required to clarify these critical aspects.

As previously discussed, combined BAFF-APRIL inhibition with atacicept results in more pronounced plasma cells and antibody decreased in preclinical models and is therefore highly appealing on theoretical grounds for the treatment of antibody-mediated human autoimmune diseases. These predictions have been confirmed in a randomized, double-blind, placebo-controlled Phase II/II of SLE patients treated in combination with corticosteroids and mycophenolate mofetil (MMF). Unfortunately, this trial had to be stopped due to severe infections in three out of four patients treated with atacicept (Ginzler et al. 2012). Strikingly, all four patients dropped their serum IgG levels to below protocol-defined discontinuation levels. This decline occurred as early as 30 days in three patients. Considering that serum IgG has a half-life of approximately 21 days, the fast and profound decline observed in atacicept treated patients, after an initial decline induced by corticosteroids and MMF, the published data suggest a profound impact of atacicept in long-lived plasma cells which represent the major contributors to serum IgG levels.

Other Cytokine Inhibitors

The list of cytokines that influence significantly the maturation, differentiation, and survival of B cells and/or plasma cells continues to grow and prominently includes both cytokines well known in this arena such as IL-6 and IL-10 as well as relative new comers such as IL-21 and IL-17. Of significant interest, some of these cytokines as well as IFNγ converge in their ability to induce abnormal germinal center reactions leading to clinical autoimmunity and therefore, their inhibition can be formally considered as a major strategy for the targeting of B cell regulation, selection, and function (Sweet et al. 2012).

IL-6 was initially identified as a B cell growth factor and has been implicated both in SLE B cell abnormalities and in the differentiation and survival of plasma cells (Naka et al. 2002; Muhammad et al. 2011; Shirota et al. 2013). Tocilizumab, an anti-IL-6R antibody has been recently approved for the treatment of RA although, given the pleiotropic effect of this cytokine on multiple cell types, whether its benefit derives from B cell inhibition remains to be determined. Recent studies have reported, however, on the impact of this agent on B cells after treatment of a small number of SLE patients(Shirota et al. 2013). In this study, IL6-blockade induced a significant decline in both CD27+ memory cells and plasmablasts while inducing an increase in naïve B cells and little change in transitional B cells thereby supporting a role for this cytokine on post-antigenic B cells.

Over the last few years, IL-21 has been recognized in multiple in vitro human systems and diverse animal models as a critical cytokine for B cell activation, expansion, survival, and differentiation into plasma cells as well as for the establishment of long-lived antibody responses in humans as illustrated by elegant studies of patients with loss-of-function STAT3 mutations (Kuchen et al. 2007; Odegard et al. 2008; Linterman et al. 2010; Zotos et al. 2010; Recher et al. 2011; Rodríguez-Bayona et al. 2012; Ding et al. 2013). While the sites and mechanisms of action of IL-21 may be diverse, strong experimental evidence points to follicular helper T cells (T_{FH}) as the main producers of direct B helping IL-21 both in the germinal centers and in the extrafollicular reactions. Accordingly, strategies aiming at the blockade or either IL-21 or its receptor hold significant promise for multiple human autoimmune diseases. A note of caution, however, should stem from the recent report that IL-21 may also be critical for the expansion of protective B regulatory cells capable of controlling T cell autoimmunity at least in animal models (Yoshizaki et al. 2012).

IL-17, a prototypical pro-inflammatory cytokine has also gained prominence as a major player in human autoimmune diseases over the last few years and this pathway is receiving great attention as a therapeutic target (Miossec and Kolls 2012). Better established in diseases such as RA and psoriasis, the role of T_H17 cells in SLE is starting to unravel as well (Isgro et al. 2013; Doreau et al. 2009; Shin et al. 2011; Hsu et al. 2008). Moreover, multiple studies have now demonstrated the powerful B cell helper function of the Th17/IL-17 axis (Mitsdoerffer et al. 2010; Doreau et al. 2009), and its ability to prolong autoreactive germinal center reactions

at least in part by inhibiting GC B cells chemotactic responses to CXCL12 (Hsu et al. 2008). Therefore, anti-Th17 agents should be considered at least in part B cell targeting agents. It will be important to understand the relative role that B cell inhibition created by these agents plays in different autoimmune diseases.

3.2 B Cell Inhibition

3.2.1 Inhibitors of B Cell Receptor Signaling

SLE is characterized by B cell hyperactivity and genetic polymorphisms of B cell signaling components are associated with disease susceptibility (Moser et al. 2009). Hence, dampening BCR stimulation represents an attractive approach further strengthened by the promising results of an inhibitor of spleen tyrosine kinase (Syk) in B cell lymphomas, rheumatoid arthritis, and NZB/NZW mice (Ghosh and Tsokos 2010). Of note however, lupus improvement was not associated with changes in autoantibodies and may be due at least in part to T cell modulation (Bahjat et al. 2008; Ghosh and Tsokos 2010). An Src family of non-receptor kinase, Syk is required for proximal BCR signaling and mediates positive selection of immature B cells, a critical checkpoint for the censoring of autoreactive B cells (Wardemann et al. 2003). This activity is consistent with the demonstration that Syk inhibition with fostamatinib induces a substantial decrease in transitional cells without impacting mature B cells. Of interest, B cell production of IL-10 was not affected (Barr et al. 2012).

These studies also provide proof-of-concept for the inhibition of signaling molecules downstream of Syk including the PI3K/AKT/mTOR pathway which may be specially relevant to the pathogenesis of SLE(Xie et al. 2007). PI3K inhibitors have shown clinical benefit in lupus-prone lpr mice in which B cell numbers and autoantibodies were significantly decreased at least in part by CD4 T cell inactivation (Barber et al. 2005). These results highlight the therapeutic potential of specific inhibitors of PI3Kδ inhibitors which has a critical and non-redundant role in B cell development (Rommel et al. 2007).

The Bruton tyrosine kinase (Btk) is a critical proximal mediator of BCR-mediated maturation and sets the threshold for B cell activation and censoring of autoreactive B cells through negative selection so that its over-expression induces spontaneous germinal center formation, pathogenic autoantibody formation, and systemic clinical autoimmunity. Therefore, the development of potent Btk inhibitors represents a promising new approach to targeting B cells in human autoimmunity (Honigberg et al. 2010; Paz and Tsokos 2013).

Antibodies to CD79 represent another promising approach to targeting B cells through a combination of depletion and interruption of B cell receptor signaling. These antibodies, which have shown promise in human B cell lymphomas, can delete efficiently a large fraction of mouse B cells including follicular, marginal zone, and IgD+ B cells and possibly IgG2a+ B cells while sparing newly formed

and IgG1 B cells which express low levels of CD79. Anti-CD79 antibodies have been shown to decrease tissue damage and antichromatin antibodies and improve survival in lupus-prone MRL/lpr mice (Bahjat et al. 2008).

Additional approaches to interfering with BCR-mediated activation and survival of B cells include the inhibition of molecules that mediate the transduction of signaling through both BCR and BAFF-R (Oleksyn et al. 2013). This approach is illustrated by the role of PKCβ in BCR signaling through NFκB and in BAFF-induced B cell metabolic fitness through AKT phosphorylation. In animal models, PKCβ is required for the development of SLE and its deficiency induces B cell anergy with decreased survival, germinal center inhibition, and a dramatic reduction in spleen and bone marrow plasma cells with preferential inhibition of autoreactive plasma cells and autoantibody reduction. These findings pave the way for the development of therapeutic inhibitors of PKCβ.

3.2.2 Engagement of Inhibitory Co-Receptors

A number of inhibitory co-receptors modulate B cell activation induced through BCR stimulation and agents directed against these receptors may therefore dampen B cell activation and improve disease. CD22 is one of the best understood B cell inhibitory receptors and the best developed agent in this class is the anti-CD22, humanized IgG1κ monoclonal antibody epratuzumab. Epratuzumab has been shown to be effective in at least two SLE studies albeit one of them had to be prematurely interrupted due to lack of drug supply (Wallace et al. 2013b). Preliminary evidence for clinical benefit of epratuzumab in other autoimmune diseases including Sjogren's syndrome is starting to accumulate as well (Dörner et al. 2012).

A member of the immunoglobulin gene superfamily, CD22 and a sialic acid-binding Ig-like lectin (SIGLEC), CD22 carries immunotyrosine inhibitory motifs (ITIMs) that attenuate BCR signaling by recruiting the tyrosine phosphatase SHP-1 (Sato et al. 1996; Otipoby et al. 1996; Doody et al. 1995; Cornall et al. 1998). CD22 surface expression is initiated in immature B cells as they acquire antigen-responsiveness at the transitional stage and is highest in mature naïve B cells, decreased in memory cells and suppressed in antibody-secreting cells (Pezzutto et al. 1988; Townsend et al. 2010). Different animal models have demonstrated a critical role for CD22 in the regulation of BCR signaling and of B cells lifespan and BCR inhibitory function has also been demonstrated for CD22 in human B cells (Pezzutto et al. 1988; Sieger et al. 2013). Animal models have also shown that CD22 deficiency promotes the development of high affinity autoantibodies (O'Keefe et al. 1999).

The mechanism of action of epratuzumab is quite distinct from that of rituximab as the former but not the latter induces growth arrest of malignant B cells. In contrast, epratuzumab is devoid of the CDC activity characteristic of rituximab but possesses a significant degree of ADCC (Dörner et al. 2012). Interestingly, the anti-proliferative activity of epratuzumab appears to be preferentially manifest against lupus B cells as compared to normal B cells. Of note, this inhibitory effect was

noted under all culture conditions including polyclonal stimulators such as CD40L and TLR agonists which are likely to play an important role in the in vivo activation of SLE B cells (Jacobi et al. 2008). Moreover, epratuzumab induces downregulation of surface CD22 expression on B cells, although it is unclear whether this phenomenon enhances its inhibitory activity or could on the contrary render B cells impervious to prolonged treatment with the antibody. Finally, CD22 also promotes integrin activity as it enhances expression of L-selectin and β7 and β1 integrins and facilitates CXCL12-induced migration of naive B cells (Daridon et al. 2010). It is therefore likely that CD22 inhibition may result in decreased B cell migration to different tissues possibly including germinal centers (Allen et al. 2004).

The available evidence from published studies indicates that in vivo administration of epratuzumab in SLE results in approximately 40 % reduction in total B cells after prolonged administration (Jacobi et al. 2008; Wallace et al. 2013a). As expected, the major reduction was observed in CD27− transitional and naïve cells which also experienced a significant reduction in the level of expression of CD22 (Jacobi et al. 2008). Consistent with the observed lack of effect on memory cells and the expected lack of impact on antibody-secreting cells, no significant changes in antibody levels were observed during the EMBLEM study in SLE (Wallace et al. 2013a). These observations provide additional evidence for antibody-independent roles for B cells and also establish, together with the belimumab observations, a paradigm for the benefit of inhibiting newly generated B cells in the treatment of autoimmune diseases even when preexisting memory cells are preserved. As it is the case with belimumab, these observations provide strong rationale for the sequential use of these agents for the maintenance of remission and potentially the restoration of tolerance following universal B cell depletion with rituximab.

The FcRγ2b (CD32b) represents another appealing agent in this class as it is a potent inhibitory receptor for B cell activation whose cross-linking may induce feedback suppression and B cell and PC apoptosis (Zhou et al. 2008; Xiang et al. 2007a). These activities may contribute to the therapeutic benefit of IVIG in multiple autoantibody-mediated conditions (Tarasenko et al. 2007), although recent animal studies indicate that the anti-inflammatory actions of IVIG may be mediated through the SIGN-R1 expressed by marginal zone macrophages (Anthony et al. 2008). Nonetheless, given the strong immunological and genetic rationale that implicates defective FcRγ2b activity in SLE, agonistic antibodies capable of differentiating the B cell-specific CD32b from other FcRγ receptors (Veri et al. 2007) could have significant potential even though their efficacy could be compromised by decreased expression of this receptor in lupus B cells and plasma cells (Xiang et al. 2007b; Mackay et al. 2006; Moser et al. 2009). Of note, agents that target this pathway in SLE will need to contend with the possibility that this intervention could potentiate a type I IFN environment (Dhodapkar et al. 2007).

3.2.3 Blockade of B Cell Co-Stimulatory Pathways

Toll-like receptors (TLR) provide critical B cell co-stimulation of major significance in SLE and therefore provide major targets for pharmacological inhibition in SLE. In mice, TLR9 is critical for the generation of anti-DNA antibodies and TLR7 controls anti-RBP antibodies and determines clinical severity (Christensen et al. 2006). Moreover, TLR signaling amplifies a pathogenic loop that integrates two other critical SLE cytokines (IFNα and BAFF) (Moisini and Davidson 2009). Thus, TLR stimulation upregulates expression of BAFF-receptors to create synergy between BAFF and BCR stimulation. Similarly, TLR-activated pDCs produce type 1 IFN that enhances TLR7 and TLR9-induced B cell activation and BAFF production by myeloid DC (Braun et al. 2002; Bekeredjian-Ding et al. 2005; Giordani et al. 2009; Thibault et al. 2009). Also of note, Type I IFN regulates the migration of marginal zone B cell precursors thereby promoting their participation in germinal center reactions (Mountz et al. 2011).

These observations provide a strong rationale for a clinical benefit of the interruption of either TLR or type I IFN stimulation that would be mediated in part through B cell inhibition. This model is also supported by the significant benefit imparted by anti-malarial drugs which represent a cornerstone of lupus therapy and can inhibit TLR9 or TLR7. More powerful and specific TLR inhibitors are under development for SLE (Barrat and Coffman 2008) and bear significant promise as indicated by clinical efficacy in murine lupus (Barrat et al. 2007). Anti-IFN agents are also currently in early phases of clinical development and a phase Ia trial of a fully human monoclonal antibody that inhibits most IFNα subtypes has shown promising biological activity and safety in patients with mild to moderate SLE when added to standard-of-care therapy (Yao et al. 2009). Two phase I studies of a fully human and a humanized anti-IFNα monoclonal antibodies (sifalimumab and rontalizumab, respectively) have demonstrated a favorable safety profile and biological activity (Petri et al. 2013; McBride et al. 2012)and are being evaluated for safety and efficacy in patients with moderate to severe active SLE.

As with all biologicals that disrupt complex regulatory circuits however, unanticipated adverse effects that could be triggered by either TLR or type IFN inhibition should be investigated. Indeed, TLR9 absence can exacerbate murine lupus (Christensen et al. 2006) and TLR activation of B cells may control autoimmune T cell responses (Lenert et al. 2005; Lampropoulou et al. 2008). Similarly, type I IFN may protect against murine lupus and dampen inflammation through IL-10 producing B cells (Hron and Peng 2004; Li et al. 2005).

Multiple other cell surface co-stimulatory molecules activate B cells upon interaction with activated T cells. Critical co-stimulatory molecules include CD40, CD27, and the inducible co-stimulatory molecule ligand (ICOS-L or B7RP1) (Liossis and Sfikakis 2004). These molecules therefore represent attractive candidates for the inhibition of B cells. Targeting the ICOS pathway is of particular interest given the role played by ICOS+ T follicular helper cells (T_{FH}) in autoimmune B cell germinal center reactions (Heinlen et al. 2007), the expression of ICOS

by rheumatoid synovial T cell and CD14+ monocytes (Ruth et al. 2007), its ability to regulate IL-17 production and the essential role it plays in collagen-induced arthritis (Nurieva et al. 2003). Anti-ICOS antibodies are currently under development for autoimmune indications.

B7-1/2 (CD80/86) transduce important activation signals to B cells and therefore members of the B7 family provide additional targets for B cell inhibitory antibodies (Rau et al. 2009). These results and the recent demonstration of the role of CD28 on plasma cell survival (Rozanski et al. 2011) suggest that interruption of CD28 signaling by CTLA4-Ig (Abatacept), while typically considered as an intervention that inhibits T cell activation, could also result in B cell inactivation and disruption of their antigen-presenting cell function and diminished plasma cells. Finally, blocking of CD70-induced CD27 stimulation has been shown to improve mouse CIA disease and provides another example of the therapeutic potential of blocking co-stimulatory TNF superfamily members that participate in B cell-T cell co-stimulation (Oflazoglu et al. 2009; Croft 2009).

3.3 Targeting Plasma Cells and Autoantibody Production

Most agents currently available in the clinic fail to directly target plasma cells (PC), and therefore, autoantibody production is only partly achieved, if at all, and only by indirect means. This effect is presumably mediated by elimination of plasma cell precursors and suppression of disease activity both decreasing the output of new plasma cells and promoting the attrition over time of short-lived but not of long-lived plasma cells. While as previously discussed, new agents targeting APRIL, IFN, TLR, IL-6, IL-21, and IL-17 may all prove to have an impact on plasma cells, the actual extent of their anti-plasma cell activity remains to be elucidated. However, agents that directly kill plasma cells, including anti-thymocyte globulin (ATG), and proteasome inhibitors are available in the clinic and may be highly effective for the treatment of autoimmune conditions. Indeed, ATG, commonly used in transplantation and autoimmunity for its anti-T cell effects has also powerful anti-B cell and PC activity due at least in part to the presence in ATG preparations of anti-CD38 and anti-CD138 antibodies (Zand et al. 2005). In addition, proteasome inhibitors (PI) are of particular interest. Original agents such as bortezomib are approved for the treatment of multiple myeloma and have shown impressive biological and clinical activity in murine lupus (Neubert et al. 2008b). A selective inhibitor of the 26S ubiquitin-proteasome, bortezomib activates the terminal unfolded protein response (UPR), which eventually leads to cell cycle arrest and apoptosis. Due to their extremely high immunoglobulin synthesis, which primes an initial UPR, plasma cells are particularly sensitive to PI (Obeng et al. 2006). In addition, PI block NFκB and can inhibit activated B cells, GC cells, and dendritic cells and the release of NFκB-induced pro-inflammatory cytokines (including TNF, IL-1 and IL-6) from RA activated T cells (Neubert et al. 2008a; van der Heijden et al. 2009). PI have shown efficacy in the treatment

of murine models of RA and SLE (Neubert et al. 2008b). In particular, an immunoproteasome-specific inhibitor (PR-957) can attenuate disease progression in experimental arthritis by a combination of cytokine inhibition, decreased cellular infiltrates and decreased autoantibody production (Muchamuel et al. 2009). Of note, immunoproteasome-specific agents such as carfilzomib, recently approved for the treatment of multiple myeloma, have a much more favorable safety profile (including decreased incidence of painful peripheral neuropathy) than bortezomib (Siegel et al. 2009; Katsnelson 2012). Moreover, the recent demonstration that carfilzomib is effective in inhibiting type I IFN production and autoantibody production in murine lupus provides a strong rationale for the investigation of these agents in human autoimmune diseases (Ichikawa et al. 2012).

Finally, the pathogenic role of PC in autoimmune diseases could be attenuated by interventions aimed at inhibiting their secretory function. This paradigm is illustrated by the ability of inhibitory CCL2 variants produced by mesenchymal stem cells (MSC) to suppress PC antibody production (Rafei et al. 2008). These interesting studies provide strong rationale for the therapeutic B cell potential of inhibitory anti-CCR2 antibodies or CCL2 inhibitors. Moreover, they suggest that plasma cell inhibition should be added to the growing number of immunoregulatory functions of MSC (François and Galipeau 2012).

3.4 Expansion of Regulatory B Cells

All the mechanisms previously discussed in this chapter deal with the elimination of B cells and/or the inhibition of pathogenic B cell function albeit in some cases, the short-term benefit created by depletion during the remission induction phase is potentiated and sustained through the indirect expansion of regulatory B cells that takes place during the repopulation phase. Under these conditions, regulatory B cell expansion should mostly contribute to the maintenance of remission. However, interventions aimed at the direct expansion of regulatory B cells could, at least in principle, be effective for both the induction and the maintenance of remission. Expanding knowledge of the biology of different subsets of human regulatory B cells (Bregs and B10s) combined with animal studies indicates that these cells can be significantly expanded with beneficial therapeutic effect (Blair et al. 2009; Yoshizaki et al. 2012). Such expansion can be achieved by CD40 stimulation alone or in combination with IL-21. Moreover, immunoregulatory IL-10-producing B cells can also be greatly expanded by stimulation with GM-CSF/IL-15 fusokines (Rafei et al. 2009). While the use of these agents in vivo might be limited by other immunostimulatory effects, these approaches provide powerful platforms for B cell-based cell therapies with reinfusion of in vitro expanded autologous regulatory B cells.

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Systemic Lupus Erythematosus: Direct B-Cell Blocking

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Abstract Systemic lupus erythematosus (SLE) is a complex autoimmune rheumatic disease with a high morbidity and mortality. Advances in the understanding of the disease have led to a growing evidence of the importance of B-cells in the origins of the disease. Two main therapeutic approaches targeting B-cells have emerged: one directed against B-cell specific molecules and the other directed against B-cell survival factors. We will discuss below the different immunosuppressant drugs targeting B-cells and we will review the results of the main trials conducted with B-cell-depleting therapies.

1 Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune rheumatic disease characterized by a deregulation of the immune system leading to multi-organ damage. Lupus mortality has significantly decreased over the last decades, improving the 5-year survival rate of 50 % in 1950, to more than 90 % today. This improvement in SLE prognosis has resulted from earlier diagnosis and better management. Nevertheless, patients with SLE have a high morbidity, notably due to renal failure, and a

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three- to fivefold increased risk of death compared to the general population, linked to infections, cardiovascular events, cancer, and active disease (Bongu et al. 2002).

Several drugs have been used in the treatment of SLE including corticosteroids, hydroxychloroquine, and major immunosuppressive medications, such as cyclophosphamide and mycophenolate, but the necessity for more effective and less toxic therapies has led to new therapeutic approaches (Sousa and Isenberg 2009). There has been an evolution from therapies directed against many different cells and proteins to therapies directed against specific targets, as a result of a better understanding of the etiopathogenesis of the disease.

In this context, there is growing evidence for the importance of B-cells in the origins of SLE leading to increased targeting of these cells in the development of new therapies.

2 Role of B-Cells in SLE

Considerable evidence supports the idea of B-cells being a central player in SLE pathogenesis; moreover, they seem to participate in the autoimmune deregulation at different levels. Different B-cell abnormalities have been found in patients with SLE including aberrant expression of costimulatory molecules or alterations in the peripheral B-cell subset with increased numbers of transitional, memory B-cells and plasma cells and naïve B-cell lymphopenia. At least three broad categories of defects can lead to B-cell hyperactivity: abnormalities in B-cell activation thresholds, increased B-cell longevity and auto-antigen processing derived from the impaired clearance of the apoptotic debris. The relationship between these defects and other immunological abnormalities and/or disease activity remains unclear (Sabahi and Anolik 2006; Ahmed and Anolik 2010). Murine models of SLE have demonstrated both auto-antibody-dependent and independent function of B-cells in SLE pathogenesis.

2.1 Auto-Antibody-Dependent Role

Patients with SLE have increased numbers of Ig-secreting B-cells when compared to normal controls, which produce antibodies with a wide variety of self-antigens. The different patterns of auto-antibodies correlate with different clinical features, for example anti-dsDNA antibodies and renal involvement. These self-reactive immunoglobulins participate in SLE development through the formation of immune complexes, direct pathogenesis and propagating the autoimmune process through immune complex mediated activation of immune cells (Sabahi and Anolik 2006; Ahmed and Anolik 2010; Ronnblom and Alm 2001). Antinuclear antibodies (ANA) have been found in the serum of SLE patients up to 9 years before the clinical onset of the disease, which indicates that the loss of B-cell tolerance occurs

very early in the disease (Arbuckle et al. 2003). There are different explanations for this finding, but it establishes that although auto-antibodies have a substantial role in SLE pathogenesis, other mechanisms are implicated.

2.2 Auto-Antibody Independent Role

As indicated above, murine models have demonstrated the importance of a B-cell auto-antibody independent function in SLE pathogens. MRL/lpr mice that expressed a mutant transgene encoding surface immunoglobulin which did not permit the secretion of circulating antibodies developed lupus nephritis. However, B-cell deprived MRL lpr/lpr mice did not develop glomerulonephritis or vasculitis (Chan et al. 1999; Shlomchik et al. 1994; Lipsky 2001). This evidence confirmed the importance of B-cells in murine SLE development, but showed their ability to produce auto-antibodies is only part of their pathogenic potential. B-cells play different roles in the immune deregulation acting as auto-antigen presenting cells or as regulators of inflammation through cytokine and chemokine secretion and regulating other immune cells.

3 B-Cells Targeted Therapies

Different B-cell targeted therapies are being investigated, and two main approaches have emerged: one directed against B-cell specific molecules, and the other directed against B-cell survival factors. We will discuss both in detail.

3.1 B-Cells Blocking Therapies

3.1.1 Anti-CD 20 Antibodies: Rituximab

Rituximab is a chimeric monoclonal antibody IgG1κ directed against CD20, which is a membrane protein expressed on the surface of mature B-cells but not on pro-B-cells or plasma cells (Anderson et al. 1984; Leandro 2009). This drug was initially approved by the Federal Drug Administration for the treatment of non-Hodgkin's lymphoma in November 1997. Its use in autoimmune rheumatic diseases was recognized shortly afterwards and it was approved in 2006 for use in patients with rheumatoid arthritis resistant to anti-tumor necrosis factor therapy. It has also shown good results in many uncontrolled studies and case reports in lupus patients (Isenberg 2012).

The mechanism of action of rituximab-mediated-killing of CD20 positive cells is not clear but may be due to a combination of complement-mediated lyses,

antibody-dependent cell-mediated cytotoxicity, and signaling-induced death. Selective depletion in the B-cell population is achieved for periods of 6–9 months while immunoglobulin production by the plasma cells is generally maintained after the first exposure to the drug (Maloney et al. 2002; Hainsworth et al. 2000; Weiner 2010).

Unfortunately the two main randomized controlled trials of rituximab in SLE treatment failed to meet their primary end-points, prompting a reevaluation of its use in SLE. However, there are significant concerns about the design of these failed trials and for the time being it continues to be used in many "hard-to-treat" patients.

3.1.2 Other Anti-CD20 Therapies: Ocrelizumab

Ocrelizumab is a humanized monoclonal antibody targeted against CD20 positive cells. The study to evaluate ocrelizumab in patients with nephritis due to SLE (BELONG trial) compared ocrelizumab to placebo in patient with lupus nephritis class III/IV with steroids and mycophenolate mofetil (MMF) or cyclophosphamide. It showed better response in the ocrelizumab group compared to placebo, unfortunately it had to be stopped in 2010 because serious infections in some oriental patients appeared in the ocrelizumab group (Mysler et al. 2010).

3.1.3 Anti-CD 22 Antibodies: Epratuzumab

CD22 is expressed mainly in the surface of mature IgM+, IgD+ B-cells, and at low levels in the cytoplasm and surface of pro-B and pre-B-cells; it is not present in plasma or memory B-cells. This molecule seems to be involved in the regulation of B-cell function and survival.

Epratuzumab is a recombinant humanized monoclonal IgG1κ antibody that binds to CD22 inducing a negative regulation of BCR activation and a decrease in the expression of CD22 on the surface of the cell. It depletes the number of circulating B-cells in SLE patients, though less than rituximab, through antibody-dependent cellular cytotoxicity without apoptotic or complement-mediated killing (Dorner and Goldenberg 2007; Traczewski and Rudnicka 2011).

The first study conducted with epratuzumab in SLE patients was a small open-label study published in 2006, in which 14 patients with moderate active lupus were treated with 360 mg/m^2 of epratuzumab every 2 weeks for four doses with good results and tolerance (Dörner et al. 2006). Subsequently two randomized controlled trials (ALLEVIATE-1 and ALLEVIATE-2) compared standard of care therapy plus epratuzumab (360 or 720 mg/m^2) with standard of care therapy plus placebo in cycles of 12 weeks during 48 weeks. These studies were interrupted because of problems in manufacturing the drug. These were overcome and are no longer an issue. The results showed that epratuzumab-treated patients required lower dose of corticosteroids compared to placebo-treated patients over the week 24 (Wallace et al. 2008). Recently, the results of the EMBLEM trial, a phase IIb randomized

controlled trial conducted in 227 patients with moderate to severe lupus have been published. They concluded that epratuzumab 2,400 mg cumulative dose was well tolerated and associated with clinical improvement (Wallace et al. 2013). Much larger trials to confirm the efficacy of the drug are on-going.

3.2 Blocking B-Cell Survival Factors

The B lymphocyte stimulator (BLyS) family, also known as B-cell-activation factor (BAFF), is a member of the TNF superfamily of biomolecules. It includes two ligands: BLyS and a proliferation inducing ligand (APRIL); and three receptors known as: BLyS receptor 3 (BR3), transmembrane activator and calcium-signaling modulating and cyclophilin ligand (CAML) interactor (TACI), and B cell maturation antigen (BCMA). BLyS can interact with the three receptors; while APRIL only interacts with BCMA and TACI.

BLyS and APRIL are produced in wide variety of cells including neutrophils, dendritic cells, monocytes, and macrophages, initially as membrane spanning monomers associated as trimers that can be cleaved to soluble form. The receptors, however, are only expressed on B lineage cells and some in activated T cells and dendritic cells. The function of the BLyS family is not completely understood, but seems to be implicated in the regulation of B-cell development at different stages. Memory B-cells seem to be BLyS-independent as anti-BLyS therapy failed to eliminate memory B-cells. Neither is its role in the pathogenesis of SLE understood, but it has been demonstrated that BLyS and APRIL can form biologically active heterotrimers whose levels are elevated in SLE patients. In murine models, mice transgenic for BAAF developed autoimmune-like manifestations including anti-DNA antibodies, rheumatoid factor, or lupus-like nephritis (Treml et al. 2009; Levesque 2009; Roschke et al. 2002; Stohl et al. 2011; Mackay et al. 1999). As a result of all this evidence the BLyS family of cytokines and receptors has become an important target in SLE trials.

Belimumab is a fully human IgGλ monoclonal antibody that neutralizes soluble BLyS. Initially it did not demonstrate significant improvement compared with placebo in a phase II trial conducted in 449 SLE patients. However, it showed improvement in many secondary activity measures in serologically active patients (notably nearly 30 % of the patients included in the study were ANA negative), and a depletion of 63–71 % of B-cell subsets was observed confirming that belimumab was biologically active. There were concerns about the training received by trial investigators in assessing lupus patient disease activity. In the more recent trials investigators had better training and a new combined end-point was developed: the SLE responder index or SRI (Wallace et al. 2009; Furie et al. 2009) which defines response as a reduction of $\geq$4 points in SELENA-SLEDAI score, no new BILAG A organ domain score or more than one BILAG B organ domain score and no worsening in the PGA score compared to baseline. Two large phase III trials (BLISS-52 and BLISS-76) met some of their primary and secondary end-points,

as a result of which the FDA has approved this drug for SLE, which constitutes a remarkable event as it has not occurred in the last 50 years. Later, we will discuss both of the trials in detail.

Atacicept is a fully human recombinant fusion protein between one of the BLyS receptors (TACI) and the Fc portion of IgG that inhibits BLyS and APRIL. A phase II/III trial on patients with lupus nephritis in treatment with high doses of steroids, MMF, and atacicept 150 mg subcutaneous twice weekly, had to be discontinued due to the occurrence of serious infections and low levels of IgG (Ginzler et al. 2012). However, careful analysis revealed this was principally the fault of the MMF not the atacicept. The APRIL-SLE trial is a randomized double-blind controlled trial with 461 moderate to severe SLE patients, comparing placebo with atacicept 75 or 150 mg subcutaneous twice a week for 4 weeks and then weekly for 48 weeks. The 52-week results from this study strongly suggest a reduced risk of flares in the 150 mg group although the primary end-points were not achieved due to the occurrence of two severe pulmonary infections within this group leading unfortunately, to the early termination of this arm of the trial. Favorable effects were observed in anti-DNA antibody and complement levels in those patients with abnormal baseline values and declines in the counts of mature and plasma B-cells and in the levels of IgG, IgM, and IgA were observed in the atacicept treated groups. Interestingly neither of the two patients who died had abnormal IgG levels (Isenberg et al. 2013; Wofsy et al. 2013).

4 Results of Uncontrolled Trials

Among all the B-cell depleting therapies rituximab has the widest clinical experience in lupus patients with numerous uncontrolled studies published over the last 10 years. Approximately 1,000 patients with SLE treated with rituximab have been reported (Ramos-Casals et al. 2012). We summarize below the characteristics of the main uncontrolled studies, focusing on those with more than 20 patients enrolled (Lu et al. 2009; Vigna-Perez et al. 2006; Garcia-Carrasco et al. 2010; Terrier et al. 2010; Lindholm et al. 2008; Vital et al. 2011; Abud-Mendoza et al. 2009; Catapano et al. 2010; Albert et al. 2008; Furtado and Isenberg 2012). Patients included were adults, 80–90 % females, and predominantly Caucasians, with African-American and Hispanic ethnicities as the second and the third more frequent. They all met the revised ACR classification criteria for SLE and had active SLE refractory to previous immunosuppressant therapies. There was a wide variety of clinical indications for B-cell depleting therapy (skin involvement, lupus nephritis, arthritis, hematological and neuropsychiatric manifestations, fever, etc.), and the dose of rituximab varied from two doses of 500 or 1,000 mg each 2 weeks apart or four doses of 375 mg/m^2 weekly. In some, retreatment was given at 24 weeks or if flare occurred. In almost all the studies steroids were maintained during rituximab treatment with variable use of concomitant immunosuppressant drugs (mainly cyclophosphamide). The results obtained of these studies were

positive with overall rates of response of 60–91 %, the percentage of patients achieving B-cell depletion was more than 90 %. The most frequently reported adverse events were infusion-related reactions and infections, predominantly pneumonia.

Good responses were observed in renal and non-renal lupus in these studies, including hematological and neuropsychiatric disease, although in one study patients with pulmonary massive hemorrhage did not improve (Abud-Mendoza et al. 2009). SLE is a complex disease characterized by a tendency to flare, which, when it occurred in these studies, was observed in most cases from 6 to 18 months after B-cell-depleting therapy. Various uncontrolled trials have shown a more sustained clinical remission after retreatment with rituximab. In one study 61 % of patients achieved remission after the first cycle of rituximab, meanwhile an 82 % did so after the second cycle, with significantly longer time to disease flare (Turner-Stokes et al. 2011). However, various concerns arise when we try to compare the results obtained in these studies. These include the absence of a common definition for flair, partial or complete response, the use of different assessment tools to measure disease activity and the duration of follow-up. In almost all the studies a direct relationship was found between depletion of B-cells and clinical response, moreover, encouragingly in most of them the time to flare was longer than the duration of B-cell depletion. These findings support the idea of B-cell repopulation as a predictor of flare and a marker of treatment response. However, in some studies patients with incomplete depletion have shown clinical improvement and the mean time for flare is extremely variable. Other factors have been analyzed to assess clinical response and to predict a flare. Anti-DNA antibody titers and complement levels are markers of disease activity, routinely measured in clinical practice. A rise in complement levels and a lowering of anti-DNA antibody levels was observed in those studies with overall clinical improvement. A full explanation for the different response to B-cell depleting therapy and the variability of its duration is awaited. Lazarus et al. (2012), analyzed B-cell phenotype in a group of patients with refractory SLE treated with rituximab, observing that patients with high anti-DNA titers flared with lower B-cell counts than those with low anti-DNA titers and had a predominance of plasmablasts in B-cell repopulation. These findings suggest the possibility of a different response to B-cell depleting therapy depending upon the cell type that repopulates. They might also help to establish the optimal time for retreatment in individual patients.

Another important issue when comparing these studies is to determine whether another immunosuppressant drug is being co-prescribed with the rituximab, notably cyclophosphamide, and whether this offers an advantage. Some reports failed to find any significant difference between rituximab as a monotherapy and rituximab combined with another immunosuppressive agent (Terrier et al. 2010). The UK-BIOGEAS registry presented the results of using rituximab combined with steroids and MMF or cyclophosphamide in 164 patients with biopsy-confirmed lupus nephritis and showed a lower complete response in the cyclophosphamide-treated patients (Díaz-Lagares et al. 2012). Thus there is no consensus about the role of concomitant immunosuppressant therapy with rituximab.

There is an increasing debate about the role of steroids in SLE treatment, which is a challenging question considering the significant morbidity associated with long-term oral steroids. Ezeonyeji and Isenberg (2012) reported eight cases of newly diagnosed lupus patients who received treatment with rituximab plus cyclophosphamide and maintenance with azathioprine with good clinical response and a significant lowering in the mean cumulative prednisolone dose at 6 months. Their work was based on the Rituxilup regimen, a new treatment protocol designed by Lightstone and colleagues to avoid the use of oral steroids in lupus nephritis, using two doses of 1,000 mg of rituximab on days 1 and 15 plus 0.5 g of methylprednisolone as induction therapy and MMF without oral steroids as maintenance therapy. The data from the first 50 patients with lupus nephritis type III–V showed good responses with 90 % of patients achieving complete or partial remission at median time of 37 weeks. These are encouraging results, and larger trials should be undertaken using the Rituxilup regimen (Condon et al. 2013).

The appearance in 2006 of two cases of progressive multifocal leukoencephalopathy (PML) in SLE patients after treatment with rituximab raised a concern about a possible relationship between this fatal infection and B-cell-depleting therapy, but no new case reports have been documented. Careful reviews have indicated that patients with SLE per se are more likely to develop PML; linked most probably to immunosuppression, however this is achieved (Molloy and Calabrese 2009; Calabrese et al. 2007).

5 Large Controlled Randomized Trials on B-Cell-Depleting Therapies

Two large phase III controlled randomized trials successfully comparing belimumab 1 or 10 mg/kg plus standard of care therapy with placebo (BLISS-52 and BLISS 76) have recently led to the approval of belimumab for SLE treatment by the FDA (Navarra et al. 2011; Furie et al. 2011). With similar inclusion and exclusion criteria, the BLISS-52 enrolled patients from Latin America, Asia Pacific, and Eastern Europe, meanwhile the BLISS-76 was conducted in patients from North America and Europe. For entry into the trial patients had to have a SELENA-SLEDAI of at least six points although patients with severe active lupus nephritis or neuropsychiatric lupus were excluded. An appreciation of the short comings of the phase II trial of belimumab described earlier led to a better study design of these phase III studies with all patients having positive titers of ANA or anti-DNA antibodies, the use of the SRI as primary efficacy end-point, the avoidance of the use of high steroid doses, better training of the investigators in assessing disease activity and lastly, stricter control of concurrent therapies. The primary end-point in both studies was the SRI response at week 52 and the SRI response rate at week 76 was a secondary end-point in the BLISS-76 trial. The other secondary end-points in both trials were: a $\geq$4 points reduction in SELENA-SLEDAI at week 52,

mean change in PGA score and in SF-36 at week 24 and an average reduction in the dose of prednisone. Both studies met their primary end-points with a significantly higher response rate in the belimumab group than in the placebo group at week 52. Moreover, a dose-response pattern was observed with belimumab 10 mg/kg resulting in greater response compared to placebo than belimumab 1 mg/kg. With respect to the secondary end-points, BLISS-76 found higher SRI response rates in the belimumab group compared to placebo at week 76 but the differences were not statistically significant. The authors of this study assumed that this lack of significance could be due to the study design to evaluate response at week 52, to an additional dropout of patients between week 52 and week 76 or to a higher used dose of steroids in the placebo group over week 76. However, patients in the belimumab 10 mg/kg group had a more sustained SRI response after week 52 than those in the placebo group, and serological improvement in anti-DNA antibodies and complement levels was documented from week 8 until week 76 with belimumab. These findings seem to support the longevity of the belimumab effect in the control of disease activity. The belimumab safety profile was similar to placebo with rates of infections, malignancies, and deaths comparables between groups. In summary, both studies concluded that belimumab was a safe and effective drug for SLE seropositive patients and that there is supportive evidence of the duration of the effect until 76 weeks.

In the case of rituximab two randomized controlled trials failed to demonstrate the superiority of this drug compared to standard of care therapy alone. The exploratory phase II/III SLE evaluation of rituximab (EXPLORER) study (Merrill et al. 2010) was conducted in North America in SLE patients with active disease defined as at least one organ system with a BILAG A score or at least two organ system with a BILAG B score despite stable treatment with an immunosuppressive agent. Patients were randomized to receive their baseline immunosuppressive agent (azathioprine, MMF or methotrexate) plus rituximab or placebo, and a 10 weeks course of high-dose steroids. The objective was to demonstrate the benefit of adding rituximab to standard of care therapy. The primary end-point was to achieve BILAG C scores or better in all organs at week 24 and maintain this without any worsening at week 52. The study showed no differences between the placebo and the rituximab group in its primary or secondary end-points. However, also at week 52, there was a lowering in anti-DNA titers and a rise in complement levels, which demonstrated that rituximab was biologically active, and the mean time to flare was higher in the rituximab-treated group. In a subgroup analysis there was a significant improvement with rituximab in African-American/Hispanic patients compared to Caucasians. These results should be interpreted with caution and suggest that populations with worse prognosis could obtain a more obvious benefit from treatment with rituximab. With respect to the safety profile, the adverse events were balanced between the rituximab and the placebo group with, interestingly, a higher rate of infections in the placebo group.

The LUNAR study (Rovin et al. 2012) was a phase III randomized placebo-controlled trial conducted in patients with lupus nephritis (LN) class III or IV, designed to evaluate rituximab as an add-on therapy to standard induction therapy

with three intravenous pulses of 1,000 mg of methylprednisolone, high oral steroids and MMF at a dosage of 3 mg/day, being its primary end-point the achievement and maintenance of complete renal response at week 52. The LUNAR study failed to show statistical significance in its primary and secondary end-points; however, the rituximab group showed a superior response compared to the placebo group in some of the parameters studied notably reduction in the proteinuria, improvement of renal function and the need for rescue therapy, as well as a greater tapering in the steroids dosage. In concordance with the EXPLORER study better responses were found in black patients, although it was not statistically significant and rituximab showed a good safety profile.

In conclusion, while rituximab failed to meet its end-points in either trial there are several encouraging indications that better designed studies will ultimately confirm a role for this drug in the treatment of patients with SLE.

Why did rituximab fail in the EXPLORER and LUNAR trials?

From the phase II and III Belimumab trials we have learnt how much the study design is vital in determining success or failure in SLE trials. The EPLORER and LUNAR trials recruited patients with mild to moderate SLE activity who had not necessarily failed standard of care therapy; in comparison with the majority of patients included in the open-label studies who were refractory to standard therapy. Moreover, half the patients included in the LUNAR trial have just had one episode of lupus nephritis. Interestingly, in both studies black patients had better responses to rituximab in the subgroup analyses, suggesting a better response for patients with a worse prognosis. Thus, the inclusion and exclusion criteria of the LUNAR and EXPLORER trials are likely to have contributed to the lack of improvement found with rituximab.

With respect to the background treatment, the use in both trials of very high doses of corticosteroids associated with a first line immunosuppressive agent could well have masked the potential benefit of rituximab. Furthermore, the fact that in the EXPLORER trials different baseline drugs were used without knowing the frequency or for how long the patients had been treated for makes the different groups difficult to compare. On the other hand, one of the combinations which has shown good results in some of the uncontrolled trials was rituximab plus cyclophosphamide and neither the EXPLORER nor the LUNAR trials employed this combination (Table 1).

Finally the primary end-point in the EXPLORER trial was perhaps too stringent as the majority of the studies evaluated the response at week 24, and responses to lupus may well take longer, i.e. an assessment at 52 weeks might have found a different outcome. In addition, the “classic” BILAG assessment tool used in the SRI is liable to accentuate the importance of minor flares.

In summary, despite the suboptimal design of both trials and the failure to meet their objectives, an improvement in some of the parameters studied as well as a clinical improvement between some of the subgroups analyzed has encouraged new and better designed trials of rituximab in lupus patients restricting the use of concomitant steroids and immunosuppressive agents.

Table 1 Main characteristics of rituximab uncontrolled and controlled trials

Variable	NCT	EXPLORER	LUNAR
Patients	418	257	144
Female sex	80–90 %	80 %	90.20 %
Mean age	27	40.4	30.6
Ethnicity			
Caucasians	13.60 %	56 %	38 %
AA	7 %	25 %	27 %
Other	17 %	19 %	41 % mainly Hispanic
NA	56.60 %		
Mean duration of SLE	6.71 years	8.6 years	30 months
Disease features	Active SLE, refractory	Active SLE. Stable Im therapy	Active LN III or IV. No refractory
Protocol treatment	RTX 375 mg/m^2 weekly RTX 500 mg days 1 and 15 RTX 1,000 mg days 1 and 15	RTX 1,000 mg days 1 and 15 + CS	RTX 1,000 mg days 1 and 15
Steroids	Yes	Yes	Yes
On-going Im	All studies except one	Yes (AZA, MTX, MMF)	Yes (MMF 1,500–3,000 mg/day)

Notes: *NCT* non-randomized trials (based on data from studies with references 33–42, "see text, Sect. 4"), *AA* Afro-American, *Im* immunosuppressant therapy, *NA* not available, *RTX* rituximab, *MMF* mycophenolate mofetil, *AZA* azathioprine, *MTX* methotrexate, *LN* lupus nephritis

6 Future Therapies

We wait with great anticipation for the outcome of trials involving agents that block interferon α, and the new trials of B-cell depletion which are being established and should provide a more definitive answer to determine the value of this approach in SLE. Ultimately the question of whether combination of biologics drugs will also have to be addressed. Although the "biologic revolution" which has transformed the lives of patients with rheumatic arthritis and psoriatic arthritis has been "slow to reach" those with SLE, we continue to believe that it will arrive, eventually!

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Systemic Lupus Erythematosus: Indirect B-Cell Blocking

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Abstract The use of B-cell depleting agents in clinical practice in systemic lupus erythematosus (SLE), overwhelmingly restricted to off-label therapy with rituximab, currently centers on patients unresponsive or intolerant to standard of care therapy, or with life-threatening presentations. Four B-cell depleting agents deserve specific mention: rituximab, use of which is reported in nearly 1,500 patients with SLE (1,000 in uncontrolled studies); ocrelizumab, trials of which were halted in 2010 owing to an association with unexpectedly high rates of severe infections; epratuzumab, which has been tested in trials that enrolled nearly 300 patients with SLE; and belimumab, tested in more than 2,000 patients with SLE overwhelmingly included in controlled trials. Belimumab has been licensed for use in SLE in 2011 by the FDA and European Medicines Agency. Forthcoming years will show the real value of B-cell targeted therapies, including the off-label use of rituximab and especially the licensed use of belimumab, as part of the standard of care in patients with SLE.

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1 Introduction

B cell targeted therapies are designed to induce the elimination either of the majority of B cells (the so-called complete B cell depletion) or of only some B cell populations (incomplete or selective B cell depletion). B cell depletion can be achieved by the several molecular etiopathogenic mechanisms (Coca and Sanz 2012):

1. Direct killing of B cells using anti-CD20 (rituximab, ocrelizumab), anti-CD19 and, at a lower level, anti-CD22 (epratuzumab) antibodies,
2. Indirect B cell attrition blocking key survival factors such as BLyS (belimumab) or APRIL (atacicept),
3. Other direct B cell targeting mechanisms include the inhibition of molecules involved in B cell receptor inhibitory signaling (Syk, Btk, PI3K), which may eliminate B cell subsets that require a sustained BCR signaling for maturation and/or survival, and
4. Other targets continue to be identified by basic research of B cell differentiation, migration and survival as illustrated by the identification of the central role played by IL-21, a cytokine produced by TFH cells, in the generation of memory cells and plasma cells. Indeed, IL-21 inhibition represents a very appealing future approach with significant specificity for the B cell compartment (Li et al. 2011).

The objective of an optimal therapeutic target of B cells in SLE is the eradication of pathogenic B cells (including autoreactive memory cells and plasma cells), while sparing cells with protective functions such as IL-10 producing B regulatory cells (Bregs). Bregs include at least three different populations: T2 transitional cells (CD38++CD24++); B10 cells (IgDhiCD5+CD27+); and IgD+CD27-naïve B cells (Mariño and Grey 2012; Mauri and Bosma 2012).

Current B-cell targeted agents differ widely in their ability to target different B cell subsets with the most widely tested category of anti-B cell agents (anti-CD20 antibodies such as rituximab and ocrelizumab), inducing broad and deep B cell depletion from pre-B cells to some early antibody-secreting plasmablasts. Anti-CD19 antibodies, which are currently in early stages of development, could target additional populations including pro-B cells and a large fraction, albeit not all, of plasma cells (Herbst et al. 2010). Unfortunately, and as has been discussed in the previous chapter, the efficacy of anti-CD20 therapy in SLE has not been confirmed in controlled trials. The two large controlled randomized trials (EXPLORER and LUNAR) of the use of rituximab in SLE failed to demonstrate superiority over the standard of care therapy (Merrill et al. 2010; Rovin et al. 2012). However and before concluding that rituximab is not a good therapy for SLE, a careful evaluation of the design of these trials is necessary (Ramos-Casals et al. 2011a). Some points should be considered, including disease severity (a significant percentage of patients included had mild-to-moderate SLE with no history of lack of response to standard therapies), the high doses of corticosteroids permitted in both arms of

these trials (that could lead to significant differences not being apparent in a short-term evaluation), or the possible synergistic effect of rituximab in combination with immunosuppressive agents (cyclophosphamide or mycophenolate) (Ramos-Casals et al. 2009a). The recently published results of the BLISS trials have marked the first positive RCT of a biologic agent in SLE, belimumab (a monoclonal antibody that induces an indirect B-cell depletion) (Scott et al. 2012).

2 Indirect B-Cell Depletion

B-lymphocyte stimulator (BLyS), also known as the B-cell activating factor belonging to the TNF family (BAFF), is a novel TNF family ligand discovered nearly 10 years ago (Stohl and Hilbert 2012). This cytokine has proven to be a key factor in the selection and survival of B cells and is expressed by a wide variety of cell types, including monocytes, activated neutrophils, T cells, and DCs (Cancro et al. 2009). BLyS is expressed as a cell surface protein organized into a trimer, furin cleaved, and released into the circulation. Although standing levels of BLyS are constitutively secreted, its expression and secretion can be potentiated by inflammatory cytokines. A splice variant of BLyS that is inefficiently cleaved from the cell surface also exists and a 60-mer form of soluble BLyS (BLyS-60) has been observed in mice (Lied and Berstad 2011).

As we have previously mentioned, the only B cell targeted therapy approved for SLE due to a positive superiority result in RTCs is belimumab, an anti-BLyS monoclonal antibody. Belimumab induces a partial and gradual depletion of B cells with a predominant impact over transitional, naïve and CD27-switched memory cells; belimumab does not significantly decrease CD27+ memory cells or plasma cells (Ramos-Casals et al. 2009a). The clinical improvement in SLE patients observed in spite of the preservation of memory cells is etiopathogenically significant, and some authors have suggested that belimumab could enforce central tolerance by restricting the recruitment of new autoreactive B cells into the mature B cell compartment (Sanz et al. 2011).

3 Belimumab: Clinical Trials

The first controlled trial (BLISS-52) tested the use of belimumab in 865 SLE patients with positive immunological markers and moderate–severe disease (Navarra et al. 2011). A clinical response at 52 weeks was achieved by 44 % of placebo-treated patients compared with 51 % of those receiving belimumab 1 mg/kg and 58 % of those treated with belimumab 10 mg/kg ($p = 0.013$ and 0.0006, respectively), with modest but consistent improvements across a range of clinical outcome measures. A second trial (BLISS-76) included 819 patients with a similar design, although patients and investigators remained blinded for additional

24 weeks (Furie et al. 2011). The results at 52 weeks showed that the percentage of patients achieving a clinical response was 34 % with placebo, 41 % with 1 mg/kg, and 43 % with 10 mg/kg ($p = 0.10$ and $p = 0.021$, respectively) (van Vollenhoven et al. 2011b). Analysis of the combined 1864 patients in both BLISS trials at 52 weeks shows reductions in disease activity and prevention of worsening in internal organ involvement (D'Cruz et al. 2011). Superiority in the BLISS trials was only observed when the clinical outcome was measured with a newly developed outcome measure, the systemic lupus erythematosus responder index (SRI) (Sanz et al. 2011).

4 Belimumab: Recommendations for Use in Clinical Practice

4.1 General Recommendations

The technical specifications for belimumab (EMEA 2013) defines that the drug "is indicated as adjunctive therapy in adult patients with active SLE, with positive autoantibodies, with a high degree of disease activity despite standard treatment." Until further post-marketing studies will be available, it seems reasonable to recommend the use of belimumab in patients having the same clinical and immunological profile as those who were enrolled in clinical trials (Navarra et al. 2011; Furie et al. 2011) and that would be defined by the following characteristics (Ramos-Casals et al. 2013):

(a) Age: all available therapeutic trials have been conducted in adult SLE patients (aged over 18 years).
(b) Criteria: patients must fulfill the current classification criteria for SLE, after discarding reasonably non-autoimmune diseases (mainly infections and neoplasia).
(c) Immunology: ANA $\geq$ 1:80 and/or anti-dsDNA $\geq$ 30 IU/mL, with at least one of these markers being positive at the time of pre-therapeutic evaluation. Recent studies have showed a greater efficacy of belimumab in the subgroup of patients immunologically "active" (positive anti-dsDNA + hypocomplementemia), with an increase in the response of 8 % in comparison with placebo (van Vollenhoven et al. 2012).
(d) Activity: score $\geq$6 in the SELENA-SLEDAI scale. A recent study (van Vollenhoven et al. 2012) identified in the combined analysis of the two trials (BLISS 52 and 76) a better response to belimumab in patients with an activity index $\geq$10 on the SELENA-SLEDAI, hypocomplementemia and treatment with corticosteroids at baseline.

(e) Refractory disease: defined as the failure to achieve an adequate therapeutic response to the standard therapy based on the association of antimalarials, corticosteroids, and immunosuppressive agents.

Belimumab may also be used in patients showing intolerance/severe adverse events to the standard therapies. The use of belimumab in SLE patients not treated with corticosteroids is not recommended; the BLISS trials included 14 % of patients who did not receive corticosteroids, in whom the efficacy of belimumab was similar to that observed in the placebo group (van Vollenhoven et al. 2012). The most common clinical situation in clinical practice will be a patient who is still active despite a steroid dose of "x" mg/day, or a patient with a relapse after tapering of steroids. In this scenario, belimumab may be used in a patient with persistent or relapsing lupus activity in spite of using a maintenance therapy with low dose of prednisone ($\leq$7.5 mg/day) (Ruiz-Irastorza et al. 2012) in association with antimalarial and immunosuppressant.

The data currently available about the duration of the therapeutic efficacy of the long-term use of belimumab are contradictory. The BLISS-76 study showed the loss of statistical significance after 76 weeks of treatment (Furie et al. 2011), while Merrill et al. have reported the maintenance of efficacy during at least 4 years of treatment (Merrill et al. 2012).

4.2 Organ by Organ Recommendations

Although SLE is a systemic disease in which various organs are frequently affected, biological therapies are usually indicated according to the specific impairment of one organ or system (often the most severe), which is considered as the "primary therapeutic target," although the patient could have other concurrent active clinical and/or immunological manifestations.

4.2.1 General Symptomatology

SLE patients presenting with fever (attributable to lupus activity), cytopenias (leukopenia and thrombocytopenia), elevated titers of anti-dsDNA, and decreased complement levels fulfilled the minimum SELENA-SLEDAI score used in the trials as inclusion criteria to be treated with belimumab (Navarra et al. 2011; Furie et al. 2011). Although this subgroup of patients has not been specifically tested in these trials, the results obtained in the SELENA-SLEDAI and BILAG constitutional domains showed no statistically significant differences. Although a post-hoc study showed a significant improvement in fatigue level assessed by FACIT (Strand et al. 2011), the use of belimumab in SLE patients only presenting with general symptoms (fever, fatigue, arthralgia) in spite of having immunological activity is not recommended.

4.2.2 Mucocutaneous Involvement

Belimumab may be used in patients with persistent mucocutaneous activity (erythema/inflammatory rash, alopecia, and/or oral/nasal ulcers) who are refractory to the use of the standard of care therapy based on topical treatments, antimalarials, corticosteroids, and immunosuppressants (methotrexate or azathioprine) (Jiménez-Alonso 2012). The level of activity recommended for belimumab administration (minimum SELENA-SLEDAI score of 6) is achieved with the simultaneous appearance of the three skin conditions above mentioned, the presence of two together with active immunological markers (increase of more than 25 % of baseline anti-dsDNA values or hypocomplementemia), or the presence of one mucocutaneous feature accompanied by two of the following features: 25 % increase of anti-dsDNA values, hypocomplementemia, cytopenias (leukopenia and thrombocytopenia), or fever with cytopenia.

Although the BLISS trials were not designed to evaluate the therapeutic efficacy organ by organ (Navarra et al. 2011; Furie et al. 2011), the combined results of the two trials showed a statistically significant improvement in the cutaneous domains (dermatological SELENA-SLEDAI and mucocutaneous BILAG) of patients treated with belimumab (Manzi et al. 2012). In addition, a post-hoc study showed a statistically significant improvement in patients with maculopapular rash, discoid lupus, and active alopecia, but not in patients with active ulcers, malar erythema, or digital erythema (Manzi et al. 2011).

4.2.3 Musculoskeletal Involvement

Belimumab may be used in patients with persistent musculoskeletal activity consisting of arthritis (at least two swollen and tender joints) and/or myositis (confirmed by raised muscular enzymes, electromyography, and/or biopsy) who are refractory to the sequential use of anti-inflammatory drugs, antimalarials, corticosteroids, and immunosuppressants (methotrexate or azathioprine) (Jiménez-Alonso 2012). The level of activity recommended for belimumab administration (minimum SELENA-SLEDAI score of 6) is achieved in patients with active arthritis or myositis together with positive immunological markers or if the patient has concomitant fever and cytopenia. The combined analysis of the results of the BLISS trials 52 and 76 showed a statistically significant improvement in SELENA-SLEDAI/BILAG musculoskeletal domains (Manzi et al. 2012), although in the SELENA-SLEDAI domain reached statistical significance only for patients treated at the dose of 1 mg/kg.

4.2.4 Hematologic Involvement

Although the SELENA-SLEDAI includes thrombocytopenia as leukopenia, only thrombocytopenia should be considered as indication for belimumab therapy. The level of activity recommended for belimumab administration (minimum SELENA-SLEDAI score of 6) is achieved in patients with thrombocytopenia (<100,000/mm^3) together with leukopenia (<3,000/mm^3) or fever and positive immunological markers of SLE activity, in spite of being treated with corticosteroids and azathioprine or intravenous immunoglobulins. The combined analysis of the BLISS trials showed no statistically significant improvement in the hematological SELENA-SLEDAI and BILAG domains (Manzi et al. 2012). Autoimmune hemolytic anemia is not included in the SELENA-SLEDAI (Navarra et al. 2011; Furie et al. 2011) and therefore no current information is available to make recommendations on the use of belimumab in these patients.

4.2.5 Serositis

Belimumab might be used in patients with persistent serositis (pleuritis and/or pericarditis) accompanied by at least two of the following features: increased levels of anti-dsDNA antibodies, hypocomplementemia, cytopenias (leukopenia and thrombocytopenia), or fever with cytopenia, who were refractory to anti-inflammatory drugs, antimalarials, corticosteroids, and immunosuppressants (azathioprine or methotrexate). However, the combined results of BLISS trials did not show a statistically significant improvement in the SELENA-SLEDAI domain corresponding to serositis (Manzi et al. 2012).

4.2.6 Vasculitis

Belimumab may be recommended for patients with persistent vasculitis (ulceration, gangrene, digital vasculitic lesions, biopsy-proven vasculitis) in spite of the use of corticosteroids and immunosuppressants (cyclophosphamide or mycophenolate). The combined results of BLISS trials (Manzi et al. 2012) showed a statistically significant improvement in the SELENA-SLEDAI vasculitis domain and a trend in the vasculitic BILAG domain.

4.2.7 Central Nervous System Involvement

The BLISS trials excluded patients with severe central nervous system (including seizures, stroke, psychosis, organic brain syndrome, or CNS vasculitis). In addition, the finding of soluble BLyS levels significantly lower in patients with SLE and neurological involvement (Petri et al. 2011) might suggest a lower efficacy of

belimumab. Therefore, it is not possible to make a solid recommendation on how to use salvage biological therapy (belimumab or rituximab) in SLE patients with severe CNS involvement, and the clinical decision must be individualized according to the characteristics of patient.

4.2.8 Renal Involvement

In addition to severe CNS involvement, the BLISS trials also excluded patients with severe renal involvement (24-h proteinuria >6 g, serum creatinine >2.5 mg/dL and current hemodialysis). However, the trials included a small subset of patients (<20 in each arm) with non-severe renal involvement and the results showed a higher numerical therapeutic efficacy but without reaching statistical significance. A post-hoc study has demonstrated a superior numerical improvement (% of response) especially in patients serologically active (high DNA and/or hypocomplementemia) and in those with proteinuria >1 g/24 h, although the results did not reach statistical significance (Dooley et al. 2011) except for a decrease of renal flares (Chiche et al. 2011).

An individualized assessment of the indication for treatment with B-cell depleting agents (belimumab or rituximab) should be made taking into account the available scientific evidence:

1. Patients with severe lupus nephropathy (see previous definition): no current evidence to support the use of belimumab in these patients waiting for the results of future clinical trials being carried out in this specific SLE subset of patients.
2. Patients with non-severe lupus nephropathy: in these patients, the use of biological therapies (belimumab or rituximab) may be considered. Although the decision should be made on an individualized basis taking into account both the characteristics of the patient and the experience of the medical team, the following considerations should be balanced:

   (a) Belimumab (not rituximab) is licensed for use in SLE patients.
   (b) The LUNAR trial did not show superiority of rituximab versus standard treatment in patients with lupus nephropathy.
   (c) The published experience in rituximab is wide but not controlled and is specially focused on severe patients.
   (d) Soluble BLyS levels are significantly higher in patients with SLE and renal involvement (Petri et al. 2011).
   (e) A statistically significant trend on the effectiveness of belimumab was found in the BLISS trials (Navarra et al. 2011; Furie et al. 2011), especially in immunologically active patients.

5 Use of Belimumab: Contraindications and Precautions

According to the drug leaflet, hypersensitivity to the active substance or to any of the excipients is the only absolute contraindication for the use of belimumab. In addition, caution is advised in the following scenarios:

- Patients with severe active central nervous system, including seizures, stroke, psychosis, organic brain syndrome, or CNS vasculitis
- Patients with severe active lupus nephritis, defined by the presence of proteinuria higher than 6 g/24 h (or equivalent), creatinine greater than 2.5 mg/dL or current hemodialysis (or during the 90 days prior initiating therapy).
- Chronic viral infections (HIV, HBV, HCV), cytomegalovirus infection (if the patient is taking immunosuppressive agents) (Hahn 2013).
- Hypogammaglobulinemia (IgG $<$ 400 mg/dL) or IgA deficiency (IgA $<$ 10 mg/dL)
- Concomitant treatment with other B-cell depleting agents (rituximab, cyclophosphamide)
- Previous history of hematopoietic stem cell, bone marrow, or solid organ transplantation
- Vaccination with live organisms 1 month before the initiation of treatment

In addition, and as is also recommended for patients with systemic autoimmune diseases candidate to be treated with any biological agent, the use of belimumab should be always carefully assessed in clinical situations associated with a high risk of infection (extensive cutaneous or mucosal ulcers, latent tuberculosis or untreated carriers, indwelling catheters) and in patients with mild neutropenia or hypogammaglobulinemia.

6 Belimumab: Tolerability and Adverse Effects

The results from phase III trials (Navarra et al. 2011; Furie et al. 2011) showed no statistically significant differences in the major adverse events among the three groups of patients (low dose, high dose, and placebo).

6.1 Infusion-Related Adverse Reactions

Any biological therapy administered intravenously may cause hypersensitivity and infusion-related reactions. In clinical trials, the incidence of infusion-related reactions that occurred during belimumab infusion was of 17 % (15 % in the placebo group), 1 % of which required permanent discontinuation of the drug. The infusion-related reactions were more frequent during the first two infusions and tended to

decrease in subsequent infusions (Navarra et al. 2011). Severe reactions were reported in 0.9 % of patients and included anaphylaxis, bradycardia, hypotension, angioedema, and dyspnea. In the BLISS-52 assay, three anaphylactic reactions were reported in the first dose, including two patients who developed severe angioedema (one considered unrelated to treatment) that were resolved with prednisone and adrenaline (Navarra et al. 2011). In the BLISS-76 trial, two cases of angioedema were reported in patients treated with belimumab 1 mg/kg (only one of which was considered drug-related), also resolved with antihistamines and/or prednisone (Furie et al. 2011).

Delayed hypersensitivity reactions have been rarely reported. They usually appear after 24 h of administration of belimumab and up to 14 days later, including symptoms such as fever, urticarial rash, arthralgia, and myalgia. Therapeutic management includes administration of second-generation antihistamine drugs together with acetaminophen 650 mg three times a day (5–7 days). In severe cases, oral corticosteroids may be required.

6.2 Infections

In the BLISS-52 trial (Navarra et al. 2011), the overall incidence of infections was 70 % in the group receiving belimumab and 67 % in the placebo group. Some infections occurred >3 % of patients and were at least 1 % more frequent than in the placebo arm, including nasopharyngitis, bronchitis, pharyngitis, cystitis, and viral gastroenteritis. Serious infections occurred in 5 % of patients receiving belimumab or placebo. Infections that led to treatment discontinuation occurred in 0.6 % of patients treated with belimumab and in 1 % of patients receiving placebo.

The combined results of BLISS trials 52 and 76 showed a numerical increase of respiratory infections, although the differences were not statistically significant. No differences in the development of herpes infections, sepsis, or opportunistic infections were found between the belimumab and placebo arms. Opportunistic infections have been reported in only three patients treated with belimumab, all of which resolved after stopping belimumab and initiating specific antibiotic treatment (Navarra et al. 2011; Furie et al. 2011). In one case, the causal relationship was considered unlikely. No opportunistic infections were reported in the placebo group. The risk of using belimumab in patients with active or latent tuberculosis is unknown.

As a B-cell depleting agent, belimumab could increase the risk of developing infections, including opportunistic infections, and patients who develop an infection during belimumab therapy should be closely monitored.

6.3 Hematological Complications

A statistically significant decrease of the IgG, IgM, and IgA levels has been reported in patients treated with belimumab included in the two BLISS trials (Navarra et al. 2011; Furie et al. 2011). The greatest reduction was reported for IgM levels (18 % versus 6 % in the placebo group), but was not associated with the development of infection. Leukopenia was reported in 4 % of patients included in the group treated with belimumab and in 2 % of those included in the placebo group.

6.4 Psychiatric Disorders

Psychiatric events were reported more frequently in the belimumab arms in comparison with the placebo arm (16 % vs 12 %) (Navarra et al. 2011; Furie et al. 2011). These events consisted of depression-related events (6.3 % vs. 4.7 %, belimumab vs. placebo), insomnia (6.0 % vs. 5.3 %, belimumab vs. placebo), and anxiety (3.9 % vs. 2.8 %, belimumab vs. placebo). Serious psychiatric events were reported in 0.8 % vs. 0.4 % of belimumab and placebo-treated patients, respectively. Severe depression was reported in 0.4 % (6/1,458) and 0.1 % (1/675) of belimumab and placebo-treated patients. Two suicides (0.1 %) were reported in patients receiving belimumab. The majority of patients who reported serious depression or suicidal behavior had a history of depression or other serious psychiatric disorder and were receiving psychoactive medications. The relationship between belimumab and these events is unknown.

6.5 Gastrointestinal Disorders

In the BLISS trials, the gastrointestinal adverse events with the greatest differences with respect to the placebo arm were nausea and diarrhea. Obese patients (BMI > 30 kg/m^2) treated with belimumab had a higher frequency of nausea, vomiting, and diarrhea in comparison with the obese patients included in the placebo arm and to patients with normal weight (BMI ≥ 18.5 to ≤ 30 kg/m^2). None of these gastrointestinal events in obese patients was severe.

6.6 Neoplasia

Patients with malignancies diagnosed 5 years before starting belimumab therapy were excluded in the BLISS trials (except for those with basal/squamous cell skin

cancers and cervical cancer who were properly treated). The BLISS trials reported ten cases of neoplasia diagnosed before initiation of belimumab therapy, including five solid neoplasms (two cases of breast cancer and one case each of stomach, cervix, and ovary cancer, respectively) and five non-melanoma skin malignancies. The incidence of malignancy per 100 patient-years of follow-up was 0.29 in the placebo group and 0.20 in the belimumab-treated group.

Data available after 4 years of treatment with belimumab (Merrill et al. 2012) showed a slight increase in the yearly prevalence of neoplasia (always less than 1.5 %/year), although no data supports that this increase may be related to the drug and not the expected prospective increase of cancer in SLE patients (Bernatsky et al. 2013a, b).

7 Atacicept: Double Block of BlyS and APRIL

Atacicept is a soluble, fully human, recombinant fusion protein that inhibits two B cell-stimulating factors, BLyS and APRIL (a proliferation-inducing ligand). Dall'Era et al. conducted in 2007 the first trial using atacicept in SLE patients (Dall'Era et al. 2007). In a multicenter, phase Ib, double-blind, placebo-controlled, dose-escalating trial including 47 patients, the authors found a dose-dependent reduction in immunoglobulin levels and in mature and total B cells, with no changes in the number of T cells, natural killer cells, or monocytes. There were no differences in the frequency or type of adverse events and no severe or serious adverse events were reported in patients treated with atacicept. Pena-Rossi et al. (2009) conducted in three centers in Russia the second double-blind, randomized, placebo-controlled, dose-escalating phase Ib study in SLE patients. The primary objective of the study was to evaluate the safety, tolerability, and pharmacodynamics of single or multiple intravenous injections of atacicept in patients with mild-to-moderate SLE. During the study, 10 patients reported 15 adverse events that were classified as mild (12 events) or moderate (three events). No deaths were reported, and no adverse event led to discontinuation of therapy.

In a recent study, Treamtrakanpon et al. (2012) have found a significant correlation between renal disease activity and APRIL serum levels in patients with lupus nephritis, especially in those who were refractory to the standard therapies (and who showed the highest APRIL serum levels). The authors have suggested that APRIL could be a potential biomarker for predicting refractory lupus nephritis and propose the use of APRIL antagonists such as atacicept in patients with lupus nephritis and with high serum levels of APRIL (Morel and Hahne 2013).

The results of the first phase II/III randomized, double-blind, placebo-controlled clinical trial have been recently published. Ginzler et al. have evaluated the efficacy and safety of atacicept in patients with active LN who were receiving newly initiated immunosuppressive therapy with corticosteroids and mycophenolate mofetil. Unfortunately, the trial was early terminated due to the unexpected severe reduction of serum immunoglobulin G (IgG) levels and the development of severe

infections. At the time of trial termination, six patients from four centers in the USA had been randomized. Of the four patients who were randomized to receive atacicept, three (75 %) developed serum IgG levels below the protocol-defined discontinuation criterion. These three patients developed severe infections, including *Haemophilus influenzae* pneumonia complicated by empyema, septicemia, and pneumothorax (IgG level of 2.9 g/L), *Legionella pneumophila* pneumonia (IgG of 2.5 g/L) and *Bacillus* bacteremia (IgG level decreased from a baseline of 20.1 to 4.7 g/L) (Ginzler et al. 2012).

Atacicept inhibits both APRIL and BLyS and has been shown to decrease levels of IgG, IgM, and IgA, as well as naive B cells and plasma cells (Genovese et al. 2011; van Vollenhoven et al. 2011a). In patients with rheumatoid arthritis, phase II trials testing atacicept showed median decreases of IgG serum levels of nearly 30 %. However, no patient with rheumatoid arthritis treated with atacicept developed IgG levels <3g/L, and the rate of serious infections was not notably increased. In patients with lupus nephritis, a possible influence of renal impairment has been suggested by the authors of the trial (Ginzler et al. 2012). Patients who were randomized to receive atacicept had higher proteinuria and UPr:Cr ratios ≥3.0 mg/mg, and proteinuria levels >2 g/day have been associated with the urine excretion of large proteins, which could include excretion of IgG. Therefore, high levels of proteinuria might affect the pharmacokinetics of atacicept, since atacicept is a protein that is secreted in the urine.

8 Conclusion

Recent studies have confirmed that SLE is a very heterogeneous disease whose outcomes and response to biological therapies may be influenced by ethnic, immunological, and genetic factors (Ramos-Casals and Khamashta 2012; Gregersen and Jayne 2012; Ramos-Casals et al. 2009b, 2011b). The treatment of SLE remains a challenge because a balance must be sought between the demonstrated efficacy of immunosuppressive agents (mostly used off-label) and the adverse effects of immunosuppression. The recent approval of belimumab for use in SLE suggests that biological agents will be increasingly used in the near future and will have a significant impact on the management of SLE patients (Burness and McCormack 2011; Ramos-Casals et al. 2012; Horowitz and Furie 2012). Unfortunately, belimumab trials excluded patients with active central nervous system involvement or severe lupus nephritis and thus provide limited data on the efficacy of this agent in the general population of patients with SLE. Indeed, belimumab should not be used in all SLE patients seems clear, and a growing body of evidence suggests that use of biological therapies should be tailored according to patient characteristics, including ethnicity, organ involvement, and the immunological profile. However, it is also clear that SLE patients display substantial heterogeneity in terms of B cell homeostasis, whose functional consequences and implications for B-cell therapy remain to be determined. New treatments and the successful application of current

ones will rest heavily on thorough understanding of these factors (Engel et al. 2011; Hahn 2013). Two open-label, parallel assignment continuation studies (NCT0072486747 and NCT0071293348) of patients completing the BLISS-52 or BLISS-76 trials are ongoing. Furthermore, a phase II multicenter, randomized, open-label trial of subcutaneously administered belimumab in SLE patients is underway (NCT00732940). Ongoing trials are testing other BLyS antagonists such as A-623 (previously known as AMG 623) and LY2127399, or a subcutaneous humanized anti-CD20 (SBI-087), but their approval for clinical use is not anticipated for at least 5 years.

In contrast to belimumab, the combined blockade of both BLyS and APRIL achieved with atacicept induces the depletion of a significantly larger swath of B cells and plasma cells possibly including long-lived plasma cells thereby inducing greater decline in antibody levels. Unfortunately, this powerful effect may also increase the risk of severe infections and the ultimate safety of this approach in combination with other immunosuppressive drugs needs to be thoroughly evaluated in SLE (Morel and Hahne 2013).

A new era is opening in the therapeutic approach to SLE, based on new drugs with more-specific mechanisms of action, of which B-cell targeted therapies are the more promising agents. However, the take-home message remains the same: a careful evaluation of the risk/benefit profile of using biological agents in SLE patients is essential.

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B-Cell Targeted Therapies in Rheumatoid Arthritis

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Abstract B cells, one of the major lymphocyte subpopulation, are known to play an important role in the pathogenesis of rheumatoid arthritis (RA). B cells can not only be found activated in central lymphoid organs and peripheral blood of patients suffering from RA but are also present in the inflamed synovial membrane, have been shown to even undergo clonal expansion there, and are responsible for the generation of autoantibodies that characterize this disease, most importantly rheumatoid factor (RF) and anti-citrullinated-peptide antibody (ACPA). However, the idea of targeting B cells to treat the disease has long been regarded as very unlikely to be successful. In 2002 the first report was published in a case series of five patients that advocated B cells as a therapeutic target, spurred by a report of Takemura et al., who even suggested that the activation of T cells in the rheumatoid synovium was B cell-dependent.

1 B Cell Depletion with Rituximab

1.1 Efficacy

It is now well established that B cells are important in the pathogenesis of RA (Aloisi and Pujol-Borrell 2006; Bugatti et al. 2007; De Vita et al. 2002; Finnegan et al. 2012; Takemura et al. 2001). The first randomized controlled trial demonstrating the efficacy of B cell depletion with rituximab in patients with long-standing RA (mean duration of disease of about 10 years), who had active disease despite treatment with methotrexate was published in 2004 (Edwards et al. 2004). The patients were randomized to four different treatment arms: (1) continue either oral methotrexate alone at a dose of at

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least 10 mg/week plus placebos for rituximab and cyclophosphamide (control group); (2) rituximab (2× 1,000 mg at day 1 and 15) plus placebos for methotrexate and cyclophosphamide; (3) rituximab plus cyclophosphamide as intravenous infusion of 750 mg on days 3 and 17 plus placebo for methotrexate; and (4) rituximab plus methotrexate at a dose of at least 10 mg/week plus placebo for cyclophosphamide. ACR 20, ACR 50, and ACR 70 response rates at week 24 and week 48 were analyzed, showing significant clinical benefit of rituximab treatment in combination with MTX compared to placebo + MTX. There was no difference in adverse events in rituximab treated patients in combination with MTX compared to the control arm. The authors further documented the depletion of B cells from peripheral blood. There also was a substantial reduction in RF titers, while serum immunglobulin levels (all classes) as well as anti-tetanus toxin titers were not significantly altered.

In an attempt to optimize the dosing regimens of rituximab, the DANCER trial compared two doses of rituximab (2× 500 mg vs. 2× 1,000 mg, on day 1 and day 15, respectively). The patients included had long-standing RA with a mean of about 9 years. There was no significant difference in the clinical efficacy between the treatment groups, although numerically more patients reached an ACR 70 response in the 2× 1,000 mg group (Emery et al. 2006). These findings were confirmed and extended in the SERENE trial, where patients were allocated to the same dosing regimen of rituximab, and followed for 48 weeks (Emery et al. 2010). Similar to efficacy, there was no difference in adverse events between the two groups in both trials. The DANCER trial also demonstrated that glucocorticoids do not account for the therapeutic effect of rituximab, since pretreatment with glucocorticoids did not affect the probability to reach an ACR 20 response (Emery et al. 2006); however, glucocorticoid pretreatment reduced infusion reactions to rituximab. The DANCER trial was also the first rituximab trial which included a significant proportion of patients who had previously been treated with TNF-blocking agents. Subanalyses showed that rituximab was similarly effective relative to placebo both in patients who had received prior biologics and those naive to these therapies. This was formally investigated in the REFLEX trial, where inadequate responders to anti-TNF therapy were treated with rituximab (Cohen et al. 2006). There was a significant reduction of clinical signs and symptoms of RA as well as of functional impairment as assessed by HAQ in the rituximab-treated patients compared to placebo. However, the response rates decreased numerically with increasing numbers of failed TNF-inhibitors. At 24 weeks, analysis of radiologic data showed a trend towards reduced progression in the Genant-modified Sharp score (Cohen et al. 2006) which reached statistical significance at 1 and 2 years of therapy regarding the total and both joint space narrowing as well as erosion scores (Cohen et al. 2010; Keystone et al. 2009).

In all studies, the concomitant use of MTX proved to convey better efficacy than rituximab monotherapy. In addition to MTX, leflunomide has been shown to provide a safe and effective alternative (Chatzidionysiou et al. 2012).

To test, whether rituximab was also effective in MTX-naïve patients with early disease, 755 patients with a mean disease duration of 1 year were randomized to receive MTX alone, rituximab 2× 500 mg + MTX or rituximab 2× 1,000 mg + MTX in the IMAGE trial. While at week 52 both doses of rituximab + MTX

conveyed significantly better clinical outcomes compared with MTX alone, as assessed by ACR20, 50, 70, and 90 responses, significant inhibition of radiographic progression was only achieved in the 2× 1,000 mg arm; however, in patients receiving 2× 500 mg rituximab the trend towards reduction of radiographic progression was close to significant and even at this lower dose the progression of damage between months 6 and 12 was almost halted (Tak et al. 2011). A summary of some rituximab trial data is provided in Table 1.

1.2 Biomarkers of Responsiveness

As with other therapeutic approaches, substantial efforts have been made towards the identification of biomarkers predicting or influencing the response of RA patients to rituximab. The ARISE trial analyzed sequential synovial biopsies of patients with long-standing RA taken 2 and 8 weeks after treatment with rituximab. Patients were divided into clinical responders and non-responders. While there was a significant reduction of CD20 staining as well as CD19 mRNA levels at week 8 compared to week 2, responders and non-responders were not different in their levels of depletion, although the authors noticed a nonsignificant trend towards greater reduction of B cells in the responder group, but also conceded profound depletion in patients with no clinical response (Kavanaugh et al. 2008). Interestingly, there was no change in the synovial expression of the proinflammatory cytokines TNF and Il-6, nor MMP-1 in patients treated with rituximab, and, again, there was no difference between responders and non-responders. Of note, a significant decrease in synovial expression of the chemokine IL-8 was observed following rituximab treatment, which was only seen in the non-responders. Here the authors stated: "this result would seem almost paradoxical, given the diverse proinflammatory actions mediated by IL-8" (Kavanaugh et al. 2008). In another study analyzing the histological changes in the synovial membrane, Thurlings et al. (2008) in addition to depletion of B cells demonstrated a significant reduction in inflammatory cells such as CD68+ macrophages as well as CD3+ T cells. However, significant differences in terms of synovial histological response between clinical responders and non-responders were observed for some cell populations, as intimal macrophages and CD138+ plasma cells were significantly more reduced in responders (Thurlings et al. 2008).

While the data on changes in synovial cell populations are not unequivocal, the findings that rituximab therapy is more efficacious in RF+ and/or ACPA+ patients than seronegative individuals are much more robust (Chatzidionysiou et al. 2011; Emery et al. 2010). Nevertheless, there is also evidence for responsiveness also in seronegative RA, although to a lesser extent than in seropositive patients.

In an attempt to characterize subsets of B cells and the kinetics of their repopulation after rituximab, Roll et al. (2008) analyzed 17 patients and found no difference between responders and non-responders with respect to the duration of B cell depletion. In contrast, repopulation with memory B cells, characterized by the

Table 1 Overview of selected major clinical trials targeting B cells

	Patents	Steroids	Treatment groups	ACR 20/50/70 responses (%)	Radiographic outcome
Efficacy of B-Cell-targeted therapy with RTX in patients with rheumatoid arthritis Edwards et al. (2004)	161 active RA despite MTX	100 mg of methylprednisolone before each RTX 60 mg prednisolone days 2–7 30 mg prednisolone days 8–14	• MTX • RTX 2× 1,000 mg day 1 and day 15 • RTX 2× 1,000 mg day 1 and day 15 + MTX • RTX 2× 1,000 mg day 1 and day 15 + cyclophosphamide 750 mg day 3 and day 17	• MTX: 38/13/5 • RTX: 65/33/15 • RTX + MTX: 73/41/15 • RTX + cyclophosphamide: 76/43/23	Not reported
Dancer Emery et al. (2006)	465 active RA despite MTX, previous aTNF allowed	1. No glucocorticoids 2. 100 mg methylprednisolone premedication 3. 100 mg methylprednisolone premedication 60 mg prednisolone days 2–7, 30 mg prednisolone days 8–14	• MTX + PBO • MTX + RTX 2× 5,00 mg day 1 and day 15 • MTX + RTX 2× 1,000 mg day 1 and day 15	Week 24 • MTX + PBO: 28/13/5 • MTX + 2× 500 mg RTX: 55/33/13 • MTX + 2× 1,000 mg: 54/34/20	Not reported
Reflex Cohen et al. (2006)	520 inadequate response to anti–tumor necrosis factor	Methylprednisolone (100 mg) 30 minutes before each infusion), and oral prednisone (60 mg on days 2–7; 30 mg on days 8–14)	• Placebo • RTX 2× 1,000 mg day 1 and day 15	Week 24 • PBO: 18/5/1 • RTX: 51/27/12	Total Genant-modified Sharp radiographic score • PBO: 1.2 ± 3.3 • RTX: 0.6 ± 1.9
Image Tak et al. (2011)	755 MTX-naïve patients with active RA	Methylprednisolone 100 mg premedication	• MTX + PBO • MTX + RTX 2× 500 mg • MTX + RTX 2× 1,000 mg	Week 52 • MTX + PBO: 64/42/25 • MTX + RTX 2× 500 mg: 77/59/17 • MTX + RTX 2× 1,000 mg: 80/65/47	Mean change TSS at week 52 • MTX + PBO: 1.08 • MTX + RTX 2× 500 mg:

					0.64 • MTX + RTX 2× 1,000 mg: 0.36
Ofatumumab Taylor et al. (2011)	Active RA despite MTX treatment	Methylprednisolone (100 mg) before each ofatumumab infusion	• MTX + PBO • MTX + Ofatumumab 700 mg day 1 and day 15	Week 24 • MTX + PBO: 27/11/2 • MTX + Ofatumumab 700 mg: 50/27/13	Not reported
Atacicept van Vollenhoven et al. (2011)	311 patients with active RA despite MTX treatment	None	• MTX + PBO • MTX + Atacicept with loading dose • MTX + Atacicept with loading dose • MTX + Adalimumab open label	• MTX + PBO: 46/15/5 • MTX + Atacicept with loading dose: 45/30/13 • MTX + Atacicept with loading dose: 58/33/13 • MTX + Adalimumab open label: 71/38/18	Not reported
Ocrelizumab Rigby et al. (2012)	1,015 patients with active RA despite MTX treatment	Methylprednisolone (100 mg) before each ocrelizumab infusion	• MTX + PBO • Ocrelizumab 200 mg day 1 and day 15 • Ocrelizumab 500 mg day 1 and day 15	• MTX + PBO: 36 • MTX + Ocrelizumab 2× 200 mg: 57 % • MTX + Ocrelizumab 2× 500 mg: 55.4	Mean change SHS at week 52 • MTX + PBO: 1.04 ± 2.84 • MTX + Ocrelizumab 2× 200 mg: 0.34 ± 2.4 • MTX + Ocrelizumab 2× 500 mg: −0.03 ± 2.53

expression of CD27 and IgD, was significantly higher in non-responders vs. responders (Roll et al. 2008). Although the biological role of these cells in the pathogenesis of RA in general and a relapse in particular is not clear, they might serve as biomarkers that could be used for tailoring rituximab therapy individually. However, the biological significance of the latter subset is unclear, especially in the light of a large study conducted in France which found this CD27+IgD+ subset even decreased in patients with RA (Sellam et al. 2011). Roll et al. (2008) also analyzed B cell repopulation after a second course of rituximab treatment and reported that, although initial numbers of B cells were still decreased at the time of retreatment, the kinetics of B cell repopulation were similar to the first depletion, starting with immature B cells (IgD+CD38+CD20+CD10+), followed by IgD+CD27+ cells. Relapse seems to correlate with early B cell repopulation.

1.3 Mechanism of Action

One of the main mechanisms of action of rituximab is antibody-dependent cellular cytotoxicity (ADCC). It has been demonstrated that a polymorphism in the FcγRIIIA (*FCGR3*-158V/F polymorphism, which affects the amino acid residue in the FcγRIIIA that directly interacts with the lower hinge region of IgG1 and therefore also rituximab) affects efficacy of rituximab in lymphoma patients (Weng and Levy 2003). In the SMART trial the influence of this polymorphism on the therapeutic response to rituximab in RA patients was analyzed; 111 patients were genotyped and indeed, the therapeutic response in patients with the polymorphism conferring lower affinity was significantly lower than in patients without this mutation (Ruyssen-Witrand et al. 2012).

Interestingly, response to rituximab has been linked with the induction of interferon-induced genes (Vosslamber et al. 2011) despite the fact that B cell depletion was comparable in all patients and, consequently, B cell-mediated immunity-related genes were downregulated in gene set enrichment analysis (GSEA). Microarray expression profiling revealed a selective increase in the expression of type I IFN-response genes at 3 months following the start of rituximab treatment in those patients who had a good clinical response, whereas the ones with no increased expression of these genes exhibited a poor response (Vosslamber et al. 2011). This response was not correlated with known disease activity parameters such as CRP levels or ESR. Of note, a similar phenomenon has been observed in patients treated with infliximab (van Baarsen et al. 2010). These data show the complexity of responses to a targeted therapy.

Rituximab has also been demonstrated to interfere with the development of T-helper cell subsets, especially Th17 cells which are thought to be the main T-helper cell subset involved in the pathogenesis of RA by secreting cytokines such as IL-22 and IL-17 (van de Veerdonk et al. 2011). Treatment with rituximab inhibited synovial expression of these cytokines, as well as ROR-γt, the transcriptional master regulator for Th17 development (Hirahara et al. 2010). In line with

previous observations (ARISE trial), there was no change in the expression levels of synovial TNF, or, for that matter, of synovial expression of the prototypic Th1 cytokine IFN-γ, suggesting a rather specific effect on Th17 polarization after treatment with rituximab.

The inhibitory effect of rituximab on radiographic progression has been clearly demonstrated in clinical trials (Cohen et al. 2010; Keystone et al. 2009; Tak et al. 2011). However, the mechanistic details of this inhibition are not entirely clear at the moment, since patients show less progression in radiographic analyses even when experiencing no or no clear-cut clinical response to the treatment (Aletaha et al. 2013). However, histological analyses of synovial biopsies of patients before and after treatment with rituximab showed significant reduction of synovial receptor activator of NF-κB (RANK) and RANK-ligand expression (Boumans et al. 2012). Since B cells express relatively high levels of RANKL (Yeo et al. 2011), the most straightforward explanation for the inhibition of radiographic damage by rituximab is the reduction of RANKL expressing cells via B-cell depletion and, therefore, prevention or reduction of the critical stimulus for osteoclastogenesis. Alternatively, it may be assumed that rituximab interferes with the proinflammatory cytokine response by either reducing autoantibody production and thus immune complex formation and immune complex-mediated macrophage activation or by inhibiting antigen presenting capacity of B-cells (Bluml et al. 2013).

1.4 Safety Aspects

While many reports showed that immunoglobulins levels as well as anti-tetanus antibody titers and anti-pneumococcal polysaccharide antibody levels remained stable after treatment with rituximab (Cambridge et al. 2006), most probably reflecting the presence of long-lived plasma cells that are not targeted by rituximab, a reduction of protective antibody levels after treatment with rituximab remained a concern. Therefore RA patients, rituximab-treated RA patients and healthy controls were vaccinated with three influenza virus antigens (NC and CAL and SHAN). There was a significant increase in geometric mean titers (GMT) for NC and CAL antigens in all subjects, but not for the SHAN antigen in the rituximab group. In general the percentage of responders was low for all three antigens tested in this study in rituximab-treated patients, suggesting compromised but not absent ability to develop humoral immunity to vaccination after treatment with rituximab. Similarly, in another study, responses to keyhole limpet hemocyanin (KLH) and T cell-independent responses to pneumococcal vaccine were decreased, but many patients were able to mount responses (Bingham et al. 2010; Oren et al. 2008). In long-term observations, a decrease of IgG and IgM was consistently noted. However, both the percentage of patients developing low IgM levels after $\geq$1 course of rituximab as well as the absolute decrease of IgM levels was more pronounced as for IgG (De La Torre et al. 2012; van Vollenhoven et al. 2013).

Treatment with immunosuppressive agents invariably increases the risk of developing infections (Bongartz et al. 2006). In this regard, a recent Cochrane review found an increased risk of serious infections in patients treated with rituximab, which did not differ from the risk observed with other biologic treatments (Singh et al. 2011); importantly, however, the risk of serious infections increases in the presence of low immunoglobulin levels. On the other hand, rituximab therapy is not associated with an increased risk for tuberculosis (Buch et al. 2011).

The occurrence of PML, a rare disease caused by the JC virus among patients with autoimmune rheumatic diseases after treatment with rituximab is a concern. However, the incidence is low, and patients with SLE seem to have a higher risk for the development of this disease (Molloy and Calabrese 2012).

Rituximab is approved for the treatment of RA patients who have had an inadequate response to conventional DMARDs and a TNF-inhibitor. However, in the presence of certain risks, such as recent malignancies including lymphoma or tuberculosis, one might consider rituximab before a TNF-inhibitor.

1.5 Novel Therapies Targeting B Cells

The success of rituximab in treating RA has initiated a search for other therapeutic agents targeting CD20 in particular and B cells in general. Some of the respective trials are depicted in Table 1.

Ocrelizumab is a novel humanized monoclonal anti-CD20 antibody, which in vitro showed enhanced ADCC and reduced complement-dependent cytotoxicity (CDC) compared to rituximab. It recognizes a distinct, but overlapping peptide of the human CD20 molecule. In a dose-ranging study, ocrelizumab showed clinical efficacy, with no safety issues arising during that trial. Interestingly, despite profound peripheral cell depletion, there was only a slight decrease of serum IgM levels, with no effect on IgA or IgG levels. The STAGE trial analyzed the effect of ocrelizumab in patients with active RA despite treatment with MTX (Rigby et al. 2012). Ocrelizumab was found to be clinically effective and, in addition, a smaller proportion of patients receiving the drug showed signs of radiographic progression compared to placebo (Rigby et al. 2012). Ocrelizumab was also tested in MTX naïve patients as well as in patients with inadequate response to TNF inhibitors (Tak et al. 2012). In both trials, the drug was effective clinically. Furthermore, its ability to inhibit structural joint damage was demonstrated in both trials, although higher doses of ocrelizumab were needed when testing patients with an inadequate response to therapy with a TNF inhibitor (Rigby et al. 2012; Tak et al. 2012). However, according to a media release in March 2010 by Roche, further development of this drug was halted due to excess mortality from opportunistic infections.

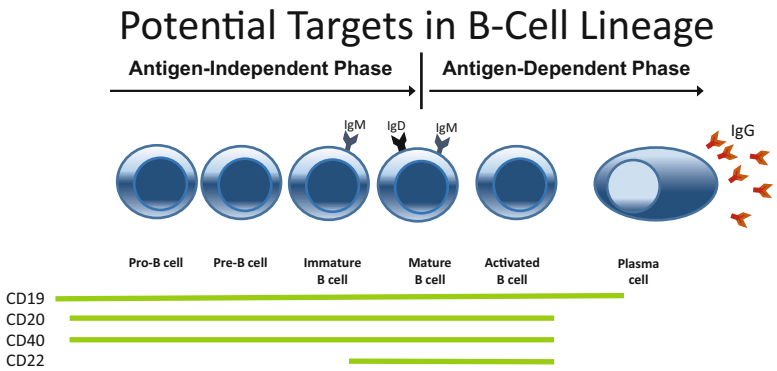

Fig. 1 This schematic representation of surface receptors on B cells in various developmental stages depicts, without being complete, either existing or potential targets for therapeutic interventions. Modified after Sell et al. *Immunology, Immunopathology, and Immunity*, 6th edn. 2001. Roitt et al. *Immunology*. 6th edn. 2001

Ofatumumab (HuMax-CD20) is a human mAb that targets a membrane-proximal epitope on the CD20 molecule. It recognizes a different epitope of CD20 than rituximab and ocrelizumab. It was tested in patients with inadequate responses to one or more synthetic DMARDs and was found to be safe and to significantly alleviate signs and symptoms of RA. Doses of up to 1,000 mg given intravenously in a 2-week interval were reported to be effective upon assessment of disease activity 24 weeks after the infusion. The authors did not find changes in the serum levels of IgA or IgG, only IgM levels were slightly reduced in patients after receiving ofatumumab (Ostergaard et al. 2010). In a phase III trial, the 2× 700 mg regimen was chosen and at week 24 a greater proportion of patients on ofatumumab compared with placebo achieved an ACR20 response and a good or moderate EULAR response (Taylor et al. 2011). No data so far are available to demonstrate beneficial effects of ofatumumab on radiographic progression in patients with RA.

While CD20 can be successfully targeted, B-cells express a variety of other molecules which are partly shown in Fig. 1. Many of these can serve as potential therapeutic targets and some are, indeed, in clinical trial.

A pathway of potential interest is the BLyS/BAFF-TACI pathway. B lymphocyte stimulator [BLyS, also called B-cell activating factor (BAFF)] and the highly related proliferation-inducing ligand (APRIL) are B cell maturation/survival factors, and thus not only regulate apoptosis of B cells, but also B-cell homoeostasis in general, maintaining peripheral tolerance and providing costimulation. Atacicept, a soluble, fully human, recombinant fusion protein comprising the extracellular portion of the TACI receptor, a member of the TNF-receptor superfamily, fused to the Fc portion of human IgG, was investigated in a phase II trial in patients suffering from RA who had an inadequate response to MTX. However, the primary endpoint of the trial was not met, since there was no significant difference in the

numbers of patients reaching an ACR20 response after treatment with atacicept compared to placebo, although the investigators did notice significant differences in the ACR50 and ACR70 responses (van Vollenhoven et al. 2011). Similarly, there was no effect of atacicept in patients with a prior inadequate response to anti-TNF therapy (Genovese et al. 2011). In both trials, some biological effects were noted, such as reduction in total serum immunoglobulin, especially not only IgM and IgA but also IgG. Furthermore an even more pronounced effect was seen on reductions of all classes of rheumatoid factor (IgM, IgA, and IgG) (Genovese et al. 2011).

Likewise, belimumab, a human anti-BLyS monclonal antibody approved for the treatment of systemic lupus erythematosus, has conveyed only small responses in RA (Stohl et al. 2013).

Tabalumab, a human anti-BAFF monoclonal antibody that neutralizes both biologically active form of BAFF (membrane-bound and soluble), was found to significantly decrease the signs and symptoms of patients with RA who experienced an inadequate response to MTX (Genovese et al. 2013b). However, in a study investigating the effect of tabalumab in patients with RA with an inadequate response to TNF-inhibitors, there was no significant clinical benefit seen when analyzing ACR20, ACR50 (which was the primary end point), or ACR 70 responses after 16 weeks of treatment. However, at earlier time points, there was a clinical benefit in patients receiving tabalumab compared to placebo (Genovese et al. 2013a). Concerning immunoglobulin levels, significant reductions from baseline were observed for IgM and IgA, but not for IgG, when combining the tabalumab groups compared to placebo. Interestingly, transient increases in blood B cell levels were noticed in both trials, indicating that efficacy of this drug is not depending on reducing B cell numbers.

In addition, novel drugs targeting B cells structures such as CD19 (MEDI-551, an affinity-optimized and afucosylated MAb targeting CD19) and epratuzumab which targets CD22, another B cell surface antigen which plays an important regulatory role and is involved in the control of B cell activation, peripheral B cell homeostasis, survival, and cell cycle progression following activation are available (Tedder et al. 2005). No clinical trial data are published yet; however, it will be very interesting to see their efficacy in RA in the years to come.

In conclusion, targeting B-cells, as seen since the advent of rituximab, is a major therapeutic breakthrough in RA. Whether by virtue of depletion of antigen presenting cells or precursors of autoantibody producing cells, targeting this pathway appears to be as efficacious and safe as targeting TNF or IL-6. Alas, not all therapeutic targeting B-cell molecules are efficacious and, therefore, the search must go on to find new and potentially even better and safer remedies that interfere with the action of this important cell population in the pathogenesis of RA and other diseases.

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B-Cell Targeted Therapies in Primary Sjögren Syndrome

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Abstract Primary Sjögren syndrome (SS) is a chronic systemic autoimmune disease characterised by sicca features and systemic manifestations and requires a multidisciplinary therapeutic approach. Treatment of sicca manifestations is symptomatic (topical therapies like saliva substitutes and preservative-free artificial tears), while the management of extraglandular features is tailored to the specific organs involved. The use of biological drugs targeting molecules and receptors involved in the etiopathogenesis of primary SS has opened up a new era in the therapeutic management of the disease. Although the evidence from controlled trials has suggested that tumour necrosis factor blockers are not efficacious in primary SS, the use of B-cell targeted agents (rituximab, epratuzumab, belimumab) may be more promising therapies. However, the potential risks and benefits of these new agents must be always weighted carefully.

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1 Introduction

Sjögren syndrome (SS) is a systemic autoimmune disease with a wide clinical spectrum that extends from sicca symptoms of the main mucosal surfaces to extraglandular manifestations. The main clinical features are dry mouth and dry eyes, which are determined by specific oral (salivary flow measurement, parotid scintigraphy) and ocular (fluorescein staining, Schirmer test) tests, respectively (Ramos-Casals et al. 2012a). The histological hallmark of SS is focal lymphocytic infiltration of the exocrine glands, determined by minor labial salivary gland biopsy (Fox 2005). Patients with SS present a broad spectrum of analytical features and a plethora of autoantibodies, of which antinuclear antibodies are the most frequently detected, anti-Ro/SS-A the most specific, and cryoglobulins and hypocomplementemia the main prognostic markers. The main complication of the disease is the development of B-cell lymphoma, with a risk 10–40 times higher than that found in the general population (Theander and Baecklund 2012). While sicca features primarily affect the quality of life and cause local complications in the mucosa involved, systemic or extraglandular involvement marks the disease prognosis (Kassan and Moutsopoulos 2004). SS patients may develop a large number of systemic manifestations (Ramos-Casals et al. 2011), either as the presenting manifestation or during the evolution.

The classic therapeutic approach to primary SS is based on symptomatic treatment of sicca manifestations and broad-spectrum immunosuppression for extraglandular disease (Ramos-Casals et al. 2012b). However, over the last decade, research has centred on new therapies with the hope of providing better management approaches (Coca and Sanz 2012). The emergence of biological therapies has recently increased the therapeutic armamentarium available to treat SS, but their use is still limited by the lack of licensing (Ramos-Casals et al. 2010a). This chapter summarises the current pharmacotherapy options and future directions on B-cell targeted therapies in patients with primary SS.

2 Therapeutic Approach of Primary SS: General Rules

The therapeutic management of patients with primary SS is often based on focusing, on the one hand, on the treatment of for sicca features, and, on the other hand, on the treatment of systemic autoimmune involvement. Unhappily, a very limited level of scientific evidence supports these two key approaches, since large RCTs are very scarce in primary SS.

The primary therapeutic approach for sicca manifestations should be symptomatic, using artificial tears and saliva substitutes, a recommendation based on reported studies which support their daily use without any kind of side effects (Ramos-Casals et al. 2012b). Available data does not show conclusively that one specific saliva substitute is superior to another (Ramos-Casals et al. 2012b), while

the evidence on eye drops supports the use of preservative-free formulations (containing hyaluronate or methylcellulose) three to four times daily, reducing the dosing interval to as little as 1 h when necessary (Ramos-Casals et al. 2012b). Patients with severe sicca features may require a more intensive therapeutic approach including oral muscarinic agonists for severe oral dryness and topical 0.05 % cyclosporine A for severe ocular dryness (Ramos-Casals et al. 2012b).

For the treatment of severe autoimmune features in primary SS, a combination of glucocorticosteroids and immunosuppressive agents is the preferred approach. However, this approach is often associated with adverse events and there is growing awareness of infections and cardiovascular involvement in these patients. In addition, new immunosuppressive agents (leflunomide, mycophenolate) have been tested in primary SS without clear clinical benefits and with unacceptably high rates of adverse effects (Ramos-Casals et al. 2012b). The primary outcomes in these studies focused on the effect of the drugs on sicca features, which is not the main indication for immunosuppressants in daily practice. Systemic life-threatening involvement has rarely been reported in primary SS (Ramos-Casals et al. 2012b) with cryoglobulinaemic vasculitis (involving the kidneys, lungs or gastrointestinal tract) being the main cause of severe SS presentation (Theander and Baecklund 2012). Other severe involvements unrelated to cryoglobulinaemia include myelitis, ataxic neuronopathy and pulmonary arterial hypertension (Theander and Baecklund 2012). Therapeutic recommendations are often based on the use of methylprednisolone and cyclophosphamide pulses, with plasma exchange being added in the most severe situations (Theander and Baecklund 2012).

In the last years, biological therapies have emerged as new therapeutic agents that are increasingly used for primary SS. B-cell targeted therapies are the most promising agents in primary SS, although their use is significantly limited by the current lack of specific licensing (Table 1).

3 B-Cell Targeted Therapeutic Approaches in Primary SS

Although not a benign condition, primary SS usually progresses very slowly, with no rapid deterioration in salivary function or dramatic changes in symptoms. The exceptions to this benign course are the development of extraglandular manifestations and the high incidence of lymphoma (Ramos-Casals et al. 2012a). B cells are central in the pathogenesis of systemic primary SS, which is a disease characterised by a marked B-cell polyclonal hyperactivity. In some patients, B-cell hyperactivity may turn on monoclonal B-cell expansion leading, in some patients, to the development of B-cell lymphoma, which is the worst complication of primary SS. Therefore, it seems etiopathogenically reasonable to postulate a potential benefit for B-cell targeted therapies (Ramos-Casals and Brito-Zerón 2007). Agents directed against B cells are designed to eliminate either the majority of B cells (general depletion) or only specific B-cell populations (selective depletion) (Ramos-Casals et al. 2012c) following two principal mechanisms: direct killing

Table 1 Use of B-cell targeted therapies in patients with primary SS: main studies

Author (year)	B-cell therapy	Study design	Number of patients with primary SS
Meijer et al. (2010)	Rituximab	RCT	30
Dass et al. (2008)	Rituximab	RCT	17
Devauchelle-Pensec et al. (2012)	Rituximab	RCT	122
Pijpe et al. (2005)	Rituximab	Open-label	15
Devauchelle-Pensec et al. (2007)	Rituximab	Open-label	16
St Clair et al. (2013)	Rituximab	Open-label	12
Gottenberg et al. (2005)	Rituximab	Retrospective	6
Seror et al. (2007)	Rituximab	Retrospective	16
Vasil'ev et al. (2009)	Rituximab	Retrospective	10
Ramos-Casals et al. (2010c)	Rituximab	Retrospective	15
Tony et al. (2011)	Rituximab	Retrospective	4
Gottenberg et al. (2013)	Rituximab	Retrospective	74
Zhou et al. (2012)	Rituximab	Retrospective	4
Mekinian et al. (2012a)	Rituximab	Retrospective	11
Mekinian et al. (2012b)	Rituximab	Retrospective	17
Pollard et al. (2011)	Rituximab	Retrospective	19
Voulgarelis et al. (2012)	Rituximab	Retrospective	17
Steinfeld et al. (2006)	Epratuzumab	Open-label	16
Mariette et al. (2012) De Vita et al. (2012)	Belimumab	Open-label	30

RCT randomised controlled trial

by monoclonal antibodies directed to B-cell surface molecules such as CD19/CD20 (rituximab, ocrelizumab) and CD22 (epratuzumab) and an indirect effect on B cells by the blockade of essential factors for B-cell survival such as BLyS/BAFF (belimumab) and APRIL (atacicept).

4 Rituximab

4.1 Randomised Controlled Studies

In 2010, Meijer et al. (2010) reported the first controlled study in primary Sjögren syndrome showing promising results on the use of rituximab not only for sicca features but also for systemic involvement (Table 2). However, their results should be interpreted with caution because: (1) the limited number of patients included (10 in the placebo group and 20 in the rituximab group); (2) the pre-therapeutic differences in baseline salivary flow rates between the two groups; and (3) benefit was assessed at weeks 5, 12, 24, and 48 and was only statistically significant at 12 weeks (Ramos-Casals et al. 2010b). A second small trial was conducted by Dass

Table 2 Randomized controlled trials evaluating rituximab in patients with primary SS

Author (year)	*N* (female)	Mean age (years)	Study design (duration)	Drug (number of patients)	Primary outcome (results)	Secondary outcomes (significant differences)
Dass et al. (2008)	17 (ND)	52	RCT-d	Rituximab 1 g/15d ($n = 8$)	Fatigue VAS improvement >20 % at 6 months (87 % vs 56 %, $p = 0.36$)	VAS: fatigue (<0.001), general health (0.021)
			6m	Placebo ($n = 9$)		SF-36: social functioning (0.01)
				Weeks 0 and 2		PROFAD VAS, FACIT-F
						Schirmer test
						Unstimulated flow rate
						ESR, CRP, abs, IgG, RF reduction (0.05)
Meijer et al. (2010)	30 (29)	43	RCT-d	Rituximab 1 g/15d ($n = 20$)	Improvement of stimulated whole salivary flow rate at 5, 12, 24 and 48 weeks ($p = 0.038$ only at 12w)	Salivary flow rates: SWS at 12w (<0.05)
			48w			Ocular tests: lissamine (<0.05)
				Placebo ($n = 10$)		Igs, decreased RF (<0.05)
				Weeks 0 and 2		VAS: dry mouth night (<0.05), dry eyes (<0.05)
						SF-36: vitality (0.013)
						MFI: reduced activity (0.023)
						Extraglandular features: decreased number (0.029), reduced vasculitis (0.03)
Devauchelle-Pensec et al. (2012)	122 (ND)	ND	RCT-d	Rituximab 1 g/15d ($n = 53$)	Composite score at 24w (VAS × 4): (21.7 % vs 20.7 %, $p = 0.9$)	Dryness VAS (<0.05)

(continued)

Table 2 (continued)

Author (year)	*N* (female)	Mean age (years)	Study design (duration)	Drug (number of patients)	Primary outcome (results)	Secondary outcomes (significant differences)
			24w			Pain VAS
				Placebo ($n = 60$)		Fatigue VAS (<0.05)
				Weeks 0 and 2		Global VAS
						ESSDAI score
						Number swollen joints
						Salivary flow rates (0.009)
						Salivary biopsy score
						Ocular tests
						Systemic/biological features

RCT randomised controlled trial, *-d* double-blind, *m* months, *w* weeks, *ND* not detailed, *d* day, *VAS* visual analogue scale, *SF*-36 medical outcomes short form, *PROFAD* profile of fatigue and discomfort, *FACIT-F* functional assessment of chronic illness therapy, *ESR* erythrosedimentation rate, *CRP* C-reactive protein, *abs* autoantibodies, *RF* rheumatoid factor, *SWS* stimulated whole salivary flow, *Igs* serum immunoglobulins, *MFI* multidimensional fatigue inventory, *ESSDAI* European activity score, *bx* biopsy

et al. (2008) in 17 patients with pSS showing a high score on fatigue visual analogue scale (VAS) who were randomised to receive either two infusions of rituximab 1 g or placebo together with oral and intravenous steroids. Although statistically significant differences were found in some secondary outcomes, no differences were found for the primary outcome (>20 % of improvement in fatigue VAS) between rituximab and placebo arms at 6 months (87 % vs 56 %, $p = 0.36$).

Larger studies testing rituximab in primary SS are needed as it is difficult to draw firm conclusions from these small randomised trials. Thus, the preliminary results of a large multicentre RCT have recently shown limited benefits. Devauchelle-Pensec et al. (2012) evaluated 122 consecutive patients who were assigned to receive either RTX infusions (1 g) or placebo (P) at weeks 0 and 2. The primary endpoint was improvement (≥30 mm) of two of four VAS that evaluate dryness, pain, fatigue and global health between weeks 0 and 24. Secondary end points included delta of improvement of all VAS separately, the ESSDAI score, swollen joints, salivary flow rates, ocular tests, laboratory parameters and extraglandular involvement. For the composite primary endpoint, 13/60 (22 %) patients treated with RTX had a favourable overall response in comparison with 11/53 (21 %) patients who received placebo ($p = 0.9$). Statistically significant differences were found in some secondary endpoints including sicca and fatigue VAS ($p < 0.05$) and salivary flow rate ($p = 0.009$). No significant differences were found in objective outcomes including Schirmer test, salivary gland biopsy or ESSDAI score.

4.2 Observational Studies

Fourteen uncontrolled studies have evaluated the use of rituximab in patients with primary SS, including three open-label prospective studies (Pijpe et al. 2005; Devauchelle-Pensec et al. 2007; St Clair et al. 2013) (Table 3) and 11 retrospective studies evaluating either systemic involvement or B-cell lymphoma (Gottenberg et al. 2005, 2013; Seror et al. 2007; Vasil'ev et al. 2009; Ramos-Casals et al. 2010c; Tony et al. 2011; Zhou et al. 2012; Mekinian et al. 2012a, b; Pollard et al. 2011; Voulgarelis et al. 2012) (Table 4).

The first open-label study was conducted by Pijpe et al. (2005). The authors designed a phase II trial including eight patients with active primary SS of short duration (<4 years) and seven patients with primary SS and mucosa-associated lymphoid tissue (MALT)-type lymphoma. Patients were treated with four infusions of rituximab (375 mg/m^2) given weekly after pretreatment with prednisone (25 mg) and clemastine. The second prospective trial (Devauchelle-Pensec et al. 2007) included 16 patients who scored >50 on at least two of four visual analog scales (VAS; 100 mm) evaluating global disease, pain, fatigue, and global dryness and who received only two infusions of rituximab (375 mg/m^2) at weeks 0 and 1 without steroid premedication. At week 36, significant improvements were noted in the four VAS scores ($p < 0.05$), tender joint count ($p = 0.017$), tender point count

Table 3 Open-label studies evaluating rituximab in patients with primary SS

Author (year)	*N* (female)	Mean age (years)	Study design (duration)	Drug	Outcomes evaluated	Significant differences
Pijpe et al. (2005)	15 (14)	50	Prospective	Rituximab 375 mg/m^2	Unstimulated flow rate	*Early pSS group*
			12w	Weeks 0–3	Sialochemical analysis	RB score (<0.05), BUT (<0.05)
					Schirmer, RB, BUT	MFI (<0.05 4/5 scales), SF-36
					Subjective VAS	(<0.05 5/9 scales)
					MFI, SF-36 questionnaire	*MALT/pSS group*
						RB score (<0.05)
Devauchelle-Pensec et al. (2007)	16 (14)	55	Prospective	Rituximab 375 mg/m^2	VAS global, pain, dryness, fatigue	Global VAS (0.03), VAS pain (0.006), fatigue VAS (0.006), dryness VAS (0.006)
			36w	Weeks 0 and 1	Tender points	Tender point (0.027) and joint (0.017) count
					Unstimulated flow rate	–
					Schirmer, van Bijsterveld	–
					ESR, CRP, abs, IgG	RF reduction (0.04)
					Salivary gland biopsy	–
					SF-36	Mental and physical components (0.03)
						Resolution pulmonary involve ($n = 1$)
St Clair et al. (2013)	12 (12)	51	Prospective	Rituximab 1 g/15 days	Safety (primary endpoint)	Clinical efficacy at 26w
			52w	Weeks 0 and 2	VAS physician/ patient	VAS physician (0.012) and patient (0.009)

VAS sicca/fatigue	VAS tongue dryness (0.007), level of thrist (0.005), oral discomfort (0.02), fatigue (0.042)
Ocular tests	–
Salivary flow rates	–
SF-36 questionnaire	Vitality (0.006)
Autoantibodies	–

w weeks, *RB* rose bengal staining, *VAS* visual analogue scale, *BUT* break-up time, *MFI* multidimensional fatigue inventory, *SF*-36 medical outcomes short form, *ESR* erythrosedimentation rate *CRP* C-reactive protein, *abs* autoantibodies, *RF* rheumatoid factor

Table 4 Retrospective studies evaluating biological agents in patients with SS

Author (year)	*N* (female)	Mean age (years)	Study design (duration)	Drug (number of patients)	Lymphoma (clinical response)	Systemic involvement (response)	Prednisone use	Serological response
Gottenberg et al. (2005)	6 (6)	57.5	Retrospective (8m)	Rituximab 375 mg/m^2 Weeks 0–3 ($n = 5$) Weeks 0,1 ($n = 1$)	MALT (1/2)	Systemic ($n = 4$) – Vasculitis (2/2) – Parotid + arthritis (2/2)	Reduction in 4/5	RF reduction 4/4 cryoglobulin negativiz 2/2
Seror et al. (2007)	16 (16)	58	Retrospective (14.5m)	Rituximab 375 mg/m^2 Weeks 0–3 ($n = 14$) Other regimens ($n = 2$)	Dryness improve 2/11 (18 %)	Systemic features 9/11 (82 %) – Cryo 4/5 – Thrombocytop 0/1 – Pulmonary + arthritis 2/2 – Arthritis 2/2 – Renal 1/1 – Parotid enlargement 3/3	Reduced median daily dose of corti-costeroids (0.003)	ESR (0.009), CRP (0.02), gamma (0.003), beta2 (0.003), cryo negativiz 4/4, reduced RF(0.004)
Vasil'ev et al. (2009)	13 (ND) 3 associated	ND	Retrospective	Rituximab ND 500 mg MP premedical	Lymphoma CR 7, PR 2	Systemic (3/4)	ND	ND
Tony et al. (2011)	4 (ND)	ND	Retrospective	ND	ND	ND PR ($n = 2$), CR ($n = 2$)	ND	ND
Gottenberg et al. (2013)	78 (67)	59.8	Retrospective	Rituximab 375 mg/m^2 Weeks 0–3 ($n = 11$)	ND	Systemic (44/74) – Articular 17/27 (63 %) – CNS 2/6 (33 %) – PN 6/12 (50 %)	Reduced median daily dose of corticosteroids mg/d (0.1)	ND

				Rituximab 1 g Weeks 0 and 2 ($n = 67$)		– Lung = 7/9 (78 %) – Vasculitis = 5/8 (63 %) – Renal = 5/6 (83 %) – Myositis = 0/3 (0 %) – Cytopenia = 2/2 (100 %) – Pancreatitis = 1/1 (100 %) – Glandular enlarge = 2/3 (67 %) – Sclera vasculitis 0/1 (0 %) ESSDAI score < 0.0001		
Ramos-Casals et al. (personal communication)	24 (24)	58	Retrospective	Rituximab 375 mg/m^2 Weeks 0–3 ($n = 20$) Rituximab 1 g Weeks 0 and 2 ($n = 4$)	Lymphoma ($n = 8$): CR in 6, PR in 2	Systemic ($n = 16$) – CNS 1/3 – PN 5/5 – Cytopenia 3/3 – GN 1/2 – Arthritis 0/1 – Muscular 1/1 – PLE 1/1	ND	ND

m month, *ND* not detailed, *CR* complete response, *PR* partial response, *cryo* cryoglobulins, *CNS* central nervous system, *PN* peripheral neuropathy, *GN* glomerulonephritis, *PLE* protein-losing enteropathy, *RF* rheumatoid factor, *ESR* erythrosedimentation rate, *CRP* C-reactive protein, *Gamma* serum gammaglobulins, *beta2* beta2 microglobulin

($p = 0.027$), and SF-36 ($p < 0.03$). The third open-label study in primary SS has recently been published (St Clair et al. 2013) and included 12 patients enrolled at the Duke University Medical Center treated with one group of rituximab (weeks 0 and 2) and prospectively followed for 1 year (eight treated with hydroxychloroquine and three with prednisone). At 26-weeks of follow-up, significant statistically improvement was reported for subjective rates of disease activity score for both the physician and the patient and for some parameters like tongue dryness, level of thirst and oral discomfort, and level of fatigue. However, no statistically significant differences were found in the results of objective diagnostic tests evaluating salivary flow outputs or corneal staining. Rituximab therapy had little effect on the serum levels of anti-Ro/La antibodies although a trend towards a decrease in the levels of serum rheumatoid factor was observed.

4.2.1 Systemic Involvement

The majority of uncontrolled studies have retrospectively collected the results of the use of rituximab in real life patients with extraglandular involvement.

The first study was conducted in 2005 by Gottenberg et al. (2005). The authors described six patients with primary SS treated with rituximab and reported therapeutic efficacy in five out of the six patients with extraglandular features, with lowering of corticosteroid dosage in four out of five patients. In 2007, Seror et al. (2007) made a retrospective analysis of 16 patients with primary SS who received rituximab for lymphoma ($n = 5$) or systemic manifestations ($n = 11$) including mixed cryoglobulinaemia ($n = 5$), refractory pulmonary disease with polysynovitis ($n = 2$), severe polysynovitis ($n = 2$), thrombocytopenia ($n = 1$) and mononeuritis multiplex ($n = 1$); treatment efficacy was observed in 9 of these 11 patients, with corticosteroid doses being lowered in all cases. Vasil'ev et al. (2009) reported the use of rituximab in 13 patients with SS (10 primary SS) with B-cell lymphoma ($n = 9$) and systemic features ($n = 4$). Complete and partial remission of lymphoma was achieved in 7 (78 %) and 2 (22 %) patients, respectively, while 3 (75 %) of four patients with systemic manifestations responded to RTX (no response was observed in a patient with cryoglubulinaemic glomerulonephritis).

Three multicentre national registries that collected patients with SAD treated with rituximab have included patients with primary SS. In the BIOGEAS Spanish registry (Ramos-Casals et al. 2010c), we have currently data from 24 patients treated due to lymphoma ($n = 8$) or systemic manifestations ($n = 16$) including peripheral neuropathies ($n = 5$), CNS involvement ($n = 3$), autoimmune cytopenias ($n = 3$), refractory arthritis ($n = 1$), protein-losing enteropathy ($n = 1$) and myasthenia gravis ($n = 1$). All patients with lymphoma showed treatment efficacy (complete response in six, partial response in two) while only three patients with systemic features had no therapeutic response (arthritis, glomerulonephritis and SNC involvement, respectively). In the Germany GRAID registry (Tony et al. 2011), four patients with primary SS were included (two had a complete

response and the other two a partial response). However, the larger uncontrolled study that has evaluated the therapeutic efficacy of rituximab on systemic involvement in primary SS has been recently reported by Gottenberg et al. (2013). The authors have reported the results obtained in 74 patients included in the French AIR Registry and who were treated due to systemic involvement (42 had more than one systemic involvement), including articular involvement ($n = 27$), CNS involvement ($n = 6$), peripheral neuropathies ($n = 12$), vasculitis ($n = 8$), pulmonary involvement ($n = 9$), renal involvement (6), myositis (3), autoimmune cytopenias ($n = 2$), autoimmune pancreatitis ($n = 1$). Four patients were treated due to severe glandular involvement. At 6 months after the first cycle of rituximab, therapeutic response assessed by the global opinion of the physician was observed in 47 patients (60 %). The ESSDAI score decreased from 11 to 7.5 after rituximab therapy ($p < 0.0001$), and the mean daily dose of prednisone decreased from 17.6 to 10.8 mg ($p = 0.1$). No significant differences were found according to the presence of anti-Ro/La antibodies or the concomitant use of immunosuppressant agents.

4.2.2 Organ-Specific Systemic Involvements

Two recent studies have focused on the efficacy and safety of rituximab in SS-related neurological involvement. The first study found stabilisation or improvement of CNS involvement in 7/11 (64 %) patients treated with rituximab (Mekinian et al. 2012a). The second study found a better response to rituximab in patients with vasculitic neuropathy in comparison with those with non-vasculitic neuropathy (Mekinian et al. 2012b). The authors evaluated 17 patients with peripheral neuropathies, 10 of whom had associated cryoglobulinaemia or vasculitis. At 3 months of follow-up, RTX was effective in 9/10 (90 %) patients in comparison with only 2 (29 %) of the seven patients without cryoglobulinaemia/vasculitis. In the first group, ESSDAI decreased from 24 to 14.5 at 6 months ($p = 0.008$), whereas no change was observed in the second group. Seven (65 %) of the 11 patients who responded to RTX experienced a relapse after a mean follow-up of 8 months, three of whom had a neurological relapse; cryoglobulinaemia reappeared in 4 (80 %) of five patients who relapsed. Six patients were retreated with an efficacy rate of 83 %.

In addition to the studies evaluating neurological SS-related involvement, Zhou et al. (2012) have reported the successful use of low-dose rituximab (100 mg in weeks 0 and 1) combined with high-dose oral prednisone (1–2 mg/kg/day) in four patients with primary SS and severe, refractory thrombocytopenia.

4.2.3 B-Cell Lymphoma Associated with Primary SS

Haematological neoplasia is the most severe complication of primary SS, with SS patients having a 10- to 40-fold higher risk of lymphoma than healthy individuals (Ramos-Casals et al. 2012a). Lymphomas that develop in primary SS patients are

extranodal in 80 % of cases, and the most common site of extranodal lymphoma development is the salivary glands, overwhelmingly the parotids (Theander and Baecklund 2012).

The first reported cases of SS patients with lymphoma treated with rituximab showed promising results. Shih et al. (2002) described the resolution of parotid NHL in a patient with SS treated with rituximab, and Somer et al. (2003) treated a patient with SS and lymphoma with rituximab, observing improvement of parotidomegaly, ocular tests and salivary flow rate, and Harner et al. (2004) successfully treated a marginal zone lymphoma associated with SS. In addition, all the retrospective studies of rituximab in patients with primary SS included patients with associated lymphoma. Gottenberg et al. (2005) observed complete remission in one of the two patients with lymphoma. Of the seven patients with MALT included in the study by Pijpe et al. (2005), complete remission was achieved in three cases, the lymphoma remained stable in three and progressed in one case. Seror et al. (2007) reported better results, with treatment efficacy in four out of the five patients with lymphoma, and we have reported a successful response in eight cases (six with complete response). Rituximab may have a beneficial effect on SS-related lymphoma (Vissink et al. 2012; Bowman 2012).

Two recent studies have been focused on the therapeutic management of SS-related lymphoma. Pollard et al. (2011) analysed 35 patients with MALT-SS, of whom 13 (37 %) were treated with rituximab only and six with R-CP. The response in the 13 patients treated only with rituximab consisted of stabilisation ($n = 7$), complete response ($n = 5$) and partial response ($n = 1$), while all patients treated with R-CP achieved a complete response. A reduction in extraglandular manifestations (arthritis, vasculitis; pulmonary, hepatic, or renal SS involvement) was also seen in all patients who had concomitant systemic involvement, while improvement in serologic measures (improvement of C4 levels, negativisation of cryoglobulins/monoclonal band) was observed in 9/13 (69 %) patients. The second study was conducted by Voulgarelis et al. (2012) and included 53 consecutive patients with primary SS and B-cell lymphoma (31 MALT, 8 NMZL and 8 DLBCL). Eleven patients were treated with rituximab only (all had a MALT lymphoma) whereas 15 patients received rituximab in combination with other chemotherapy agents (mainly R-CHOP in 11). The response was not detailed for each type of lymphoma, but the authors found an overall response rate of about 75 %. High response rates were observed in patients newly diagnosed with MALT lymphoma and especially in those with localised MALT lymphoma and associated cryoglobulinaemia. In addition, R-CHOP induced sustained CR in all SS patients after a mean follow-up of 3.5 years.

4.3 Isolated Reported Cases

Rituximab has been used to treat other SS-related systemic features in isolated cases, including principally haematological, neurological, digestive, pulmonary or

Table 5 Isolated cases of the successful use of rituximab in severe involvements rarely found in primary SS

a) Haematological involvement
– Thrombotic thrombocytopenic purpura (Toumeh et al. 2012)
– Symptomatic thrombocytopenia (Zhou et al. 2012)
– Acquired C1 inhibitor deficiency (Sánchez-Cano et al. 2008)
– Acquired von Willebrand disease (Iwabuchi et al. 2011)
– Acquired factor VIII inhibitor (Vintimilla et al. 2010)
b) Gastrointestinal involvement
– Protein-losing enteropathy (Uraoka et al. 2012)
– Autoimmune pancreatitis (Rueda et al. 2009)
c) Pulmonary involvement
– Lymphocytic interstitial pneumonitis (Swartz and Vivino 2011)
– Shrinking lung (Langenskiöld et al. 2012)
d) Ocular involvement
– Severe keratoconjunctivitis sicca (Zapata et al. 2007)
– Anterior scleritis (Ahmadi-Simab et al. 2005)
e) Neurological involvement
– Mental nerve neuropathy (Sève et al. 2007)
– Severe refractory polyneuropathy (Pertovaara and Korpela 2012)
– Demyelinating neuropathy (Botez and Herrmann 2010)

ocular autoimmune involvements (Table 5) (Toumeh et al. 2012; Zhou et al. 2012; Sánchez-Cano et al. 2008; Iwabuchi et al. 2011; Vintimilla et al. 2010; Uraoka et al. 2012; Rueda et al. 2009; Swartz and Vivino 2011; Langenskiöld et al. 2012; Zapata et al. 2007; Ahmadi-Simab et al. 2005; Sève et al. 2007; Pertovaara and Korpela 2012; Botez and Herrmann 2010).

4.4 Management of Relapses

Few data are available about the therapeutic management of patients with primary SS who relapsed after receiving rituximab. The first study (Meijer et al. 2009) reported five patients who were retreated with four infusions of rituximab and followed for 48 weeks due to decrease of salivary flow, increase of rheumatoid factor and return of B cells and subjective symptoms. Retreatment had a significant effect on RF levels, stimulated salivary flows, VAS scores for dry mouth and quality of life questionnaires (MFI, SF-36). One patient developed serum sickness-like disease (purpura, arthralgia, myalgia) after the second rituximab infusion.

A recent study by Gottenberg et al. (2013) has described the results of retreatment in a large number of patients. In this study, 41 patients were retreated with rituximab receiving two ($n = 21$), three ($n = 8$), four ($n = 3$) or five or more ($n = 9$) RTX cycles. RTX efficacy evaluated according to the physician was

observed in 16/21 (76 %) patients treated with two cycles, 7/8 (87 %) of patients treated with three cycles and 11/12 (92 %) patients treated with four or more cycles. After a mean follow-up of nearly 3 years, rituximab was discontinued in 41 patients due to inefficacy ($n = 35$) or serious adverse events ($n = 6$).

4.5 Evaluation of Response

The most recent studies are using successfully the ESSDAI activity score to evaluate the therapeutic efficacy of rituximab (Devauchelle-Pensec et al. 2012; Gottenberg et al. 2013; Mekinian et al. 2012b; Meiners et al. 2012). Other studies are investigating how to predict a successful response before initiating rituximab therapy. Devauchelle-Pensec et al. (2010) have elaborated a set of genes that may help to predict rituximab efficacy, analysing the differential expression of B-cell and IFN pathway signalling molecules. Another study (Abdulahad et al. 2011) have analysed the characteristics of reconstituted B cells following depletion by rituximab treatment and found a predominance of transitional B cells during the early recovery phase, while B-cell depletion did not influence the balance of peripheral regulatory/effector T cells. Hamza et al. (2012) have also demonstrated that rituximab treatment does not alter the characteristic features of increased clonal expansions seen in the parotid salivary glands of patients with pSS and that Ig-producing cells may persist in salivary glands despite B-cell depletion; this finding may explain the clinical relapses that are often reported in primary SS patients treated with RTX. Finally, Pollard et al. (2013) found that RTX has a differential effect on BAFF and APRIL levels in pSS patients, and the authors suggested that a potential combination of RTX and BAFF/BLyS blockade might achieve a prolonged reduction of (autoreactive) B cells and thus may reduce the frequent clinical relapses. However, this double B-cell blockade should be carefully evaluated due to the anticipated higher risk of development of infections and hypogammaglobulinaemia.

4.6 Side Effects

Table 6 summarises the main side effects reported in both controlled and uncontrolled studies of rituximab in primary SS. An overall analysis of adverse events related to rituximab in primary SS patients found early and late infusion-related reactions in 36/205 (18 %) patients (with delayed/serum sickness-like reactions being more frequent than immediate reactions) and infections in 17/205 (8 %). The most frequent infection site was respiratory in seven patients. With respect to the development of human anti-chimeric antibodies (HACAs), contrasting results have been found in two studies. Four of the 15 patients (27 %) included in the study by Pijpe et al. (2005) developed human anti-chimeric

Table 6 Adverse events reported in controlled and uncontrolled studies using rituximab in patients with primary SS

Author (year)	*N* (female)	Study design (duration)	Drug (number of patients)	Related to infusion	Infection	Cancer	HACA	Others
Gottenberg et al. (2005)	6 (6)	Retrospective (8m)	Rituximab 375 mg/m^2 Weeks 0–3	Infusion related ($n = 1$) Serum sickness ($n = 1$)	–	–	–	–
Dass et al. (2008)	17 (ND)	RCT-d (6m)	Rituximab 1g/15d ($n = 8$) Weeks 0 and 2	Infusion related ($n = 2$) Serum sickness ($n = 1$)	Gastroenteritis ($n = 1$)	–	–	–
Meijer et al. (2010)	30 (29)	RCT-d (48w)	Rituximab 1g/15d ($n = 20$) Weeks 0 and 2	Infusion related ($n = 4$) Serum sickness ($n = 1$)	Otitis ($n = 2$) Upper respiratory ($n = 4$) Ocular toxoplasm ($n = 1$) Parotid gland ($n = 3$)	–	–	–
Devauchelle-Pensec et al. (2007)	16 (14)	Prospective (36w)	Rituximab 375 mg/m^2 Weeks 0 and 1	Infusion-related ($n = 2$) Delayed reactions ($n = 8$) Serum sickness ($n = 4$)	–	Lymphoma ($n = 1$)	–	–
Pijpe et al. (2005)	15 (14)	Prospective (12w)	Rituximab 375 mg/m^2 Weeks 0–3	Infusion-related ($n = 2$) Serum sickness ($n = 3$), all HACA+	Herpes zoster ($n = 1$)	–	HACAs: 4/8 of early SS, 0/7 in the other group	–
Seror et al. (2007)	16 (16)	Retrospective (14.5m)	Rituximab 375 mg/m^2 Weeks 0–3	Serum sickness ($n = 2$)	Herpes ($n = 1$)	–	HACAs in 1/8	One patient with PN-cryo worsened

(continued)

Table 6 (continued)

Author (year)	*N* (female)	Study design (duration)	Drug (number of patients)	Related to infusion	Infection	Cancer	HACA	Others
			($n = 14$) Other regimens ($n = 2$)					
St Clair et al. (2013)	12 (12)	Prospective (52w)	Rituximab 1 g/15 days Weeks 0 and 2	Severe AE reaction to pneumococcal vaccine ($n = 1$); non-severe ($n = 2$)	–	Squamous cell carcinoma ($n = 1$)	–	–
Ramos-Casals et al. (2010c)	15 (15)	Retrospective	Rituximab 375 mg/m^2 Weeks 0–3 ($n = 20$) Rituximab 1 g Weeks 0.2 ($n = 4$)	–	Urinary infection ($n = 1$)	–	–	Interstitial pneumonitis ($n = 1$)
Tony et al. (2011)	4 (ND)	Retrospective	ND	Infusion-related ($n = 1$)	–	–	–	–
Gottenberg et al. (2013)	74 (ND)	Retrospective	Rituximab 375 mg/m^2 Weeks 0–3 ($n = 11$) Rituximab 1 g Weeks 0 and 2 ($n = 67$)	Immediate infusion reactions (severe = 3) Delayed serum sickness-like (severe = 1)	Pulmonary infection ($n = 1$) CMV lung infection ($n = 1$) SA lung infection ($n = 1$)	Squamous cell carcinoma Mx ($n = 1$) Paget's cancer of the nipple Mx ($n = 1$)	–	Hypogammaglobulinaemia ($n = 4$)

RCT randomised controlled trial, *-d* double-blind, *m* months, *w* weeks, *ND* not detailed, *HACA* human anti-chimeric antibodies, *AE* annular erythema, *CMV* cytomegalovirus, *SA* staphylococcus aureus, *Mx* metastasis, *PN-cryo* cryoglobulinaemic polyneuropathy

antibodies (HACAs). All had early primary SS and three of the four patients developed a serum sickness-like disorder. Three (19 %) of the 16 patients treated by Seror et al. (2007) experienced adverse events, although only one developed a mild serum sickness-like reaction with positive HACAs. Four cancers (2 %) have been reported, including two patients with squamous cell carcinoma (St Clair et al. 2013; Gottenberg et al. 2013), one with a Paget's cancer of the nipple (Gottenberg et al. 2013) and one with lymphoma (Devauchelle-Pensec et al. 2007). Other reported adverse events included worsening of cryoglobulinaemic neuropathy (Seror et al. 2007), interstitial pneumonitis (Ramos-Casals et al. 2010c) and development of hypogammaglobulinaemia (Gottenberg et al. 2013). Gottenberg et al. (2013) have reported hypogammaglobulinaemia (gammaglobulins <6g/L) after the first cycle of rituximab in 4/22 (18 %) patients, two of whom developed severe infection. A very recent adverse event has been reported by St Clair et al. (2013) in the three first patients included in the trial, who presented a grade 2 reaction to pneumococcal vaccine (one severe), a fact that lead to the discontinuation of this vaccine in the following patients included in this trial.

5 Epratuzumab

CD22 is a 135-kDa B-lymphocyte restricted type-I transmembrane sialoglycoprotein of the immunoglobulin (Ig) superfamily (Engel et al. 1993). CD22 appears intracellularly during the late pro-B-cell stage of ontogeny, shifting to the plasma membrane with B-cell maturation. CD22 is expressed at low levels on immature B cells and at higher levels on mature IgM+, IgD+ B cells, and absent on differentiated plasma cells. Selective modulation of B cells has recently been achieved using a humanised monoclonal antibody against CD22. This antibody (epratuzumab) was originally developed for the treatment of B-cell lymphoma and was found to be effective, with a very good safety profile (Leonard et al. 2003). Preliminary promising results have been obtained with the use of epratuzumab in patients with SLE (Ramos-Casals et al. 2012c).

In 2006, Steinfeld et al. (2006) conducted an open-label, phase I/II study investigating the safety and efficacy of epratuzumab in the treatment of patients with active primary SS. Sixteen patients received four infusions of 360-mg/m^2 epratuzumab once every 2 weeks, with 6 months follow-up. Fourteen patients received all infusions without significant reactions, one patient received three infusions and one discontinued due to a mild acute reaction to the first infusion. Three patients showed moderately raised levels of HACA unrelated to clinical manifestations. B-cell levels were reduced by 54 and 39 % at 6 and 18 weeks, respectively, but T-cell levels, immunoglobulins, and routine laboratory tests did not change significantly. Fifty-three percent of patients achieved a clinical response at 6 weeks, with 53, 47, and 67 % responding at 10, 18 and 32 weeks, respectively. Additionally, statistically significant improvements were observed in fatigue, and patient and physician global assessments. The authors also found an over-expression

of CD22 in peripheral B cells, a finding that was down-regulated by epratuzumab after 12 weeks of therapy. According to this preliminary study, epratuzumab appears to be a promising therapy in active primary SS, but unfortunately no further clinical trials have been conducted in primary SS.

6 Belimumab

6.1 Targeting the BAFF/BLyS Molecule in Primary SS

The discovery of a molecule that plays a key pathogenic role in a specific autoimmune disease always leads to consideration of its use as a potential therapeutic target. The best (and most recent) example in autoimmune diseases is BAFF/BLyS (B lymphocyte stimulator), a soluble ligand of the TNF cytokine family, which is a prominent factor in B-cell differentiation, homeostasis, and selection (Cancro et al. 2009). Although the biologically active form of BLyS is trimeric, a multimeric (60-mer) form has also been detected in mice (Karpusas et al. 2002). The bind of BLyS to their receptors inhibits apoptotic pathways, providing essential and non-redundant survival signals for B cells (Cancro et al. 2009). Therefore, investigation about the potential therapeutic role of blocking BAFF in primary SS, a disease characterised by a marked B-cell hyperactivity, may be considered pathogenically of common sense.

6.2 Pathogenic Role of BAFF/BLyS in Primary SS

A possible role of BLyS in the pathogenesis of pSS was firstly suggested by the findings observed in a BAFF-transgenic murine model (Mariette and Gottenberg 2010). The excess of serum BAFF levels was associated with the development of a murine autoimmune-related disease resembling human lupus and SS, also including the development of lymphoma. Subsequent studies were focused on the pathogenic role of BAFF in patients with primary SS (Brkic et al. 2013; Youinou et al. 2012; Cornec et al. 2012).

Groom et al. (2002) found elevated levels of circulating BAFF, together with a significant upregulation of BAFF expression in salivary glands, suggesting an altered differentiation and tolerance of B cells induced by excess of BAFF. Szodoray et al. (2003) found a reduced level of apoptosis among BAFF-expressing cells that might lead to a longer BAFF expression in these cells, which maintained positive signals for the infiltrating B cells to proliferate and mature. Ittah et al. (2006) and Gottenberg et al. (2006) have demonstrated the capacity of epithelial cells to express and secrete BAFF after IFN stimulation. An abnormal, aberrant expression of BAFF in B cells infiltrating the salivary glands have also

been reported (Youinou and Pers 2011), and BLyS has also been implicated in SS in the formation of ectopic germinal centres (Szodoray et al. 2005), which have been recently associated with a high risk of lymphoma development (Theander et al. 2011). Although production of BAFF by B cells is not usual, malignant B cells may produce BAFF (Youinou and Pers 2011), a fact that promotes their abnormal survival following an autocrine-like mechanism. In addition, excess of BAFF also increases the survival of self-reactive B cells and facilitates infiltration to "forbidden" follicle or marginal zone niches (Youinou and Pers 2011). All these experimental studies suggest a key role for BAFF in the development of autoimmune and lymphoproliferative processes in primary SS.

Clinically, Mariette et al. (2003) demonstrated in SS patients a correlation between BAFF levels and circulating levels of autoantibodies (IgG, RF, anti-Ro and anti-La), while Pers et al. (2005) found increased serum levels of BAFF in 43 patients with SLE, 58 with primary SS and 28 with RA, in comparison with 68 controls. High levels of BAFF were associated with the presence of autoantibodies (anti-double-stranded DNA antibodies in SLE, anti-SSA antibodies in SS, and rheumatoid factor in RA), suggesting that high levels of BAFF may be directly associated with the B-cell hyperactivity/proliferation usually observed in patients with SAD.

A recent study by Quartuccio et al. (2013) has demonstrated a close association between sBLyS levels and the key features of primary SS. Although the study had a retrospective design and included a limited number of pSS patients, the close association reported between BLyS levels and the main SS-related features (immunological markers, disease activity, histopathological B-cell clonality and B-cell lymphoma) allows to think that BLyS is a crucial molecule in the pathogenesis of primary SS (Ramos-Casals 2013). Firstly, Quartuccio et al. (2013) found that serum BLyS levels were significantly higher in pSS patients in comparison with controls and were closely associated not only with the main immunological markers but also with the disease activity measured by the ESSDAI score (Seror et al. 2012). This suggests that BLyS, which is a good soluble biomarker of B-cell hyperactivity, may also be a good soluble biomarker linking B cells and SS disease activity. Secondly, the study found a close association between sBLyS levels and SS-related lymphoma, not only with the diagnosis of an overt lymphoma but also with histopathological findings that poses pSS patients at high risk of lymphoma development, such as monoclonal lymphocytic infiltration and MESA. An attractive hypothesis might be that as much higher are the sBLyS levels, as much higher may be the potential "malignant" role of B cells in the glandular damage (Ramos-Casals 2013). Finally, Quartuccio et al. (2013) have found a close association between sBLyS levels and the three main prognostic biomarkers in primary SS (Brito-Zerón et al. 2007; Theander et al. 2004; Baimpa et al. 2009), supporting their possible inclusion as an additional biomarker tool in classifying patients at higher or lower risk when a lymphoma is suspected.

6.3 Use of Belimumab in Patients with Primary SS

All the above-mentioned studies are paving the way for further investigations of the therapeutic utilities of blocking BLyS in the daily management of patients with primary SS (Ramos-Casals 2013). Unfortunately, data of the use of BAFF-targeted therapies in patients with primary SS are not yet available, and two clinical trials with belimumab are underway (NCT01160666 and NCT01008982) (Brito-Zerón et al. 2013). Since not all the available BAFF-targeting agents seem to bind the membrane and the soluble forms of BAFF with a similar affinity, the therapeutic results obtained in future trials might vary (Abdulahad et al. 2012).

The recently revealed preliminary results of the BELISS trial (leader by Salvatore de Vita and Xavier Mariette), the first open-label study of belimumab in pSS patients, are promising (Mariette et al. 2012; De Vita et al. 2012). Thirty patients (all female, mean age = 49.5 years) were included in two simultaneous and identical studies in two European centres. Inclusion criteria were the fulfilment of the 2002 criteria (including as mandatory criteria positivity to anti-Ro/La antibodies) and had to have at the time of inclusion either systemic complications ($n = 15$), early disease ($\leq$5 years) ($n = 11$) and/or the presence of at least one of the following biomarkers (increased serum IgG, free light chains, beta2-microglobulin, low C4 levels, cryoglobulins or monoclonal band) ($n = 20$). Patients were treated with 10 mg/kg of belimumab (weeks 0, 2 and 4, and then every 4 weeks until week 24). The primary endpoint was evaluated at week 28 and consisted of improvement of at least two of the five following items: $\geq$30 % reduction of VAS for dryness, fatigue, musculoskeletal pain and physician's systemic activity, and $\geq$25 % reduction of any of the above-mentioned B-cell activation biomarkers. The percentage of responders was 8/11 (73 %) in patients with early disease and 7/15 (47 %) in those with systemic disease. The ESSDAI score decreased from 8.8 to 5.59 ($p < 0.0001$) and the ESSPRI score from 6.44 to 5.56 ($p = 0.01$). There was no significant change of salivary flow and Schirmer test. The treatment was associated with reduction of serum IgG ($p < 0.001$) and IgA ($p = 0.001$) levels and rheumatoid factor levels ($p < 0.001$). Only one severe adverse event was reported (pneumococcus meningitis) after six infusions of the drug. A specific subanalysis of the therapeutic response in patients with parotid involvement at week 28 found that the glandular domain improved in 10/13 (77 %) patients (De Vita et al. 2012), while no improvement was reported in two patients with parotid low-grade lymphoma. Data from one of these centres available at 52 weeks (De Vita et al. 2012) in 13 patients found that the median ESSDAI score was two in comparison with three at week 28 and 8 at baseline ($p = 0.003$).

7 Conclusions

In patients with systemic autoimmune diseases, the most recent therapeutic advances are searching for new highly selective biological therapies without the adverse effects often associated with the standard, less-selective current therapeutic options (corticosteroids, immunosuppressants).The emergence of biological agents targeting molecules and receptors involved in the etiopathogenesis of primary SS has opened up a new era in the therapeutic management of the disease. The excellent results of TNF-targeted therapies in rheumatoid arthritis led to these agents being tested in patients with primary SS (Ramos-Casals et al. 2010a), although RCTs showed a lack of efficacy. In contrast, B-cell targeted therapies, including epratuzumab, belimumab and, especially, rituximab, seem to be the most promising agents tested so far (Engel et al. 2011). The amount and quality of evidence on the off-label use of B-cell targeted therapies (especially rituximab) in SS-related extraglandular features is higher than that reported for the use of the above-mentioned standard options. Rituximab has been used in more than 400 patients included in either controlled or uncontrolled studies, with a wide range of outcomes evaluated, including sicca features, fatigue and, especially, systemic features and lymphoma. Although some studies have reported significant improvements in sicca features and fatigue, we consider that the off-label use of these new drugs to treat only these symptoms (even when severe) is not currently warranted (Ramos-Casals et al. 2012b). In contrast, rituximab is the most widely used biological agent in patients with severe involvements refractory to standard treatment in an off-label context and is increasingly used in patients with associated B-cell lymphoma. Finally, the close association between sBlyS levels and the key features of pSS are paving the ground for further investigations about the therapeutic utilities around this molecule. The current off-license use of biological agents should be accompanied by a reasonable assessment of the risk of serious adverse events versus the potential benefits of treatment.

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Targeting B Cells in ANCA-Associated Vasculitides

Xavier Bosch and John H. Stone

Abstract ANCA-associated vasculitides (AAV) is defined as vasculitides associated with anti-neutrophil cytoplasmic antibody (ANCA) serum positivity affecting small and medium-sized vessels. Glomerulonephritis in AAV is typified by focal necrosis, crescent formation, and few or no immunoglobulin deposits. In vitro and animal evidence suggests that ANCA play a pathogenic role in AAV. Specific gene expression signatures are reported to predict long-term prognosis in AAV, suggesting that therapy might be personalized and new therapeutic targets identified. Although immunosuppressant agents and glucocorticosteroids are the basis of AAV therapy, the results of randomized controlled trials show that rituximab is not inferior to cyclophosphamide (CYC) in inducing remission in patients with severe AAV. In 2011, the US Food and Drug Administration (FDA) approved rituximab plus glucocorticosteroids as a front-line therapy for adults with granulomatosis with polyangiitis (Wegener's granulomatosis) and microscopic polyangiitis. This new indication for rituximab represents the first ever FDA-approved therapy for these two diseases and the first alternative to CYC for the treatment of severe disease in nearly four decades. However, questions regarding the use of maintenance therapy after rituximab, the concurrent use of CYC and the toxicity of rituximab remain to be answered in current and future randomized trials.

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1 Introduction

ANCA-associated vasculitides (AAV), which are vasculitides associated with anti-neutrophil cytoplasmic antibody (ANCA) serum positivity and which affect the small and medium-sized vessels, include granulomatosis with polyangiitis (GPA; formerly Wegener's granulomatosis), microscopic polyangiitis (MPA), and Churg–Strauss syndrome (CSS). In these patients, glomerulonephritis is typified by focal necrosis, crescent formation, and few or no immunoglobulin deposits. Lung involvement ranges from transient focal infiltrates or interstitial disease to massive pulmonary hemorrhagic alveolar capillaritis, which is the severest life-threatening presentation of small-vessel vasculitis. ANCA against proteinase 3 (PR3-ANCA) are detected primarily in GPA, and anti-myeloperoxidase antibodies (MPO-ANCA) in MPA and CSS. Nevertheless, there is substantial overlap, with some GPA patients being MPO-ANCA positive and some MPA and CSS patients being PR3-ANCA positive. In addition, a small number of patients classified as AAV have no detectable serum ANCA. In vitro and animal evidence suggests ANCA play a role in AAV pathogenesis and are not just epiphenomena. In vivo studies in 2002 showed that murine MPO-ANCA lead to intrinsic pauci-immune renal vasculitis. In spite of mounting evidence of the pathogenic role of ANCA, especially MPO-ANCA, much remains unclear, with no convincing animal model yet being postulated for PR3-ANCA glomerulonephritis or vasculitis (Bosch et al. 2006; Gómez-Puerta et al. 2012).

Immunosuppressants of varying toxicity, in combination with glucocorticosteroids, remain the basis of AAV treatment. Recent results show that rituximab (RTX) is an alternative to cyclophosphamide (CYC) for inducing remission in severe AAV, and regimens based on RTX are preferable in cases of relapsing disease (Gómez-Puerta et al. 2012). This chapter focuses on the most recent data from clinical trials of B-cell-depleting agents in AAV, a novel treatment approach that could change the therapeutic possibilities of patients with GPA and MPA.

2 Pathogenesis of AAV

Increased understanding of the principal pathogenic mechanisms of AAV could lead to safer therapies that target the main disease-mediating immune pathways. Although the pathogenesis of AAV is not completely elucidated, reports have explained many of the processes that lead to autoimmunity, the action of ANCA on neutrophils, the role of T and B cells and the development of tissue injury in AAV.

2.1 Role of ANCA

Since 1990, when Falk et al. showed that ANCA stimulate respiratory bursts in neutrophils and trigger the release of primary granule constituents, it has been thought that ANCA play a pathogenic role (Falk et al. 1990). In vitro studies demonstrate that ANCA incite vascular damage by inducing neutrophil effector functions such as cytokine and chemokine release, and induce lysis through adhesion to cultured endothelial cells. In 2002, a pathogenic role of ANCA in a murine model was demonstrated by Xiao et al. (2002), who passively administered murine anti-MPO-IgG to Rag2−/− mice lacking functioning T and B cells, resulting in focal necrotizing glomerulonephritis (FNGN) with no immune deposits, and damage to ~15 % of glomeruli. However, no studies have confirmed a similar pathogenic role for PR3-ANCA, with experiments in animal models failing to show the induction of granulomatous inflammation typical of GPA, or provided a model for the development of vasculitis (Gomez-Puerta and Bosch 2009). Various explanations for these experimental differences in the effects of MPO- and PR3-ANCA have been suggested.

It is suggested that GPA is initiated by aberrant cell-mediated immune responses to exogenous or endogenous respiratory tract antigens, which lead to granuloma formation and humoral autoimmunity to PR3 (Bosch et al. 2008). The autoantigen complementarity hypothesis of PR3-directed autoimmunity involves the PR3 complementary peptide, which is encoded by the PR3 gene antisense strand (Pendergraft et al. 2004) and suggests that the inciting immunogen which elicits a cascade of immunological events is a protein that is complementary in surface structure to the autoantigen and not the autoantigen itself or its mimic. In other words, a protein homologous or identical to the amino acid sequence of translated antisense RNA from the noncoding strand of the autoantigen gene. The cascade could be initiated when the complementary protein triggers antibody production, in turn eliciting an anti-antibody or anti-idiotypic response. Anti-idiotypic antibodies may then react with the autoantigen. Possible complementary proteins include microbial and fungal proteins, which is consistent with the idea that invading microorganisms can be the vehicle for the inciting immunogen. The complementary protein could be derived endogenously by aberrant antisense transcription, or exogenously by a germ like *Staphylococcus aureus* using a complementary, antisense, mimicking protein that binds to and possibly inhibits the antimicrobial properties of PR3 or MPO (Falk and Jennette 2010). PR3 anti-complementary specificity has been demonstrated in human antibodies and T cells. The endogenous protein, plasminogen, might be complementary to the middle portion of PR3, with dual specificity of antibodies to plasminogen and complementary PR3 providing support for this hypothesis (Bautz et al. 2008). Plasminogen antibodies, which are present in ANCA disease, inhibit fibrinolysis and are associated with an increased risk of thrombosis.

2.2 New Autoantibodies

Kain et al. suggested molecular mimicry as the fundamental mechanism underlying pauci-immune FNGN in patients with AAV, although the antigen involved is lysosome-associated membrane protein-2 (LAMP-2) and not PR3 or MPO. In neutrophils, LAMP-2 is located on the membranes of intracellular vesicles containing MPO and PR3, and is abundantly found on endothelial cell surfaces. LAMP-2 is involved in antigen presentation and the adhesion of peripheral blood mononuclear cells to vascular endothelium. Kain et al. suggested an alternative rationalization for the source and development of pauci-immune FNGN, with possible clinical implications after discovering that infection by fimbriated bacteria could generate cross-reactive autoimmunity to LAMP-2, leading to the production of autoantibodies that activate neutrophils and damage endothelium in vitro, and causing pauci-immune FNGN in rodents. With respect to human disease, the authors suggest that ANCA might produce tissue damage by acting in synergy with anti-LAMP-2 antibodies or that anti-LAMP-2 antibodies could change the role of LAMP-2 in the presentation of cytoplasmic antigens such as MPO and PR3, resulting in antibodies against them being synthesized (Kain et al. 2008). Kain's study also pointed at the potential usefulness of a diagnostic test to determine anti-LAMP-2 antibody specificity and sensitivity, as 80/84 (95 %) patients were positive for anti-LAMP-2 antibodies compared with 83 % of patients who were ELISA-positive for either anti-MPO or anti-PR3 antibodies. However, the results of Kain et al. have not yet been reproduced by an independent laboratory. In fact, in 2012, two studies (one of them by Kain) published in the *Journal of the American Society of Nephrology* reported highly discordant results regarding the pathogenic potential and prevalence of anti-LAMP-2 antibodies in AAV (Roth et al. 2012; Kain et al. 2012). In a reconciling Editorial, Flint and Savage (2012) indicated that "there are enough differences in the enrolled cohorts and assay design to provide a potential explanation for these results, but a priority in the ongoing evaluation of anti-LAMP-2 antibodies for use as a clinical biomarker needs to be a robust and reproducible assay validated across multiple laboratories."

Berden et al. (2010) showed that around 25 % of PR3- and MPO-ANCA-positive patients have anti-plasminogen antibodies. In addition, anti-tissue plasminogen activator antibodies have been found in some AAV patients, especially those with anti-plasminogen antibodies. This study demonstrated that patients with anti-plasminogen antibodies had significantly greater percentages of glomeruli with fibrinoid necrosis and cellular crescents and more severely reduced renal function, suggesting that some AAV patients may benefit from fibrinolysis-enhancing therapies.

2.3 Neutrophil Extracellular Traps: Their Role in AAV

Neutrophil extracellular traps (NETs) are neutrophil-released decondensed chromatin fibers containing cytoplasmic proteins including PR3, MPO, elastase, and LL-37. NETs are formed as a host defense mechanism that allows the efficient containment and elimination of pathogens but also have immune-modulating functions that play a part in autoimmunity and AAV. NET formation in lesions is due to neutrophil apoptosis and degranulation in which DNA and serine proteases are deployed. Products derived from NETs activate dendritic and B cells through sensing via Toll-like receptors. In AAV, NETs have been implicated in NETosis, a novel form of cell death that differs from apoptosis (Bosch 2011).

In AAV, NETs seem to construct a platform from which MPO and PR3 are made available to initiate autoimmune responses. Kessenbrock et al. found that NET formation, which is implicated in neutrophil granulocyte apoptosis during infection, could cause endothelial damage and possibly perpetuate autoimmune responses to neutrophil components in AAV (Kessenbrock et al. 2009). The authors demonstrated that, after neutrophils were primed with tumor necrosis factor-alpha (TNF-α) and incubated with purified IgG, ANCA-containing IgG, but not IgG from healthy controls, induced NET formation by neutrophils. After 180 min, 23 % of neutrophils incubated with ANCA-IgG produced NETs, in comparison with 11 % of IgG-treated neutrophils in controls. The authors also induced NETs with a PR3-specific mouse monoclonal antibody and demonstrated that PR3 and MPO both localized with extracellular chromatin fibers and interacted directly with NET DNA. The study showed in vivo evidence of NET formation by the finding of typical NET components close to neutrophil infiltrates in affected glomeruli from renal biopsies of AAV patients, and acutely worsened renal function. In samples with higher neutrophil infiltration, NETs were more prominent, suggesting that NET formation may occur mainly during active disease. However, the study examined no control biopsies. Using MPO-specific capture and subsequent DNA-specific detection antibodies, the study identified circulating MPO–DNA complexes in AAV patients but not in healthy controls. The authors suggested that ANCA could perpetuate a vicious circle of NET production that maintained the delivery of antigen-chromatin complexes to the immune system. However, while these results suggest TNF-α may be a key pro-inflammatory cytokine in AAV, a controlled trial of etanercept, a TNF-α inhibitor, showed no effectiveness in maintaining remission in GPA patients (WGET Research Group 2005). Future studies may evaluate whether suppressing NET formation with reactive oxygen species scavengers or other agents might improve AAV and halt chronic autoimmunity.

2.4 B Cells in AAV

B-lymphocytes express cell-surface immunoglobulin receptors which recognize antigens and the generation of antibody-secreting plasma cells and memory B cells in response to specific antigens is one of their prime roles. Secreted antibodies are the main molecules implicated in humoral immunity, as they both neutralize pathogens and their toxins and facilitate their elimination through the activation of effector mechanisms including phagocytosis and proteins of the complement system.

The idea that B cells may negatively regulate cell immune responses emerged in the 1970s. Experimental models of autoimmunity, infection, and cancer support the concept that B cells with suppressor or regulatory functions (Breg) are vital component in maintaining peripheral tolerance, with Breg cells being analogous to regulatory T cells (Engel et al. 2011). Although B-cell depletion therapies are reported to have been used with success in AAV (see below), it remains possible that these therapies could deplete both pathogenic and regulatory B cells.

Reports suggest that mouse B-cell subsets with different phenotypes and origins may have regulatory functions that seem to be mediated directly by their capacity to produce interleukin-10 (IL-10) and/or tumor growth factor-beta (TGF-β), and which interact directly with pathogenic T cells (Mauri and Ehrenstein 2008). Stimulation of human CD25+ B cells is reported to produce IL-10, suggesting these cells participate in regulatory B-cell responses. Memory B cells express CD27 molecules and regulatory B cells promote the secretion of IL-10. Although CD25 is not a specific marker of B cells, elevated CD25 expression reflects B-cell activation.

Using flow cytometry, Eriksson et al. (2010) assessed the numbers and proportions of circulating lymphocytes in 34 AAV patients (24 GPA and 10 MPA; 16 in remission and 18 with active disease) and 20 healthy blood-donors. The proportion of CD25-expressing B cells was significantly elevated in patients in remission (48 %) compared with controls (29 %) and patients with active disease (23 %), while the proportion of CD27+ memory B cells showed no between-group differences. However, the proportion of CD27+ memory B cells that also expressed CD5 (CD27+5+) was less in patients in remission and those with active disease than in controls. Eriksson et al. suggested that elevated CD25+27-B-cell levels in patients in remission could be a result of a successful immunoregulatory response, but the direct influence of the remission induction regimen (CYC) could not be excluded.

3 Treatment of AAV

3.1 Established Therapy

Established immunosuppressive agents and corticosteroids remain the basis of AAV therapy. However, although these drugs have increased survival considerably, about a quarter of patients suffer treatment-related side effects including infections and malignancy. In addition, while repeated immunosuppressive therapies may

lead to disease control, remission is not necessarily achieved. Relapses and a chronic, often-relapsing disease evolution, typified by severe cumulative organ damage and the requirement for repeated immunosuppression are experienced by 50–80 % of patients, while 5–10 % of patients suffer disease manifestations refractory to standard immunosuppressive agents or drug-related adverse effects that lead to withdrawal from therapy (Gómez-Puerta et al. 2012; Bosch et al. 2007).

3.2 B-Cell Targeted Therapies: Justification for Their Use

The majority of systemic autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus (SLE), progressive systemic sclerosis, primary Sjögren syndrome (SS), inflammatory myopathies, and AAV, have been treated principally with cytotoxic agents and corticosteroids, which, although effective in improving disease manifestations and survival, produce severe adverse events and relapses, while a varying proportion of patients are refractory to treatment. The need for safer, more effective drugs, together with increased knowledge of the pathogenesis of autoimmune diseases is reflected by the interest shown in biologicals, with clinical trials of RTX arousing great hope. RTX has been successfully deployed in many autoimmune diseases, including SLE, mixed cryoglobulinemia, primary SS, inflammatory ocular disorders, hematological autoimmune disorders, and others and has been shown to be suitable for patients refractory to conventional immunosuppressant agents (Bosch et al. 2008; Gómez-Puerta et al. 2012; Ramos-Casals et al. 2012). Initially, the rationale for using RTX in AAV was that the elimination of the immediate plasma-cell precursors could inhibit their replacement, resulting in the transient removal of pathogenic antibodies, thus curing the vasculitis. However, although most RTX-treated AAV patients achieve remission, less than 50 % became ANCA negative (Wilde et al. 2011). In addition to their role as plasma-cell precursors, B cells play other physiopathological roles in AAV: they can produce pro-inflammatory cytokines and present antigens to T cells; they activate T cells and stimulate their proliferation, differentiation, and polarization, enhancing primed T-cell activation (Bouaziz et al. 2007). Inhibition of co-stimulation between B and T cells is another potential RTX target. Likewise, it has also been shown that activated B cells correlate closely with disease activity and that autoantigen-specific B cells are present at inflammation sites of tertiary lymphoid-like organ production (Lamprecht et al. 2007). Recent studies suggest that RTX induces changes in T-cells, such as T helper 17 cells, which play a role in AAV pathogenesis (van de Veerdonk et al. 2011). Taken together, these results strongly suggest that RTX therapy may be effective in patients with AAV.

3.3 Rituximab: Compelling Evidence from Controlled Trials

Two prospective, randomized controlled trials (RCT) have provided the best evidence for the use of RTX in AAV (Stone et al. 2010; Jones et al. 2010). In 2011, the FDA

approved RTX plus glucocorticosteroids for the treatment of both GPA and MPA, making RTX the first-ever FDA-approved drug for the treatment of AAV. Nevertheless, while RTX may probably replace current standard therapy with CYC, some questions remain to be elucidated by current and future RCT, especially the question of maintenance therapy after RTX therapy. Results at 6 months of the placebo (PBO)-controlled RAVE trial of the effectiveness of RTX compared with CYC in inducing disease remission in PR3- or MPO-ANCA positive patients with severe GPA or MPA in 2010 (Stone et al. 2010) showed that patients with severe renal disease had a median creatinine clearance of 53.8 (RTX group) and 68.9 mL/min (control group) at entry, and patients with creatinine >4 mg/dL or pulmonary hemorrhage were excluded. Ninety-nine patients received intravenous RTX (375 mg/m^2 once weekly for 4 weeks) plus daily PBO-CYC, and ninety-eight controls received PBO-RTX infusions plus daily CYC (2 mg/kg of body weight, adjusted for renal failure). Controls achieving remission between 3 and 6 months were switched from CYC to azathioprine (2 mg/kg/day). RTX patients achieving remission during the same period were changed from PBO-CYC to PBO-azathioprine. A glucocorticosteroid regimen of 1–3 methylprednisolone pulses of 1,000 mg were followed by prednisone (1 mg/kg/day) in both groups, with tapering in order that all patients who achieved remission without disease flares by 5 months had discontinued glucocorticosteroids. The main end point—a Birmingham Vasculitis Assessment Score for Wegener's Granulomatosis (BVAS/WG) of 0 and successful prednisone tapering at 6 months—was achieved by 64 % of RTX patients and 53 % of controls, fulfilling the non-inferiority criterion ($p < 0.001$). RTX displayed greater efficacy in inducing remission of relapsing disease, with 67 % of RTX patients and 42 % of controls reaching the primary end point ($p = 0.01$). RTX was as effective as CYC in treating patients with major renal disease or alveolar hemorrhage. No significant differences in disease flare rates, total, serious, non-disease-related adverse events, or patients with ≥1 non-disease-related adverse event were found. Thirty-three percent of patients in the CYC group had ≥1 of the predefined selected adverse events, compared with 22 % of RTX patients ($p = 0.01$), principally due to more episodes of severe leukopenia in controls. Concurrently, the open-label, two-group, parallel-design RITUXVAS trial involving patients with newly diagnosed AAV and renal involvement found that RTX was not inferior to standard intravenous CYC in inducing disease remission (Jones et al. 2010). Patients were randomly assigned to standard glucocorticosteroids plus either RTX (375 mg/m^2 of body-surface once weekly for 4 weeks) with two intravenous CYC pulses (33 patients) (RTX group), or intravenous CYC for 3–6 months followed by azathioprine (11 controls) (control group). Primary end points were sustained remission at 12 months and severe adverse events. Sustained remission was achieved by 76 % of RTX patients and 82 % of controls ($p = 0.68$), and 6 RTX patients (18 %) and two controls (18 %) died ($p = 1.00$). There were no significant differences in severe adverse events. The two trials highlighted the risk of serious adverse effects after administration of RTX and other immunosuppressive drugs in AAV. In the RAVE trial, the rate of adverse events was similar in the two study groups, although oral CYC was used in controls instead of less toxic intravenous CYC. Similarly, in the RITUXVAS trial, 6 out of 33 RTX patients and 2 out of 11 controls died. While the

between-group mortality rate was similar, such a high rate so early in the disease evolution is of concern.

3.4 Rituximab: Long-Term Results

The results of the larger RAVE trial at 6 months clearly show that RTX may be considered a first-line therapeutic option for inducing disease remission in AAV, especially in patients presenting with relapsing disease as opposed to newly diagnosed disease, as RAVE trial patients with relapsing disease had clearly superior outcomes when randomized to RTX rather than to CYC. Long-term follow-up data from the RAVE trial may also aid understanding of the best extended management and treatment of AAV patients, and especially in identifying patients with the highest risk for disease flares and the appropriate timing of RTX re-treatment. Mechanistic studies from this trial may also provide data on unanswered questions relating to the pathogenesis of AAV.

Preliminary long-term follow-up data from the RAVE and RITUXVAS trials presented at the 15th International Vasculitis and ANCA Workshop in Chapel Hill (Jones et al. 2011; Specks et al. 2011) showed that after two years, and in spite of no maintenance therapy, relapses occurred in only 7 out of 33 (21 %) patients treated with RTX in the RITUXVAS study compared with 2 out of 11 (18 %) patients treated with CYC, who were on maintenance therapy with azathioprine for the two-year follow-up. After 18 months follow-up, 36 % of the patients in the RTX arm were still in remission with no drugs in the RAVE study vs. 31 % of patients receiving CYC and azathioprine as maintenance therapy. There was no difference in the number of flares between treatment arms. These results suggest that RTX in newly diagnosed AAV seems to be an effective alternative to CYC, especially when CYC is contraindicated due to special circumstances such as an elevated risk of infertility. The RAVE trial data also suggest relapsing patients seem to have a better response to RTX compared with restarting CYC therapy, including patients with renal relapses, retro-orbital granulomata and/or severe pulmonary and/or ear, nose and throat relapses. In addition, RTX is currently the first-choice therapy in patients with refractory disease (i.e., those who do not respond to standard therapy, have common relapses, and/or are intolerant to standard therapy). In these patients, RTX 375 mg/m^2/week for 4 weeks or two infusions of 1,000 mg each 2 weeks apart are given. However, it is unclear whether RTX should be combined with CYC to induce remission. While two doses of CYC (15 mg/kg) were administered with the first RTX course in the RITUXVAS trial, no CYC was added in the RAVE trial.

The results of these trials suggest that physicians should consider how to treat AAV patients in the future, while it remains to be seen whether concomitant CYC is required. It has been suggested that CYC may be added in patients with severe and/or life threatening AAV, but that it may not be required for less-severe disease (Cohen Tervaert 2011). Remission with RTX is also unclear, as maintenance therapy was not administered in the RITUXVAS or RAVE trials, and, given a large number of patients with experience relapse, additional therapy may be

necessary. Three main schemes can be used: (1) prevention of relapses through maintenance therapy with immunosuppressants such as azathioprine or methotrexate; (2) RTX re-treatment when a relapse occurs; and (3) prevention of relapses with RTX.

3.5 Controlling Relapses

3.5.1 Rituximab for Chronic Relapsing Refractory GPA

A recent single-center follow-up study of a historic cohort of all patients with chronic relapsing GPA treated with at least two courses of RTX between January 2000 and May 2010 evaluated the efficacy and safety of repeated, prolonged RTX therapy for the maintenance of long-term remission in patients with chronic, relapsing GPA. Patients included in the RAVE trial were excluded from the analysis. Fifty-three patients with refractory GPA (median age 46 years, 53 % female) received $\geq$2 courses of RTX for the treatment of GPA relapses or to maintain remission. All patients except one were PR3-ANCA positive. Patients, all of whom had B-cell depletion, received a median of 4 courses of RTX: the median time to return of B cells was 8.5 months. All relapses observed happened after B-cell reconstitution and were accompanied or preceded by increased ANCA (except for the one ANCA-negative patient). Sixteen patients had infusion-related adverse events. During B-cell depletion, 30 infections requiring antibiotic therapy occurred. The 10-year follow-up shows that repeated RTX therapy effectively and safely reestablishes and maintains remission in patients with chronic, relapsing refractory GPA. No evidence was found for resistance to RTX over time, even after repeated courses of RTX, but relapsing PR3-ANCA-positive patients remained at risk of disease recurrence after B-cell reconstitution (Cartin-Ceba et al. 2012).

Repeated B-lymphocyte depletion appears to incur a low risk of infection. Preemptive re-treatment decisions in individual patients may be based on serial B-lymphocyte and PR3-ANCA monitoring. All patients in this cohort achieved complete remission with the RTX-based remission induction regimen, and no disease flares were observed when peripheral blood B-lymphocytes in peripheral blood were lacking, supporting a central role of B-lymphocytes in the pathogenesis of GPA. The few, mild infusion-related adverse events did not preclude RTX re-treatment. Few infections needed antibiotic therapy, considering the long follow-up and the large amount of sequential RTX courses received.

A recent retrospective study had the objective of reducing rates of refractory or relapsing AAV using a fixed-interval RTX re-treatment protocol. Twenty-eight patients (Group A) received RTX induction therapy (four infusions of 375 mg/m^2 or two infusions of 1 g) and further RTX at relapse and 45 patients (Group B) received routine RTX re-treatment for 2 years: two doses of 1 g each for remission induction, then 1 g every 6 months (total of 6 g). Nineteen patients from group A who relapsed and began routine re-treatment for 2 years were included as group C. Twenty-six of the 28 patients (93 %) in group A, 43/45 patients (96 %) in

group B, and 18/19 patients (95 %) in group C achieved a response, defined as complete or partial remission. Relapses occurred in 19/26 patients (73 %) in group A, 5/43 (12 %) in group B ($p < 0.001$), and 2/18 (11 %) in group C after two years ($p < 0.001$). At the last follow-up, with a median of 44 months, 22/26 (85 %) of patients in group A, 11/43 (26 %; $p < 0.001$) patients in group B, and 10/18 (56 %; $p = 0.001$) patients win group C had suffered relapses. Glucocorticosteroid dosages were decreased and immunosuppression therapy was withdrawn in most patients. Routine RTX re-treatment was well tolerated, with no new safety issues being identified. Therefore, the results showed that a 2-year, fixed-interval routine RTX re-treatment regimen had lower rates of relapse compared with a single RTX course, during both the 2-year treatment regimen and the early phase after RTX withdrawal. Reduction of the glucocorticosteroid dosage and withdrawal of immunosuppressive agents was possible, and no new safety issues were identified. Therefore, in the absence of biomarkers that accurately predict relapse, routine RTX re-treatment appears to be effective in maintaining remission in patients with refractory/relapsing AAV (Smith et al. 2012).

However, relapse prevention is being tested in an ongoing RCT comparing RTX re-treatment with azathioprine/ methotrexate maintenance treatment (MAINtenance of Remission Using RITuximab in Systemic ANCA-associated vasculitides = MAINRITSAN) (http://www.clinicaltrials.gov/ct2/show/NCT00748644?term=MAINRITSAN&rank=1).

3.6 Other Anti-CD20 Therapies

There have been no published trials of other B-cell targeted therapies in AAV, even though humanized anti-CD20 antibodies such as ocrelizumab and ofatumumab might provide more effective and longer-lasting B-cell depletion without the development of neutralizing anti-chimeric antibodies, as reported with RTX. Neither are there trials with drugs with other mechanisms of action such as belimumab and atacicept in AAV.

Belimumab is a human monoclonal antibody that inhibits B-cell activating factor (BAFF), also known as B-lymphocyte stimulator (BLyS), a factor used to determine B-cell survival, especially murine B cells, but with little effect on the survival of developing human B cells, consistent with the relatively modest effects of anti-BLyS antibody therapies in humoral autoimmune conditions.

Atacicept is a designer recombinant fusion protein that inhibits B cells and suppresses autoimmune disease. It combines the binding site for 2 cytokines that regulate the maturation, function, and survival of B cells, B-lymphocyte stimulator (BLyS), and a proliferation-inducing ligand (APRIL), with the constant immunoglobulin region. Atacicept blocks B-cell activation by the TNF receptor superfamily member 13B (TACI), a transmembrane receptor protein mainly found on B-cell surfaces. Like belimumab, atacicept blocks BLyS binding, and also APRIL, thus inducing B-cell proliferation, activation, and longevity and therefore, autoantibody production. It is suggested that atacicept selectively impairs mature B cells and

plasma cells but has less impact on progenitor and memory B cells. It inhibits B-cell maturation, differentiation, and survival, as well as immunoglobulin production, by depriving B cells of needed growth and development signals (Engel et al. 2011).

Increased BAFF is found in patients with active vasculitis (Krumbholz et al. 2005) and it is suggested that belimumab could modulate T- and B-cell interactions in AAV without causing B-cell depletion. RTX treatment raises BAFF levels, and therefore the addition of belimumab might enhance B-cell suppression (Golbin and Specks 2007).

4 Conclusions

RTX can now be considered a first-line option for the induction of remission in severe AVV, but its role in routine and maintenance therapy is unclear. It has been suggested that, based on current evidence, patients with newly developed AAV who are candidates for RTX treatment should not receive maintenance therapy (Cohen Tervaert 2011). A possible tailored solution might be administering maintenance therapy (azathioprine or re-treatment) together with RTX only to patients with a high risk of relapse (i.e., patients who are PR3-ANCA positive, have extensive ear, nose and throat disease, and are nasal carriers of *S. aureus*). The frequent adverse effects observed during RTX therapy, which may be due to the high doses of corticosteroids administered, are another controversial issue. Limitation of corticosteroid therapy and possible induction therapy with plasma exchange or additional anti-TNF blocker therapy may be advisable. As the RTX doses administered in AAV patients are mainly extrapolated from those given for hematologic malignancies (i.e., lymphomas), RCT using smaller doses of RTX to assess whether efficacy is maintained at the expense of fewer adverse events might be useful. Further studies of the efficacy and toxicity of B cell-depleting drugs are required to minimize the rate of relapses and exposure to steroids and CYC in AAV.

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B-Cell Targeted Therapies in Systemic Sclerosis and Inflammatory Myopathies

Rohit Aggarwal, Vivek Nagaraja, and Dinesh Khanna

Abstract Systemic sclerosis (SSc) and idiopathic inflammatory myopathies (IIM) are autoimmune diseases with multisystem involvement. Of late, there is a tremendous interest around the role of B-cells in the early inflammation, autoimmunity, and subsequent fibrosis both in animal and human models. There have been promising results from B-cell depletion therapy in clinical trials in both SSc and IIM. This review will concentrate on the role of B-cells in pathogenesis and the potential of B-cell targeted therapy in SSc and IIM.

1 Introduction

Progressive systemic sclerosis (SSc) is a chronic connective tissue disorder characterized by extensive cutaneous and visceral fibrosis. The pathogenesis of SSc remains unknown. The interplay of autoimmunity, vascular injury, and extracellular matrix (collagen) deposition results in fibrosis of the skin, lungs, and other organs.

Many alterations in the immune system have been noted in both animal and human models with SSc. Increased antibody production (Gu et al. 2008),

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hyper-gammaglobulinemia, and polyclonal B-cell hyperactivity (Famularo et al. 1989) are noted. Studies in animal models over the past decade have shown increasing body of evidence supporting the role of B-cells and their interaction with different cell surface receptors and signaling molecules, in the pathogenesis of SSc. The tight skin (TSK) mice have intrinsic B-cell activation (Saito et al. 2002). The chronic B-cell activation in TSK mice is due to augmented CD-19 (Cluster of Differentiation 19) signaling. Humoral immune responses are normally regulated by signal transduction molecules that influence B-cell antigen receptor (BCR) signaling during responses to self and foreign antigens (Tedder et al. 1997). CD-19 is one such regulatory molecule and belongs to a subset of functionally interrelated cell surface receptors. Altered function or expression of these molecules can influence susceptibility for autoimmunity.

In addition to chronic B-cell activation, a favorable cytokine environment drives the proliferation and differentiation of the B-cells. T-helper cell-1 (Th1) and T-helper cell-2 (Th2) imbalance causes a preponderance of Th2 cytokine profile with increased IL-4, IL-5, IL-6, IL10, and IL-13 production in SSc (Baraut et al. 2010). These cytokines heighten the autoantibody production by B-cells. IL-6 can stimulate collagen production by fibroblasts and hence fibrosis, through autocrine and paracrine pathways (Duncan and Berman 1991). The levels of another cytokine belonging to the tumor necrosis factor super family called B-cell activating factor (BAFF, also called BlyS or B-lymphocyte stimulator) were found to be markedly elevated in TSK mice (Matsushita et al. 2007a). BAFF is best known for its role in the survival and maturation of B-cells (Mackay and Browning 2002). BAFF is produced by several cell types, including monocytes, macrophages, neutrophils, dendritic cells, and T-cells (Schneider et al. 1999). BAFF/BAFF-receptor family in association with APRIL—a proliferation-inducing ligand—appears to traverse nearly all stages of B-lineage differentiation (O'Connor et al. 2004). Furthermore, BAFF has a strong co-stimulatory function for B-cell activation in vitro (Moore et al. 1999).

Analogous to a positive feedback, activated B-cells induce a Th2-dominant immune response and hence cause Th1/Th2 imbalance. B-cells promote the development and differentiation of Th2 cells with their antigen presenting activity (Stockinger et al. 1996) and by producing IL-10, which inhibits IL-12. These findings suggest that communication between activated B-cells and Th2 cells in a suitable cytokine environment induces and perpetuates fibrosis in SSc (Fig. 1).

2 Animal Models

For many decades animal models have helped in understanding different aspects of human disease and development of new therapies. Currently, there is no single animal model that exhibits all the features of SSc. Bleomycin (BLM)-induced scleroderma, TSK 1 and TSK 2 mouse models are have been used commonly in SSc research.

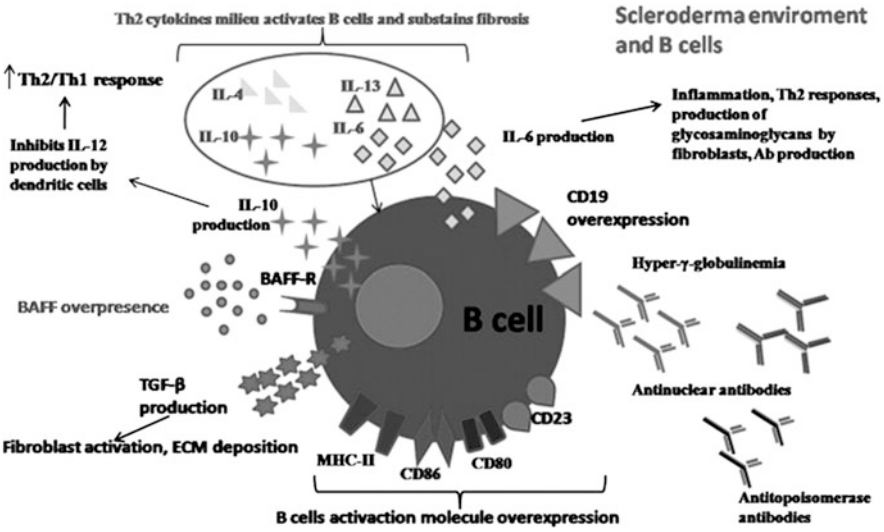

Fig. 1 B-cells and cytokine environment in SSc patients. B-cells in SSc have the characteristics of activated cells, with increased expression of CD19, MHCII, CD80, CD86, and CD23. The enhanced amount of BAFF and Th2 cytokines (IL-4, IL-6, IL-10, IL-13) in SSc patients can maintain the activation of B-cells that lead to a consequent increased production of (**a**) IL-6 that sustains inflammation, Th2 responses, production of glycosaminoglycans by fibroblast and antibodies production; (**b**) TGF-β that induces the fibroblast activation and ECM deposition, leading to fibrosis; (**c**) autoantibodies. *IL* interleukin, *Ab* antibodies, *BAFF* B-cell activating factor belonging to the TNF family, *BAFF-R* BAFF receptor, *TGF-β* transforming growth factor-β, *ECM* extracellular matrix, *MHC* major histocompatibility complex. Reproduced with permission from Bosello et al. (2011). Copyright: Elsevier

2.1 Tight Skin Models

TSK-1 has a spontaneous mutation in fibrillin-1 gene and TSK-2 has an induced mutation in mouse chromosome 1. The gene mutation in TSK-1 leads to overexpression of the fibrillin, which causes excessive extracellular matrix deposition in skin (Pablos et al. 2004). The most striking feature is the presence of thickened fibrotic skin that is firmly bound to the subcutaneous and deep muscular tissue; however, dermal sclerosis is not recognized, and the fibrosis is much deeper in comparison with human SSc. TSK mice produce autoantibodies against SSc-specific target auto-antigens including topoisomerase-I, fibrillin-1 (Fbn-1), RNA polymerase-I, collagen type-I, and Fcγ receptors (Bona and Rothfield 1994; Murai et al. 1998; Tan et al. 1999).

In TSK mice, B-cells exhibit a hyper-responsive phenotype with decreased surface IgM expression, enhanced serum immunoglobulin production, and spontaneous autoantibody production (Saito et al. 2002). CD-19 mediated intra-cellular signaling is similarly enhanced. CD-19 deficiency inhibits IL-6 production in the B-cells of TSK mice. This suggests that dermal sclerosis in TSK mice due to

chronic B-cell activation resulting from augmented CD-19 is possibly through IL-6 overproduction as well as autoimmunity (Saito et al. 2002). CD-19 is a major target of CD22-negative regulation. Reduced inhibitory signal provided by CD-22 in TSK mice results in abnormal activation of signaling pathways including CD19 and also disrupts the B-cell signaling contributing to specific autoantibody production (Asano et al. 2004). Serum BAFF levels are significantly elevated in TSK mice. When newborn TSK mice were treated with BAFF antagonist, it inhibited the development of skin fibrosis, hyper-globulinemia, and the autoantibody production (assessed after 8 weeks). Their skin showed up-regulated expressions of fibrogenic cytokines, such as IL-6 and IL-10, while BAFF antagonist significantly suppressed them. TSK/+ B-cells with BAFF stimulation had a significantly enhanced ability to produce IL-6. These results suggest that BAFF/BAFF receptor system has a role in the development of skin fibrosis in TSK mice and could be a potent therapeutic target (Matsushita et al. 2007a).

B-cell depletion during disease onset, suppresses skin fibrosis (Hasegawa et al. 2006). Skin thickness and autoimmunity were assessed in TSK mice after circulating and tissue B-cell depletion using an anti-mouse CD20 monoclonal antibody before (day 3 after birth) and after disease development (day 56). CD20 monoclonal antibody treatment depleted majority (85–99 %) of circulating and tissue B-cells in newborn and adult TSK mice. Development of skin fibrosis, autoantibody production, and hyper-gammaglobulinemia were markedly suppressed in newborn TSK mice. B-cell depletion also restored a more normal balance between Th1 and Th2 cytokine mRNA expression in the skin. By contrast, these effects were not seen with B-cell depletion in adult mice with established disease. Thus, B-cells may contribute to the initiation of systemic sclerosis pathogenesis in TSK mice but are not required for disease maintenance.

2.2 Bleomycin Mouse Model

Bleomycin exerts its pro-fibrotic activity by influencing various cells (T-cells, mast cells, macrophages, eosinophils, endothelial cells) through cytokines (transforming growth factor-β, type 2 cytokines, tumor necrosis factor-α) and chemokines like CCL-2 (Yamamoto 2006). Bleomycin-treated wild-type mice exhibit dermal and lung fibrosis, hyper-gammaglobulinemia, autoantibody production, and enhanced serum and skin expression of various cytokines (fibrogenic IL-4, IL-6, and transforming growth factor-β1), all of which were inhibited by CD19 deficiency. These findings are quite similar to that in TSK mouse models. Further, bleomycin induces fibrosis by enhancing hyaluronan production, which activates B-cells to produce fibrogenic cytokines and induce autoantibody production, and these processes may be potentiated by CD19 (Yoshizaki et al. 2008).

3 B-Cells in SSc

As with SSc animal models, chronic B-cell activation is suggested to play a pivotal role in the pathogenesis of SSc in patients and resultant clinical manifestations. SSc patients demonstrate increased autoantibody production, hypergammaglobulinemia, and polyclonal B-cell hyperactivity (Famularo et al. 1989).

SSc patients also have distinct abnormalities of B-cells, characterized by expanded naive B-cells and activated but decreased memory B-cells (Sato et al. 2004b). Long-lived memory B-cells play a crucial role as auto-reactive B-cells. CD 27, a type I glycoprotein is a useful cell surface marker of human memory B-cell (Agematsu et al. 2000; Klein et al. 1998). B-cells with high levels of CD-27 produce low levels of CD-19 and surface immunoglobins (Odendahl et al. 2000). When the cell surface marker expression of CD-27 was determined by flow cytometry, the numbers of blood CD-27 negative naive B-cells from SSc patients were increased compared with normal control cells, while memory B-cells expressing medium levels of CD-27 and plasmablasts expressing high levels of CD-27 were reduced (Sato et al. 2004a). Besides, memory B-cells in SSc showed increased expression of activation markers, including CD80, CD86, and CD95; CD-19 expression was increased in both naïve and memory B-cells.

SSc patients are found to have increased cell surface expression (~20 %) of CD-19 compared to normal controls (Sato et al. 2000). It has been demonstrated that a single nucleotide polymorphism in the upstream region of CD19, $-499G > T$, is genetically associated with susceptibility to SSc. Reduced cell surface expressions of CD-22 due to gene polymorphisms was found in SSc patients (Hitomi et al. 2007). Genetic polymorphisms have also been reported in genes such as HLA, fibrillin 1, interleukin-1α, toll-like receptor 2 (Tan et al. 2001; Kawaguchi et al. 2003; Broen et al. 2012).

CD-19/CD-22 loop is of interest in SSc. This loop has been found to be disrupted in many autoimmune diseases (Fujimoto and Sato 2007). CD-22 is a B cell-specific inhibitory receptor that dampens BCR signaling. Circulating antibodies reacting with CD22 were found in 22 % of SSc patients (Odaka et al. 2010). Furthermore, these patients had diffuse cutaneous SSc with higher modified Rodnan skin score (mRSS), higher titers of anti-topoisomerase antibodies, lower percentage vital capacity, and higher esophageal involvement compared to patients negative for CD-22 antibodies.

Serum BAFF levels were found to be significantly elevated in SSc patients compared to healthy controls (Matsushita et al. 2006). These patients more commonly had diffuse cutaneous SSc with higher mRSS, arthralgia/arthritis, myositis, elevated erythrocyte sedimentation rate, and decreased forced vital capacity. Further, SSc B-cells that were stimulated by BAFF exhibited an enhanced ability to produce immunoglobulin-G and IL-6. BAFF appears to be a potential therapeutic target especially in patients with diffuse cutaneous SSc. Another member of the tumor necrosis factor superfamily is APRIL (a proliferation-inducing ligand), which has been shown to be elevated in the peripheral blood of patients with SSc

(Matsushita et al. 2007b). APRIL works in concert with BAFF and stimulates B-cell and plasma survival. Increased production of APRIL in SSc patients was associated with the presence of diffuse skin involvement, scleroderma lung disease, peripheral vasculopathy, greater capillary damage on capillaroscopy, and presence of anti-topoisomerase I antibodies (Bielecki et al. 2010).

4 Targeted Therapy on B-Cell and B-Cell Signaling in SSc

4.1 Rituximab (Anti-CD 20 Treatment)

In 1997, FDA approved the use of rituximab to treat B-cell non-Hodgkin's lymphomas resistant to other chemotherapy regimens. Since then, it generated tremendous interest for the treatment of autoimmune diseases. It is now one of the key therapeutic options in ANCA-associated vasculitides and rheumatoid arthritis. Rituximab is a chimeric monoclonal antibody against the CD-20 molecule found on B-cells and hence inhibit the B-cells. So far, there have been four open labeled trials looking into rituximab therapy in SSc patients (Table 1).

An open-label pilot trial of 15 patients with diffuse cutaneous SSc (their first non-Raynaud's associated disease manifestation within 18 months of trial entry) was recruited to receive two intravenous doses of 1,000 mg rituximab 2 weeks apart (Lafyatis et al. 2009). The mRSS (range 0–51 where 51 is severe skin thickness) did not have a statistically significant change between baseline and 6months [range: −14.5 (improvement) to +14.0 (progression), $p = 0.82$]. The average FVC (92.7 % predicted at baseline) and DLCO (77.9 % predicted at baseline) showed no significant differences at 6months (FVC: 89.2 % predicted and DLCO: 79.7 % predicted). None of the patients showed evidence of progressive major end-organ involvement. B-cells were quantified on skin biopsies. At baseline, the number of B-cells in the skin biopsies of patients (average 10.4 per biopsy, $n = 15$) was significantly higher than the controls who showed no B-cells ($n = 8$, $p < 0.0005$). Six months after rituximab administration, patients showed near complete depletion of B-cells on dermal biopsy (average 3.4 per biopsy). Myofibroblasts in the deep dermis were measured and the average myofibroblast score in patients treated with rituximab improved from 49.5 units at baseline to 36.6 units at 6 months. Autoantibodies commonly seen in diffuse cutaneous SSc (RNA-polymerase III, topoisomerase-I, U1RNP, PM-Scl, Ro60) were measured and did not show consistent changes during the course of the study. (Lafyatis et al. 2009).

The change in mRSS and correlation of clinical characteristics with the levels of IL-6 after rituximab therapy was evaluated in nine patients with diffuse cutaneous SSc in another open-label study (Bosello et al. 2010). These patients had diffuse disease with truncal involvement and a worsening mRSS > 10 % after conventional cyclophosphamide therapy. Their median disease duration was 2 years (range: 1.0–12 years). All nine patients continued with their vasodilator therapy

Table 1 B-cell targeted drug trials in SSc

Author	Journal and year	Study design/organ	Inclusion criteria	Drug/dosing/how often	Change in MRSS	Change in PFT	Other comments
Lafyatis et al.	Arthritis and Rheumatism, 2009	Open-label/skin	Early dcSSc with first non-Raynaud's disease manifestation within 18 months of trial entry	Two doses of rituximab 1,000 mg intravenously 2 weeks apart. No pre-medication was given	Mean change −0.37, $p = 0.82$	• Mean FVC (89.2 % at baseline to 92.7 % at 6 months) • Mean DLCO (79.7 % at baseline to 77.9 % at 6 months)	• Improvement in myofibroblast score • No consistent changes in autoantibody levels
Bosello et al.	Arthritis Research and Therapy, 2010	Open-label/skin	• Age >18 years • dcSSc with truncal involvement • Worsening in skin score greater than 10 % after the conventional cyclophosphamide therapy	Two doses of rituximab 1,000 mg intravenously 2 weeks apart, together with 100 mg methylprednisolone at each infusion	Mean ± SD 21.1 ± 9.0 (at baseline) to 12.0 ± 6.1 (at 6 months)	• Mean ± SD FVC (91.6 ± 20.7 % at baseline to 96.8 ± 18.9 % at 6 months) • DLCO (58.0 ± 15.8 % at baseline to 58.4 ± 14.2 % at 6 months)	High levels of IL-6 at baseline, decreased after 6 months
Smith et al. (2010)	Annals of Rheumatic Disease, 2010	Open-label/skin	• Age >18 years • Disease duration ≤4 years • mRSS ≥14 or disease activity score ≥3	Two doses of rituximab 1,000 mg intravenously 2 weeks apart, together with 100 mg methylprednisolone at each infusion	Mean ± SD mRSS of 24.8 ± 3.4 at baseline to 14.3 ±3.5 at 24 weeks, ($p < 0.001$)	Not assessed	Significant improvements in dermal hyalinized collagen content ($p = 0.014$) and dermal myo-fibroblast numbers ($p = 0.011$)

(continued)

Table 1 (continued)

Author	Journal and year	Study design/organ	Inclusion criteria	Drug/dosing/how often	Change in MRSS	Change in PFT	Other comments
Smith et al. (2013)	Journal of Rheumatology, 2013	Two year follow-up of Smith et al. (2010)/ skin	See Smith et al. 2010	See Smith et al. 2010	Mean ± SD MRSS of 24.8 (SD 3.4) at baseline versus 13.6 (SD 5.6) at Month 24 ($p < 0.0001$)	• Mean FVC % (92.8 % at baseline to 84.7 % at 24 months) • DLCO remained stable for 24 months	Significant improvements in dermal hyalinized collagen content ($p = 0.009$) and dermal myo-fibroblast numbers ($p = 0.005$)
Daoussis et al. (2010)	Rheumatology, 2010	Open-label/ lung	• Positive anti-Scl-70 • Presence of SSc-associated ILD (based on HRCT and PFT) • Absence of any changes in medications and/or dosage of treatment administered (12 months prior to enrolment)	Four weekly pulses of rituximab (375 mg/m^2) at baseline and at 6 months in addition to already administered treatment	Mean ± SD mRSS 13.5 ± 6.84 at baseline versus 8.37 ± 6.45 at 1 year ($p = 0.0003$)	Mean ± SD FVC (68.13 ± 19.69 at baseline versus 75.63 ± 19.73, 1 year, $p = 0.0018$) DLCO (mean ± SD: 52.25 ± 20.71 at baseline versus 62 ± 23.21, at 1 year, $p = 0.017$)	Marked improvement in collagen deposition in papillary dermis at 24 weeks
Daoussis et al. (2012)	Clinical and Experimental Rheumatology, 2012	Two year follow-up of Daoussis et al. 2010/ lung	See Daoussis et al. 2010	See Daoussis et al. 2010	Mean ± SD (4.87 ± 0.83 at 2 years, $p < 0.0001$)	Mean ± SD FVC (77.13 ± 7.13 at 2 years, $p < 0.0001$) DLCO (mean ± SD: 63.13 ± 7.65 at 2 years $p < 0.001$)	Significant improvement in the myofibroblast score at 2 years Significant improvement in the functional status based on HAQ scores

(iloprost, nifedipine and acetylsalicylic acid). They received two intravenous doses of 1,000 mg rituximab 2 weeks apart. After 6 months, the mRSS decreased in all patients from mean ± SD 21.1 ± 9.0 (at baseline) to 12.0 ± 6.1 (at 6 months). The FVC and DLCO values showed no significant differences at 6-month follow-up (96.8 ± 18.9 % and 58.4 ± 14.2 % of predicted value, respectively) compared with baseline (91.6 ± 20.7 % and 58.0 ± 15.8 % of the predicted value, respectively) (Bosello et al. 2010). The role of IL-6 in promoting fibrosis and augmenting inflammation has been shown in many studies focusing on the pathogenesis of SSc (Hasegawa et al. 1998; Scala et al. 2004; De Santis et al. 2005). Elevated levels of IL-6 were found in the skin, serum, and bronchoalveolar lavage fluid. Further, the SSc lung-derived B-cells induce the secretion of IL-6 by lung fibroblasts (Kondo et al. 2001). In the above trial, Bosello et al. showed that SSc patients presented high levels of IL-6 (3.7 ± 5.3 pg/ml) at baseline had a significant decline, after 6 months (0.6 ± 0.9 pg/ml, $p = 0.02$) following rituximab therapy. Also, there was depletion of circulating B-cells at 3 months following rituximab therapy as measured by flow cytometry and then re-population between 6 and 12 months. However, the BAFF increased relative to the baseline (baseline: 1,233.5 ± 683.3 pg/ml versus 6 months: 3,257.8 ± 1,571.8 pg/ml) (Bosello et al. 2010).

In a third 24-week open-label clinical and histopathological study focusing on safety and efficacy of anti-CD-20 treatment, eight patients with diffuse cutaneous SSc were enrolled (Smith et al. 2010). These patients had disease duration (the first non-Raynaud's disease manifestation) of 4 years or less, an mRSS of 14 or greater and a disease activity score of 3 or greater. They received an infusion of 1,000 mg rituximab 2 weeks apart, on months 0 and 6, together with 100 mg methylprednisolone at each infusion. Six-month follow-up results were initially reported. Rituximab induced effective B-cell depletion in all patients. There was a significant change in mRSS at week 24 (mean mRSS of 24.8 (SD 3.4) to 14.3 (SD 3.5), $p < 0.001$). Also, significant improvements were measured in the dermal hyalinized collagen content ($p = 0.014$) and dermal myofibroblast numbers ($p = 0.011$) (Smith et al. 2010).

These patients were reevaluated at a two-year follow-up (Smith et al. 2013). There was both a statistically and clinically significant decrease in mRSS [mean MRSS of 24.8 (SD 3.4) at baseline versus 13.6 (SD 5.6) at Month 24, $p < 0.0001$] and disease activity score (median of 4.5 at baseline and 0.5 at Month 24; $p < 0.0001$). Again, there was significant decrease in the dermal hyalinized collagen content ($p = 0.009$) and dermal myofibroblast numbers ($p = 0.005$). A statistically significant decrease in FVC was reported [mean FVC 92.8 % of normal at baseline (SD 8.6) versus 84.7 % of normal at Month 24 (SD 13.3); $p = 0.047$]. DLCO remained stable over the 2 years of follow-up (Smith et al. 2013).

Daoussis et al. (2010) performed a proof-of-principle study where 14 patients with diffuse cutaneous SSc who had positive anti-Scl 70, high-resolution CT (HRCT) scan, and pulmonary function tests (PFT) confirmed SSc-associated ILD were enrolled. The mean disease duration was 6.87 ± 4.88 years. Skin involvement was assessed both clinically and histologically. The patients born on an even-numbered date ($n = 8$) were assigned to the rituximab and those born on an

odd-numbered date ($n = 6$) to the control group. In addition to the already administered treatment prior to study enrollment, patients in the rituximab group received four weekly infusions of rituximab at 375 mg/m^2. PFT and mRSS were assessed at baseline, 24 weeks and 1 year. HRCT of the chest and skin histology was assessed at baseline and 24 weeks. There was a significant increase of forced vital capacity (68.13 ± 19.69 versus 75.63 ± 19.73, at baseline versus 1 year, respectively, $p = 0.0018$) and diffusing capacity of carbon monoxide (52.25 ± 20.71 versus 62 ± 23.21, at baseline versus 1 year, respectively, $p = 0.017$) in the rituximab group. HRCT remained unchanged at 24 weeks. There was also an improvement in the mRSS (13.5 ± 6.84 versus 8.37 ± 6.45 at baseline versus 1 year, respectively, $p = 0.0003$) and collagen deposition in papillary dermis at 24 weeks (51.75 ± 19.78 versus 31.68 ± 14.02 at Week 0 versus Week 24, respectively, $p = 0.030$) (Daoussis et al. 2010). Eight patients who received rituximab in the above study had follow up for 2 years. There was significant improvement in the forced vital capacity, forced vital capacity, mRSS, and reduction in the myofibroblast score (on skin histology) at 2 years (Daoussis et al. 2012).

4.2 Anti-CD 25-Treatment

Recently, in an open labeled trial, ten patients with rapidly progressive SSc received six infusions of 20 mg basiliximab (CD-25 monoclonal antibody targeting CD-25 positive lymphocytes) every 4 weeks. The median mRSS was reduced from 26/51 to 11/51 at week 68 ($p = 0.015$), the mean forced vital capacity increased from 82.1 to 88.4 % expected (week 44, $p = 0.078$) and the mean carbon monoxide diffusing capacity improved slightly to 57.8 % expected (week 44, $p = 0.578$) (Smith et al. 2013).

4.3 Other Potential Targets

Cytokines and interleukins that can alter the B-cell activity are possible targets to develop future therapies. CD-19, which has been shown to be vital in its influence on the B-cells, is an attractive focus. Other targets include the BAFF-APRIL, CD-19/CD-22 loop, IL-6, and CD-27.

In summary, there have been five open-label trials (four with anti-CD-20 and one with anti-CD25) in patients with diffuse cutaneous SSc or ILD and there was stabilization and/or improvement in mean/median mRSS and pulmonary function tests. These preliminary data provide impetus for well-designed, randomized controlled trials to test this hypothesis (Table 1.1).

5 Conclusion

B-lymphocytes are pleiotropic cells with multiple functions, in antigen presentation, production of cytokines (IL-6 among others), lymphoid organogenesis, differentiation of T-effector cells and modulation of dendritic cell function. Ongoing research is necessary to better understand the B-cell, its signaling molecules and its role in SSc. The results from the small open labeled trials with rituximab have been quite encouraging. Given the phenotypic heterogeneity of SSc, larger scale, multicenter, randomized, controlled studies are needed to further explore the efficacy of rituximab. In parallel, we need to continue exploring other potential targets and develop novel therapies for this complex and disabling disease.

6 B-Cell Depletion Therapies in Idiopathic Inflammatory Myopathies

Idiopathic inflammatory myopathies (IIM) are a group of acquired, heterogeneous, systemic connective tissue diseases that include adult polymyositis (PM), adult dermatomyositis (DM), childhood myositis mainly juvenile dermatomyositis (JDM), myositis associated with cancer or another connective tissue disease, and inclusion body myositis (IBM). The treatment of myositis is difficult due to rarity of these syndromes and their heterogeneous presentations. However, until recently, the main hurdle in advancement of treatment of myositis was lack of controlled trials utilizing validated outcome measures. Most studies in the past were single referral center cross-sectional retrospective studies on small numbers of treatment-refractory patients observed for relatively short periods of time. The general expert consensus confirms the use of corticosteroids as first-line medication for myositis. However, in many patients, corticosteroid toxicity leads to significant disability, or these agents are ineffective, which then requires additional immunosuppression like methotrexate and azathioprine. Despite conventional use of these immunosuppressive agents, they have not been tested in randomized controlled trial and many patients failed to respond adequately even with two to three immunosuppressive agents. Thus, there is a significant need for novel therapeutic agents that target disease pathogenic pathway in myositis. Recently there have been many advancements in the of treatment of myositis, including large multicenter controlled clinical trials and validated outcome measures (Rider et al. 2003, 2004; Sultan et al. 2008a). Rituximab, a B-cell depleting agent, with increasing use in autoimmune diseases and given that B-cells play a critical role in the initiation and propagation of the immune response and are implicated in the pathogenesis of myositis (Chiu and Co 2011a), many studies have evaluated the use of rituximab in myositis in last 5–7 years. In this chapter, we will discuss mechanism of action, efficacy and safety of B-cell depleting therapy in myositis.

Basic Science Aspect of B-Cell Depletion in IIM: B-cells appear to play a critical role in the initiation and propagation of the immune response in myositis. B-cells are found in inflammatory infiltrates and tend to localize in peri-vascular regions in myositis especially DM patients (Khanna and Reed 2010; Chiu and Co 2011b). Moreover, differentiated B-lymphocytes in the form of CD138+ plasma cells were seen predominantly in the endomysium of muscle tissue of PM and IBM patients (Greenberg et al. 2005). In addition, B-cells function as antigen presenting and co-stimulatory to T cell and secrete pro-inflammatory cytokines that are implicated in myositis (Chiu and Co 2011b). Clonal expansion, class-switched significant somatic mutation, and variation of local B-lymphocyte and plasma cell maturation could occur within the skeletal muscle (Grundtman et al. 2007). These characteristics are hallmarks of B cell antigen-driven autoimmune response in inflammatory myopathies.

Dermatomyositis and anti-Jo-1 antibody positive patients have high serum levels of BAFF which also suggest role of B-cells in myositis and represent another molecular target (Krystufkova et al. 2009). Moreover, BAFF expression was markedly increased at muscle fibers in the perifascicular area in DM patients and was associated with an increased number of CD4 (+) T-cells and CD19 (+) B-cells in DM (Baek et al. 2012).

A role of B-cells in the pathophysiology of IIM is also supported by evidence that suggests possible role of autoantibodies in pathogenesis in myositis (Ascherman 2003). Different myositis-specific autoantibodies (MSA) have been identified and are strongly associated with distinct clinical phenotypes. Serum levels of anti-synthetase autoantibodies like anti-Jo-1 (most common MSA seen in 30 % of IIM patients) correlate with myositis disease activity and are predictive of responsiveness in IIM (Stone et al. 2007; Hervier et al. 2012; Miller et al. 1990; Aggarwal et al. 2012). Histidyl-transfer RNA synthetase (HisRS; a target for anti-Jo-1 antibody) has been shown to trigger both innate and adaptive immune responses through activating the type I Interferon resulting in severe muscle inflammation that is the hallmark of IIM (Soejima et al. 2011). B-cells in peripheral blood have been reported to show a significant increase in DM compared with healthy subjects or PM (Aleksza et al. 2005; Ishida et al. 1993; Kikuchi et al. 2001). However, a recent flow cytometric analysis of peripheral blood lymphocytes showed increased levels of activated B-cells in peripheral blood of both PM and DM (Ishii et al. 2008).

Definite role of B-cell in pathogenesis of JDM is still unclear but increasing evidence of humoral immunity and autoantibodies in JDM has thrown a spot light on B cell-mediated disease process in JDM. Moreover, complement-mediated injury in pathogenesis of JDM also suggests possible role of B-cells and autoantibodies. More and more autoantibodies are being discovered in JDM, for example, transcriptional intermediary factor 1-γ (TIF1-γ) and each representing clinically distinct phenotypes of JDM. Biopsy studies have shown lymphoid follicles (neolymphogenesis) in the muscle of patients with JDM and that it correlated with more severe disease requiring more aggressive therapies like IVIG and rituximab (Lopez De Padilla et al. 2009). In contrast, patients with diffuse infiltrates

or lymphocytic aggregates were responsive to standard therapy with steroids and methotrexate. Given the above evidence of B-cells-mediated process in JDM, there has been increasing interest in evaluating the utility of B-cell depletion therapy in JDM over last few years.

6.1 Current Treatment Paradigm in IIM

Corticosteroids remain the empiric agents of choice for the initial treatment of inflammatory myopathy despite lack of randomized controlled trial. Major limitations of corticosteroids are associated side effects and that the reduction in doses often leads to relapse (van de Vlekkert et al. 2010). Therefore, many experts recommend adding a first-line immunosuppressive agent like methotrexate (MTX) or azathioprine in combination with corticosteroids, at least in severe or refractory cases of myositis. Several uncontrolled studies have confirmed the efficacy of MTX and azathioprine in steroid-refractory myositis (Joffe et al. 1993; Miller et al. 2002); however, there are no prospective, blinded, controlled studies of MTX or azathioprine in myositis. Intravenous immunoglobulin is a reasonable short-term treatment with proven benefit in a controlled trials, but its long-term effectiveness remains unknown and cost can be prohibitive. The evidence for other immunosuppressive therapies has been derived mainly from case reports and open studies. Cyclosporine or tacrolimus particularly for antisynthetase antibody–positive patients, mycophenolate mofetil for refractory rash, and both for interstitial lung disease have been used with some success. Overall balance of evidence suggests that immunosuppressive drugs are effective in dermatomyositis and polymyositis, although randomized controlled trials are lacking, and many patients still continue to face refractory progressive course despite maximal conventional immunosuppression. Therefore, newer therapies (e.g., rituximab) are encouraging and may change the current treatment paradigm in myositis.

6.2 B-Cell Depletion Therapy in Adult Polymyositis and Dermatomyositis

Until the Rituximab in Myositis trial was completed, the evidence of efficacy of rituximab in PM was limited to mainly small case series and case reports (Table 2). A single open-label prospective study of the efficacy of rituximab in four patients with PM who were refractory to multiple conventional therapies including IVIG showed complete remission in 50 % (2/4) patients and partial improvement in the other 50 % (Mok et al. 2007). This study also showed trend towards improvement in disability and health-related quality of life parameters. Several other case reports

Table 2 Summary of all studies of rituximab treatment in adult polymyositis and dermatomyositis

Author	Journal and year	Type of study	Number of subjects	Improved/stabilized	Worse/failed	Adverse events
Oddis et al.	Arthritis and Rheumatism, 2013	Randomized, double-blind, control trial	76 PM, 76 DM, 48 JDM	Primary and secondary outcome of efficacy were not achieved. 83 % patients improved at the end of the RCT	27 % failed	One death, 26 drug-related serious adverse events mostly infections
Sem et al.	Rheumatology, 2009	Retrospective case series	11 anti-syn (10 jo-1, 1 PL12	7 out of 11 improved/ stabilized	Three worsening, one death	One died 3 months after rituximab –Pneumocystis jirovecii infection
Sultan et al.	Clinical and Experimental Rheumatology, 2008	Five cases in open labeled trial and three as retrospective	5 DM (2 Jo-1), 2 PM (later diagnosed as IBM, muscle dystrophy), 1 JDM	2 DM (2 Jo-1) with improvement in muscle enzyme but not in muscle strength. Both had two stabilization of ILD	Six failed (including IBM, muscle dystrophy and Nodular sclerosing lymphoma)	One diagnosed with Nodular sclerosing lymphoma, 1 died of unrelated cause
Mok et al.	The Journal of Rheumatology, 2007	Open labeled trial	4 PM (1 anti Jo-1)	Two complete response, two partial response	None	None, no retreatment
Valiyil et al.	Arthritis Care and Research, 2010	Retrospective	8 PM (8 anti-SRP)	Six complete and partial response	Two	One death – pneumonia/ CHF; two infections (Herpes zoster infection, facial abscess); three re-treatment 3, 6, and 9 months)
Chung et al.	Arch Dermatol., 2007	Open labeled trial	8 DM	Three with partial muscular response; none with skin response	Five with no response	No infections One died of metastatic cancer Nine months after his last infusion

Levine et al.	Arthritis and Rheumatism, 2005	Open labeled trial	7 DM	6/6 patients had good muscular response, 5/5 had good skin response	None	One minor skin infection; no re-treatment in 1 year
Dinh et al.	J Am Acad Dermatol, 2007	Retrospective	2 JDM, 1 DM	Three with skin improvement; muscle were normal at baseline	None	None
Frikha et al.	Rheumatology 2009	Case series: two cases	2 PM (1 with ILD)	Two improvement in muscle strength and muscle enzyme and ILD	None	None. Two re-treatment (>12 months)
Gottenberg et al.	Ann Rheum Dis, 2005	Retrospective	2 PM (2 anti-Jo1)	One complete response, one partial response	None	None. One re-treatment (4 months)
Noss et al.	The Journal of Rheumatology, 2006	Retrospective	2 PM, 1 DM	Two complete response, one partial response	None	None. Two re-treatment (6 and 10 months)
Majmudar et al.	Journal of Clin Rheumatol, 2009	Retrospective	2 DM, 1 PM (1 anti-Jo1)	Three complete response	None	None. Two re-treatment (11 and 12 months)
Arlet et al.	Neuromuscul Disord, 2006	Retrospective	2 PM (anti-SRP)	Complete response but also got plasma exchange and high dose steroids	None	Flare-up of Hepatitis B. Two re-treatment (4 months)
Delingy et al.	Arthritis Care and Research, 2011	Retrospective	2 PM (anti-SRP)	Complete response	None	None
Whelan et al.	Rheumatology, 2009	Retrospective	2 PM (anti-SRP)	None	Two failed	None

and small series of two to three patients have described benefits of rituximab in the management of refractory PM and DM (Lambotte et al. 2005; Majmudar et al. 2009; Noss et al. 2006; Gottenberg et al. 2005). Even the most refractory of myositis subset, i.e. anti-SRP antibodies positive necrotizing myopathy patients who had severe muscle weakness and high CK levels, unresponsive to many agents, showed a robust clinical response to B-cell depletion therapy in about 70 % patients based on four case series (Arlet et al. 2006; Valiyil et al. 2010; Deligny et al. 2011; Whelan and Isenberg, 2009). Valiyil et al. (2010) reported retrospective study of eight anti-SRP patients with six of eight patients who had been refractory to standard immunosuppressive therapy demonstrating improved manual muscle strength and/or decline in CK levels, with three showing sustained response 12–18 months post treatment, and three requiring re-dosing at 3, 6, and 9 months. There was robust decrease in anti-SRP levels post-rituximab in four of the five patients studied.

In terms of DM, first open-label uncontrolled trial of 7 DM patients suggested improvement of muscle disease after rituximab with skin improvement, although four patients had muscular relapse that coincided with the return of B-cells (Levine 2005). In contrast, another open-label uncontrolled trial of 8 DM patients failed to show response after rituximab treatment for recalcitrant skin disease, but had partial muscular improvement in three of eight patients (Chung et al. 2007). Muscle enzyme levels and skin scores were not significantly changed at 6 months from those at baseline in this study. There are other small case reports of favorable outcome in DM with rituximab (Dinh et al. 2007). Overall, based on the above reports, the evidence of efficacy of rituximab in muscular aspect of DM is good, but in recalcitrant skin disease data is mixed, and further studies are warranted.

6.3 B-Cell Depletion Therapy in Juvenile Dermatomyositis

Levine et al. first reported the use of rituximab in 2 JDM patients in a prospective open-label trial of five DM patients. Since then there have been many more case reports and series of use of rituximab in JDM with mostly favorable results (Cooper et al. 2007; Dinh et al. 2007; Holzer et al. 2010; Tzaribachev et al. 2009; Endo 2005; Levine 2002). Recently, Chiu reviewed cases of JDM treated with rituximab in a single center until 2011 and reported that overall 75 % (9 out of 12) showed improvement in cutaneous or muscular manifestations (Chiu and Co 2011b). Most patients had failed conventional immunosuppression including steroids and methotrexate, and some also failed IVIG. Rituximab was given to children aged 8–22 years and mostly 375 mg/m^2 weekly for 4 weeks was used. Five of 12 patients went into remission and two had relapses requiring re-treatment with rituximab. No major adverse events were reported and 3 of the 12 patients eventually required autologous stem cell transplantation.

6.4 *Rituximab in Myositis Trial in Adult and Juvenile Myositis*

Given the autoimmune characteristics of myositis, the aforementioned immunopathogenic role of the B-cell in myositis, and favorable data from open-label studies/case series, the Rituximab in Myositis (RIM) Study was recently completed to evaluate safety and efficacy of rituximab in a randomized, double-blind, placebo-phase, trial of adult and pediatric myositis using validated measures of myositis disease activity and damage and a consensus-driven definition of improvement (Rider et al. 2004; Rider et al. 2003; Sultan et al. 2008a; Oddis et al. 2012). This was a large multinational National Institutes of Health (NIH)-funded 44-week clinical trial evaluating 200 refractory myositis patents (76 PM, 76 DM, and 48 JDM) in a unique randomized placebo-phase design (Feldman et al. 2001). Patients were randomized to either "rituximab early" (drug at weeks 0 and 1, placebo at weeks 8 and 9) or "rituximab late" arm (placebo at week 0 and 1, drug at week 8 and 9), such that all patients were treated with rituximab. Refractory myositis was defined as failure to glucocorticoids and at least one other immunosuppressive agent and patients enrolled in this trial had failed average of three immunosuppressive agents. The primary end point of the trial was time to improvement with a hypothesis that patients with rituximab early will improve earlier than patients with rituximab late. The improvement was International Myositis Assessment and Clinical Studies Group (IMACS) preliminary validated response criterion (Rider et al. 2004) defined as $\geq$20 % improvement in three of any six core set measures (CSM) with no more than two worsening by $\geq$25 % (manual muscle testing (MMT) could not be worsening CSM). The 6 CSM for this trial were (Miller et al. 2001): (1) Patient (or parent) global 10 cm visual analog scale (VAS); (2) MD global VAS; (3) Health Assessment Questionnaire (HAQ) or Childhood HAQ (CHAQ); (4) Muscle enzyme; (5) Global extramuscular disease activity based on the investigator's composite assessment of disease activity on the constitutional, cutaneous, skeletal, gastrointestinal, pulmonary, and cardiac scales of the Myositis Disease Activity Assessment Tool (MDAAT) (Sultan et al. 2008a); and (6) manual muscle testing (MMT) assessed using a validated measure, the MMT-8 (Rider et al. 2010). Rituximab dosing was based on the body surface area (BSA); children with a BSA $\leq$ 1.5 m^2 received 575 mg/m^2 at each infusion, and adults and children with a BSA $>$ 1.5 m^2 received 750 mg/m^2 up to 1 g/infusion. There were no significant differences between the time to improvement in rituximab late ($n = 102$) and rituximab early ($n = 93$) groups, $p < 0.05$. However, in post-hoc analysis, 83 % of patients who had previously failed corticosteroids and multiple other immunosuppressive agents improved by IMACS criteria after rituximab at a median of 20 weeks from the drug. Myositis subsets of PM and DM showed no significant difference in the two arms except JDM subset showed an 8-week difference in the median time to improvement in both arms but was not statistically significant probably as the study was not powered for detecting difference in each subsets.

Rituximab also had a significant steroid-sparing effect with mean prednisone dose decreasing from 20 to 14 mg over the course of the study ($p < 0.05$). Moreover, ten patients were re-treated with rituximab due to initial response and then worsening, out of which nine were reevaluated longitudinally and eight of them again met the criteria of improvement. B-cell depletion in this study was complete and appropriate. Lowest B-cell counts on an average were at 4 weeks after rituximab with a return of median B-cells > 5 B-cells/μl at 32–36 weeks after rituximab. In conclusion, although the RIM trial failed to prove efficacy of rituximab in myositis patients due to failure of its primary and secondary end points, which is possibly due to trial design issues (de Visser 2013), it gave some exciting information regarding possible efficacy of rituximab in myositis with high response rate in a very refractory cohort, significant steroid-sparing effect, and response rate of re-treatment.

6.5 Special Treatment Considerations

6.5.1 B-Cell Depletion Therapy in Treatment of Anti-Synthetase Syndrome

Anti-tRNA synthetase autoantibodies (anti-synAbs) target aminoacyl-tRNA synthetase enzymes, a family of cytoplasmic proteins that participate in protein synthesis by catalyzing the attachment of amino acids to their specific tRNA. To date there are autoantibodies to eight distinct aminoacyl-tRNA synthetases which, as a group, are the most common of the myositis-specific autoantibodies (MSA) and are seen in up to 35–40 % of patients with idiopathic inflammatory myopathy (IIM) (Hirakata 2005). While anti-Jo-1 is the most commonly detected anti-synAb, occurring in up to 30 % of IIM patients, the other anti-synAb (non-Jo-1) are collectively found in 10–20 % of myositis patients (Mammen 2010; Targoff 2000). The "anti-synthetase syndrome" (ASS) (Lambotte et al. 2005) refers to a collection of some or all of the following features—myositis, interstitial lung disease (ILD), inflammatory arthropathy, Raynaud phenomenon, fever, and "mechanic's hands"(Love et al. 1991) along with one of the anti-synAbs. A major cause of morbidity in ASS is ILD, and the concurrence of antibodies to non-Jo-1 and anti-Ro/SS-A is associated with more severe ILD and worse prognosis (Aggarwal et al. 2013; La Corte et al. 2006). Conventional management of ASS has been with corticosteroids and other immunosuppressive medications, such as methotrexate, azathioprine, or cyclophosphamide similar to any IIM patients. Many case reports have successfully used rituximab in refractory patients with ASS with severe ILD associated with anti-Jo1 antibody (Lambotte et al. 2005; Limaye et al. 2012; Vandenbroucke et al. 2009). In some of these reports patients have failed multiple immunosuppressive agents. A retrospective review of 11 patients with ASS and severe progressive ILD showed stabilization or improvement in 7 out of 11 patients after 6 months of rituximab (Sem et al. 2009). These patients were

refractory to other immunosuppressive agents including cyclophosphamide. Anti-Jo-1 levels decreased after rituximab but decrease was modest. Overall treatment was well tolerated except one fatal outcome due to infection.

6.5.2 B-Cell Depletion Therapy in Treatment of Pulmonary Disease in Myositis

Pulmonary manifestations, particularly ILD and respiratory muscle weakness, are the most common cause of morbidity and mortality in patients with myositis warranting aggressive combination therapy. ILD is very common in patients with anti-synthetase antibody syndrome and is a predictor of poor survival in IIM patients. Levine (2005) first showed improvement of FVC in 3 DM patients with impaired pulmonary function at baseline (two of them with ILD) after rituximab therapy. In a retrospective study of 11 patients with severe progressive ILD (10 anti-Jo-1, 1 PL-12) who were refractory to conventional immunosuppression (including CYC in many cases), 7 (65 %) showed a good response to rituximab (Sem et al. 2009). In five of nine patients, there was reduction of ground glass opacities, six showed a greater than 10 % improvement in forced vital capacity, and three had greater than 15 % improvement in diffusion capacity of carbon monoxide 3–6 months after use of rituximab. This suggests that B-cell depletion may be a valuable adjunct in the treatment of refractory ILD in IIM. Other evidence supportive of rituximab's efficacy in ILD comes from case reports with six of six patients with ILD showing favorable response (improvement or stabilization of lung function or chest imaging (Frikha et al. 2009)). Sultan et al. (2008b) showed stabilization or improvement in ILD associated anti-Jo-1 in 2 DM patients with rituximab; however, the other six patients without ILD/Jo-1 failed to respond including one unrelated death and one development of nodular sclerosing lymphoma. Larger, prospective, controlled studies are needed to validate this finding and further assess safety issues.

6.5.3 B-Cell Depletion Therapy in Treatment of Refractory Skin Disease

The rash of DM or JDM can be quite stubborn, and its course and response to treatment may be discordant with the underlying muscle disease. Moreover, patients with JDM and occasionally adult DM can have severe vasculitis rashes including ulcerations as well as have subcutaneous or soft tissue calcification that is very difficult to treat. Often conventional treatments from hydroxychloroquine and topical tacrolimus to IVIG fail to improve these recalcitrant lesions or rashes. With the growing interest of B-cell depletion therapies in myositis, some case reports and retrospective studies have focused on evaluating the efficacy of rituximab in refractory rashes of DM/JDM. An open-label uncontrolled trial of 8 DM patients with refractory rashes failed to show response after rituximab treatment for

recalcitrant skin disease with partial muscular improvement in three of eight patients (Chung et al. 2007). Another open-label uncontrolled trial of 7 DM patients suggested improvement of all five patients with rash and two patients with alopecia, although rash was not noted to be refractory in this study (Levine 2005). Another case series of three patients and few case reports of DM with resistant skin disease showed improvement with rituximab (Dinh et al. 2007). Combination of rituximab and IVIG has also been used with success in one case (Sanchez-Ramon et al.). Overall, based on the above reports the evidence of efficacy of rituximab in refractory skin lesions is rather limited and further studies are warranted.

6.5.4 B-Cell Depletion Therapy in Treatment of Dysphagia in Myositis

Esophageal involvement in myositis causing pharyngeal dysphagia is often slow to respond and is a poor prognostic feature that leads to recurrent aspiration pneumonia. Although oral corticosteroids are the first line of therapy, treatment with other IS agents and/or IVIG is necessary. To date, no reports of B-cell depletion therapy for treatment proximal dysphagia due to IIM has been reported.

6.5.5 B-Cell Depletion Therapy in Inclusion Body Myositis

Although the pathophysiology in IBM is primarily considered to be either antigen-driven T cell response or more neurodegenerative (Aggarwal and Oddis 2012), some studies have suggested B-cell-mediated inflammation to contribute to the pathogenesis of IBM (Bradshaw et al. 2007). The reports of B-cell depletion therapy in IBM are limited to a few case reports (Sultan et al. 2008b; Vordenbaumen et al. 2010). A patient of PM, who was later on found to be IBM, was treated with rituximab and continued to have progressive muscle weakness. In another case report of IBM and rheumatoid arthritis (RA), treated with rituximab for active arthritis, no amelioration of muscle weakness was noted despite improvement in RA (Vordenbaumen et al. 2010). Currently there is no evidence of response of B-cell therapies in IBM; however, more conclusive evidence is needed. Also given encouraging results on rituximab efficacy for refractory PM and DM, more studies may be pursued in IBM. Lerario et al. (2010) showed improvement in muscle strength after rituximab in two patients with dysferlin-deficient muscular dystrophy, again suggesting there may be a role of rituximab in muscle disease beyond immune mediated diseases.

6.6 Re-treatment and Sustained Efficacy with Rituximab

The response to rituximab has been short-lived in many patients which might correspond to return of B-cell in few months. Therefore, retreatment is often required in many patients on flare-up with most patients showing recovery on

re-treatment (Rios Fernandez et al. 2009; Levine 2005; Brulhart et al. 2006). In one study, four of the six patients with initial response to rituximab had muscular relapse that coincided with the return of B-cells, while two others showed sustained response despite return of B-cells (Levine 2005). In the RIM trial, ten patients met stringent criteria of flare-up (disease worsening) after initial response and eight of nine patients responded to re-treatment after relapse.

6.7 *Safety of B-Cell Depletion Therapy*

Most of the safety data of B-cell depletion in rheumatology comes from studies in rheumatoid arthritis and some in vasculitis. In general rituximab is well tolerated except minor infusion reactions and has low rate of serious adverse events in rheumatology. However, it is associated with an increased risk for infection as well as the rare but serious potential side effect of progressive multifocal leukoencephalopathy (PML). All small retrospective series of cases reported that rituximab in myositis was well tolerated and safe with few studies showing rare serious adverse events mostly serious infection and a rare case of death or malignancy which may not be directly related to the drug (Table 1). However, more robust safety and tolerability data comes from the largest randomized clinical trial in myositis on rituximab, the RIM trial (Oddis et al. 2012). In this trial, rituximab was well tolerated and considered safe given that a trial of 200 severe refractory myositis patients with long systematic follow-up had one death of an elderly female with lung mass suspicious of malignancy followed by stroke and severe hemiparesis. There were only 26 drug-related serious adverse events, mostly infections that were treated [pneumonia ($n = 6$), cellulitis ($n = 6$), urosepsis ($n = 2$), herpes zoster ($n = 2$) and one each of septic arthritis, histoplasmosis, urinary tract infection] and some other serious adverse events like one case each of respiratory failure, heart failure, dysrhythmia, venous thrombosis, syncope, rash, and neurologic symptoms. There was no difference in serious or non-serious adverse events observed at week 8, the randomized placebo controlled time-point, between the placebo treatment and rituximab treatment. There has been no case of PML reported in myositis patient receiving rituximab in several case series and in the RIM trial.

6.8 *Predictor of Response in Rituximab Treated Myositis Patients*

In a follow-up study of RIM trial, presence of myositis-associated autoantibodies (MAS) was the strongest predictor of improvement in rituximab treated myositis patients (Aggarwal et al. 2012). Presence of anti-Syn (primarily anti-Jo-1 antibody)

and anti-Mi-2 were strongly related to higher chances of achieving improvement (two to three times as compared to no autoantibodies, $p < 0.002$), while no autoantibody group was associated with the lower chances of improvement. Patients with other autoantibodies combined had higher chances of improvement (hazard ratio of 1.386) although not statistically different from that for patients with no autoantibodies ($p = 0.14$). There were no meaningful differences among subgroups of patients with other autoantibodies: Anti-SRP, Anti- TIF1-γ, Anti-MJ, other and undefined ($p = 0.74$). Similarly, lower levels of global damage and JDM subtype (as compared to the adult subtypes) were strongly associated with better chances of improvement early on, with effects disappearing with time. Similarly, lower muscle damage and absence of muscle atrophy predicted better outcome univariately but were not significant in final multivariate model, as global damage explained their effect adequately and was a stronger predictor.

7 Conclusions

Many myositis patients have refractory and progressive course leading high morbidity and mortality despite aggressive regimen of corticosteroids and other conventional immunosuppressive agents. Biological therapies like rituximab provide a novel therapeutic option and might be a better target of disease pathogenesis. Rituximab appears to be effective in severe and refractory myositis in uncontrolled studies and over 80 % of myositis patients improved in a large myositis randomized controlled trial completed despite the fact that primary and secondary end points in this trial were not met. Overall, studies on rituximab in myositis show significant improvement in muscle strength even in most severe subtypes like anti-SRP antibody positive patients; however, effect on cutaneous manifestation is modest and needs further study. Different studies used different dosing regimen of rituximab making it difficult to conclude an optimal dosing regime should be used in the future. Moreover, the effect of rituximab in some patients appears to be short-lived, requiring re-treatment when B-cells return. The drug appears to be safe and well tolerated in myositis patients. In the era of biologic drugs for the autoimmune disease there will be more agents like rituximab that will be studied in myositis in the near future and hopefully improve the outcomes of our patients.

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B-Cell Targeted Therapies in Cryoglobulinemia

Salvatore De Vita

Abstract Mixed cryoglobulinemia, or cryoglobulinemic syndrome or vasculitis (CV) is a systemic vasculitis prevalently mediated by immune-complexes, associated with hepatitis C virus infection in 80–90 % of cases, and with non-neoplastic B-cell lymphoproliferation.

The treatment of CV remains a challenge and requires the optimal integration, in the individual case, of clinical expertise with the knowledge of disease biology, based on the close relationship between chronic infection, autoimmunity, and lymphoproliferation in this disease. The issue of B-cell depletion in CV must be then analyzed in the context of these three concomitant biologic events linked one to each other, i.e., chronic infection, autoimmunity, and B-cell lymphoproliferation. Since rituximab is used for B-cell depletion in CV, this drug will be herein discussed.

1 Introduction

Mixed cryoglobulinemia, or cryoglobulinemic syndrome or vasculitis (CV) is a systemic vasculitis prevalently mediated by immune-complexes, associated with hepatitis C virus (HCV) infection in 80–90 % of cases, and with non-neoplastic B-cell lymphoproliferation (Meltzer and Franklin 1966; Ferri et al. 1991; De Vita et al. 2000, 2011).

The treatment of CV remains a challenge and requires the optimal integration, in the individual case, of clinical expertise with the knowledge of disease biology, based on the close relationship between chronic infection, autoimmunity, and lymphoproliferation in this disease (Della Rossa et al. 2009; De Vita et al. 2008).

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The issue of B-cell depletion in CV must be then analyzed in the context of these three concomitant biologic events linked one to each other, i.e., chronic infection, autoimmunity, and B-cell lymphoproliferation. Since rituximab is used for B-cell depletion in CV, this drug will be herein discussed.

As a first point, treatment should be tailored to the single patient with CV, taking into account the severity of the disease. Concomitantly, the previous history and therapies administered, including previous treatments for CV and for hepatitis HCV infection, as well as comorbidities, should be considered. For life-threatening and severe disease manifestations, the rapidity of a given treatment to be effective is of primary importance. Low-dose aspirin should be considered, whenever possible, to reduce the cardiovascular risk.

Secondly, in CV there is evidence of an antigen-driven proliferation of rheumatoid factor-positive B-cell clones leading to cryoglobulin production. Since rheumatoid factor-positive B-cells may be stimulated by immune-complexes containing quite different antigens (Carson et al. 1991), HCV infection might be crucial for the induction of CV. On the other hand, once the autoimmune diseases CV had developed, the persistence of viral infection may play a different pathogenetic role: it may still be relevant for disease persistence, but it may also be irrelevant if the process is totally autoimmune-oriented (De Vita et al. 2008). Thus, treatment options for HCV-associated CV could attack both the viral trigger, when detected, and the autoimmune downstream events (De Vita et al. 2008). This distinction is clinically relevant and supported by experiments in the animal (De Re et al. 2006), and this is crucial to define the importance of B-cell depletion in CV.

Overall, while biologic issues must be considered to optimize the treatment, it is the clinical picture that should be taken in primary consideration for treatment choices. Clinical issues have the absolute priority for treatment decisions in life-threatening and severe manifestations of CV. The question of whether treatment approaches should focus on either the infectious trigger or on downstream immune activation (mainly B-cell hyperactivation) is more important for the long-term management of the disease, when stabilized.

Thirdly, treatment decisions should be taken also thinking in the long term, whenever possible. This means that a particular induction therapy could be chosen if it may facilitate, rather than complicate, decisions to be taken subsequently, when the disease is less active. In this light, a sequential therapy starting with B-cell depletion as a monotherapy may offer substantial advantages over a combination therapy (i.e., B-cell depletion plus antiviral therapy) given ab initio in severe CV.

Fourth, the background of the physician proves also relevant in determining the final treatment decision. A better integration of specialists is needed. The rheumatologist is usually more dedicated to severe cases of CV and has a larger experience with B-cell depletion and with immunosuppressive treatments in general, supported by the experience in treating similar manifestations in other autoimmune diseases. Hepatologists, gastroenterologists, and infectivologists may have larger experience with antiviral therapy and with less severe CV cases. The experience of internal medicine and hematology specialists may be more variable and based on their personal interest. Interestingly, in a recent survey, infectious disease specialists,

gastroenterologists, and hepatologists resulted more prone to use antiviral therapy than rheumatologists (Pietrogrande et al. 2011).

With regard to these issues, a recent consensus joined physicians from different medical specialities (Pietrogrande et al. 2011). In the final recommendations, the bias of single-center, single-speciality-oriented opinion was prevented. We herein will focus on B-cell depletion with RTX and on antiviral therapy based on the perspective of a rheumatologist after previous consensus discussion (Pietrogrande et al. 2011), while for the other treatments currently used in HCV-related CV, which may also act on B-cells (e.g., glucocorticoids, cyclophosphamide, plasmapheresis) a recent consensus is recommended (Pietrogrande et al. 2011). For what concerns B-cell depletion of HCV-unrelated CV, fewer data are available. Understanding the biologic bases of the underlying disorder is crucial to improve the treatment approach.

2 Treatment of Acute and Life-Threatening Conditions

Early diagnosis and prompt treatment of CV acute and life-threatening manifestations, such as abdominal vasculitis, hemorrhagic alveolitis, and complicated hyperviscosity syndrome, is mandatory.

If a very early diagnosis is not formulated, even in few hours and based only on clinical suspicion in selected cases, early treatment is impossible and further complications may occur, rendering subsequent therapy much more difficult. High dose corticosteroids and plasmapheresis represent the most rapid approaches currently available (Fig. 1). By contrast, antiviral therapy should be not considered as a priority in acute and very severe cases of CV. Cyclophosphamide may be used after plasmapheresis or associated with high dose steroids. Recently, RTX was used successfully in patients with CV and severe gatrointestinal vasculitis refractory to plasmapheresis and cyclophosphamide (Quartuccio et al. 2010). Overall, while RTX has not a role as monotherapy for very severe CV where a very rapid therapeutic effect is needed, its role in early association therapy deserves investigation.

A major problem in very severe CV is represented by the heavy immunosuppression, drug-induced, which may also antedate and the clinical setting. The risk of infections is high, and additional B-cell depletion further increases this risk. The early recognition, prolonged treatment and the prophylaxis, whenever possible, of infectious complications, is underscored.

Acute motor neuropathy and rapidly progressive glomerulonephritis in CV may deserve an aggressive approach, as described above. However, according to the clinical setting and rapidity of progression, induction may be very short, and B-cell depletion with RTX may be planned early in a fraction of cases, as reported below.

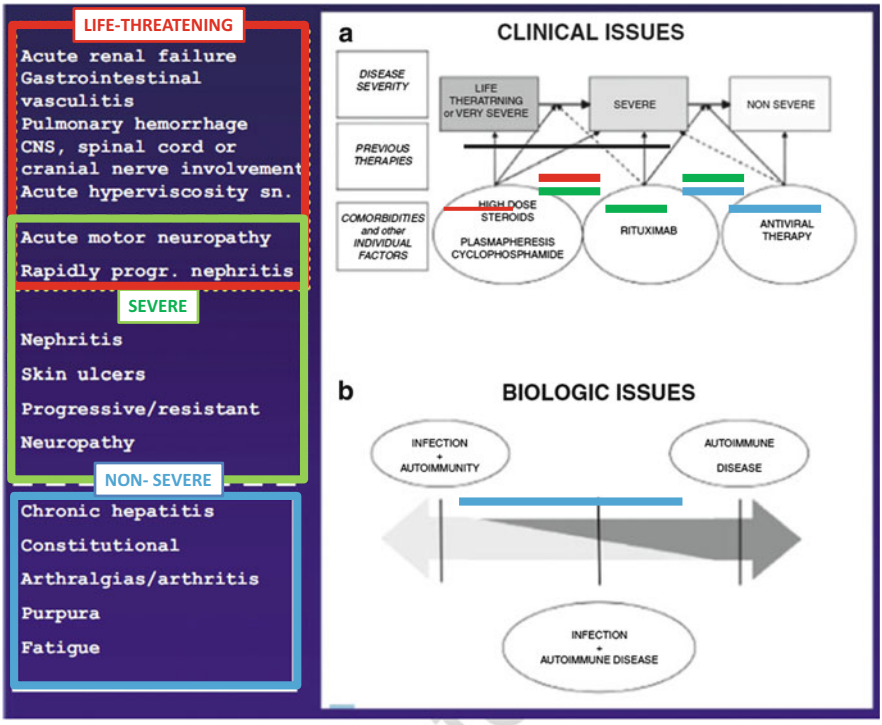

Fig. 1 Both clinical and biologic issues should be evaluated to decide the treatment of cryoglobulinemica vasculitis, but priority should be given to the clinical issues. Treatment should be tailored to the single patient taking into account disease severity, previous therapies, comorbidities, and other relevant individual factors (*panel A*). Biologic issues are also relevant (*panel B*), since infection and the downstream autoimmune response may play a different role in different disease stages. There may be a rationale for using antiviral monotherapy, monoterapy directed to the autoimmune and lymphoproliferative response, or the combination of the two

3 Treatment of Severe Manifestations

The more common and severe manifestations of CV include active glomerulonephritis, skin ulcers, and peripheral neuropathy (motor, or sensory refractory to symptomatic therapy, or evolving) (Tarantino et al. 1995; Ferri et al. 2004; De Vita et al. 2012a). In addition, CV is also severe in patients recovering from acute and life-threatening CV manifestations effectively managed, where additional, though less intense treatment may be advisable to stabilize the disease and to avoid the risk of hazardous relapses.

Rituximab often represents the best current option for severe CV (Fig. 1). Other approaches targeting the immune activation downstream to viral infection such as steroids, plasmapheresis, and cytotoxic drugs may also prove effective, but

rituximab appears superior based on recent open experience in different centers (Pietrogrande et al. 2011) and on the results of two recent, independent controlled studies (De Vita et al. 2012a; Sneller et al. 2012).

RTX is a monoclonal antibody against the CD20 antigen, which is selectively expressed on B-cells. CD20-positive cells are expanded and activated in CV, may harbor and present viral antigens, and play a crucial pathogenetic role in cryoglobulin production. The rationale underlying RTX treatment is to intervene downstream of the infectious trigger more selectively than with conventional immunosuppression. RTX has led to very encouraging results in open studies and single case reports (Pietrogrande et al. 2011; Ferri et al. 2010). Glomerulonephritis and skin ulcers usually respond within the first 1–3 months, but complete healing of skin ulcers requires a longer time. Both sensitive and motor neuropathy improve within 1–5 months, with stable electromyography.

A recent multicenter RCT involving 59 CV patients who had failed or were not eligible for antiviral therapy compared RTX monotherapy (at the dose recommended in rheumatoid arthritis, i.e., 1 g every two weeks for a total of two infusions, with or without low-dose steroids) with the best conventional immunosuppressive treatment (corticosteroids, cyclophosphamide, azathioprine or plasma exchange, as chosen by the clinician) (De Vita et al. 2012a). Importantly, the results of the trial supported the usefulness of RTX not only in the short term but also in the long term (24 months). Furthermore, RTX allowed steroid sparing, and the study regimen with low-dose or no steroids associated with RTX ab initio proved effective (De Vita et al. 2012a). The activity of RTX is supported by the restoration of some CV-related immune abnormalities (Saadoun et al. 2008) and the disappearance of bone marrow B-cell clonal expansion (Quartuccio et al. 2008).

The duration of the response to a single cycle of RTX may frequently lasts more than 1 year, but earlier relapses also occur. Retreatment with RTX after relapse has proved to be effective in most cases (Pietrogrande et al. 2011; De Vita et al. 2012a), and maintenance schedules may be advisable, at present, only when a relapse would be very hazardous (Quartuccio et al. 2010). The issue of maintenance treatment with RTX, however, remains open and surely deserves additional investigation. Of note, the issue was addressed in a recent trial in ANCA-associated vasculitides, where maintenance treatment with 1 g of RX every 6 months for 2 years greatly decreased the risk of disease relapse, in compared to no maintenance treatment (12 % vs. 73 %; $p < 0.01$) (Smith et al. 2012). In addition, in the same trial the RTX induction dose of 1 g every two weeks, for a total of two infusions, proved as effective and safe as the hematological induction dose of 375 mg/m^2 every week for 4 weeks.

Short-term reactions to RTX infusions do not seem to be more frequent in CV than in rheumatoid arthritis, systemic lupus erythematosus, or Sjögren's syndrome (Pietrogrande et al. 2011). Severe infectious complications are not increased in CV after RTX, but, as in the case of other biologics, these appear more frequent in patients heavily immunosuppressed by previous treatments, taking corticosteroids, or with hypogammaglobulinemia. Up to now, RTX did not worsen liver function also after retreatment during a follow-up of 2 years (Pietrogrande et al. 2011;

De Vita et al. 2012a) and has been recently given to CV patients with liver cirrhosis with improvement in both CV symptoms and in liver function (Petrarca et al. 2010).

By contrast, RTX may induce the severe reactivation of hepatitis B virus infection and then should be used in HbsAg-positive and in potential occult HBV carriers (HBsAg-negative/anti-HBc-positive) only when strictly needed and in combination with antiviral therapy (Pietrogrande et al. 2011).

Serum sickness has been rarely reported in CV after RTX (about 1 % in pooling data) (Pietrogrande et al. 2011). A French Group, on the other hand, reported a higher incidence of serum sickness (Sene et al. 2009). Therefore, patients should be carefully monitored, and pre-medication with 100 mg of methylprednisolone, antihistamine drugs, and paracetamol may reduce the risk. We and others never performed plasmapheresis before rituximab to reduce the risk of serum sickness, although this approach has been proposed (Sene et al. 2009). In patients with a history of heart failure or arrhythmia, the administration of half a dose per day on two consecutive days, and/or prolonging the administration of each infusion, may be considered.

Antiviral therapy is a cornerstone for the management of CV in HCV-related cases and has the strongest biologic rationale, in general, in this disease.

However, with regard to the issue of a possible etiologic therapy for severe CV, the lower rapidity of the antiviral approach does not support its priority. Thus, antiviral monotherapy should be not considered as a first step approach, in general, for severe cases (Fig. 1). The possible persistence or onset of CV features in patients despite serum HCV RNA negativization, although not frequent, should also be remembered (Quartuccio et al. 2006). This suggests that the autoimmune process can become independent of viral triggering or may play a dominant pathogenetic role at some disease stages. Antiviral therapy may also be counterindicated or not tolerated.

In sequential schedules RTX should then be given before antiviral therapy in severe CV (Fig. 1).

Whether antiviral therapy may be of value given ab initio in severe CV as a combination, i.e., directly in conjunction with immunosuppressive therapy, and more effectively than immunosuppressive therapy alone, remains to be demonstrated. The combination might show some synergistic effects, but might as well prove unadvisable for safety and subsequent treatment decision issues.

The combination of antiviral and rituximab has recently been employed by French and Italian authors (Saadoun et al. 2010; Dammacco et al. 2010), showing a superiority to antiviral therapy alone, as expected. In the French study (Saadoun et al. 2010) the combination therapy reduced the time to clinical remission, improved renal response rates (but not those of other organic manifestations), and led to higher rates of cryoglobulin clearance and clonal VH1-69+ B-cell suppression than the monotherapy with PegIFN plus ribavirin. In the Italian study (Dammacco et al. 2010), a higher rate of complete response was achieved with the combination (12/22 cases: 54.5 %) than with antiviral monotherapy (vs. 5/15 cases, 33.3 %; $p < 0.05$).

No study was designed however, up to now, to define whether and when the combination of antivirals plus rituximab, given ab initio, is superior to rituximab monotherapy in terms of both efficacy and safety. Furthermore, starting directly with the combination regimen does not allow to distinguish between the efficacy and safety of the single regimen, while this is crucial for subsequent, long-term treatment choices. A sequential rather than a combined approach as a first step, with RTX monotherapy having the priority, appears then rationale. This also considering that the beneficial effects of RTX monotherapy can be usually detected rather early, i.e., within 2 months (De Vita et al. 2012a; Zaja et al. 2003). Thus, we suggest RTX monotherapy followed by evaluation. Antiviral therapy can be added to rituximab or may substitute rituximab, as a second step, for instance when no improvement is observed within 2 months. The combination of rituximab plus antiviral therapy deserve attention in future studies, and may eventually prove the treatment of choice ab initio in some cases, however to be defined (Fig. 1).

A more recent issue is the definition of predictors of response to RTX in CV. Recently, the FF haplotype of the Fcgamma IIIa receptor, related to a decreased response to RTX in lymphoid malignancies, lupus erythematosus and rheumatoid arthritis, was also linked to a decreased response to RTX in CV (Zaja et al. 2003; Ghany et al. 2009). However, how the response is evaluated may greatly change the results, and this was not defined in the only published paper (e.g., partial vs. complete, and how this is evaluated; response in the disease manifestation for whom RTX was administered vs response in different manifestations). Unpublished observations by our group, by using published criteria for response (De Vita et al. 2012a), confirm that the Fcgamma IIIa FF polymorphism is linked to a lower frequency of response to RTX in CV only when a complete response is considered, but not in the case of a partial response. Furthermore, we recently reported that also the pattern of B-cell clonal expansion in the bone marrow of CV patients may predict response to RTX. A significantly better response to RTX was associated with a clonal pattern of B-cell expansion, if compared to a polyclonal B-cell pattern (Quartuccio et al. 2011).

4 Treatment of Non-Severe Manifestations

Many patients present non-severe CV manifestations, such as constitutional features, purpura, or arthritis. Other patients may show very mild renal and neurologic features. A high variability in the whole clinical picture may be observed, leading to very different treatment decisions. These also depend on either the chronicity or the frequency of relapsing manifestations, on the age and social activity of the patient, the concomitant liver disease, and comorbidities. Cases recovered from severe CV treated with rituximab or immunosuppressors, where a different treatment plan is needed for the long term, after an adequate period of disease stabilization, may also be considered as non-severe (Fig. 1).

In general, there is no indication to B-cell depletion with rituximab in this setting, while antiviral therapy may be crucial (Fig. 1). However, a maintenance

therapy with rituximab may be considered in selected CV patients with previous very severe or severe manifestations, as previously discussed (Quartuccio et al. 2010; Smith et al. 2012). The optimal duration of this maintenance therapy is however unknown and could be currently decided in the single case.

For what concerns antiviral therapy in non-severe CV, while it could be more obvious for younger patients and for those with active hepatitis, it has no place at all in other patients where it is contraindicated, where the best antiviral regimen already failed or was not tolerated in the past, and in the elderly patients with very mild or inconstant CV manifestations easily managed with symptomatic treatment. These situations represent the extremes of a spectrum of clinical pictures where treatment should be individualized.

Interestingly, IL28B genotyping (rs12979860 CC or rs8099917 TT) of the CV patient might be helpful to predict response to antiviral therapy, when difficult-to-treat HCV genotypes 1 and 4 are considered (Piluso et al. 2013).

On the other hand, the use of novel antiviral agents may lead to substantial side effects. A triple antiviral therapy with pegilated interferon plus ribavirin plus a recent protease inhibitor (either boceprevir or telaprevir) was used in 23 pts with CV, and in four of them RTX was also employed (Saadoun et al. 2013). While data on clinical response and HCV RNA negativization were encouraging, severe adverse events, mainly hematological and cutaneous, were frequently noticed. The large majority of patients needed eythropoietin, and about 40 % should be transfused. Additional antiviral drugs are being studied, and should be more effective and safer, likely changing in the future the current approach.

5 Treatment of HCV-Unrelated Cryoglobulinemic Vasculitis

B-cell depletion may be used in the same way in CV where an underlying viral infection by HCV is not detected. In general, rituximab should represent a good therapeutic option also for severe, HCV-unrelated CV (De Vita et al. 2012a; Terrier et al. 2012). Caution should be, however, paid to reduce or to avoid the concomitant glucocorticoid therapy as much as possible, since it represents a risk factor for infectious complications, especially in the elderly patients.

The concept that the definition of the pathogenetic events underlying each type of HCV-unrelated CV is, however, underscored. If CV is secondary to a lymphoma, then lymphoma is the key target. Sjögren's syndrome is very often encountered in HCV-unrelated CV (De Vita et al. 2011). If CV develops in Sjögren's syndrome, the comprehension of the biologic events leading to chronic inflammation of mucosa-associated lymphoid tissue (MALT) in this disease may also be helpful to understand and possibly to treat the associated CV. For instance, targeting additional factors implicated in MALT lymphoproliferation, such as the B lymphocyte stimulator (BlyS or BAFF), has a strong rationale and thus could be explored in selected patients (De Vita et al. 2012c). Subjects with primary Sjögren's syndrome

and mixed cryoglobulinaemia, HCV-unrelated, were recently compared to HCV-related patients CV without Sjögren's syndrome (De Vita et al. 2012b). Lymphoproliferation of MALT appears as the biologic background of cryoglobulinaemia in Sjögren's syndrome, differently from HCV-related cryoglobulinaemia, where lymphoproliferation primarily occurs in the bone marrow and in the liver (De Vita et al. 2012b). Importantly, the presence of CV is a red flag for concomitant lymphoma or a high risk to develop lymphoma in Sjögren's syndrome (De Vita et al. 2012b).

6 Conclusions

Treatment of CV requires clinical expertise, knowledge of disease biology, and a multispecialistic approach with an open mind to novelties. It is the clinical picture, i.e., the degree of disease severity that mainly guides the choice. In addition, the choice should also reflect a strategy for the long term, whenever possible. B-cell depletion with RTX therapy is probably the greatest advance for the treatment of CV in the last few years. It has a primary role in severe CV, deserves further study in very severe CV as a possible induction therapy in conjunction with other faster approaches, and may be planned as a maintenance therapy in very selected patients. Importantly, the concomitant use of glucocorticoids could be minimized. A dosage of 1 year every two weeks (2 grams in total) appears effective and safe also in the long term up to now.

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B-Cell Targeted Therapies in Autoimmune Cytopenias and Thrombosis

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Abstract Ever since the advent of Rituximab and subsequently the emergence of other compounds targeting B cells, a cornucopia of medical applications have been found for this family of compounds. After their establishment as standard of care in many conditions such as rituximab in lymphoma and rheumatoid arthritis, they have been progressively found to aid in the treatment of many other conditions. This area constituted a fertile area of research in the past 12 years. Physicians have investigated the B-cell depleting agents use in cases of autoimmune hematologic cytopenias such as immune thrombocytopenia, Evans syndrome, cold and warm autoimmune hemolytic anemia, and other thrombophilic disorders such as the antiphospholipid syndrome and thrombocytopenic purpura. This chapter presents a historical perspective reviewing the various studies that have been published in this field. In addition, it offers a current assessment of the evidence regarding the use of B-cell depleting agents in the aforementioned conditions.

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1 Introduction

Ever since the advent of Rituximab and subsequently the emergence of other compounds targeting B cells, a cornucopia of medical applications have been found for this family of compounds. After their establishment as standard of care in many conditions such as rituximab in lymphoma, they have been progressively found to aid in the treatment of many other conditions. This area constituted a fertile area of research in the past 12 years (Zimmer et al. 2004). Physicians have investigated the B-cell depleting agents use in cases of autoimmune hematologic cytopenias such as ITP (immune thrombocytopenia), Evans syndrome, cold and warm autoimmune hemolytic anemia, and other thrombophilic disorders such as the antiphospholipid syndrome and TTP (thrombocytopenic purpura).

2 B-Cell Depleting Agents in Primary ITP

2.1 ITP at a Glance

Immune thrombocytopenia is an autoimmune disease of platelet destruction and subsequent thrombocytopenia. The disease has been witnessed to many changes all the way from understanding its pathophysiology to the evolution in its management options. Initially called idiopathic thrombocytopenic purpura and thus the acronym ITP, it went on to be named immune thrombocytopenic purpura, and more recently immune thrombocytopenia (Stasi et al. 1995). Changes in nomenclature emerged due to advances in understanding the disease. It was primarily thought of as a disease of peripheral platelet destruction of unknown origin. ITP's definition progressively evolved to include peripheral platelet destruction due to autoantibodies in addition to an inadequate production by megakaryocytes through disturbed level of erythropoietin (Neunert et al. 2011; Nugent et al. 2009). ITP is classified into primary and secondary. No test has been yet developed that can accurately pinpoint the diagnosis. The primary form is usually diagnosed by exclusion of a secondary form which is usually part of another disease process such as: antiphospholipid syndrome, systemic lupus erythematosus, a hematologic or non-hematologic malignancy, common variable immune deficiency or might be due to hepatitis C or Helicobacter pylori, cytomegalovirus, varicella zoster or human immunodeficiency virus infection or to a vaccination side effect (Cines et al. 2009). Secondary ITP accounts for around 20 % of the total number of patients diagnosed with immune thrombocytopenia (Cines et al. 2009). Of relevance to this chapter is the primary form since this is where B-cell depleting agents come into play.

2.2 Standard of Care and Available Treatments

The management of ITP varies between children and adults. This stems from the fact that children have a more acute disease with a higher tendency for spontaneous remission as opposed to adults (Neunert et al. 2011; Stasi et al. 1995).

2.2.1 Therapy in Children

Initial therapy in an emergency setting for symptomatic patients mainly consists of glucocorticoids, IVIG, or alternatively anti-D in select cases (non-splenectomized, Rh-positive patients). High dose dexamethasone can also be considered in patients who do not respond to the above-mentioned treatments or as an alternative to splenectomy in chronic ITP patients. Splenectomy is exclusively a second-line therapy however, should be delayed until 12 months after the diagnosis since patients might spontaneously remit within this period (Sailer et al. 2006).

2.2.2 Therapy in Adults

Treatment is usually administered in this population when patients are bleeding or are at increased risk of bleeding, such as premenopausal women or when they have coexistent risk factors, lifestyle, preference and pros versus cons of treatment (Stasi et al. 1995; Cohen et al. 2000; Daou et al. 2008). It has been proposed that a platelet count of $30 \times 10^9\ L^{-1}$ can be adopted as a threshold for treatment as it was shown to improve mortality in newly diagnosed ITP patients (Li et al. 2005; Neylon et al. 2003; Neunert et al. 2011). Glucocorticoids, IVIG, and anti-D have all been proposed as treatment options for ITP and are given alone or in combination based on the patients' tolerability of every treatment or the need for a quicker response in some cases (Nugent et al. 2009; Thota et al. 2012; Godeau et al. 1999; Newman et al. 2001; Zimmer et al. 2004). High dose dexamethasone has also been reported to be highly effective however, due to paucity of head to head studies with other treatments, it is not yet considered a standard of care (Cheng et al. 2003). Splenectomy, rituximab, and TPO agonists are all considered adequate second-line therapies (Provan et al. 2010; Arnold 2013; Neunert et al. 2011). Splenectomy is the option physicians have the most experience with not to mention the fact that it has the most studies to support it since it was the first and only second-line treatment option for a long time (Ghanima et al. 2012).

2.3 Rituximab in ITP

The first study exploring B depleting agents in ITP was reported in 2000. It was a retrospective one that included patients who had already been treated with steroids. It showed a 30 % response rate to rituximab when given at 375 mg/m^2 weekly for 4 weeks (as per the lymphoma protocol) (Saleh et al. 2000). The first prospective trial using rituximab in ITP was reported in 2001 (Stasi et al. 2001). This study included 25 patients who had chronic ITP and had failed prior treatments. All of the patients received the 375 mg/m^2 dose. The results from that trial showed an encouraging 52 % response rates in otherwise treatment refractory patients. With the minimal adverse event profile of this drug, it was found to be a safe alternative to more traditional treatments. A large systematic review exploring the efficacy of rituximab as a second-line treatment in patients older than 15 years of age with roughly half of them having undergone splenectomy found that treatment with rituximab gave a complete response rate (CR) of 46.3 % (95 % CI: 29.5–57.7) and a partial response rate (PR) of 24.0 % (95 % CI: 15.2–32.7). In that study PR and CR were defined as exceeding 50×10^9 cells/L and 150×10^9 cells/L, respectively (Arnold et al. 2007). This is different than the most recent ASH guidelines that define a CR as one that leads to a platelet count $\geq 100 \times 10^9$ cells/L without bleeding and a response as one that leads platelet count $\geq 30 \times 10^9$ cells/L accompanied with a greater than twofold increase of platelet count from baseline with absence of bleeding (Neunert et al. 2011). The median time to response and response duration in the above-mentioned review were 5.5 weeks and 10.5 months, respectively. A recent meta-analysis confirmed rituximab's efficacy in ITP revealing an overall response and complete response of 59.7 and 45.7 %, respectively (Barcellini and Zanella 2011). Previous studies have shown that the response rate does not vary whether rituximab is used before or after splenectomy or used after a previous trial of rituximab (Auger et al. 2012). It has also been shown that the 100 mg/m^2 dose can be as effective as the much higher ones used in the lymphoma protocol giving an overall response rate of 71 % (Stasi 2010). A recent systematic review recently published that included 18 observational studies with a total number 323 children with ITP concluded that CR for these patients was 39 % (CI: 30–49 %). The response rate was, however, 68 % (CI: 58–77 %). This is very similar to the response rates observed in adults; however, the definitions of CR ($\geq 100 \times 10^9$ cells/L) and response ($\geq 30 \times 10^9$ cells/L) differed from their adult counterparts (Liang et al. 2012). A multicenter prospective trial of chronic ITP patients, who are candidates for splenectomy and were treated with rituximab, has shown promising results. At 1 year, 40 % of patients had responded (95 % CI: 28–52 %). This group, however, decreased over time with only 6.7 % of the patients experiencing sustained response after a single course of rituximab using the lymphoma protocol. This proved that rituximab could be used to delay or prevent splenectomy (Godeau et al. 2008) A recent large meta-analysis exploring rituximab as an option prior to splenectomy demonstrated a 57 % overall response rate after rituximab treatment with younger patients responding the most (Auger et al. 2012).

Another study using rituximab earlier in the course of treatment of patients with immune thrombocytopenia evaluated 103 patients that were treatment naïve. It consisted of randomizing patients to either dexamethasone alone or to dexamethasone with concomitant rituximab (lymphoma protocol). Results showed a significantly better sustained response by 27 % in patients receiving the steroid and rituximab regiment; 63 % versus 36 % in the steroid only arm. The occurrence of side effects nonetheless (grade 3 and 4) was much more pronounced however in the combined treatment group (Zaja et al. 2010). A newer trial demonstrated that there was no significant difference in the composite outcome of reaching a platelet count less than 50×10^9 cells/L, significant bleeding or need for rescue treatment, between a group of untreated ITP patients receiving their standard treatment and another receiving additional adjuvant rituximab (Arnold et al. 2012). One prohibitive factor in the use of rituximab has been the elevated cost. On average, a splenectomy would cost $20,000 whereas thrombopoietin and rituximab would each cost $2,500–$4,500 per month, and $10,000–$50,000, respectively (Ghanima et al. 2012).

2.4 Proposed Mechanism of Action of Rituximab in ITP

Three mechanisms of action of rituximab have been proposed. The first involves a decrease in macrophage phagocytosis and peripheral destruction of platelet coated with autoantibodies, when the latter are bound by rituximab. This would explain the early response (after 4 weeks) which occurs in the majority of cases after rituximab treatment. It is thought to be mediated by an inhibition of the Fc receptor portion of macrophages. The second mechanism involves B-cell depletion and accounts for the late response by decreasing the number of autoantibody producing B cells. The third mechanism is probably through T cell modulation effect of the drug since in some cases no correlation is observed between the disease severity and the antibody level (Stasi 2010).

2.5 Other B-Cell Depleting Agents in ITP

It seems that rituximab dominates the biologics field in ITP. A recent noteworthy trial has combined low rituximab with alemtuzumab yielded an impressive 100 % overall response rate and a complete response rate of 56 % (Gomez-Almaguer et al. 2010).

3 B-Cell Depleting Agents in TTP

3.1 TTP at a Glance

Thrombotic cytopenic purpura is a rare hematological disease that has an estimated annual incidence of 11.3 cases/1,000,000 people. It has been classically defined as patients presenting with the pentad of thrombocytopenia, uremia, microangiopathic hemolytic anemia, fever, and neurologic symptoms. This continues to be the classic teaching even though these symptoms are present all together in a minority of patients: 5 % of 64 patients from the Oklahoma registry of TTP patients (George 2010). Although TTP shares the same histological appearance with the hemolytic uremic syndrome, it is important to differentiate it from that latter since the management differs radically (Claus et al. 2010). It is considered to be an ancient disease that was first described as early as 1923 (Moschcowitz 2003). It has been proposed that it is due to aggregation of single von Willebrand factors units into a large multimer due to the absence of a metalloproteinase that usually breaks it down in order to keep the system in check (Galbusera et al. 1999). Later on, this metalloproteinase was discovered to be ADAMTS 13 (Zheng et al. 2001). The direct result of this abnormally large multimer is platelet aggregation, injury to RBCs, thrombosis, and subsequent end organ damage. ADAMTS 13's deficiency was considered the culprit of the disease up until recently when some TTP patients were discovered not to have a deficiency in this metalloproteinase (Kremer Hovinga et al. 2010; Vesely et al. 2003). TTP has been classified as either congenital or acquired. Patients having the congenital form of the disease (Upshaw–Schulman Syndrome) constitute 5 % of people with TTP whereas the people with the acquired type make up the remaining 95 %. The acquired type is further subdivided into an autoimmune idiopathic subtype that makes up 70–80 % of those patients versus a secondary subtype that constitutes the rest (Rizzo et al. 2012). Some of the secondary causes that have been described are infections such as HIV, tumors, autoimmune diseases, pregnancy, and stem cell transplant recipients (Kremer Hovinga et al. 2010).

The diagnosis of the disease continues to be mainly a clinical one due to the absence of a specific lab test that could pinpoint the diagnosis. ADAMTS 13 as mentioned earlier maybe negative in a subset of patients due mainly to the variability in the testing methods however, when present, it offers supporting evidence of diagnosis (Shah and Sarode 2013; George 2010).

3.2 Standard of Care and Available Treatments

Plasma exchange is the sinequanone of treatment in TTP since untreated, this disease is usually fatal (Ghanima et al. 2012). It has been shown to be superior to plasma infusion and can reduce mortality to 20 % or less (Rock et al. 1991).

Nonetheless, plasma exchange is not without risk. With a death rate that can reach 3 % not to mention fatal arrests, hypotension, catheter-related complications, and venous thrombosis, plasmapheresis should be used with caution (Cohen et al. 2000; McMinn et al. 2003). In plasma exchange non-responders glucocorticoids and other immunosuppressive agents such as vincristine, cyclosporine, cyclophosphamide, and even splenectomy have been tried with varying degrees of success. In addition, a large numbers of initial responders (>30 %) relapsed at a later time (George 2000, 2010; Sadler et al. 2004).

3.3 Rituximab in TTP

The investigation of rituximab as a potential treatment for TTP started in 2002 after two women with refractory TTP were reported to respond after addition of rituximab to their plasma exchange therapy (Chemnitz et al. 2002). Many studies later on observed the same effect of rituximab in treating refractory TTP patients in conjunction with plasmapheresis. A recent review reviewing six studies each of which included five or more patients reported clinical remission in 97 % from a total of 67 patients treated with rituximab given as per lymphoma protocol (Stasi 2010). This result should be interpreted with caution, since due to the rarity of the disease, many of the studies included represented small case series with no adequate controls (Sallah et al. 2004; Reddy et al. 2005; Heidel et al. 2007; Ling et al. 2009). Another review that included 118 patients with either refractory of relapsing TTP treated with rituximab came to a conclusion that 85 % of patients achieved remission and considered rituximab to be a safe and efficient treatment option in this subset of patients (Caramazza et al. 2010). Rituximab has also been shown to be a good first-line treatment in conjunction with plasma exchange. It has been shown to decrease hospital stay by a mean of 7 days in non-ICU admitted patients. Moreover, it markedly decreased relapse to 10 % versus 56 % in controls (Scully et al. 2011). Rituximab has successfully been used as a preemptive maintenance therapy in patients with recurrent disease and has also been shown to be equally effective in patients with long-standing and recently diagnosed TTP (Herbei and Venugopal 2006; Stasi 2010).

It has been postulated that rituximab acts not only by depleting antibodies against ADAMTS 113 but also by decreasing cytokine production. This stems from the fact that patients with normal ADAMTS 13 levels still respond to the biologic therapy (Kameda et al. 2007; Reddy et al. 2005).

The regular lymphoma dose of Rituximab has been used in most studies however, some have used lower or more numerous dosing regiments with success (Newman et al. 2001; Kivity and Agmon-Levin 2011). Furthermore, some authors advise for performing plasma exchange 24 h after rituximab infusion whereas others recommend doing it after 72 h (Boctor and Smith 2006).

3.4 Other B-Cell Depleting Agents in TTP

No other B-cell depleting agent has been investigated in TTP, but given the success of rituximab, we should be expecting increasing interest in this field in the near future.

4 B-Cell Depleting Agents in Evan's Syndrome

4.1 Definition and Available Therapy

Evan's syndrome is defined as autoimmune hemolytic anemia coexisting with ITP. Patients usually suffer from intermittent exacerbations and remissions in their lifetime. Its diagnosis is usually confirmed by the direct antiglobulin test which is usually positive (Norton and Roberts 2006). Corticosteroids were historically found to be the cornerstone of treatment. This poses a challenge to the treating physician especially when considering corticosteroids side effects on the long term owing to the chronicity of the disease. Research tackling second-line treatments has been scarce in this field and relied mostly on immunosuppressant such as danazol, mycophenolate mofetil, cyclosporine, or splenectomy (Norton and Roberts 2006). We have recently witnessed an emergence of studies employing rituximab as a second-line or even first-line treatment (Barcellini and Zanella 2011).

4.2 Rituximab in Evan's Syndrome

Early reports have shown encouraging results. A response rate of 83 % was first reported which subsequently increased to 94 % in new reports (Norton and Roberts 2006; Barcellini and Zanella 2011). The problem with those numbers however is that they are based on case reports and case series with no adequate controls not to mention the eventual publication bias that predominates such studies. Most of the studies deal with relapsed patients with Evan's syndrome but dosing has been highly variable between different patients. A recent retrospective study looked at the charts of 11 patients having received rituximab, seven for refractory ITP, three for relapsing hemolytic anemia, and one for refractory ITP and hemolytic anemia. An encouraging 82 % response rate was achieved with a 64 % long-term response rate after a 1 year mean follow-up (Michel et al. 2009).

Table 1 The updated (Sapporo) classification criteria for antiphospholipid antibody syndrome

Clinical criteria	Vascular thrombosis	≥1 clinical episodes of arterial, venous or small-vessel thrombosis in any tissue or organ
	Pregnancy morbidity (one of the following)	≥1 fetal death (at or beyond the 10th week of gestation)
		≥1 premature birth before the 34th week of gestation because of eclampsia, severe preeclampsia or placental insufficiency
		≥3 consecutive (pre) embryonic losses (before the 10th week of gestation)
Laboratory criteria	Lupus anticoagulant positivity on ≥2 occasions at least 12 weeks apart	
	Anticardiolipin antibody (IgG and/or IgM) in medium or high titer (i.e., >40, or above the 99th percentile), on two or more occasions at least 12 weeks apart	
	Anti-β_2-glycoprotein-1-antibody (IgG and/or IgM) in medium or high titer (i.e., above the 99th percentile) on two or more occasions at least 12 weeks apart	
Definite APS is present if at least one of the clinical criteria and one of the laboratory criteria are met		

5 B-Cell Depleting Agents in Antiphospholipid Syndrome

5.1 APS at a Glance

The antiphospholipid syndrome (APS) is an acquired autoimmune disease characterized by a hypercoagulable state that leads to arterial and/or venous thrombosis, recurrent pregnancy loss, and persistently positive antiphospholipid (aPL) antibodies, namely anticardiolipin (aCL), lupus anticoagulant (LA), and anti-β_2-glycoprotein I antibodies (anti-β2GPI). The latest classification criteria for diagnosing APS are the 2006 updated Sapporo criteria that require the presence of at least one clinical manifestation and one positive laboratory criteria (Table 1) (Miyakis et al. 2006).

In a small subset of APS patients, the disease can have an accelerated progression resulting in multiorgan failure, called "catastrophic" APS (CAPS). CAPS is characterized by multiple organ involvement with histopathologic evidence of small-vessel thrombosis developing over a very short period of time, in the presence of laboratory confirmation of aPL antibodies (Asherson et al. 2003).

In 2003, Hughes and Khamashta described another group of patients who present with clinical manifestations highly suggestive of APS but with persistently negative LA, aCL, and anti-β2GPI antibodies. This group was collectively referred to as seronegative APS (SNAPS) (Hughes and Khamashta 2003; Nayfe et al. 2013).

Taking into consideration the diverse clinical manifestations of APS, it is suggested that more than one pathological process may be involved. Despite this fact, the current therapeutic approaches are mostly restricted to anticoagulation therapy, which does not happen to benefit all patients (Pierangeli et al. 1995, 1999). Moreover, recurrent thrombotic events can occur in up to 30 % of APS patients (Gharavi et al. 1999), and 2–3 % might experience bleeding complications (Pierangeli et al. 1996). The best treatment for these APS patients who are

intolerant or resistant to long-term anticoagulation remains unclear. New pathogenic mechanisms in APS are under investigation by ongoing research, including aPL-induced activation of platelets, endothelial cells, monocytes, complement and coagulation cascade, leading to the discovery of potential targets and therapies for APS (Comarmond and Cacoub 2012). New data indicates a link between high titers of aPL antibodies and elevated circulating CD5+ B cells, suggesting that APS may be responsive to B cell targeted therapies (Youinou and Renaudineau 2004).

5.2 Pathogenesis of APS

The pathogenic mechanisms behind the clinical symptoms of APS are not fully understood, and many factors contribute in the aPL-induced manifestations of the disease (Comarmond and Cacoub 2012).

5.2.1 aPL Antibodies

aPL antibodies promote thrombus formation in both the venous and arterial circulation, and their thrombogenic properties have been shown in several in vitro and in vivo animal studies to be responsible for the pathogenesis of APS (Domenico Sebastiani et al. 2003; Dagenais et al. 1992; Zhou et al. 2011). Nonetheless, how these antibodies are produced and the exact mechanism by which they mediate thrombosis is not fully elucidated. aPLs comprise a heterogeneous family of autoantibodies; yet, similar HLA class alleles are identified to be consistent with APS patients, namely HLA-DR4, -DR7, and -DRw53 (Doring et al. 2010). aPL antibodies bind to their target cells (i.e., monocytes, platelets, endothelial cells, and trophoblasts) through a mediator plasma apolipoprotein called β2GPI, the main autoantigen for aPL antibodies. Consequently, up-regulation of tissue factor expression on monocytes and endothelial cells takes place leading to thrombosis and fetal loss through a series of signal transduction events (Romay-Penabad et al. 2007). Since aPLs play a central role in the pathogenesis of APS, special interest has been put on B cells, as they are the source of these pathogenic autoantibodies. Over the past 10 years, the role of autoreactive B cells in APS and the breakdown of B-cell tolerance have been extensively studied (Rand et al. 2008; Edwards et al. 1997). In addition to antibody production, B cells have other pathogenic mechanisms in APS, such as modifying their B-cell receptor specificity and acting as antigen presenting cells for self-antigens; besides differentiating into B effector cells (Be-1 and Be-2) which regulate helper T cells and their functions (Wallace 1994; Khattri et al. 2012).

5.3 *Immunomodulatory Approach: B-Cell Targeted Therapies*

Because accumulated data support the pathogenic role of B cells in the development and progression of APS, B-cell targeting therapies have been investigated in both human and murine APS.

5.3.1 B-Cell Depletion in APS

To date, literature review reveals only a limited number of case reports and series published regarding the use of rituximab in the treatment of APS. No randomized clinical trials were retrieved. Rituximab is a chimeric (murine/human) monoclonal antibody that targets CD20 on peripheral B cells, depleting them from the circulation and consequently decreasing disease activity (Willems et al. 2006; Youinou et al. 2009). Rituximab is FDA approved for the treatment of rheumatoid arthritis, B-cell non-Hodgkin's lymphoma (Higashida et al. 2005) and recently for anti-neutrophilic cytoplasmic antibody (ANCA)-associated vasculitis (Cohen Tervaert 2011). In addition to these indications, rituximab is being used as off-label treatment in a number of inflammatory and systemic autoimmune diseases (Butterly et al. 2010).

No reports on other B-cell directed therapies in APS patients were found.

Rituximab was used in the treatment of 27 reported cases of APS (primary, secondary, and APS with concomitant malignancies), including 18 females and 9 male patients, whose age ranged from 3 months to 69 years (Khattri et al. 2012). Of the 27 APS patients, four suffered from concomitant lymphomas; two had non-Hodgkin's lymphoma for which rituximab was administered as part of the R-CHOP chemotherapy regimen (Veneri et al. 2005; Erre et al. 2008), and two received rituximab for marginal zone lymphoma (Manner et al. 2008; Harner et al. 2004), one of which had Sjogren's syndrome also (Harner et al. 2004). Five patients had SLE with secondary APS, where rituximab was used after failure of treatment with anticoagulation and/or immunosuppression for lupus (Cianciulli et al. 2008; Weide et al. 2003; Tomietto et al. 2004; Ahn et al. 2005; Anandacoomarasamy et al. 2006). One patient had Evans syndrome (Ruckert et al. 2008). The rest of the patients had primary APS (Iglesias-Jimenez et al. 2010; Nageswara Rao et al. 2009; Tsagalis et al. 2010; Adamson et al. 2008; van Wissen et al. 2008; Chalam et al. 2007; Ames et al. 2007; Rubenstein et al. 2006; Trappe et al. 2006; Binstadt et al. 2003; Erdozain et al. 2004; Asherson et al. 2008; Sciascia et al. 2011). Table 2 summarizes the clinical and serological manifestations of the reported APS patients along with their outcomes after treatment with rituximab (Khattri et al. 2012). The rituximab dosing regimen used in the majority of the cases was 375 mg/m^2 body surface area, given weekly for 4 weeks. Four patients received rituximab 1,000 mg given 15 days apart. The reported patients were not treatment naïve prior to rituximab administration; most received anticoagulation unless

otherwise contraindicated, eight patients were treated with cyclophosphamide earlier (Ahn et al. 2005; Anandacoomarasamy et al. 2006; Iglesias-Jimenez et al. 2010; Ames et al. 2007; Rubenstein et al. 2006; Binstadt et al. 2003; Asherson et al. 2008), and all but one case (Tsagalis et al. 2010) used corticosteroids. The treatment regimen contained other immunosuppressants too, including azathiprine, mycophenolate mofetil, dapsone, and cyclosporine (Khattri et al. 2012). Improvement in the serological markers of APS was noticed in the majority of the cases, where decrease or normalization of LA, aCL, and anti-β2GPI antibodies was seen. Furthermore, multiple systemic clinical manifestations associated with APS improved after starting rituximab regardless whether the patient suffered from primary or secondary APS. There was a uniformly good response to rituximab in primary APS and in APS associated with SLE (Weide et al. 2003; Cianciulli et al. 2008; Tomietto et al. 2004; Ahn et al. 2005; Anandacoomarasamy et al. 2006; Ruckert et al. 2008; Tsagalis et al. 2010; Adamson et al. 2008; Chalam et al. 2007; Ames et al. 2007; Rubenstein et al. 2006; Trappe et al. 2006; Binstadt et al. 2003; Erdozain et al. 2004; Sciascia et al. 2011; Vianna et al. 1994; Danowski et al. 2009) or lymphoma (Erre et al. 2008; Veneri et al. 2005; Manner et al. 2008; Harner et al. 2004) in the case reports reviewed. Moreover, six out of seven CAPS patients benefited from rituximab treatment, knowing the severity and frequent fatality faced in this entity in spite of standard treatment with anticoagulants and immunosuppressant agents (Manner et al. 2008; Iglesias-Jimenez et al. 2010; Nageswara Rao et al. 2009; van Wissen et al. 2008; Rubenstein et al. 2006; Asherson et al. 2008).

In the BIOGEAS registry, a multicenter, national registry in Spain, rituximab was shown to have beneficial therapeutic effects in APS, with 92 % response rate in 12 APS patients (Ramos-Casals et al. 2008).

Despite the promising data from case reports and the BIOGEAS registry on the beneficial effect of rituximab in APS patients, the literature is still limited on this topic as there are no clinical trial data available yet. Moreover, the above-mentioned published case reports have several limitations including their small number, besides the fact that in all the cases, other immunosuppressants were used including steroids and cyclophosphamide, which creates confusion on whether it was rituximab-induced B-cell depletion by itself or the combination of immunosuppressants used that caused improvement in APS patients. Also, it is worth mentioning that the treated population was diverse including patients with primary or secondary APS or malignancy (Khattri et al. 2012). Finally, in a pilot open-label phase II trial aimed primarily to evaluate the safety of rituximab in aPL-positive patients with non-criteria manifestations of APS, and secondarily to evaluate the effect on the aPL profile and efficacy of treatment, it was suggested that rituximab may be effective in controlling some but not all non-criteria manifestations of APS with a safety profile in aPL-positive patients consistent with that of rituximab (Erkan et al. 2013).

Table 2 Review of the clinical and serological manifestations and outcomes in APS patients treated with rituximab

Clinical manifestations	Serological manifestations	Serological outcome	Clinical outcome
Venous thrombosis (Weide et al. 2003; Ahn et al. 2005; Anandacoomarasamy et al. 2006; Ruckert et al. 2008; Erre et al. 2008; Veneri et al. 2005; Manner et al. 2008; Harner et al. 2004; Iglesias-Jimenez et al. 2010; Nageswara Rao et al. 2009; Tsagalis et al. 2010; Adamson et al. 2008; van Wissen et al. 2008; Chalam et al. 2007; Ames et al. 2007; Rubenstein et al. 2006; Trappe et al. 2006)	aCL, LA, anti-β2GPI	Normalization of aCL, LA, anti-β2GPI	No new thrombotic events
Arterial thrombosis (van Wissen et al. 2008; Asherson et al. 2008; Ruckert et al. 2008)	aCL, LA	LA normalized	Minor self-limiting relapses (Asherson et al. 2008), clinical improvement in others
Hematological (thrombocytopenia, AIHA) (Ahn et al. 2005; Anandacoomarasamy et al. 2006; Ruckert et al. 2008; Erre et al. 2008; Manner et al. 2008; Iglesias-Jimenez et al. 2010; Tsagalis et al. 2010; van Wissen et al. 2008; Chalam et al. 2007; Ames et al. 2007; Rubenstein et al. 2006; Trappe et al. 2006; Erdozain et al. 2004; Asherson et al. 2008; Sciascia et al. 2011)	aCL, LA, anti-β2GPI	LA and anti-β 2GPI normalized, aCL decreased	No new bleeding episodes, thrombocytopenia improved
Neurological (seizures, chorea, cerebral vasculitis, CVA) (Tsagalis et al. 2010; Erdozain et al. 2004; Sciascia et al. 2011; Binstadt et al. 2003; Nageswara Rao et al. 2009; Weide et al. 2003; Tomietto et al. 2004)	aCL, LA, anti-β2GPI	aCL, LA, anti-β2GPI decreased	Seizures resolved

(continued)

Table 2 (continued)

Clinical manifestations	Serological manifestations	Serological outcome	Clinical outcome
Pulmonary (ARDS) (Asherson et al. 2008)	aCL		Restoration of lung function and discontinuation of respiratory support
Renal (acute renal failure) (Tsagalis et al. 2010; Asherson et al. 2008)	aCL	aCL normalized	Improvement in serum creatinine
Gastrointestinal (ischemic bowel, mesenteric and celiac artery occlusion) (van Wissen et al. 2008; Asherson et al. 2008)	aCL, LA		One patient died of sepsis (Asherson et al. 2008), the second had no further thromboembolic events (van Wissen et al. 2008)
Cardiovascular (right atrial thrombus, MI) (Rubenstein et al. 2006; Anandacoo-marasamy et al. 2006; Cianciulli et al. 2008)	aCL, LA		No further intra-cardiac thrombi formation
Adrenal (adrenal hemorrhage) (Nageswara Rao et al. 2009)	LA		Decrease in size of hemorrhage
Cutaneous (vasculitis, livedo, necrosis) (Ruckert et al. 2008; Iglesias-Jimenez et al. 2010; Asherson et al. 2008; Binstadt et al. 2003; Anandacoo-marasamy et al. 2006)	aCL, LA, anti-β2GPI	aCL and anti-β 2GPI normalized	Clinical improvement in skin involvement
Pregnancy loss (Tsagalis et al. 2010)	aCL	aCL normalized	No further pregnancy losses
CAPS cases (Manner et al. 2008; Iglesias-Jimenez et al. 2010; van Wissen et al. 2008; Rubenstein et al. 2006; Asherson et al. 2008; Nageswara Rao et al. 2009)	aCL, LA, anti-β2GPI	aCL, LA, anti-β2GPI normalized	All patients improved except for one death due to complications (sepsis, subdural hematoma) (Asherson et al. 2008) and one had minor self-limiting episodes associated with thrombocytopenia (Asherson et al. 2008)
Primary APS (Ruckert et al. 2008; Tsagalis et al. 2010; Chalam et al. 2007; Ames et al. 2007; Rubenstein et al. 2006; Trappe et al. 2006; Erdozain et al. 2004; Sciascia et al. 2011; Binstadt et al. 2003; Adamson et al. 2008)	aCL, LA, anti-β2GPI	aCL, LA, anti-β2GPI normalized	Improvement in thrombocytopenia, no further thrombotic events

(continued)

Table 2 (continued)

Clinical manifestations	Serological manifestations	Serological outcome	Clinical outcome
APS associated with SLE (Weide et al. 2003; Tomietto et al. 2004; Ahn et al. 2005; Cianciulli et al. 2008; Anandacoomarasamy et al. 2006)	aCL, LA, anti-β2GPI	aCL, LA, anti-β2GPI normalized	No new thrombotic events, seizures resolved
APS associated with lymphoma (Erre et al. 2008; Manner et al. 2008; Veneri et al. 2005; Harner et al. 2004)	aCL, LA, anti-β2GPI	aCL, LA, anti-β2GPI normalized	No new thrombotic events

aCL anticardiolipin antibody, *β2GPI* β2 glycoprotein 1, *LA* lupus anticoagulant, *CVA* cerebrovascular accident, *MI* myocardial infarction, *AIHA* autoimmune hemolytic anemia, *ARDS* acute respiratory distress syndrome

5.4 Conclusion

Rituximab, a chimeric anti-CD20 monoclonal antibody, is FDA approved for the treatment of rheumatoid arthritis, B-cell non-Hodgkin's lymphoma and ANCA-associated vasculitis. The off-label use of B-cell depleting agents in several systemic autoimmune diseases has been studied. Data on the use of rituximab in the treatment of APS is limited to case reports, the BIOGEAS registry and a pilot open-label phase II trial, and suggests a beneficial role in the therapeutic approach of APS. However, well-designed randomized clinical trials are needed to evaluate the use of rituximab, alone or in combination with other immunosuppressants, in improving the clinical and serological manifestations of the disease.

6 B-Cell Depleting Agents in Autoimmune Hemolytic Anemia

6.1 AIHA at a Glance

Autoimmune hemolytic anemia (AIHA) is an uncommon disorder characterized by autoantibodies directed against self red blood cells (RBCs) (Gehrs and Friedberg 2002). Consequently, the normal 100–120 days lifetime of the RBCs is reduced to just a few days in serious cases (Sawitsky and Ozaeta 1970). AIHA can be idiopathic or secondary to infections, other autoimmune conditions or lymphoproliferative disorders, and depending on the thermal range of the autoantibodies involved, the disease can be classified into warm, cold (which includes cold agglutinin disease and paroxysmal nocturnal hemoglobinuria) or mixed (Gehrs

and Friedberg 2002). Whether warm- or cold- type secondary AIHA, each can result from its own more common secondary causes. For instance, secondary warm-type AIHA mostly results from lymphoproliferative disorders (e.g., chronic lymphocytic leukemia, lymphoma) and other autoimmune disorders, including SLE, RA, scleroderma, and ulcerative colitis. Less commonly, it can be caused by neoplasms other than lymphoid and infection. Similarly, secondary cold-type AIHA is primarily caused by lymphoproliferative disorders, but also occurs secondary to infection, especially by mycoplasma, viral pneumonia, infectious mononucleosis, and other respiratory infections, and infrequently due to concomitant autoimmune disorders (Sokol et al. 1981).

6.2 Treatment of AIHA

6.2.1 Standard Treatment

In warm AIHA, the first-line therapy has been the administration of corticosteroids, where the response rate reaches 70–85 %, of which only one third remain in long-term remission after drug discontinuation, 50 % necessitate maintenance doses, and around 20–30 % require second-line therapies including immunosuppressants and splenectomy (Wahl et al. 2008). Splenectomy is probably the most effective second-line treatment with a response rate of 50 %, especially in relapsing patients on corticosteroids or those requiring the equivalent of 10–15 mg prednisone per day to maintain adequate hemoglobin levels. We have to bear in mind the surgical and infective complications, particularly gram-negative sepsis in patients above 65 (Gehrs and Friedberg 2002; Valent and Lechner 2008; Newland et al. 2005). Moreover, patients who are unresponsive to or do not fit for splenectomy, have limited options including cytotoxic or immunosuppressive medications such as azathioprine, cyclophosphamide, or cyclosporine, with a response rate of 40–60 % and associated side effects (52). On the other hand, cold-type AIHA has failed to demonstrate a convincing response to standard therapy, particularly cold hemaglutinin disease (Barcellini and Zanella 2011).

6.2.2 Rituximab: Treatment of Cases Refractory to Standard Therapy

In a recent review carried by Garvey et al. collecting data from studies using rituximab in the treatment of AIHA, rituximab (375 mg/m^2 given weekly for 4 weeks) was found to be effective in treating both warm AIHA and cold hemaglutinin disease, with a median response rate of 60 % and lasting responses for more than 3 years (Garvey 2008). Furthermore, in three more recent studies (Dierickx et al. 2009; Bussone et al. 2009; Penalver et al. 2010), the response rate was higher and ranged from 77 to 93 % with a disease-free survival at 1 and 2 years in 72 and 56 % of cases, respectively (Dierickx et al. 2009). In several case series,

rituximab has been shown to be effective in both patient groups with idiopathic and secondary AIHA, even in those associated with autoimmune and lymphoproliferative disorders, and bone marrow transplant (Shanafelt et al. 2003; Penalver et al. 2010; Quartier et al. 2001; Zecca et al. 2003; Narat et al. 2005; Berentsen et al. 2006; Schollkopf et al. 2006; D'Arena et al. 2007; Gupta et al. 2002; Trape et al. 2003; D'Arena et al. 2006). Moreover, rituximab proved to be effective whether used as a monotherapy or in combination with corticosteroids, immunosuppressants and interferon (Zecca et al. 2003; Narat et al. 2005; Berentsen et al. 2006; D'Arena et al. 2007; Gupta et al. 2002), and irrespective or prior therapy (Penalver et al. 2010; Shanafelt et al. 2003; Quartier et al. 2001; Zecca et al. 2003; Narat et al. 2005; Berentsen et al. 2006; Schollkopf et al. 2006; Gupta et al. 2002; Trape et al. 2003; D'Arena et al. 2006). Time to maximum response varied between the studies from quick response to weeks or even months, where in two recent studies, the median time to response was 3 and 6 weeks, respectively (Bussone et al. 2009; Penalver et al. 2010). It is noteworthy that re-treatment with rituximab is effective with both warm AIHA and cold hemaglutinin disease (Rao et al. 2008; Zecca et al. 2003; Berentsen et al. 2006; Gupta et al. 2002), with re-treatment benefiting some patients more than once (Zecca et al. 2003; Berentsen et al. 2006; Penalver et al. 2010). When comparing rituximab to the next best therapeutic regimen which includes alkylating agents with or without corticosteroids, rituximab was the only treatment able to induce a complete response in cold hemaglutinin disease with a response of 60 % (10 % complete response and 50 % partial response), compared with 16 % (all partial responses) (Berentsen et al. 2006; Berentsen et al. 2004; Barcellini and Zanella 2011). Regarding rituximab safety when given to patients with AIHA, the drug was well tolerated and no adverse events were reported in most cases, besides mild to moderate infusion-related side effects (e.g., fever, chills, hypotension, and upper airway edema) (Rao et al. 2008; Zecca et al. 2003; Schollkopf et al. 2006; Gupta et al. 2002; Trape et al. 2003). Some patients (around 7 %) experienced possible rituximab-related infections (Quartier et al. 2001; Zecca et al. 2003; Narat et al. 2005; Trape et al. 2003; Schollkopf et al. 2006), and few (roughly 2 %) had grade 4 neutropenia (Berentsen et al. 2006; Gupta et al. 2002). Moreover, low dose rituximab (100 mg $\times$ 4 weeks) was tried in patients unresponsive to standard therapy in an attempt to reduce side effects and costs, and it was found effective as a monotherapy (Provan et al. 2007) and in combination with alemtuzumab (Gomez-Almaguer et al. 2010). In a study conducted by Barcellini et al, low dose rituximab along with standard oral prednisone was able to induce an overall response rate of 86 % (complete 67 % and partial 19 %) in 21 patients with warm and cold AIHA; response was sustained (Hb $>$10 g/dl) in 13/14 patients at 6 months and in 11/11 evaluable patients at 1 year (BARCELLINI et al. 2010).

6.3 Conclusion

Rituximab proved to be an effective therapeutic alternative to standard therapy of splenectomy and/or chemotherapy in patients with primary or secondary warm AIHA with a significant response rate and sustained remissions. Furthermore, rituximab induced durable responses in patients with cold hemaglutinin disease, once a disease with very limited therapeutic options (Barcellini and Zanella 2011).

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Targeting B Cells in Neurological Autoimmune Diseases

Maria Sepúlveda, Albert Saiz, and Francesc Graus

Abstract As B cells are thought to play an active role in the pathophysiology of a large number of neurological disorders through the secretion of antibodies, the activation-dependent release of cytokines and the mutual activation of T cells, B-cell-directed therapy has emerged as a promising tool in the therapeutic strategies of a range of autoimmune neurological diseases, such as multiple sclerosis (MS), neuromyelitis optica, autoimmune encephalitis, chronic inflammatory demyelinating polineuropathies, myasthenia gravis, and Lambert–Eaton syndrome. Rituximab, an anti-CD20 monoclonal antibody, has been the most frequently reported therapy in this context. However, evidence of efficacy is limited to case reports or short small series, most of them retrospective, and with a few exceptions, e.g. MS, randomized controlled trials are lacking. In this article, we review and discuss the available literature on B cell-targeted therapies in autoimmune neurologic diseases.

1 Introduction

Over the past few years, the benefit of therapies targeting B cells in patients with autoimmune diseases has led to increased interest in the role of B cells not only in autoimmune neurological disorders associated with the presence of autoantibodies but also in diseases that have been thought to be largely mediated by T cells. B cells and autoantibodies contribute to the pathophysiology of a large number of neurological disorders that affect the central nervous system, peripheral nerves, neuromuscular junction, and muscle. B cells act as effector cells through their secreted antibodies, the activation-dependent release of cytokines, and the mutual activation

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of T cells (Archelos et al. 2000; Dalakas 2008). Although the presence of autoantibodies produced from plasma cells, the end products of B-cell differentiation, has been linked historically to the idea of a pathogenic role of B cells, the latter has only been demonstrated in a few neurological diseases such as myasthenia gravis, Lambert–Eaton syndrome, and certain neuropathies (Moscato et al. 2010). A prerequisite to mediate the antibodies a pathological effect is the recognition of the native protein at the membrane surface. The binding of the antibody causes structural and/or functional alterations of the target antigen, or initiates an acute inflammatory cascade by complement activation on the targeted tissues. On the other hand, antibodies and immune complexes can also produce tissue damage activating Fc receptors on macrophages, neutrophils, and NK cells and inducing an antibody-dependent cell-mediated cytotoxic process (Archelos et al. 2000; Dalakas 2008). However, in most of the autoimmune neurologic diseases the autoantibodies are directed against intracellular target antigens and the process appears to be mediated by cytotoxic T-cell mechanisms. The presence of autoantibodies in these cases indicates the involvement of B cells in the autoimmune process and serves in most instances as useful marker of the disease (Moscato et al. 2010; Graus et al. 2010). In such cases, the contribution of B cells appears to be mediated by antibody-independent mechanisms including antigen presentation that leads to clonal expansion of cytotoxic T cells and cytokine production. Activated B cells produce proinflammatory cytokines, IL-4, IL-6, IL-10, IL-12, IL-16, IL-23, tumor necrosis factor alfa and interferon gamma, which results in the activation of macrophages and dysfunction of other immunoregulatory cells, including T cells. In this context, the costimulation due to B-cell–T-cell interactions perpetuates or enhances the immune response (Dalakas 2008). Altogether, the advances in the understanding of B cells participation at multiple levels of the immune response provide a strong rationale for B cell-targeted therapies in autoimmune neurologic diseases.

2 B Cell-Targeted Therapies in Neurological Diseases

2.1 Central Nervous System Autoimmune Disorders

2.1.1 Demyelinating Diseases

Multiple Sclerosis

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the CNS and represents one of the main causes of neurological disability in young adults. Although MS is not generally a fatal disease, the social impact of the disability caused by MS is substantial (Boster et al. 2010). The majority of MS patients suffer a disease characterized by relapses followed by partial or complete

periods of remission (relapsing–remitting MS, RRMS). After several years, this clinical presentation is continued by a progressive phase (secondary progressive MS, SPMS). In some patients the relapsing phase is missed and the disease is progressive from the onset without interim clinical improvement (primary progressive MS, PPMS) (Lassman et al. 2007; Bartok and Silverman 2011). Although the exact etiology is unknown, available evidence points to a multifactorial origin that includes genetic predisposition and environmental factors (Lassman et al. 2007).

Hypotheses of MS pathogenesis have been dominated by T-cell-mediated immunity as a result from pathological studies and accumulate data from animal models of experimental autoimmune encephalomyelitis (Lassman et al. 2007). Evidence for an important role of B cells and autoantibodies in the pathogenesis of MS, however, has been accumulating for the past few years. Oligoclonal IgG bands in the cerebrospinal fluid (CSF) are present in almost 90 % of MS patients, indicating an intrathecal synthesis of immunoglobulins. The presence of B cells, plasma cells, complement and myelin-specific antibodies in chronic and active MS lesions (Lassman et al. 2007; Cross and Waubant 2011), as well as memory B cells and plasma cells in the CSF of patients with MS, suggests an antigen-driven humoral immune response in the CNS in MS. Memory B cells, which cross the blood–brain barrier, are believed to undergo restimulation, antigen-driven affinity maturation, clonal expansion, and differentiation into antibody-secreting plasma cells in the CNS. Although multiple candidate antigens have been identified in different studies, the presence and role of the autoantibodies against these antigens in the disease process are still lacking and in several cases their involvement is not specific to MS (Racke 2008; Fraussen et al. 2009). In addition, B-cell follicle-like structures have been described in the brain meninges of patients with MS, especially with secondary progressive form of the disease (Serafini et al. 2004). The presence of such follicles indicates that a microenvironment promoting B-cell expansion, maturation, and autoantibody production can exist within the CNS (Franciotta et al. 2008). The proximity of these ectopic follicles to large demyelinated subpial lesions supports the notion that these structures are important for the development of the cortical damage observed in these patients, likely by the release of soluble factors (Magliozzi et al. 2007). The beneficial effect of plasma exchange that affects antibody clearance (Llufriu et al. 2009; Weinshenker et al. 1999) and the positive results of several trials with B-cell depleting therapies provide further support for the participation of B cells in the MS pathogenesis.

Rituximab

Rituximab (RTX), a human/murine chimeric monoclonal antibody initially approved for the treatment of non-Hodgkin B-cell lymphomas, was the first to be used in MS. RTX binds to the CD20 antigen, a transmembrane phosphoprotein expressed on a broad range of cells of the human B-cell lineage, from pre-B through naïve and memory B cells. Stem cells, pro-B cells, and fully differentiated plasma cells are CD20 negative (Bar-Or et al. 2008). A combination of cell-mediated and complement-dependent cytotoxicity and possibly the induction of apoptosis have

been postulated as the mechanisms through RTX to efficiently achieve the depletion of circulating B-cell populations (Kitsos et al. 2012). RTX induces a near complete depletion of CD20+ peripheral B cells by week 2, and the depletion is sustained for 6–8 months. Up to 40 % of the treated patients recover peripheral B cells after 48 weeks from their last dose (Hawker et al. 2009), and most of them are naïve (CD27-) rather than memory (CD27+) B cells (Bar-Or et al. 2008). Functional studies have shown that B cells reconstitution after RTX therapy produces higher levels of IL-10 and lower levels of proinflammatory cytokines including Lymphotoxin beta and TNF alfa (Bar-Or et al. 2008). As expected, total circulating immunoglobulin (Ig) levels in the periphery do not significantly change after a single treatment course and the CSF IgG index, IgG concentration, and oligoclonal bands remain unchanged. In the CSF, RTX is detectable after IV administration for up to 24 weeks (Petereit et al. 2008) and causes not only an almost complete depletion of B cells but also reduction in the percentage of T cells (Cross et al. 2006) and in the levels of B-cell chemokines such as CXCL13 and CCL19 (Bartok and Silverman 2011). The correlation between levels of CXCL13 and the decline of total T cells in CSF suggests that recruitment of disease-associated T cells would be directly linked to B-cell recruitment into CSF of MS patients and supports the idea that B cells are critical for T-cell trafficking into the CNS (Bartok and Silverman 2011).

Clinical Trials in Relapsing-Remitting MS A phase I, open-label, multicenter study of 26 patients with active RRMS was conducted over a 72-week period with 1 g of RTX on days 1 and 15 and repeated courses on weeks 24 and 26. The primary endpoint was safety and secondary endpoints included clinical and MRI parameters. Over the 72 weeks, 21 (80.8 %) patients remained relapse free, and patients experienced fewer new gadolinium-enhancing (Gd-enhancing) or T2 hyper-intense brain MRI lesions as compared to the year before therapy (Bar-Or et al. 2008).

The HERMES study was a phase II, double-blind, 48-week trial involving 104 RRMS patients; 69 patients received 1 g of RTX and 35 placebo on days 1 and 15. The primary endpoint was the total number of Gd-enhancing lesions. Clinical outcomes included safety, and the annualized rate of relapse. Patients treated with RTX had less Gd-enhancing lesions and new Gd-enhancing lesions at weeks 12, 16, 20, and 24, and the results were sustained for 48 weeks ($p < 0.001$). The proportion of patients with relapses was significantly lower in the RTX group at weeks 24 (14.5 % vs 34.3 %) and 48 (20.3 % vs 40 %) compared to placebo (Hauser et al. 2008).

A phase II unblinded clinical trial but with blinded radiologic endpoints of 30 patients with RRMS and inadequate response to standard disease-modifying therapies (interferon beta or glatiramer acetate) was conducted to evaluate the efficacy, safety, and tolerability of add-on therapy with RTX. Patients received RTX administered at 375 mg/m^2 weekly $\times$ 4 doses. Gd-enhancing lesions were significantly reduced after treatment with RTX. Up to 74 % of post-treatment MRI scans were free of Gd-enhancing activity compared with 26 % at baseline. Expanded Disability Status Scale (EDSS) score remained stable 32 weeks after

treatment. The individual reduction in enhancing lesions was not predicted by the reduction of CSF B-cells or T-cells after treatment. This study provided Class III evidence that add-on RTX reduces Gd-enhancing brain lesions in MS (Naismith et al. 2010).

Clinical Trial in Primary Progressive MS The OLYMPUS study was a phase II/III randomized, double-blind, multicenter placebo-controlled trial to assess the efficacy of RTX in PPMS using time to confirmed disease progression (CDP) as primary endpoint. In the study, 439 patients received two infusions of RTX (1 g) or placebo every 24 weeks through 96 weeks. The primary endpoint was not met in this study because time to CDP between groups was not significant at completion of the 96-week study. RTX-patients treated had significant less increase in T2 lesion volume but brain volume change was similar to placebo. However, a subgroup analysis showed that time to CDP was delayed in RTX-treated patients who were <51 years and had Gd-enhancing lesions on MRI (Hawker et al. 2009). This data suggested that B-cell depletion could be beneficial in younger patients with evidence of acute blood–brain barrier breakdown on neuroimaging and it was the basis for initiating a trial with ocrelizumab in PPMS (see "Clinical Trial in Primary Progressive MS" in ocrelizumab section).

Clinical Trial in Secondary Progressive MS Clinical benefit from RTX administration in SPMS has also been reported in three patients who were treated for at least 15 months. In all of them, clinical progression subsided and no new or Gd-enhancing lesions were detected after initiation of RTX (Rommer et al. 2011). A recent double-blind, phase I/II trial evaluates RTX vs placebo in patients with "low-inflammatory" SPMS using a combination of both intravenous and intrathecal injections administered the same day. The primary endpoint measure is progression of brain atrophy (ClinicalTrials.gov identifier: NCT01212094).

Safety and Adverse-Event Profile RTX has shown an attractive safety profile with no significant differences in the incidence of serious adverse events or infections at short-term compared with placebo. Moreover, the combination of RTX with standard injectable disease-modifying therapy was well tolerated, and there were no serious adverse events (Naismith et al. 2010). The most common side effects are infusion-associated events including fever, chills, flushing, itching, hypotension, and general flu-like symptoms (headache, fatigue, muscle weakness). Most are classified as mild or moderate, do not represent hypersensitivity or allergic responses, they tend to occur during the first infusion, and decrease both in frequency and in intensity with subsequent infusions. Infections-associated events are more frequent among patients treated with RTX than with placebo but most of them are mild to moderate severity, including nasopharyngitis, bronchitis, upper respiratory tract and urinary tract infection. In the OLIMPUS study there were three deaths, one of them in the RTX group due to a brainstem lesion and aspiration (Hawker et al. 2009). The incidence of human antichimeric antibodies (HACA) during treatment or follow-up period has been reported in these trials in a variable proportion, from 7 % in the PPMS study to 24.6 % in the RRMS study. However,

there was no apparent association between positivity for HACA and the type or severity of adverse events or the clinical efficacy; therefore, the biological relevance of their presence remains to be determined. So far, no cases of progressive multifocal leukoencephalopathy (PML) have been reported in patients with MS.

Considerations RTX is not officially approved for use in MS patients. Nonetheless, it is currently used off-label by some physicians for MS patients who have failed approved therapies. The optimal frequency of RTX administration and how long subjects should be retreated is unclear. Moreover, the risks of long-term B-cell depletion in MS patients are unknown. Whether RTX itself or in combination with current disease modifying therapy increases risk of PML or other opportunistic infection in the MS population is unknown. Nevertheless, the RTX development program in MS was stopped and no phase III is ongoing.

Ocrelizumab

Ocrelizumab (OCR) is a humanized monoclonal antibody designed to selectively target CD20 B cells. Compared with RTX, OCR binds to a different but overlapping epitope of the extracellular domain of CD20 and is associated with increased antibody-dependent cell-mediated cytotoxicity (ADCC) and reduced complement-dependent cytotoxic effects in vitro (Lulu and Waubant 2013). By increasing ADCC, OCR might modulate tissue-dependent mechanisms of pathogenic response more effectively than does RTX. As a humanized molecule it is expected to be less immunogenic with repeated infusions and to have a more favorable benefit-risk profile than RTX.

Clinical Trial in Relapsing-Remitting MS OCR was evaluated in a 48-week, randomized, placebo-controlled and active comparator, international multicenter Phase II study that included 220 RRMS patients. Patients were randomized to receive either placebo, low dose (600 mg) or high-dose (2,000 mg) OCR in two doses on days 1 and 15, or intramuscular interferon beta-1a (IFNβ1a), 30 μg once a week. All groups were double blinded to group assignment, except for the IFNβ1a group who were rater masked. At week 24, patients in the initial placebo, 600 mg OCR and INFβ1a groups received OCR 600 mg, and the 2,000 mg group received 1,000 mg for another 24 weeks. The mean number of Gd-enhancing lesions was reduced by 89 % in the low-dose and 96 % with high-dose group compared to placebo. Annualized relapse rates over 24 weeks were 80 % lower in the 600 mg OCR group than in the placebo group and 73 % lower in the 2,000 mg group. From week 24 to week 48, the level of relapses in the OCR groups remained low. Placebo and IFNβ1a groups reached similar low disease activity after one treatment cycle with OCR. Change in total volume of T2 lesions was not significantly different between groups at week 24 (Kappos et al. 2011). Because the study did not provide data on progression of disability and MRI markers do not correlate reliably with clinical progression, the efficacy of OCR in long-term disability is uncertain.

Both doses of OCR were overall well tolerated. Most infusion-related events occurred during first infusion and were mild to moderate and decreased to rates

comparable to the placebo group in the second part of the dual infusion. The incidence of serious adverse events was similar in all groups (2 % low-dose, 6 % high-dose, 4 % placebo, and 4 % IFNβ1a treated groups). Serious infections occurred at similar rates in all groups, and no opportunistic infections were reported. However, a 41-year-old woman with a 10-year history of MS, previously treated with interferon, developed a systemic inflammatory reaction syndrome that resulted in multi-organ failure, brain edema, and death. The contribution of OCR cannot be excluded. By week 2 after injection, B-cell counts were nearly completely depleted for both OCR groups, which persisted until week 24. The number of patients who developed antibodies to OCR was similar in all groups, although baseline antibodies were not checked in the IFNβ1a group.

A phase III trial in RRMS patients is currently recruiting patients. Patients will be randomized in a double-blind (with respect to OCR dose) and rater-blind (versus active comparator), parallel group study to evaluate the efficacy and safety of OCR in comparison with high dose IFNβ1a. Patients will receive either OCR 600 mg or 400 mg IV every 24 weeks, or IFNβ1a 44 mcg subcutaneously three times weekly. The primary endpoint will be annualized relapse rate at 96 weeks (ClinicalTrials.gov Identifier: NCT01247324).

Clinical Trial in Primary Progressive MS The currently ongoing ORATORIO study is a 120-week, phase III, multicenter, randomized, double-blind, placebo-controlled trial to evaluate the efficacy and safety of OCR in patients with PPMS. This trial consists of five cycles of IV OCR 600 mg. Primary outcome measure is time to onset of sustained disability progression (ClinicalTrials.gov identifier: NCT01194570).

Considerations In Phase III trials for rheumatoid arthritis (RA) with OCR, high rates of serious and opportunistic infections were identified, and some of them resulted in death. As consequence, the RA clinical program was discontinued. However, it is important to bear in mind that the increased risk in RA trials was mainly seen in the group treated with 1,000 mg of OCR and in patients recruited in Asia. Moreover, all patients received concomitant methotrexate or leflunomide and half of them low-dose of corticosteroids, immunosuppressants that likely contributed to increased susceptibility to infectious adverse events. The minor rate of serious infections and the absence of opportunistic infections reported so far in MS patients could be explained by the lower morbidity of MS compared to RA patients, and the use of OCR as monotherapy in MS rather than add-on therapy. In addition, in phase III trials in patients with MS the dose of OCR has been reduced to mitigate safety concerns related to OCR 1,000 mg. Nevertheless, the long-term safety profile of OCR in MS has yet to be established.

Ofatumumab

Ofatumumab (OFA) is a fully human recombinant anti-CD20 antibody (IgG1k) which, compared to RTX and OCR, binds to a completely distinct epitope. OFA has different pharmacodynamic properties, appears to dissociate more slowly from the

CD20, exhibits pronounced complement-dependent cytotoxicity activity and relatively decreased antibody-dependent cell-mediated cytotoxicity (Gensicke et al. 2012). The drug was approved for treatment of patients with chronic lymphocytic leukemia refractory to fludarabine.

Results of a phase II, 24-week, multicenter, randomized, double-blind, placebo-controlled trial of OFA in patients with RRMS was communicated but awaits publication (Sorensen et al. 2010). Thirty-eight patients were randomized to receive two courses (weeks 0 and 2) of 100 mg (8 patients), 300 mg (11 patients) or 700 mg (7 patients) of OFA or placebo (12 patients) and were followed by monthly MRI for 24 weeks. The mean cumulative number of new Gd-enhancing lesions, total number of Gd-enhancing lesions, and new and/or enlarging T2 lesions were significantly lower in the combined OFA group than in the combined placebo-group. Peripheral CD19+ B cells were depleted with a mean count reduction of 78 % (100 mg), 95 % (300 mg) and 98 % (700 mg) at week 24. No dose limiting toxicities and no safety signals were reported. Similar to other anti-CD20 monoclonal antibodies, infusion-related reactions were frequent but the number and severity decreased after the first infusion. A full-size phase IIb study with RRMS patients treated for 48 weeks was planned (ClinicalTrials.gov identifier: NCT00640328), but the sponsor recently announced its intention to refocus the development of OFA in autoimmune indications to a subcutaneous delivery and to stop the development of the IV route of administration (Gensicke et al. 2012).

Atacicept

Atacicept is a human recombinant fusion protein that contains the extracellular ligand binding domain of the transmembrane activator and calcium modulator and cycophilin ligand interactor (TACI) receptor and a modified Fc portion of human IgG (Gross et al. 2001). TACI receptors bind two members of the TNF-α family: APRIL, a proliferation-inducing ligand, and BAFF, the B-cell activating factor of TNF family, also called BLyS (B-lymphocyte stimulator). Both APRIL and BAFF have been recognized as the lead players in B-cell survival and proliferation (Dillon et al. 2006). Atacicept neutralizes all forms of APRIL and BAFF inhibiting their effects on B-cell survival and function. Atacicept acts on mature B cells and plasma cells, but spares B-cell progenitors and memory cells, and therefore does not result in generalized depletion of B cells (Lulu and Waubant 2013). Atacicept demonstrated some efficacy in systemic lupus erythematosus and RA (Dall'Era et al. 2007; Tak et al. 2008). ATAMS was a phase II study to investigate efficacy, safety, and tolerability in RRMS, and ATON in patients with clinically isolated syndrome and optic neuritis (Plitz 2008; Sergott 2008). Despite a clear rationale for atacicept being effective in MS, the drug in fact led to an increase in inflammatory disease activity, and both trials were stopped early (Hartung 2009). The reasons underlying increased inflammatory activity in MS patients are not clear.

Neuromyelitis Optica

Neuromyelitis optica (NMO) is an inflammatory demyelinating disease of the CNS characterized by selective involvement of the optic nerves and spinal cord. Although the disease is over-represented in non-Caucasian populations, NMO has a worldwide distribution with an estimated prevalence of 1–2/100,000. The disease is up to nine times more frequent in women than in men, and the median age of onset is 39 years, but also occurs in children and elderly people (Wingerchuk et al. 2007). Almost 90 % of the patients have a relapsing course usually with incomplete recovery and increased disability due to frequent and severe attacks (Wingerchuk et al. 1999, 2007). More than 50 % of the patients are blind in one or both eyes or need ambulatory help within 5 years onset, and a 20 % of mortality is reported due to respiratory failure from cervical myelitis (Wingerchuk et al. 2007). Although NMO was considered a variant of MS for long time, a role for humoral immunity in its pathogenesis was suggested based on the presence of Ig and complement deposits in a vasculocentric pattern and the extensive macrophage infiltration associated with neutrophils and eosinophils in immunopathological studies (Lucchinetti et al. 2002). The hypothesis was strengthened by the identification of a highly sensitive (73 %) and specific serum autoantibody called NMO-IgG (Lennon et al. 2004) that bound to aquaporin-4 (AQP-4), a water channel highly expressed in the polarized foot processes of astrocytes at the blood–brain barrier (Lennon et al. 2005). The identification of NMO-IgG/AQP-4 antibodies led to expand the clinical spectrum of NMO to limited forms of the disease, i.e. recurrent longitudinally extensive transverse myelitis and recurrent optic neuritis, also known as NMO spectrun disorders (NMOsd), and to define a new set of diagnostic criteria (Wingerchuk et al. 2006). The pathogenic role of NMO-IgG/AQP-4 has been suggested in recent studies on animal models. The systemic injections of NMO-IgG from NMO patients into rats with experimental autoimmune encephalomyelitis (Kinoshita et al. 2009; Bennett et al. 2009; Bradl et al. 2009), or the intra-cerebral injection of NMO-IgG along with human complement into a mouse brain, reproduced the NMO-like pathological features (Saadoun et al. 2010). Altogether, suggesting that NMO lesions are initiated by the NMO-IgG binding to AQP-4 leading to complement-dependent destruction of astrocytes.

Although controlled clinical trials for NMO are still lacking, the available data support that immunosupressive therapies are more effective than immunomodulatory drugs.

Rituximab

The first report was an open-label study of eight patients treated with rituximab (RTX) and six of them remained relapse-free for 12 months (Cree et al. 2005). To date, over 100 NMO and NMOsd patients have been treated with RTX. Most patients were included in small retrospective case series (Jacob et al. 2008; Bedi et al. 2011; Lindsey et al. 2012; Ip et al. 2012), and a few in two prospective

uncontrolled studies (Kim et al. 2011; Pellkofer et al. 2011). Two RTX regimens were used in these studies, 375 mg/m^2 weekly × 4 doses or 1,000 mg administered 2 weeks apart, mostly used as a monotherapy with few exceptions (Jacob et al. 2008). Most patients were non-responders to a wide variety of immunotherapies, and a few were naive (Jacob et al. 2008; Bedi et al. 2011). The rate of NMO-IgG seropositivity ranged from 70 % (Jacob et al. 2008; Kim et al. 2011) to 100 % of the patients included (Pellkofer et al. 2011). The number of infusions was variable between studies and between patients from one dose (Cree et al. 2005) to up to five (Pellkofer et al. 2011; Kim et al. 2011).

Good results in reducing the attack frequency and increasing the proportion of patients relapse free have been reported in all but one study (Lindsey et al. 2012). For example, in one study with 25 patients and median follow-up of 19 months, the median annualized post-treatment relapse rate was significantly lower than the pre-treatment rate (0 vs 1.7) and the disability improved or stabilized in 20 of the 25 patients (Jacob et al. 2008). Similar results were observed in other study with 30 patients. The relapse rate was reduced by 88 %, 70 % of the patients became relapse-free over 24 months, and the disability improved or stabilized in 97 % of them (Kim et al. 2011). Finally, in the study with ten patients and up to five consecutive treatment courses, eight patients had a pronounced reduction in the relapse rate, and RTX was more effective than the respective previous treatment (Pellkofer et al. 2011). Despite the beneficial profile described for RTX, these studies results must be interpreted with caution. They are uncontrolled studies, and without an appropriate placebo-controlled group it is impossible to determine the contribution of regression to the mean in the decline of relapses and if changes in neurologic function were due to treatment. Moreover, most patients are from selected populations with treatment-refractory NMO. Interestingly, it seems that the rate of clinical response is similar between seropositive and seronegative NMO-IgG patients (Bedi et al. 2011; Kim et al. 2011).

As expected, B cells become undetectable within 14 days after the first dose and although time of B-cell repopulation varies interindividually, B-cell depletion is sustained during 6–12 months (Cree et al. 2005; Pellkofer et al. 2011). Although the information provided by these studies is limited, relapses often occurred after reappearance of B cells and most patients stabilized during phases of complete B-cell depletion. Therefore, readministration of RTX is usually considered at fixed intervals of 6–9 months, or immediately after B-cell reappearance (Cree et al. 2005; Jacob et al. 2008; Pellkofer et al. 2011). The influence of RTX on NMO-IgG titers and disease activity is controversial. Whereas in one study the titers did not decline significantly and some patients could have an increase in absence of clinical activity (Pellkofer et al. 2011), other found a significant reduction in the titer following RTX therapy consistent with the clinical response (Kim et al. 2011). In fact, RTX does not eliminate plasma cells in the bone marrow and presumably has few effects on preexisting antibodies. Therefore, other mechanisms beyond antibody reduction such as inhibition of B-cell–T-cell interactions, increase of regulatory T cells, decrease of CD20 + cells with reduction of proinflammatory cytokines, and

modulation of the T-cell compartment may contribute to clinical stabilization (Kim et al. 2011; Pellkofer et al. 2011).

Consistent with previous experiences, RTX appears to be well tolerated and safety of readministration of RTX seems acceptable given the clinical severity of NMO. The most common adverse events are quite similar to those describe in MS. Three deaths have been reported. One patient died 9 months after the last dose following a severe relapse; the second, 6 months after the last dose following a suspected septicemia in the setting of reduced lymphocytes counts and immunoglobulin concentrations (Jacob et al. 2008); and the third, due to a cardiovascular failure 3 days after the second infusion (Pellkofer et al. 2011). A posterior reversible encephalopathy syndrome 24 h after the first infusion of RTX has been reported in an NMO patient (Sánchez-Carteyron et al. 2010). Long-term safety concerns regarding RTX therapy exist, especially due to the possibility of PML. According to the experience in MS, ocrelizumab and ofatumumab could be selected for use in NMO in the future.

Others Monoclonal Antibodies

Eculizumab, a C5-specific humanized monoclonal antibody that inhibits C5a generation and membrane attack complex formation approved for the treatment of paroxysmal nocturnal hemoglobinuria, is currently being tested in a phase I/II study in NMO-IgG seropositive patients at the Mayo Clinic. The aim of the study is to prevent relapses during a 12-month follow-up. Patients will receive eculizumab at a dose of 500 mg IV each week for 4 weeks, 900 mg the fifth week, and thereafter 900 mg every 2 weeks for 48 weeks (ClinicalTrials.gov identifier: NCT00904826). *Tocilizumab* is a recombinant humanized monoclonal antibody against the interleukin 6 (IL-6) receptor approved for the treatment of RA. IL-6 levels are increased in the serum and CSF of active NMO, and IL-6 was found to enhance the production of NMO-IgG/AQP4 antibodies and promote the survival of plasmablasts (Yamamura and Miyake 2012). Because tocilizumab, in vitro, inhibits the NMO-IgG production and survival of plasmablasts, it has been hypothesized that NMO could be partly driven by this proinflammatory IL-6, which would propagate the survival of disease-specific B-cell subclasses, and would deviate $CD4^+$ T-helper cell differentiation toward IL-17-producing T-helper 17 cells (Yamamura and Miyake 2012). Clinical improvement after receiving tocilizumab in an NMO patient who did not respond to leukocyte depletion has been recently reported (Kieseier et al. 2012).

2.1.2 Paraneoplastic Neurological Syndromes and Autoimmune Encephalitis

Some neurologic disorders are thought to be caused by antineuronal immune mechanisms. When these disorders occur associated with cancer they are known as paraneoplastic neurological syndromes (PNS). The term “onconeuronal”

antibodies refers to antibodies that target neural antigens expressed by the tumor. These antibodies are present in approximately 60 % of the patients with PNS; therefore, PNS may occur without onconeuronal antibodies, and conversely the antibodies can occur without a neurological syndrome (Graus et al. 2004, 2010). On the basis of their clinical relevance, antibodies can be classified into two categories: well-characterized onconeuronal antibodies and cancer-related onconeuronal antibodies. Well-characterized onconeuronal antibodies, in addition to reacting with neural antigens, serve to establish the associated neurological disorder as definite PNS. Thus, their detection confirms the paraneoplastic origin of the neurologic dysfunction and their characterization directs the search for the tumor to one or few organs because they associate with limited subsets of tumors, usually small cell lung cancer (SCLC), breast, ovarian, and testicular cancer (Graus et al. 2004, 2010; Dalmau and Rosenfeld 2008). However, the intracellular location of the antigen and the fail to reproduce the neurologic syndrome in animal models suggest that these antibodies are not pathogenic. They probably represent the humoral component of a complex immune response, likely mediated by cytotoxic T-cell mechanisms against the same neural antigen. The second category of antibodies, cancer-related onconeuronal antibodies, even though recognize tumor antigens also expressed in the CNS, there is no evidence that the immune response is pathogenically related to the PNS. They include SOX and ZIC antibodies, both recognize antigen expressed in SCLC, and can be useful in indicating an underlying tumor in patients with a neurologic syndrome. For example, the detection of SOX1 antibodies in patients with Lambert–Eaton syndrome predicts the presence of SCLC, and may be useful to follow those patients with no evidence of tumor at the initial workup (Dalmau and Rosenfeld 2008; Graus et al. 2010).

The fact that patients with PNS associated with antibodies to intracellular antigens do not improve usually with immunotherapy is further evidenced against their pathogenic role. Clinical experience suggests that tumor treatment is necessary to stabilize or improve the symptoms and the combination with immunosuppressors, corticosteroids, IV immunoglobulin (IVIg), and cyclophosphamide may help to achieve this outcome (Dalmau and Rosenfeld 2008). In general better responses to immunotherapies have been described in patients with limbic encephalitis, opsoclonus-myoclonus syndrome (OMS), and paraneoplastic cerebellar degeneration (PCD) in Hodgkin disease, but neurologic improvement is unlikely to occur if the tumor is not treated (Dalmau and Rosenfeld 2008). In this context, the use of B-cell depleting therapy is very limited. In one study, 9 PNS patients with Hu or anti-Yo antibodies were treated with rituximab (RTX). Three patients improved $\geq$1 point on the Rankin Scale (RS) and one of them with limbic encephalitis improved dramatically (RS from 5 to 1) (Shams'ili et al. 2006). In other report, a 10-year-old boy was diagnosed with PCD in association with Hodgkin disease and Tr antibodies. He was treated with chemotherapy and radiation therapy, and then with RTX with resolution of his lymphoma and marked improvement of the cerebellar syndrome (Yeo et al. 2012). More recently, several reports have shown favorable effects of RTX in combination with other immunosuppressors or in monotherapy in children with paraneoplastic and

non-paraneoplastic OMS, including the reduction of relapses (Pranzatelli et al. 2010; Battaglia et al. 2012; Alavi et al. 2012). Ofatumumab has also been used in a child with OMS associated with ganglioneuroblastoma resistant to multiple therapies. The therapy was well tolerated and the patient remained free of relapses for 3 years (Pranzatelli et al. 2012). As expected, improvements occur in those PNS with functional rather than structural irreversible neuronal damage.

Another group of immune-mediated neurologic disorders are the encephalitis associated with antibodies against neuronal cell surface or synaptic proteins. The target antigens are receptors, including NMDA, AMPA, $GABA_{B,}$ and glycine, and proteins such as leucine-rich glioma-inactivated 1 (LGI1) and contactin-associated protein-like 2 (Caspr2), the latter antigens of antibodies previously attributed to voltage-gated potassium channels (Lancaster et al. 2011). The antibodies of this group share several characteristics: the epitopes are extracellular, the antibody binding is visible in cells transfected with the target antigen, the antibodies alter the structure or function of the corresponding neuronal antigen, the effects of the antibodies are often reversible, and the clinical picture resembles that of pharmacologic or genetic models in which the antigen is disrupted (Lancaster et al. 2011). In addition, these disorders may or may not be associated with cancer and are usually responsive to immunotherapy, altogether suggesting that they are pathogenic (Lancaster et al. 2011; Zuliani et al. 2012). The syndromes associated with these antibodies can be similar at presentation to classical PNS, such as limbic encephalitis, but one is a distinct and well-characterized disorder, anti-NMDA receptor encephalitis (Dalmau et al. 2011). Several studies support that this disorder is the most common cause of autoimmune encephalitis after acute demyelinating encephalomyelitis (Dalmau et al. 2011; Titulaer et al. 2013). The encephalitis mainly affects young women and children (only 5 % older than 45 years). Patients often have prodromal symptoms resembling a viral process followed in a few days by severe psychiatric symptoms, memory loss, seizures, decline of consciousness, abnormal movements, autonomic instability, and frequent hypoventilation that requires admission to intensive care units. The presence of a tumor (38 %) predominates in females between 12 and 45 years. In males and children the frequency of tumors is very low (6 %). The tumor is almost always (94 %) an ovarian teratoma (Dalmau et al. 2011; Titulaer et al. 2013). Despite the severity of the syndrome, prompt immunotherapy and, in patients with teratoma, tumor removal is associated with faster and more complete neurological recovery. A recent review with 577 patients showed that up to 50 % of the patients did not respond to first-line immunotherapy (corticosteroids, IVIg or plasma exchange alone or combined). However, the administration of a second-line immunotherapy with RTX, cyclophosphamide or both, resulted in complete recovery or with minimal deficits in 80 % of those patients. In addition, second-line therapy decreased the frequency of relapses, which are more frequent in patients without tumor (Titulaer et al. 2013). This study provides level II-2 evidence of the usefulness of immunotherapy and tumor removal in anti-NMDAR encephalitis, although which type of immunotherapy and duration of the treatment remains to be elucidated.

2.1.3 Neurological Disorders Associated with Glutamic Acid Decarboxylase Antibodies

Antibodies to glutamic acid decarboxylase (GAD), the rate-limiting enzyme for the synthesis of the inhibitory neurotransmitter γ-aminobutyric acid (GABA), were initially recognized in the serum and CSF of patients with stiff person syndrome (SPS), a rare CNS disorder characterized by progressive muscular rigidity, predominantly of the trunk muscles, with superimposed spasms. The syndrome is frequently associated with other autoimmune diseases, mainly type 1 diabetes mellitus (DM1) and with a high incidence of organ-specific autoantibodies. Later, autoantibodies to GAD (GAD-ab) were identified in about 80 % of newly diagnosed DM1 patients, although in patients with SPS the levels were more than 100-fold higher (Saiz et al. 2008). At the time GAD-ab determination became more available to neurologists, high levels of GAD-ab were reported in a subgroup of patients with cerebellar ataxia, and a few patients with epilepsy, brainstem dysfunction, and limbic encephalitis (Peltola et al. 2000; Honnorat et al. 2001; Pittock et al. 2006; Saiz et al. 2008; Malter et al. 2010). One study that analyzed the significance of high GAD-ab levels in patients with neurological syndromes found that patients with SPS and cerebellar ataxia, the second most frequent syndrome, shared the same demographic, clinical, and immunological features. The frequency of increased intrathecal synthesis of GAD-ab was 85 % in SPS, 100 % in cerebellar ataxia, and 86 % in other associated neurological disorders (Saiz et al. 2008). Although GAD-ab could just reflect the presence of DM1, which in many instances occurs in the setting of the neurological syndrome, the high frequency of a specific intrathecal synthesis of GAD-ab in these patients supports a possible humoral immunopathogenesis of the neurological syndrome. The main argument against the pathogenic role of GAD-ab is the intracellular location of GAD. However, in vitro, GAD-ab from patients with neurological syndromes induce a suppression of GABA release (Ishida et al. 1999), and in vivo the injection of IgG isolated from patients with GAD-ab and SPS or cerebellar ataxia induce neurophysiological and neurochemical changes that are not observed with the injection of IgG from GAD-ab-positive subjects and no neurological symptoms (Manto et al. 2007). Further support for a role comes from the fact that immunotherapy can improve or stabilize some of the syndromes associated with GAD-ab (Dalakas 2009).

Depletion of B cells with rituximab (RTX) has been performed in a few patients, most of them with SPS, with conflicting results. The clinical benefit described in 8 SPS open-label treated patients (Baker et al. 2005; Bacorro and Tehrani 2010; Dupond et al. 2010; Qureshi and Hennessy 2012; Katoh et al. 2010; Sevy et al. 2012; Fekete and Jankovic 2012) contrast with the lack of efficacy in the only two cases (monozygotic twins with SPS) included in a double-blind placebo-controlled trial of RTX (Venhoff et al. 2009). Overall, GAD-ab remained positive in four of the nine reported patients. The absence of reliable, responsive, and validated outcome measures in SPS and the low number of patients included may account for these discrepancies.

In other neurological associations, such as cerebellar ataxia and limbic encephalitis, the information is even more limited. It includes the stabilization of the cerebellar ataxia in a 48-year-old woman with coincidental hepatitis C infection (Awad et al. 2011) and the inefficacy of several immunotherapies, including RTX, in an 11-year-old-girl who developed cerebellar ataxia 5 months after the presentation with limbic encephalitis (Mirabelli-Badenier et al. 2012). The last patient reported, a 6-year-old girl who had developed refractory seizures, development regression and DM1 at age 25 months, had a major clinical improvement, rapid decrease of seizures and improvement in behavior, in parallel with a decrease in the levels of serum and CSF GAD-ab after treatment with plasma exchange and RTX (Korff et al. 2011). In fact, the lack of response to several immunotherapies, IVIg, corticosteroids or cyclophosphamide was recently described in nine patients with limbic encephalitis and GAD-ab in contrast to the ten patients with limbic encephalitis and voltage-gated potassium channel antibodies (VGKC-ab) who became seizure free (Malter et al. 2010). In addition, it has been reported that immunotherapy improves the SPS but not the cerebellar symptoms of patients in who coexist SPS and cerebellar ataxia (Rakocevic et al. 2006).

From these reports it is not clear whether different pathophysiology mechanisms, such as functional blockade or more destructive processes, are involved in the different neurological syndromes associated with GAD-ab. It is likely that GAD-ab only reflect the presence of a more complex immune response, probably T cell-mediated, in a subset of patients. Nevertheless, although the direct pathogenic role of GAD-ab remains to be proved in these disorders, evidences exist on their autoimmune basis and trials with immunotherapies, including B cell-targeted therapies, are warranted.

2.2 Peripheral Nervous System Autoimmune Disorders

2.2.1 Chronic Inflammatory Demyelinating Polyneuropathy and Other Chronic Neuropathies

Autoimmune neuropathies encompass a broad spectrum of acquired immune-mediated inflammatory disorders of the peripheral nervous system. Chronic inflammatory demyelinating polyneuropathy (CIDP) is a common and potentially treatable disease, which shares clinical similarities and the beneficial effect of immunosuppressive therapies with Guillain–Barré syndrome. It has been defined other forms of acquired demyelinating neuropathies with presumed autoimmune or dysimmune pathogenesis different from the classic CIDP, with respect both to clinical presentation and to the response to treatment (Köller et al. 2005). In this review we will focus on those disorders in which the experiences with B cell-targeted therapies, mainly rituximab (RTX) are not limited to case reports, such as CIDP, multifocal motor neuropathy, and anti-myelin-associated glycoprotein demyelinating neuropathy.

Chronic Inflammatory Demyelinating Polyneuropathy

Classic CIDP is characterized by symmetrical weakness affecting proximal and distal muscles associated with sensory disturbances that progressively increases for more than 2 months. The course can be relapsing or chronic and progressive, and the disorder is associated with elevated CSF protein level and demyelinating nerve-conduction studies. The contribution of autoantibodies to the pathogenesis is suggested by the demonstration of immunoglobulin and complement deposition on myelinated nerves, and studies of passive transference (Köller et al. 2005). The 28-kD myelin protein zero, gangliosides, and related glycolipids have been suggested as possible putative target antigens (Dalakas 2006). Corticosteroids, plasma exchange, and IVIg have demonstrated efficacy in short-term prospective randomized trials (Finsterer 2005) although about one-third of the patients do not respond well to these therapies. Other immunosuppressant drugs have shown to be beneficial in CIDP, but there is no evidence enough to recommend a particular drug among others (Joint Task Force of the EFNS and the PNS. Guidelines 2010). The experience with RTX is very limited and significant clinical improvement has been especially reported in those patients who have associated hematological diseases (Benedetti et al. 2008). A pilot study conducted with 6 patients treated with RTX, two of them with CIDP, showed no benefit in reducing the dose of IVIg (Gorson et al. 2007). In other retrospective multicenter observational study with 13 refractory CIDP patients, clinical response, defined by improvement of at least two points in standard clinical scales and not requirement of new dose of IVIg or steroids, was achieved in nine (69 %) of them. Seven of the responders had a concomitant hematologic disease. Median time response from RXT administration was 2 months, and the improvement lasted at least 1 year (Benedetti et al. 2011). An extension of the study analyzed the response to different immunosuppressive agents in 110 IVIg and steroids refractory CIDP patients. A good response was reported in 6 of the 18 (33 %) patients treated with RTX. Allergic reaction and transient rise in transaminases were reported in one of the patients. However, the study did not find significant differences in the response between RTX and other immunosuppressors (azathioprine, cyclophosphamide, mycophenolate mofetil, cyclosporine, methotrexate, interferon-alpha) (Cocito et al. 2011).

Multifocal Motor Neuropathy

Multifocal motor neuropathy (MMN) is an infrequent purely motor neuropathy characterized by asymmetric distal limb weakness and the presence of motor conduction blocks in the electrophysiological study (Stieglbauer et al. 2009; Vlam et al. 2013). In 80 % of the patients, symptoms present before the age of 50 years and have a slow and often stepwise progression. The exact pathogenesis of MMN is still unclear, but 30–50 % of the patients have IgM anti-GM1 antibodies (Rüegg et al. 2004; Nobile-Orazio et al. 2000). Studies in vitro have shown that IgG and IgM anti-GM1 antibodies can trigger complement activation after binding its

target (Vlam et al. 2013). The assumption that IgM anti-GM1 antibodies could be pathogenic is further supported by the finding that patients with IgM anti-GM1 antibodies have more severe weakness, disability, and axonal loss than patients without anti-GM1 IgM antibodies, and high antibody titers are associated with more weakness (Vlam et al. 2013). Most patients respond well to IVIg (Vlam et al. 2013) but require frequent infusions (Rüegg et al. 2004). For those patients who respond less well, other immunosuppressants such as cyclophosphamide are tried (Rüegg et al. 2004). Uncontrolled studies indicate that RTX may be effective in some patients but the grade of evidence is low. Improvement in the muscle function and reduction in the serum IgM anti-GM1 titer was described in the first four patients reported at 3–6 months after the initial treatment (Levine and Pestronk 1999). However, improvement was not observed in a patient who had anti-GM1 and anti-GD1b antibodies. In this patient, the increase in the antiganglioside levels correlated with the period of clinical worsening, supporting the possible pathogenic role of the autoantibodies (Rojas-García et al. 2003). One of the largest reported case series was a 2-year open-label study of RTX that included 21 cases of IgM-associated polyneuropathies, 14 of them with MMN and IgM anti-GM1 antibodies, and 13 untreated patients as control. Quantitative strength measures improved by 13–22 % in the RTX group at 1 and 2 years, and antibodies titers decreased to 45 % at 2 years, whereas control subjects remained unchanged but the assessment of the outcome measures were not blinding. However, most patients with initial benefit experienced recurrent weakness 3–9 months after the first infusion and received a second set of treatment (375 mg/m^2 every week for 2 weeks and then one infusion every 10 weeks). No major side effects were described. The authors concluded that continued treatment might often be necessary to optimize clinical improvement (Pestronk et al. 2003). The effect of RTX therapy on the IVIg dose requirement in patients whose neuropathy was responsive to IVIg but required chronic therapy has been assessed in few studies with controversial results. Two studies reported clinical stabilization and a decreasing frequency of IVIg courses in four MMN and anti-GM1 antibodies negative patients (Rüegg et al. 2004; Stieglbauer et al. 2009), but negative results were found in two other studies (Gorson et al. 2007; Chaudhry and Cornblath 2010). In a prospective uncontrolled 12-month trial of six patients treated with RTX, two of them with MMN, one patient reached the primary outcome (a reduction of cumulative IVIG dosage by at least 25 % at 1 year after RTX therapy compared with the previous year) but the other patient required increased IVIg (Gorson et al. 2007). The dose of RTX used in these studies was 375 mg/m^2 every week for 4 weeks. One recent open-label trial involved six MMN and anti-GM1 antibodies negative patients on periodic IVIg treatment. The patients were treated with RTX 1,000 mg administered 2 weeks apart. There were no statistically significant changes in IVIg use, medical research council sum score, grip strength, overall disability sum score, or Rotterdam handicap scale during 12 months (Chaudhry and Cornblath 2010).

Eculizumab (see "Others Monoclonal Antibodies" in neuromyelitis optica section) was tested in a recent open-label study that included 13 MMN patients. The therapy resulted well tolerated and safe. Although improvement in some motor

assessment scores was reported, the IVIg requirements were not reduced (Fitzpatrick et al. 2011).

Anti-Myelin-Associated Glycoprotein Neuropathy

In almost 50 % of patients with neuropathy associated with IgM monoclonal gammopathy, the paraprotein reacts with the CD57/HNK-1 carbohydrate epitope found on myelin-associated glycoprotein (MAG). The clinical demyelinating neuropathy associated with antibodies against MAG is an entity that presents with progressive symmetric sensory ataxia or sensorimotor deficits. Considerable evidence exists supporting that anti-MAG antibodies are pathogenic (Dalakas 2009; Lunn and Nobile-Orazio 2012). Although the prognosis of neuropathy associated with IgM monoclonal gammopathy is relatively favorable, almost half of the patients with neuropathy and anti-MAG antibodies will reach at least a moderate disability (Rankin disability score higher than 2) 10–15 years later (Nobile-Orazio 2010). A recent Cochrane review, however, concluded that results from available trials do not provide evidence to recommend any particular immunotherapy treatment, and there is very low quality evidence of benefit from RTX (Lunn and Nobile-Orazio 2012).

In the study of Pestronk et al. (2003) seven of the 21 patients treated with RTX had neuropathy with anti-MAG antibodies and all of them improved compared with none of the 13 untreated patients. However, two studies failed to show any benefit in five patients (Rojas-García et al. 2003; Barohn et al. 2005). In one open phase II study with nine patients, two had an important improvement ($\geq$10 points on the Neurologic Disability Score), four a marginal improvement (2–5 points), two remained the same and one deteriorated (Renaud et al. 2003). This was followed by a study with a high RTX doses, 750 mg/m^2 weekly $\times$ 4 weeks, in eight of the patients. Clinical improvement along with reduction in anti-MAG antibodies was observed in four of the patients (Renaud et al. 2006). Improvement at 1 year was also observed in eight of the 13 (62 %) patients included in an uncontrolled open-label study. Responders improved in their Inflammatory Neuropathy Course and Treatment (INCAT), sensory sum score, and the Medical Research Council sum score for muscle strength. Seven (54 %) also improved in the INCAT disability score (Benedetti et al. 2007a, b). Ten of the patients from this study who initially responded well were followed. The improvement lasted 24 months in eight patients, and 36 months in six after a single course of RTX (375 mg/m^2 every week for 4 weeks) (Benedetti et al. 2008). The only published double-blind placebo-controlled randomized trial included 13 RTX and 13 control cases. The primary outcome measure (change of one point in the INCAT leg score at month 8) using the intention-to-treat population did not reach statistical significance (4 of 13 RTX-treated patients improved compared with 0 of 13 placebo-treated patients). However a post hoc analysis, after excluding one RTX-treated patient who initially entered as having a normal INCAT leg, found a statistically significant difference between the treated group and the placebo group. At 8 months, IgM was reduced by

34 % and anti-MAG titers by 50 %. From this study, it is unclear if the clinical improvement observed was related to the depletion of B cells or to the reduction of anti-MAG antibodies (Dalakas et al. 2009). A recent observational study of seven patients treated with a single course of RTX (two of them were retreated with one or more additional cycles) and followed for 24 months found significant improvement in pinprick sensation and discrimination. However, there were no correlations between anti-MAG antibodies titers and clinical scales or electrophysiological findings. Moreover, reduction in antibodies titers was only detected in those patients treated with a second or a third cycle (Zara et al. 2011). Finally, the use of RTX in combination with fludarabine (RTX 375 mg/ m^2 on day 1 and oral fludarabine at 40 mg/m from days 1–5) was reported in five patients. Four patients improved clinically and electrophysiologically, and there was a decrease in anti-MAG titers in three and clearing in one. Improvement was sustained in the four patients without associated toxicity (Gruson et al. 2011).

2.3 Neuromuscular Transmission Autoimmune Disorders

2.3.1 Myasthenia Gravis

Myasthenia gravis (MG) is an autoimmune disease of the neuromuscular junction that causes painless fatigable muscle weakness. Although the pattern of muscle involvement varies, 80 % of the patients presenting with ocular symptoms will eventually develop generalized MG, whereas early prominent bulbar symptoms will be present in up to 20 % of the patients. The course is highly variable, but 39 % of patients will have a severe MG, and half of them will require invasive ventilation during the course of their disease. Approximately 80 % of patients with generalized MG have autoantibodies directed against the acetylcholine receptor (AChR-ab). They are predominantly of the IgG1 and IgG3 subclasses and cause severe loss of the receptor via a number of mechanisms, including complement-mediated damage to the postsynaptic membrane, antigenic modulation resulting in receptor endocytosis and occasionally by direct block (Vincent et al. 2000; Dalakas 2006). Around 20 % of patients, and a higher proportion of those with purely ocular MG, do not have AChR-ab (seronegative MG). A variable proportion of these patients, 0–64 %, have antibodies against the muscle-specific tyrosine kinase (MuSK-ab). They are predominantly of the noncomplement-fixing IgG4 subclass and adversely affect the maintenance of AChR clustering at the muscle end-plate, leading to reduced numbers of functional AChRs (Guptill and Sanders 2010). MuSK-ab-positive patients are more likely to have early bulbar and respiratory symptoms and to be more refractory to standard therapies (Illa 2005; Vincent and Leite 2005). Similar to AChR-ab, the successful passive transference of MuSK-ab to mice indicates that they are pathogenic. Most of the patients with generalized or ocular MG need immunosuppressant drug at some time during the progression of the disease, and drugs such as corticosteroids, azathioprine, methotrexate, cyclophosphamide,

mycophenolate, or tacrolimus have been used in MG patients, although there is a lack of evidence-based studies of efficacy for most of them.

Rituximab

To date, almost 100 MG patients have been treated with rituximab (RTX), most of them included in single case reports and short retrospective series (Baek et al. 2007; Gajra et al. 2004; Hain et al. 2006; Thakre et al. 2007; Zaja et al. 2000; Illa et al. 2008; Stieglbauer et al. 2009; Lebrun et al. 2009; Maddison et al. 2011; Zebardast et al. 2010; Blum et al. 2011; Stein and Bird 2011; Nowak et al. 2011; Collongues et al. 2012). Most patients were considered resistant to conventional therapy, had undergone thymectomy, oral steroids plus scheduled cycles of IVIg, plasma exchange, or treated with one or more lines of immunosuppressors. Data on non-refractory MG patients and long-term use of RTX is very limited (Collongues et al. 2012; Diaz-Manera et al. 2012a, b). RTX was administered at standard doses (375 mg/m^2 weekly $\times$ 4 doses, and some 1,000 mg weekly $\times$ 2) except in one study that used single or repeated doses of 500 mg/m^2 (Blum et al. 2011). Most patients were moderately to severely affect with respiratory failure or with bulbar dysfunction (stages IVB or V of the Myasthenia Gravis Foundation America classification). Approximately 60 % of the reported patients were positive for AChR-ab, 35 % for MuSK-ab, and 6 % seronegative for both antibodies. The number of infusions was variable, but all patients received at least one infusion. A good clinical response has been reported in almost all patients with MuSK-ab and more than 80 % of the patients with AChR-ab. Although improvement with decrease in the number of exacerbations and reduction or withdraw of concomitant immunosuppressant drugs have been reported, remission status and longer duration to retreatment have been most frequently described in MuSK-ab patients (Diaz-Manera et al. 2012a, b). Moreover, whereas MuSK-ab titers decreased as early as 3 months after the first doses and remained low during a mean follow-up of 31 months, AChR-ab levels did not significantly change and remained at the same levels during the same period of time (Diaz-Manera et al. 2012a, b). The reason why MuSK-ab positive patients have a better and persistent clinical benefit as compared with AChR-ab positive patients is unknown, but it is likely related to the different pathological mechanisms involved (McConville et al. 2004; Diaz-Manera et al. 2012a, b). Although the clear benefit reported, the results have to be taken with caution considering the bias of reporting positive results and especially the lack of randomized controlled trials.

RTX appears to be well tolerated and, similarly as in others diseases, the most common adverse events are mild infusion-related reactions. Although PML has not been reported in any MG case, there is concern related to the use of previous immunosuppressant drugs in MG patients. Currently two open label trials are ongoing (ClinicalTrials.gov identifier: NCT00619671 and NCT00774462).

Others Monoclonal Antibodies

Belimumab, a humanized monoclonal antibody targeted against B lymphocyte stimulator (BLyS), recently approved for the treatment of systemic lupus erythematosus (Chugh and Kalra 2013), will be tested in a phase II placebo-controlled trial in AChR-ab or MuSK-ab positive MG patients refractory to standard therapies. The primary objective will be to assess the efficacy evaluating the change in the quantitative myasthenia gravis score during a 36-month follow-up (ClinicalTrials.gov identifier: NCT01480596).

2.3.2 Lambert–Eaton Myasthenic Syndrome

Lambert–Eaton myasthenic syndrome (LEMS) is an autoimmune disease mediated by autoantibodies against the voltage-gated calcium channels (VGCC) present on the presynaptic nerve terminal at the neuromuscular junction and the autonomic ganglia. Although it was first described in patients with lung cancer, LEMS also occurs as an idiopathic organ-specific autoimmune disorder in absence of cancer. The main symptom is proximal muscle weakness, usually of the legs, frequently accompanied by muscle aching or stiffness. During the course of the disease, oculobulbar involvement can occur but it is mostly mild and transient. Most patients have signs of autonomic dysfunction (dry mouth, postural hypotension, erectile dysfunction, constipation) (Smith and Wald 1996). Similar to MG, the pathogenic role of antibodies to VGCC has been established (Dalakas 2006). Treatment of LEMS is individualized and based on drugs which act at the neuromuscular synapse, drugs which suppress the immune response and the treatment of the underlying malignancy. IVIg and plasma exchange have been reported to have a swift, but rather short-lasting effect. There are isolated case reports describing improvement at medium term from RTX therapy. Improvement after plasma exchange and RTX was reported in a patient with LEMS and concomitant severe ataxia and no evidence of cancer over 5 years who had failed to conventional immunosuppressive therapy (Pellkofer et al. 2009). Other two LEMS VGCC-ab positive patients improved but did not achieve remission during a follow-up of 18-month (Maddison et al. 2011).

3 Conclusions

B cell-targeted therapies have emerged as a promising tool in the therapeutic strategies of a range of autoimmune neurological diseases, such as multiple sclerosis (MS), neuromyelitis optica, autoimmune encephalitis, chronic inflammatory demyelinating polineuropathies, myasthenia gravis, and Lambert–Eaton syndrome. Rituximab, an anti-CD20 monoclonal antibody, has been the most frequently reported therapy in this context. Although it has been suggested to be effective

and relatively safe, most of the available data are derived from case reports or short uncontrolled series and, with a few exceptions, e.g. MS, randomized controlled trials are lacking. There is a need for randomized controlled trials to unambiguously establish the efficacy and long-term safety of new or former B-cell-directed therapy, in the meantime the promising results described must be interpreted with caution.

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Targeting B Cells in Other Systemic Autoimmune Diseases

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Abstract In this chapter, we discuss the use of B-cell directed therapies in entities that cannot be covered separately in single chapters. In all of these conditions, evidence on the use of these therapies is restricted to rituximab and it mainly relies on individual case reports or short series. In general, the underlying pathophysiology of the disease and/or the successful use of biological agents with a different mechanism of action seem to justify the paucity of evidence on the use of agents targeting B-cell. Nevertheless, there is anecdotal indication of successful use of rituximab in several conditions such as steroid-resistant and dependent sarcoidosis, Behçet disease with retinal vasculitis, chronic hepatitis C virus-associated polyarteritis nodosa, highly active refractory Takayasu arteritis, and Henoch–Schönlein purpura nephritis.

1 Sarcoidosis

Steroid-resistant sarcoidosis, a chronic, inflammatory granulomatous disease of the lung in which various cytokines have a key pathogenetic role, may be treated with immunosuppressive drugs, antimalarial therapies, and, most recently, with anti-tumor necrosis factor (TNF)-α agents. The administration of biologicals to treat

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sarcoidosis is based on the investigation in disease pathogenesis and the use of these agents in other chronic inflammatory diseases (Bargagli et al. 2011).

The most recent therapeutic guidelines, the American Thoracic Society/European Respiratory Society consensus, date from 1999 and were updated in the 2008 UK guidelines on interstitial lung disease (Hunninghake et al. 1999; Bradley et al. 2008). The guidelines maintain steroids as first-line treatment, but propose fresh approaches, especially for patients with relapses, extra-pulmonary sites, complications, and corticosteroid resistance. The aim of biological treatment is to modify the disease course in addition to improving clinical symptoms. Study of the immunoinflammatory pathways of sarcoidosis, and especially the identification of cytokines produced by lymphocytes and macrophages involved in granuloma formation, is the basis for new forms of treatment. TNFα is the principal cytokine involved in granuloma maintenance and development and is produced mainly by alveolar macrophages, activated T-cells, and pulmonary epithelial cells (Fehrenbach et al. 2003). In sarcoidosis, alveolar macrophages release exaggerated amounts of TNFα (Ziegenhagen et al. 2002), with more being produced in patients with active disease than in those with stable disease (Ziegenhagen et al. 1997). Based on this reasoning, TNFα inhibitors were developed to block the cytokine cascade and inflammatory events in sarcoidosis.

Anecdotal case reports of anti-CD20 treatment of sarcoidosis, especially steroid-dependent and refractory disease are few. There are no randomized trials that have assessed the safety and efficacy of rituximab. Symptoms improved with prednisone therapy in one patient with stage IIA lung and joint sarcoidosis, but prednisone dependency at a daily dose of 40 mg/day developed. Administration of methotrexate had no effect, and two 1 g infusions of rituximab at a 2-week interval were well tolerated. Positive effects were first noted 3 months after treatment, with a reduction in pain (visual analog scale) and improved lung function. Prednisone was withdrawn, and 12 months after rituximab administration, the patient was on methotrexate 10 mg/week and two 1 g rituximab infusions at 2-week intervals and had resumed normal activities for 1 year (Belkhou et al. 2008).

Dasilva et al (2010) reported that rituximab was effective in one patient with sarcoidosis non-responsive to infliximab or other biologicals and suggested this might be because rituximab inhibits the CD20 antigen of B-lymphocytes located in the periphery of the granuloma, thereby inducing apoptosis.

In a further case, stability was maintained in a patient with neurosarcoidosis who was non-responsive to steroids and cyclophosphamide after rituximab therapy. About 5 % of cases of systemic sarcoidosis are estimated to have central nervous system involvement, and less than 50 % present isolated central nervous system disease (Bomprezzi et al. 2010; Burns 2003). While the mechanism of action of rituximab in neurosarcoidosis is unclear, and B-lymphocytes acting as antigen-presenting cells could contribute to inflammation, the authors suggested that rituximab might be considered a therapeutic option in patients with neurosarcoidosis refractory to both corticosteroids and other frequently administered immune therapies.

2 Behçet Disease

Behçet disease is a rare vasculitis characterized by oral aphthosis (98 %), genital aphthosis (77 %), skin manifestations (73 %) and ocular lesions (54 %) and progressive cycles of attacks, healing and remission. Mucocutaneous manifestations normally have a short attack and healing course, resulting in healing of the lesion with sequelae in most cases. Ocular lesions have a longer healing process, with fresh attacks occurring before complete healing, with lesions accumulating from one attack to another, leading to severe vision loss and blindness in a high percentage of cases. The first line of treatment is cytotoxic drugs, combined with corticosteroids for efficiency. Ocular lesions are the most difficult to treat, with cytotoxic drugs only improving visual acuity in 52 % of patients and maintaining baseline vision in 18 %, despite their long-term efficiency. Visual acuity is worsened compared to baseline in 30 % of eyes (Davatchi et al. 2010).

Although not tested in longitudinal series or studies with a long follow-up (with the exception of interferon-α2a), biologicals might be of use in these patients. The rationale is that Behçet disease is principally a T-cell-driven disorder (Turan et al. 1997), analogous to uveitis, where high levels of T-cells are found in serum and the aqueous humor (Santos Lacomba et al. 2001), and thus T-cells may be of use in the treatment of the ocular manifestations of Behçet disease (Ohno et al. 2004; Tugal-Tutkun et al. 2005).

In Behçet disease, while the total B-cell count is normal, raised levels of spontaneous immunoglobulin-secreting B cells and immunoglobulin are reported. Raised levels of activated and memory B cells, in addition to changes in T-cell numbers and activity, suggest a modified B-cell function. CD13- and CD33-positive B cells are greater in Behçet disease than in healthy controls and rheumatoid arthritis and systemic lupus erythematosus patients, perhaps related to stimulation by unknown external antigens or internal antigens like heat-shock proteins, although B-cell numbers are reduced in tissue lesions (aphthous ulcers and ocular lesions). Patients with Behçet disease with uveitis have higher serum interleukin (IL)-6 levels, which are higher still in the aqueous humor. As activated B-lymphocytes are known to be a source of IL-6, rituximab administration in these patients has a logical rationale (Davatchi et al. 2010; Sadreddini et al. 2008).

There are some reports on the use of rituximab in patients with Behçet disease and retinal vasculitis. Rituximab clearly improved visual acuity and retinal vasculitis at 6 weeks in a patient with retinal vasculitis, who was resistant to azathioprine 150 mg/daily and 1 mg/kg prednisolone daily with sustained remission of 24 months (Sadreddini et al. 2008).

Twenty patients with Behçet disease with retinal vasculitis and edema who were resistant to cytotoxic drugs were randomized to rituximab (rituximab group: RG) or cytotoxic combination therapy (cytotoxic combination therapy group: CCTG). Rituximab, in two 1,000-mg courses separated by 15-days, methotrexate (15 mg/weekly), and prednisolone (0.5 mg/kg per day) were administered. CCTG patients were administered pulse cyclophosphamide (1,000 mg/monthly), azathioprine

(2–3 mg/kg per day), and prednisolone (0.5 mg/kg per day). Primary endpoints were overall eye state and total adjusted disease activity index (TADAI), and secondary endpoints included visual acuity, posterior uveitis, and retinal vasculitis. At 6 months of treatment, the TADAI improved significantly in the RG ($t = 3.340$, $P = 0.009$) but not the CCTG ($t = 2.241$, $P = 0.052$). Mean visual acuity improved in two RG patients versus three CCTG patients (2/3) was unaltered in 1/1 and was worse in 7/6 patients, respectively. Mean posterior uveitis improved significantly in the RG ($t = 3.943$, $P = 0.001$) but not in the CCTG ($t = 2.371$, $P = 0.028$). Although retinal vasculitis improved more in RG patients, the difference was not significant. Edema of the retina, disk, and macula improved significantly in both groups but was much greater in RG patients ($t = 2.781$, $P = 0.012$ vs. $t = 2.707$, $P = 0.014$). Thus, in Behçet disease patients non-responsive to cytotoxic drugs who all had retinal vasculitis and edema (retina, disc, macula), rituximab efficiently treated severe ocular manifestations, and the TADAI improved significantly at 6 months of rituximab, but did not improve with combination cytotoxic therapy (Davatchi et al. 2010). These results suggest rituximab is an effective alternative in Behçet disease patients with intractable ocular lesions who are refractory to long-term cytotoxic and prednisolone therapy.

3 Relapsing Polychondritis

Relapsing polychondritis (RP) is a rare inflammatory disease characterized by recurrent inflammation and cartilage destruction, especially of not only the pinnae, nose, and respiratory tract but also the joints, inner ears, eyes, skin, heart valves, and blood vessels. There are reports of an association with myelodysplastic syndromes and, possibly, with lymphoma. The latest evidence on the pathogenesis of RP suggests that an inappropriate immune response involving B cells may play a role (Leroux et al. 2009; Hansson et al. 2004). Although corticosteroids remain the mainstay of treatment, no one single treatment is effective in symptom relief or preventing disease progression.

Leroux et al. (2009) retrospectively evaluated the addition of various rituximab regimes to current therapy in nine patients with severe, refractory RP. Disease activity was measured by clinical, laboratory, physiologic, and radiologic indicators. Corticosteroid doses and changes in immunosuppressive agents were measured and disease activity assessed in the 6 months before and 6 and 12 months after rituximab administration. At 6 months of treatment, although no patient achieved full remission, two patients presented partial improvement, four were stable, and three had worse disease. After the three patients with worse disease at 6 months were excluded, two patients remained stable and four had worse disease at 12 months, with no patients with partial or complete remission. B-cell depletion was found in all eight patients in whom this parameter was counted at 6 months after treatment.

A systematic review of the efficacy and/or safety of biologic agent in RP until December 2010 found only cases series or single case reports, with no randomized controlled trials. The review included 30 reports with 62 patients, who were administered TNFα blockers ($n = 43$) and rituximab ($n = 11$). Rituximab was ineffective in the nine patients from the Leroux study and partially effective in two other patients. In one case, rituximab was administered after infliximab and adalimumab were not efficacious in a patient with nasal and auricular chondritis, hypoacusia, arthralgia, and urticaria. There was a rapid therapeutic response that persisted for more than 16 months, although total remission was not achieved. In the second case, a patient previously treated with four immunosuppressant agents, including infliximab, was administered rituximab 1 month before the appearance of severe, acute aortic insufficiency, which was successfully treated by aortic valve repair and coronary artery grafting. Histologically, the aortic valve had a bland appearance with no inflammatory infiltrate. Steroids could be decreased postoperatively, and at 18 months the patient remained in clinical remission with some hearing gain after successful cochlear implantation (Kemta Lekpa et al. 2012).

4 Giant-Cell Arteritis

Giant-cell arteritis (or temporal arteritis) is the most frequent primary vasculitis in adults and is characterized by preferential involvement of the extra-cranial branches of the carotid artery, although there is frequently inflammation of the whole aorta and its branches. Biopsy of the temporal artery often shows granulomatous vasculitis, although negative biopsies do not exclude the diagnosis. The most frequent severe consequence is non-reversible visual loss due to ischemia of the optic nerve (Schäfer and Zwerina 2012).

To prevent visual loss, rapid immunosuppression with glucocorticoids is obligatory, although this therapy often results in severe adverse events, with disease recurrence being observed during glucocorticoid tapering. Severe ischemic events are rare during relapses. To spare glucocorticoid therapy and prevent relapsing disease, various immunosuppressive therapies have been tried, including methotrexate as adjunctive treatment which was tested in three randomized controlled trials (Jover et al. 2001; Spiera et al. 2001; Hoffman et al. 2002).

Although the results were discordant, a meta-analysis showed a 35 % reduction in relapses and a lower cumulated dose of glucocorticoids (Mahr et al. 2007). Although there is no clear evidence that giant-cell arteritis is a B-cell-mediated disease, rituximab was administered in one patient with glucocorticoid-dependent giant-cell arteritis (Bhatia et al. 2005) with a good response; however, the efficacy of rituximab could not be assessed as the patient was receiving concomitant cyclophosphamide.

Likewise, a patient with polymyalgia rheumatica/giant-cell arteritis refractory to reduction of glucocorticoids to acceptable levels improved after B lymphocyte depletion but developed respiratory problems (Bhatia et al. 2005).

5 Polyarteritis Nodosa

Polyarteritis nodosa, a systemic necrotizing vasculitis that mainly affects the medium-size vessels, is mostly idiopathic, but has frequently been related to acute hepatitis B virus infection and has been associated with chronic hepatitis C virus (HCV) infection in 5–12 % of cases (Néel et al. 2011). Polyarteritis nodosa-type vasculitis accounts for 19 % of HCV-associated vasculitis. Corticosteroids and/or antiviral therapy (interferon α and/or ribavirin) have been used to treat HCV-associated polyarteritis nodosa until recently, with severe cases treated with plasma exchange and/or cyclophosphamide (de Dios García-Díaz et al. 2005; Pateron et al. 1996).

Néel et al. (2011) reported a patient with severe, life-threatening HCV-associated polyarteritis nodosa who achieved rapid, sustained full remission after treatment with rituximab and a short low-dose corticosteroid course without antiviral therapy. Saadoun et al. (2011) recently reported the successful use of rituximab in 11 patients with HCV-associated polyarteritis nodosa, although seven patients also received antiviral therapy. This treatment controlled the disease in several patients, although the authors do not state whether the four patients treated with rituximab without antiviral therapy received plasma exchange.

The pathophysiology of HCV-associated polyarteritis nodosa has received little attention but the reports by Saadoun et al. (2011) and Néel et al (2011) suggest that, as in HCV-related cryoglobulinemic vasculitis, there is B-cell involvement, with the rapid, sustained response to B-cell depletion found by Néel et al (2011) suggesting that the role of B cells may go further than just antibody production.

6 Takayasu Arteritis

Takayasu arteritis, a rare form of chronic large-vessel vasculitis that involves the aorta and its main branches, most frequently affects young females, with a higher incidence in Asia and Eastern Europe, in contrast to giant-cell arteritis. Glucocorticoids supplemented or not by alternative immunosuppressive agents and/or TNF blockade in refractory patients is the current treatment. Although the etiology of Takayasu arteritis remains unknown, evidence suggests that T cell-mediated autoimmunity is involved, with antiendothelial antibodies and B-cell infiltrates in inflamed vessels indicating a pathogenic role for B cells (Hoyer et al. 2012).

A report in 2008 by Galarza et al. (2008) found a good clinical response to rituximab in one of the two patients with Takayasu arteritis refractory to methotrexate and TNFα inhibitors with clinical improvement. The patients were 25- and 29-year-old females, with disease duration of 84 and 96 months, respectively, both of whom had had no response to methotrexate and anti-TNF treatment (Unizony et al. 2013).

Hoyer et al. (2012) were the first to report B-cell disturbances in Takayasu arteritis resulting in raised amounts of newly generated plasmablasts in peripheral blood that correlated with disease activity. Three patients with highly active refractory Takayasu arteritis in spite of treatment with prednisone, mycophenolic acid, cyclosporine and adalimumab were treated with rituximab according to the rheumatoid arthritis rituximab protocol, resulting in clinical remission and normalization of the number of peripheral plasma cell precursors, although these later increased during relapse in two patients who were successfully retreated with rituximab. The correlation found between plasmablast levels and active Takayasu arteritis, together with the beneficial effects of rituximab treatment, suggests that plasmablasts may potentially be used as biomarkers of disease activity and for B-cell targeting. In the two patients successfully retreated with rituximab, relapse was accompanied by renewed rises in plasmablast levels: these patients had previously developed resistance to agents considered as effective in therapy for Takayasu arteritis, including TNF blockers. The results suggest the need to reconsider the role of B cells in this disease.

In a more recent report, induction therapy with 1 mg/kg oral prednisolone and 500 mg/m^2 intravenous cyclophosphamide was started in a patient diagnosed with Takayasu arteritis. Due to recurrent disease activity, prednisolone could not be lowered to <15 mg/day, and adjuvant azathioprine, 100 mg/day, was added. Some weeks later, the patient was hospitalized with left homonymous hemianopia and was discharged on azathioprine, 150 mg/day, and prednisolone, 30 mg/day, after surgery for a right carotid artery lesion. The patient reported worsening exertional dyspnea and angina pectoris and new right pulmonary artery stenosis was confirmed by imaging, with surgery not being an option. Rituximab was administered based on the report by Hoyer et al. (2012) due to evident disease activity that did not respond to maximum standard treatment, and sustained remission was achieved, as shown by corticosteroid requirements, stabilization on MRI, and impressive clinical and functional improvement (Ernst et al. 2012).

7 Henoch–Schönlein Purpura

Henoch–Schönlein purpura is an immunoglobulin A vasculitis that affects the small vessels. It is a multiorgan system disease that may include cutaneous purpura, arthralgia, acute enteritis, and nephritis (Pillebout et al. 2011). Histologically, it is characterized by perivascular deposition of IgA around dermal blood vessels. In over 90 % of cases, the disease is found in children less than 10 years old with a peak occurrence at 3–5 years. Whereas the etiology of Henoch–Schönlein purpura is unknown, it may be triggered by infection, particularly in children, malignancies, and medications. Generally, it is a benign, self-limiting process in children with an excellent prognosis. The major morbidity is related to renal involvement with progression to renal insufficiency occurring in approximately 5–15 % of children. Whereas adults are much less commonly affected, they display higher morbidity

and mortality (up to 25 %). This is often related to renal insufficiency, which is seen in about 30 % of adult cases (Miniter et al. 2012).

In fact, Henoch–Schönlein purpura nephritis is a rare kidney disease leading to end-stage renal disease in up to 30 % of adult patients during long-term follow-up. Management of Henoch–Schönlein purpura remains controversial. In children, various treatment regimens have been proposed in cases of severe digestive involvement or nephritis, including corticosteroids, azathioprine, cyclophosphamide, cyclosporine, and mycophenolate mofetil (Pillebout et al. 2011). In a prospective randomized trial, the addition of cyclophosphamide provided no additional benefit for adults with severe Henoch–Schönlein purpura compared to steroids alone (Pillebout et al. 2010). The efficacy of rituximab in standard treatment-refractory chronic Henoch–Schönlein purpura has recently been reported in three children (Donnithorne et al. 2009).

It was recently reported a case report of an adult with Henoch–Schönlein purpura with several episodes of purpuric rash in the legs, arthralgia, persistent hematuria and proteinuria (1.1 g/day), and mild renal insufficiency. Skin biopsy revealed a small-vessel neutrophilic vasculitis with IgA deposition. A renal biopsy revealed focal proliferative glomerulonephritis with a segmental crescent formation in 15 % of the glomeruli, with an immunofluorescence study showing mainly mesangial deposits of IgA and fibrin. The patient, who never received any previous immunosuppressive treatment or steroids, was administered, as a single therapy, only two doses of 1,000 mg of rituximab at 2-week intervals. Three months later, the patient noticed no new rash and had no more hematuria or proteinuria and the renal function was normal. Twenty-two months later, he was symptom-free (Pillebout et al. 2011).

More recently, two single case reports described the successful use of rituximab in two adults with nephrotic syndrome who failed to respond to steroids, cyclophosphamide (and plasmapheresis in one case). In one case, the patient needed three doses of 1,000 mg rituximab by infusion, 2 weeks apart (El-Husseini et al. 2013) and in the other patient, rituximab therapy (375 mg/m^2 body surface area per time) was administered once weekly for 4 weeks and a complete remission was achieved and maintained after the 4 weeks (Ishiguro et al. 2013).

Notably, IgA plays a central role in the pathogenesis of Henoch–Schönlein purpura. Collectively, these case reports suggest that the inhibition of IgA production may be a good approach to treat Henoch–Schönlein purpura nephritis.

8 IgG4-Related Aortitis

A multiorgan disease recently named IgG4-related disease by researchers in Japan has been increasingly recognized in the last decade. Conditions in many organs previously considered disparate entities are now considered to be connected by tissue infiltration with IgG4-positive plasma cells and serum IgG4 elevation in some patients. The organs involved present a characteristic histopathology and

immunohistochemical staining pattern. IgG4-related disease may be differentiated from possible mimicking disorders by some clinical and pathologic features. Overall, IgG4-related disease is a fibroinflammatory disorder characterized by a propensity to the formation of tumefactive lesions, dense lymphoplasmacytic infiltrate containing many IgG4-positive plasma cells, storiform fibrosis, raised serum IgG4 levels in most but not all cases, and a rapid initial response to glucocorticoids when there is no tissue fibrosis. Patients with IgG4-related disease often require sustained glucocorticoid treatment that frequently cannot be tapered. Traditional DMARDs are usually not effective (Stone et al. 2012).

Aortic involvement in IgG4-related disease leads to lymphoplasmacytic aortitis or periaortitis (Unizony et al. 2013). Khosroshahi et al. (2012) recently reported ten patients with IgG4-related disease who were treated with rituximab, including three patients with aortitis, the largest case series to date of this type of patient. The three patients with aortitis had responded positively to rituximab, with clinical improvement and reductions in acute phase reactants and serum IgG4 levels. Prednisone administration, ranging between 10 and 60 mg/daily before rituximab initiation, was withdrawn in all three patients after rituximab therapy. However, two of the three patients suffered disease recurrences, typified by worsening of clinical symptoms and raised serum IgG4 levels, some 6 months after rituximab initiation, which responded well to rituximab retreatment.

9 Hemophagocytic Syndrome

Hemophagocytic syndrome is an immune-mediated life-threatening disease caused by impaired natural killer and cytotoxic T-cell function. Clinically it is characterized by fever, hepatosplenomegaly, and cytopenia and the finding of macrophages in hematopoietic organs. Hemophagocytic syndrome has traditionally been classified according to the etiology and is divided into primary (genetic) and secondary (reactive), which has been subclassified as viral, autoimmune, or neoplasia-related (Henzan et al. 2006). However, clinically, a significant number of patients do not fit this classification exactly due to the frequent etiological overlap.

Etiopathogenetically, the theoretical basis for treating hemophagocytic syndrome requires a triple, simultaneous approach. Firstly, support measures are essential, due to the frequent life-threatening presentation. Secondly, the elimination of triggers (mainly infection) is crucial to remove the stimuli that initiate abnormal immune system activation. Thirdly, suppression of the inflammatory response and/or cell proliferation by immunosuppressive and cytotoxic drugs, respectively, is necessary.

There are isolated reports of success using biological therapies for refractory hemophagocytic syndrome in adults, including rituximab, infliximab, and etanercept, after failure with cyclosporine A, intravenous immunoglobulin, and/or etoposide. The evidence is too limited to support solid recommendations, but common sense, and consideration of the underlying disease may help select the

most appropriate biological agent. Thus, the use of B-cell depleting agents such as rituximab or belimumab in patients with hemophagocytic syndrome related to systemic lupus erythematosus, Sjögren's syndrome, or antineutrophil cytoplasmic antibody-associated vasculitides may be postulated. In addition, rituximab may be a salvage therapeutic option in patients with Epstein–Barr virus-related hemophagocytic syndrome or B-cell lymphoma (Ramos-Casals et al. 2013).

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Safety of B-Cell Targeted Therapies

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Abstract B-cell targeted therapies are relatively new therapeutic agents, although they are increasingly widely used for the treatment of systemic autoimmune diseases such as SLE. Rituximab also has a longer established role in the treatment of B-cell lymphoproliferative disorders and rheumatoid arthritis. In this chapter we review the available data on the safety aspects of B-cell targeted therapies. A common adverse event with this family of agents is infusion reactions, most of which are early in the treatment course and are generally manageable with simple preventative measures. The risk of infection is complex and depends on whether we consider all common infections or specific rare but serious infectious complications. There is also variation between short- and long-term exposure, some of which may also be modulated by the potential beneficial effects of better disease control and reduced concomitant immunosuppressive and steroid use. Specific infectious complications such as hepatitis B reactivation and PML also remain a concern with the widespread use of these drugs.

For these reasons, long-term safety data is needed and large international registry efforts will be required to fully understand the short-, medium-, and long-term safety issues associated with this class of agent.

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1 Introduction

B-cell targeted therapies are now widely used for the treatment of systemic autoimmune diseases such as SLE. A number of these agents also have been used in other clinical contexts. Most notably, rituximab is licensed for the treatment of B-cell lymphoproliferative disorders and is also approved for use in rheumatoid arthritis. Other agents such as ocrelizumab are being developed for the treatment of multiple sclerosis. As a result, there is a wide experience of using these agents across a range of contexts from which safety reporting can be reviewed. A number of these agents are, however, still in the clinical development stage and therefore safety reporting to date has been limited. For the purposes of this chapter we will firstly consider how we can best learn about safety related to biological agents in general, and we will then review the safety data currently available on five of the key agents currently in use or under consideration within systemic autoimmune diseases namely rituximab, belimumab, ocrelizumab, epratuzumab, and atacicept.

2 Approaches to Studying Biological Drug Safety

The traditional method of examining early safety signals for most drugs is through the clinical trial program in which the drug is evaluated. Clinical trials are extremely useful to identify significant early safety problems that may halt the agent's subsequent development into clinical use. Their particular strength is to identify common major adverse events that would stop the agent being widely used in clinical practice. It should, however, be understood that early phase (Phase I and II) clinical trials are usually established to determine the initial safety and efficacy of an agent. The later Phase III trials generally focus on efficacy with safety signals being a secondary endpoint. Rare and uncommon adverse events that will limit an agent's widespread use cannot always be assessed and fully accounted for even in a Phase III trial program. The long-term extension studies which usually follow Phase III trials are also a routine source of safety data. Generally, such long-term extension studies allow responders to continue on the drug and offer the drug in an open label fashion to patients initially exposed to the placebo arm of studies. As such, whilst long-term extension studies examine patients exposed to the agent over a prolonged period of time, often the control data is limited to the placebo treated patients over the shorter duration of the randomized part of the trial itself. In order to make inferences about any safety signals that arise, a historical data set or a comparative patient cohort is often used to understand these results. One limitation of long-term extension studies is that they are enriched for good responders to the drug. If patients have an adverse event and are then withdrawn from the study, they may not contribute further to the data analysis.

When drugs get licensed, most countries have spontaneous reporting systems such as the Medicines and Healthcare products Regulatory Agency (MHRA) "yellow card" system in the United Kingdom. Such spontaneous reporting systems allow doctors treating patients with new therapies to report any adverse event seen with a particular

drug. It is well recognized that such systems are very poorly adhered to and significantly underestimate the actual frequency of any adverse events. They do, however, have the advantage that they give a snapshot of the range of potential adverse events that may occur in routine practice and may prompt further investigation of particularly rare adverse events as well as particular interactions that may occur in routine practice. In recent times, a number of major initiatives have been developed to try and better understand real-world usage and exposure to biological agents. In the context of rheumatoid arthritis (RA), a number of countries now have large national biologics registers exemplified by the British Society for Rheumatology Biologics Register (BSRBR) (Hyrich et al. 2006), the BIOBADSER register in Spain (Carmona et al. 2007) and the RABBIT register in Germany (Listing et al. 2005). These post-licensing registers provide a formal pharmacovigilance programme that allows patients to be registered and followed up prospectively according to the drug therapy they are exposed to. Several such registers have alongside a control register of patients with the same disease, treated with conventional disease modifying therapies. A control register allows adjustments to be made for the baseline risk of particular adverse events related to the disease itself so that there is no false attribution made to drug therapy. For example, in RA it is recognized that non-Hodgkin's lymphoma and other lymphoproliferative disorders appear at an increased risk in patients with RA compared to the background population (Franklin et al. 2006). Therefore, any development of lymphoproliferative disorders in the context of a drug exposed population needs to take into account the baseline risk of that complication in the disease itself rather than in a healthy control population. The other advantage of such registries is that they can be powered to detect less common adverse events and are essentially primarily focused on prescribing patterns and safety. The data from such registries can therefore change practice to more safe prescribing as has been seen with the modification of tuberculosis screening for anti-TNF drugs and the food safety warnings which have reduced the incidence of listeriosis and salmonella in TNF treated patients (Carmona et al. 2005; Davies et al. 2013).

With regard to the B-cell targeted therapies for systemic autoimmune diseases, only belimumab has been licensed for use in active SLE. The other agents have been parts of clinical trial programs for these conditions. Rituximab has a unique position of having a large wealth of post-licensing and post-approval safety data gathered already however such data has been gathered in the context of lymphoproliferative disorders and rheumatoid arthritis. There is, however, both open label experience and clinical trial data informing its use in systemic autoimmune diseases. Within the past 5 years several biologics registers have been established for these systemic autoimmune conditions particularly in SLE (Terrier et al. 2010).

3 Rituximab

There is now a large global experience of using rituximab for its licensed indications in lymphoproliferative disorders and rheumatoid arthritis. As a result, a great deal is known about the profile of this drug and in particular safety aspects of its use.

Open label experience and clinical trial experience is also now emerging in systemic autoimmune diseases, particularly SLE and Sjögren's syndrome. A number of safety issues have been considered during follow-up in the studies. In addition, case series and data from observational registries have added to the knowledge base regarding rituximab.

3.1 Infusion Reactions

One of the commonest adverse events associated with rituximab use is infusion reactions. A recent study reviewed the long-term safety of rituximab in 3,194 patients from the global RA clinical trial program (Van Vollenhoven et al. 2012). Amongst this population, infusion reactions occurred in 23 % of patients. The majority of these reactions occurred with the first infusion and were mild-to-moderate in intensity. A small number of patients (19 [0.5 %]) experienced a severe infusion reaction including drug hypersensitivity or anaphylaxis with 10 of these 19 events occurring with the first infusion. No serious infusion reactions occurred after the sixth course of rituximab. All patients had received premedication for the infusion which included 100 mg of intravenous methylprednisolone usually taken with acetaminophen and an antihistamine (Van Vollenhoven et al. 2012). In view of the risk of infusion reactions rituximab has traditionally been given as a slow intravenous infusion over approximately 3–4 h. Recent experience both in rheumatology and hematology has suggested that a more rapid infusion protocol may be equally safe. For example, Sehn et al. (2007) employed a 90-min infusion schedule for patients with hematological malignancy (20 % of dose in the first 30 min and 80 % in the next 60 min). In a formal review of 206 patients treated with this regime there was no increase in minor or serious infusion reactions. This regime has now been used in over 1,200 patients without any major increase in adverse events. Similarly, in patients with rheumatic diseases, more rapid infusion times have also been reported to have no increased risk of severe events. Can et al. (2013) used a 120-min infusion rate in 68 patients with no increased risk of adverse events whilst Larsen and Jacobsen (2013) reported no excess risk of adverse events using a 90-min infusion time.

With regard to SLE, in the EXPLORER and LUNAR trials infusion reactions occurred in 13.6 and 16.4 % of rituximab treated patients, respectively (Merrill et al. 2008; Rovin et al. 2012). The majority of these were mild to moderate reactions. In the EXPLORER trial, four of 127 patients also experienced a serum sickness reaction; three of these four patients had anti-chimeric antibodies detectable. These reactions, however, were transient and resolved after 1–3 weeks. In an open label study of 136 patients treated with SLE in the French AIR register two patients developed major acute infusion reactions and five episodes of serum sickness were observed in the cohort (Terrier et al. 2010).

3.2 Infections

Analysis of clinical trial data in RA patients, which represents the largest autoimmune disease experience of using rituximab, has not identified any overall excess risk of infection in patients treated with this agent. For example, a meta-analysis of three clinical trials that involved rituximab compared to placebo, found an overall odds ratio for infection of 1.45 (95 % CI: 0.56, 3.74) (Salliot et al. 2009). Similarly, in a review of long-term safety in the overall rituximab clinical trial program, the rate of serious infections was 3.94 (95 % CI: 3.60, 4.31)/100 patient-years for all rituximab treated patients and 3.79 (2.80, 5.13)/100 patient-years in the placebo group (Van Vollenhoven et al. 2012). Also, a network meta-analysis that included all patients in rituximab clinical trials and long-term extension studies compared to a pooled placebo group from a large number of biologics clinical trials estimated the relative risk of infection with rituximab compared to the pooled placebo group to be 0.97 (95 % CI: 0.64, 1.48) (Singh et al. 2011).

The majority of the data from the above sources were of course during clinical trials lasting 6 to 12 months. An initial follow-up study of 1,039 patients treated with more than one rituximab course showed stable rates of infection up to the fourth cycle of therapy (Keystone et al. 2007). Further analysis of this cohort by van Vollenhoven et al. (2012) estimated the infection risk in patients treated with rituximab for more than 5 years to be 3.26 (2.77, 3.84)/100 patient-years; again, this is comparable to the placebo rates in RA trials. As has been noted previously, clinical trial populations may represent a highly selected population in which patients at particularly high risk of infection are excluded. Registry data in patients treated with autoimmune diseases have also begun to examine infection risk in real-world settings. In a French registry, 1,303 patients treated with rituximab for RA had a mean follow-up of 1.2 years (SD = 0.8). The rate of severe infection was 5.0 per 100 patient-years (Gottenberg et al. 2010). Within this population there was only one opportunistic infection (fungal septic arthritis). No cases of TB were reported. Of the 82 infections reported, 42 (51.2 %) occurred within 3 months of the most recent rituximab infusion (Gottenberg et al. 2010). Risk factors for severe infection in the 12 months following a rituximab infusion included chronic lung disease and/or cardiac insufficiency (OR 3.0 [95 % CI: 1.3, 7.3]), RA-related extra-articular disease (OR 2.9 [1.3, 6.7]), and low IgG levels (<6 g/l) prior to rituximab infusion (OR 4.9 [1.6, 15.2]) (Gottenberg et al. 2010).

In the context of SLE, the EXPLORER and LUNAR trials both use rituximab with 1,000 mg infusions in two "courses" (i.e., at days 1, 15 and days 168, and 182). Patients in both the active arm and placebo arm were also given concomitant high-dose steroids and allowed to remain on their background immunosuppressant drug (Merrill et al. 2008; Rovin et al. 2012). In the EXPLORER trial, herpes infections were more frequent in patients treated with rituximab (15.4 % vs 8 %) with herpes zoster occurring in 9.5 % of rituximab treated patients compared to 3.4 % of placebo exposed patients. Serious infections overall were more frequent in the placebo group (17 % vs 9.5 %) (Merrill et al. 2008). In the LUNAR trial, rates of

serious infection were comparable between groups (19.9 and 16.6 per 100 patient-years in the placebo and rituximab groups, respectively), with similar hospitalization rates (Rovin et al. 2012). This trial data, albeit restricted to a 12-month analysis, suggests no excess risk of infection related to rituximab therapy even when added to significant additional immunosuppression. Real-world use of rituximab in SLE can be confounded by a lack of a control group particularly in a disease treated with high-dose corticosteroids during flares and in which there is an intrinsic excess risk of infection noted. In the French registry that reported experience from 136 SLE treated patients, 9 % experienced a severe infection (6.6/100 patient-years), the majority of these (83 %) occurred in the first 3 months following a rituximab infusion and 9 of 12 severe infections occurred after the first rituximab course (Terrier et al. 2010). Of 136 patients followed 5 (3.7 %) died. Three of the deaths were due to severe infection namely endocarditis, septicemia, and cholangitis (Terrier et al. 2010). Within a UK cohort of 50 patients treated with rituximab in one center, one patient had a severe infection following rituximab (Lu et al. 2009). A follow-up from this cohort which included 18 patients treated with more than one cycle of rituximab also reported a further patient with varicella pneumonia (Turner-Stokes et al. 2011).

3.3 Hepatitis B

Several reports have emerged of reactivation of hepatitis B infection in patients treated with rituximab. Initial reports were from the oncology literature and a fatal outcome was noted in some cases. A meta-analysis of 387 patients with hepatitis B reactivation found that the relative risk (RR) is particularly increased in patients with positive HBcAb (RR = 5.52; 95 % CI: 2.05, 14.85) (Dong et al. 2013). As a result, guidelines for screening and monitoring patients being treated for lymphoproliferative malignancies have now been developed (Oketani et al. 2012). Hepatitis B reactivation has also been reported in RA following rituximab therapy (Pyrpasopoulou et al. 2011). With regard to hepatitis C, there is ongoing controversy. Rituximab therapy can increase hepatitis C viral replication, which itself is associated with flares of hepatitis, as well as reactivation of hepatitis C (Sagnelli et al. 2012). Nevertheless there is also evidence of a favorable outcome of hepatitis C related cryoglobulinemia treated with rituximab along with PEG-IFN and ribavirin (Geri et al. 2012) with no excess increase in hepatitis C viral load or biomarkers of hepatic decompensation in the group treated with rituximab. Best practice is therefore to screen patients for viral hepatitis *prior* to treatment with rituximab and seek advice from a hepatologist in patients with evidence of previous or current infection.

3.4 Progressive Multifocal Leukoencephalopathy

Progressive multifocal leukoencephalopathy (PML) is a progressive neurological disorder with an extremely high mortality rate caused by reactivation of the ubiquitous JC (John Cunningham) polyomavirus. For many years, PML has been noted to occur in the context of HIV infection, and post chemotherapy related to hematological and solid cancers. Sporadic reports of PML occurring in the context of rheumatic diseases have occurred over the last few years and a retrospective review of the national discharge database in the USA noted that the risk of PML appears disproportionately increased in patients with SLE compared to patients with RA and other connective tissue diseases (Molloy and Calabrese 2008). Indeed, in the context of RA, additional risk factors such as HIV infection or concomitant malignancy are frequently seen. In contrast, in many cases of SLE no additional major risk factor may be present (Molloy and Calabrese 2008; Nived et al. 2008). Further analysis from a large administrative database in the USA estimated that overall patients with any rheumatic disease had an annual incidence of PML of 2.6/100,000. The majority of these had an additional explanation either HIV or cancer and therefore the risk in rheumatic disease patients without these risk factors was estimated to be 0.2/100,000 (Bharat et al. 2012). A number of biological agents over the past few years have been associated with PML and natalizumab and efalizumab were both withdrawn because of this complication although natalizumab has subsequently been re-instated with an active management program in place (Calabrese and Molloy 2008). A number of cases of PML associated with rituximab therapy have also been reported and an FDA warning has been issued for this agent (http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm126519.htm). In addition, patient information regarding this potential complication has also been developed. More recent evidence from the adverse event reporting system in the USA has also identified a number of agents as being associated with PML over and above their use in SLE. In addition to rituximab, natalizumab and efalizumab; nonbiological agents such as mycophenolatemofetil, tacrolimus, and cyclophosphamide were also associated with PML (Schmedt et al. 2012). Therefore in the context of treating systemic autoimmune diseases, a thorough discussion about the rare risk of PML is necessary prior to offering treatment however, such an informed discussion also needs to take into account the small but appreciable risk of PML associated with lupus itself, other immunosuppressive drugs and comorbidities. All this of course also needs to be considered in the context of the risks associated with uncontrolled disease activity.

3.5 Late-Onset Neutropenia

Late-onset neutropenia (LON) is a recognized complication of rituximab therapy for malignancy. Several case series also noted this complication in the treatment of rheumatic diseases. LON was reported to occur in 5.8 % of patients in one series (Besada et al. 2012) and in 5.4 % of patients in a larger cohort (Tesfa et al. 2011). LON is more common in patients with granulomatous polyangiitis (23 %) and SLE (20 %) (Tesfa et al. 2011). The median time to occurrence in one series was 23 (7–48) weeks after rituximab and there is evidence that it is occurrence coincides with B-cell depletion and low immunoglobulin levels. As a result, up to half of patients will have an infective complication around the time of neutropenia. Nevertheless, recovery has been shown to be the norm and in a number of cases re-treatment of rituximab has occurred without recurrence of the complication (Besada et al. 2012; Tesfa et al. 2011).

3.6 Hypogammaglobulinemia

Hypogammaglobulinemia is a risk factor for infection in patients treated with rituximab (Gottenberg et al. 2010). Also, in view of its ability to deplete B cells there are concerns that low immunoglobulin levels may occur after prolonged treatment with B-cell depletion therapy. In a long-term follow-up study of RA patients treated in clinical trials, 717 (22.4 %) patients developed low IgM had at least one time point. Low IgG developed in 3.5 % of patients and before and during episodes of low IgG lasting 4 months or more the rates (95 % CI) of serous infection per 100 patient years of follow-up were 8.06 (5.08, 12.8) and 9.13 (6.3, 13.22), respectively, compared to 3.73 (3.38, 4.09)/100 pyrs in patients who did not develop prolonged low IgG (Van Vollenhoven et al. 2012). Patients more at risk of developing low IgG included patients who were older, with longer disease duration, a lower mean CD 19 and IgG level at baseline and these patients had also received more conventional disease modifying drugs prior to starting rituximab (Van Vollenhoven et al. 2012).

In prospective studies of patient cohorts, the mean levels of immunoglobulin in a rituximab treated cohort reduces over time and is particularly marked in patients exposed to more than 5 g of rituximab (Isvy et al. 2012). In a study of 137 patients from a single center 18 patients received five cycles of rituximab and in this population eight (38 %) had low IgM and (22 %) had low IgG levels. Lower baseline immunoglobulins were associated with risk of developing hypogammaglobulinemia (de la Torre et al. 2012). Hypogammaglobulinemia associated with rituximab therapy may require replacement immunoglobulin to reduce the risk of subsequent infection and help manage emergent chronic infection in such patients (Otremba et al. 2012).

3.7 Vaccination Responses

Early studies of rituximab therapy in RA demonstrated that preexisting levels of antibodies against tetanus and pneumococcus were not affected by a single infusion (Cambridge et al. 2006). Several recent studies have, however, studied antibody responses following active immunization in patients previously treated with rituximab. Humoral responses to influenza vaccination have been shown to be impaired in rituximab treated patients (Arad et al. 2011; Oren et al. 2008; van Assen et al. 2010). The impaired response is particularly seen soon after rituximab therapy. Patients vaccinated 6 months after their previous dose appear to have a modest response to vaccination (van Assen et al. 2010). The cellular responses to influenza vaccine, however, appear to be maintained following rituximab therapy (Arad et al. 2011) and this may explain why some protection remains in these patients. Others have found that the responses to the 23-valent pneumococcal vaccine are better than those seen with influenza vaccination (Rehnberg et al. 2010); however in a controlled clinical trial, pneumococcal responses were significantly impaired following rituximab (Bingham et al. 2010). Similarly, in a renal transplant population, responses to tetanus vaccination while impaired compared to controls did still produce satisfactory levels of protection in patients (Puissant-Lubrano et al. 2010). Recent European guidelines for vaccination of patients with inflammatory rheumatic diseases recommend early consideration and administration of vaccines in this population. Specifically however it also recommends that vaccination, where possible, should be given prior to rituximab therapy. If given after rituximab, then preferably this should be 6 months following the most recent dose and more than 4 weeks before the next dose is due (van Assen et al. 2010).

3.8 Miscellaneous AEs

A number of additional warnings have also been issued regarding rituximab use in rheumatoid arthritis. These include rare reports of severe mucocutaneous reactions within the toxic epidermolysis and Stevens Johnson Syndrome categories (http://www.mhra.gov.uk/home/groups/pl-p/documents/drugsafetymessage/con270719.pdf). In addition, caution has been recommended in patients who have previously developed cardiac dysrhythmias with rituximab.

4 Belimumab

Belimumab recently received its license from the FDA and European regulators for use in active SLE. The clinical trial program included a large Phase II clinical trial (Wallace et al. 2009) and two global Phase III trials (Furie et al. 2011; Navarra et al. 2011). In this clinical trial program 1,458 patients received belimumab therapy at either 1, 4 or 10 mg/kg (Wallace et al. 2013c). The licensed dose is 10 mg/kg. In this overall program, the overall rates of adverse events and serious adverse events were comparable across all treatment arms (Wallace et al. 2013c).

All infusion reactions, group together, occurred and 14.7 % of placebo treated patients and 16.9 % of patients treated with the 10 mg/kg dose. Of these, 0.4 and 0.9 %, respectively, were defined as serious. Hypersensitivity reactions resulting in discontinuation of the drug occurred in no placebo treated patients and in 0.3 % of belimumab treated patients. Infusion reactions were most common during the first infusion (7.3 % of belimumab treated patients) (Wallace et al. 2013c). Only one serious infusion reactions occurred beyond the second dose in a patient treated with 1 mg/kg of belimumab. Infusion reactions included headache, nausea, and skin rashes however two patients in the 10 mg/kg treated group suffered anaphylaxis-angioedema during the 2-hour administration of the initial dose (Wallace et al. 2013c). Recently, a further warning has been issued regarding belimumab infusions indicating that a very small number of cases have been reported where patients have had a serious infusion reaction several hours following the infusion. This is usually occurred following the first or second dose and may be more likely in patients with a history of multiple drug allergies and hypersensitivity (http://www.fda.gov/Safety/MedWatch/SafetyInformation/ucm299628.htm) .

It is now advised that during early infusions, patients are observed for a longer period of time in hospital to monitor for such a reaction.

The overall rates of infection in the belimumab trial program were 5.35 (3.77, 7.37)/100 patient-years in the placebo group and 6.00 (4.83, 7.37)/100 patient-years in the belimumab exposed patients. Infection leading to discontinuation of the drug occurred in 1.2 % of placebo and 0.6 % of belimumab 10 mg/kg treated patients. In addition, 0.1 % of placebo exposed patients and 0.4 % of belimumab 10 mg/kg treated patients died from infection (Wallace et al. 2013c). There was no particular imbalance in types of infection seen in patients treated with belimumab. Fungal infections occurred in 3.4 % of placebo patients and 2.5 % of belimumab 10 mg/kg treated patients and herpes virus infections in 8.0 % vs 6.8 %, respectively (Wallace et al. 2013c). Immunoglobulin levels fell significantly during treatment with belimumab compared to placebo in the clinical trial. For example, in the BLISS-52 trial, the median change in IgG levels in the placebo treated group was −3.6 % (IQR: −14.5, 6.10) compared to −15.6 % (−23.92, −6.64) in patients treated with 10 mg/kg belimumab (Navarra et al. 2011). In the overall trial population 2.9 % of placebo treated patients had a reduction in IgG levels to below the lower limit of normal with 0.2 % having a Grade 3 hypogammaglobulinemia (<400 − 250 mg/dL). In the belimumab 10 mg/kg group 6.6 % develop low IgG with 0.2 % also

developing Grade 3 hypogammaglobulinemia. IgM levels fell below the lower limit of normal in 6.3 % of placebo and 19.9 % of belimumab 10 mg/kg treated patients (Navarra et al. 2011).

Malignancy rates did not differ between groups (0.29 [95 % CI: 0.04, 1.04] and 0.20 [0.04, 0.58]/100 patient-years in placebo and all belimumab treated patients) (Wallace et al. 2013c). With regard to mortality 14 deaths occurred in the entire trial population 3 in placebo treated patients and 11 in belimumab treated patients (Wallace et al. 2013c). The 11 deaths in the belimumab exposed patients included four due to infection, two suicides, and individual cases of SLE, cardiovascular disease, cerebrovascular disease, malignancy, and one unknown death. The mortality rates were 0.43 (95 % CI: 0.09, 1.27)/100 patient-years in placebo and 0.73 (0.36, 1.3)/100 patient years in belimumab patients (Wallace et al. 2013c). Overall, 12.4 % of placebo patients and 15.9 % of belimumab 10 mg/kg treated patients had a psychiatric adverse event reported and serious psychiatric disorders occurred in 0.4 and 1.2 % of these populations, respectively. Serious depression also occurred in 0.1 and 0.4 % of these two groups (Wallace et al. 2013c).

Long-term safety data has also been reported for belimumab from the Phase II clinical trial where patients who completed the 52-week blinded treatment period were offered the option of entering a 24-week open label extension phase (Merrill et al. 2012). Patients could continue their dose of belimumab and placebo treated patients were offered treatment with belimumab 10 mg/kg. After 24 weeks, patients could be entered into a long-term extension study on an open label basis. The Phase II clinical trial recruited 449 patients with active SLE. Of these, 364 completed the 52-week blinded trial and 345 entered the 24-week extension study; 296 of these continued into the long-term extension study. From October 2003 to August 2009, 1,165 patient years of exposure to belimumab was therefore analyzed. The overall mortality rate in this population treated with belimumab was 0.4/100 patient-years (95 % CI: 0.14, 1.0) (Merrill et al. 2012). In the post trial period of the long-term study, three further deaths occurred from coronary heart disease, suicide and CMV pneumonia (Merrill et al. 2012). No hypersensitivity reactions occurred beyond the first year of belimumab exposure; however, two further infusion reactions did occur during the long-term extension. One patient developed vertigo in the second year and a further patient had an acute episode of chest pain and dyspnea which resulted in discontinuing belimumab in the third year of therapy (Merrill et al. 2012). Infection rates remained stable in the extension study with two opportunistic infections noted including 1 case of CMV pneumonia and 1 case of coccidioidomycosis in an endemic area. It was also noted that over at the period of time patients were exposed to belimumab, the cumulative rates of serious infections was approximately 1.5-fold higher in patients taking MMF with belimumab compared to patients taking other immunosuppressants with belimumab (9.4 vs 6.3/100 patient years). Similarly, serious infection rates were 2.5-fold greater in patients receiving corticosteroids compared to those not receiving steroids at baseline (Merrill et al. 2012). Beyond the randomized trial period, four patients developed malignancies including two solid tumors and two hematological malignancies. There were also seven non-melanoma skin cancers during the overall period of time

patients were exposed to belimumab. Immunoglobulin levels did not show any evidence of any further reductions over 4 years of exposure for example, the median percentage change in IgG level from baseline in year 1 was −9.1 % and was −12.9 % in year 4 (Merrill et al. 2012).

5 Epratuzumab

To date experience with epratuzumab has been reported in several short-term Phase I and II trials (Dorner et al. 2006) in SLE as well as a single long-term extension experience of a small number of SLE patients. The ALLEVIATE trial programme was terminated early because of issues with supply of the drug and so these were reported together (Wallace et al. 2013a). Ninety SLE patients were randomized across this programme and 53 received active drug over a 48-week period; either 360 mg/m^2 (42 patients) or 720 mg/m^2 (11 patients). Mild-moderate infusion reactions occurred in 9 (18 %) epratuzumab treated patients which did not differ to the placebo rates (7 [19 %]). Similarly there was no excess of infection or serious infection seen with 8 (22 %) of placebo patients and 9 (15 %) of epratuzuamb treated patients having a serious infection (Wallace et al. 2013c). In the SL006 long-term extension study of 29 patients treated for a median of 120 weeks, 3 (10 %) also experienced a serious infection. There was only one death in the study which occurred 16 weeks after the last dose of epratuzumab in a patient who had pneumococcal infection and their course was complicated by a cerebral hemorrhage (Wallace et al. 2013a).

The EMBLEM trial was a 12-week dose ranging study of 227 patients randomized to receive placebo or epratuzumab at a cumulative dose of 200–3,600 mg. In this programme, 187 patients received active drug (Wallace et al. 2013b) Overall 24 (12.8 %) experienced an infusion reaction most of which were mild and this was comparable to the placebo group (4 [10.5 %]) (Wallace et al. 2013b). There were 11 serious adverse events in the epratuzumab treated population including two infections and one case of anaphylaxis. Further large Phase III trials are underway.

A small trial in 16 patients with Sjögren's syndrome also suggested good safety of this agent over a 32-week evaluation in which patients received 360 mg/m^2 in four infusions over the first 6 weeks. In this population, two stopped the drug because of a moderate–severe infusion reaction (one with the first dose and the other with the third dose). Two infections were also reported (sinusitis and a dental abscess) (Steinfeld et al. 2006).

6 Ocrelizumab

Ocrelizumab has not been taken forward in SLE or RA owing to unfavorable safety to benefit considerations. In 2 RA studies first dose infusion related AEs occurred at a higher rate with ocrelizumab than in placebo patients (Rigby et al. 2012; Stohl et al. 2012). In a 24-week trial of RA patients who were methotrexate inadequate responders, 7 (6.5 %) serious infections occurred in 114 patients treated with ocrelizumab including 2 pneumocystis jiroveci pneumonias, 1 herpes zoster and 1 herpes simplex infection (Harigai, Tanaka, & Maisawa 2012). Also in a 52-week trial of 603 MTX-naïve RA patients, serious infections rates were higher in the patients treated with 500 mg OCR (7.1 [95 % CI: 3.9, 11.9]/100 patient years), compared to patients treated with 200 mg 2.6 (0.9, 6.1) and placebo 3.0 (1.1, 6.5). In this trial it was also noted that there was a particularly high rate of infection in patients treated with 500 mg ocrelizumab from the Asia/Pacific region (30.1 vs 4.5 per 100 patient-years) (Stohl et al. 2012). In a Phase II trial of MS, ocrelizumab was administered in cumulative doses of 600–2,000 mg. The AE rates were comparable to placebo exposed patients and there were no opportunisitic infections. One patient treated with a cumulative dose of 2,000 mg ocrelizumab, however, died from a systemic inflammatory syndrome at week 14 in the trial (Kappos et al. 2011).

In the BELONG trial of lupus nephritis, patients were randomized to 400 or 1,000 mg ocrelizumab at days 0, 15, and 16 weekly thereafter as add-on therapy to SOC therapy which was either mycophenolatemofetil (MMF) 3 g/day or cyclophosphamide, according to the EuroLupus Nephritis Trial regime. The trial was terminated early by which time 378 patients had been enrolled. Serious Infection Events (SIEs) occurred in 18 (14 %) of placebo patients who had just received SOC therapy. In the group treated with MMF 3 g/day plus ocrelizumab 400 mg infusions SIEs occurred in 25 (32.9 %) (Reddy et al. 2013).

7 Atacicept

In an early Phase Ib dose finding study in SLE patients, atacicept dosing was associated with reductions in IgM and IgG with 6/32 patients developing IgM below the lower limit of normal (LLN) and 1/32 developing IgG below LLN. Some recovery was noted in patients after dosing ceased. In this trial, patients were permitted to be on steroids and antimalarials but not on immunosuppression (Pena-Rossi et al. 2009). In a trial of lupus nephritis patients, all enrolled patients were initiated on high-dose prednisolone (60 mg/day or 0.8 mg/kg daily which ever was lower) along with MMF escalating over 14 days to 3 g/day. After this run-in phase, patients were randomized to atacicept 150 mg twice weekly for 4 weeks then weekly thereafter, with the aim of continuing therapy to 48 weeks. The trial was terminated after six patients had been entered (four atacicept and two placebo) (Ginzler et al. 2012). Three atacicept treated patients developed low IgG. Three

serious infections also occurred in these patients. Of interest the IgG levels began to fall in the 14 day run-in period when patients were initiating high-dose steroids and MMF. The two placebo treated patients also experienced a less profound reduction in IgG which gradually recovered during the study. The combination of high-dose steroids and MMF may have contributed to early falls in IgG but the precise explanation why this unexpected and serious AE occurred with this treatment regime remains unclear (Ginzler et al. 2012).

8 Conclusions

We are still in the early days of B-cell targeted therapies in the clinic. With the exception of rituximab, experience of these agents remain limited in general clinical practice. Several drugs have been withdrawn from clinical development within rheumatology; however, some of these have continued development in other indications such as lymphoproliferative disorders and multiple sclerosis. While a number of these agents were withdrawn because of an unacceptable risk-benefit ratio within the trial program, it remains unclear for some of these agents whether the limiting factor was the drug itself or the dose chosen or whether there were particular interactions with additional concomitant medications. Better understanding of these limiting factors may allow further use of these agents in a more targeted and specific way.

A common adverse event with this family of agents is infusion reactions, most of these tend to be early in the treatment cycle and are generally manageable with simple preventative measures. A key question for these drugs is whether they contributed to an excess risk of infection overall or to an excess risk of specific rare but serious infectious complications. Short-term experience with most of these agents has been favorable in this regard but the relationship between drug exposure and infection risk with these agents is likely to be complex. Factors that appear to be of importance include the drug dose used as well is the actual effect of the drug whether they are cell-depleting agents or modulating agents. In addition, several agents notably rituximab have been associated with reductions in immunoglobulin levels over the long-term and may also produce neutropenias and affect responses to vaccinations. All of these factors may contribute to increased infection rates over the long term with these agents. Specific infectious complications such as hepatitis B reactivation and PML also remain a concern with the widespread use of these drugs. On the other hand, better control of underlying inflammatory disease particularly in lupus may actually improve infection risk in this population as may the promise of reduced steroid exposure and reduced overall immunosuppression burden in these patients. For this reason, long-term safety data is needed in these patients and large international registry efforts will be required to fully understand the short medium and long-term safety issues associated with this class of agent.

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The Future Potential of Biosimilars Targeting B-Cells

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Abstract The patent and regulatory data protection periods for the first and second waves of biological agents based on recombinant proteins have started to expire, leaving open the potential for development and regulatory approval of 1 or more "similar" versions of these biologic therapies, termed biosimilars in Europe (BS)—the term that will be used in this chapter—subsequent entry biologics in Canada, or follow-on-biologics in the USA. The development of BS therapies could lead to a substantial saving for patients/health systems and, therefore, increased availability of effective treatments. BSs are similar but not identical to their reference products, because their chemical characteristics are directly related to the manufacturing process, which cannot be precisely duplicated. An exact replica of a protein molecule is extremely difficult if not impossible. Thus, major concerns about short- and long-term safety and efficacy have been raised and should be addressed

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in the approval process by regulatory agencies. For these reason, BSs require an approach to grant the marketing authorization, different from generics.

1 Introduction

Treatment in inflammatory conditions in rheumatology has experienced a revolution during the last two decades with the introduction of biological agents. These drugs have led to a completely new approach to the management of patients with inflammatory autoimmune conditions. The advent of biological therapy for rheumatic diseases provided a more effective control of the disease and sustained amelioration of activity compared to the pre-biologic era when only anti-inflammatory and immunosuppressant drugs were available. Treat to target remission is now the aim. Nevertheless, biologic agents are expensive and rheumatologic diseases are common. Even the wealthiest societies are unable to support the widespread use of biologic agents in patients requiring this type of drugs.

Although the value to the patient is priceless, efficacious treatments for rheumatic diseases do have a quantifiable cost. Recently a systematic review of cost-of-illness studies in rheumatoid arthritis found mean annual health care cost of 4,170€ per patient, with indirect costs (sick leave, lost productivity) taking total cost to 14.906€ per patient per year (Huscher et al. 2006; Franke et al. 2009).

It has been anticipated that by 2016, 10 of the top-selling 20 drugs will be biologics and of these, three (Adalimumab, Rituximab, Infliximab) are monoclonal antibodies or (etanercept) a fusion protein containing antibody components currently widely used to treat rheumatic disease (Cuadrado et al. 2013; Dorner et al. 2013).

Now, the patent and regulatory data protection periods for the first and second waves of biological agents based on recombinant proteins have started to expire, leaving open the potential for development and regulatory approval of one or more "similar" versions of these biologic therapies, termed biosimilars in Europe (BS)—the term that will be used herein—subsequent entry biologics in Canada, or follow-on-biologics in the USA. The development of BS therapies could lead to a substantial saving for patients/health systems and, therefore, increased availability of effective treatments.

BSs are similar but not identical to their reference products, because their chemical characteristics are directly related to the manufacturing process, which cannot be precisely duplicated. An exact replica of a protein molecule is extremely difficult if not impossible.

Thus, major concerns about short- and long-term safety and efficacy have been raised and should be addressed in the approval process by regulatory agencies. For these reason, BSs require an approach to grant the marketing authorization, different from generics.

BSs potentially applicable in rheumatologic field as BS of etanercept or rituximab have been already approved in countries such as India, China, and

South Korea and their possible emergence on European and US markets is being a matter of discussion by the European Medicines Agency (EMEA) and Food and Drug Administration (FDA) (Cuadrado et al. 2013; Dorner et al. 2013).

2 Definition of BSs

BSs are defined as biological products similar, but not identical, to biological medicine that has been already authorized (Crommelin et al. 2005; Hughes 2010). Thus, BSs are not generic versions of biological products. Conventional generics are considered to be therapeutically equivalent to the reference drug. Once pharmaceutical equivalence (i.e., identical active substances) and bioequivalence (i.e., comparable pharmacokinetics) have been established, generic drugs do not require formal clinical efficacy and safety studies (Schellekens 2004).

This is not the case with BS. The active substance of a biologic agent such as those ones used in the treatment of rheumatic diseases is a collection of large protein isoforms and not a single molecular entity, which is generally the case with conventional small-molecule drugs. Thus, it is highly unlikely that the active substances are identical between two products, and there are currently no analytical techniques to establish biopharmaceutical equivalence (Minghetti et al. 2011).

Table 1 shows general agreed standard definitions for conventional generic agents, biologic agents, and BS based on terminology used by the EMEA.

3 Regulatory Approval

Limited documentation is required to obtain marketing authorization for a conventional small-molecule generic drug (European Medicines Agency 2005). In general, it is sufficient to show pharmaceutical equivalence and bioequivalence of a generic drug compared with the original product in a small study of volunteers, via an abbreviated procedure (Minghetti et al. 2011). However, this approach cannot be extrapolated to the majority of biological agents currently used in rheumatology. Physicochemical and biological methods for characterization of biological agents such as monoclonal antibodies (mAb) are becoming increasingly sophisticated, but the ability to compare a BS mAb to a reference mAb on an analytical level remains limited (Hughes 2010). Thus, the amount of data required for market approval of BS will be more than for a typical generic drug application (Dorner et al. 2013). At present the EMEA guidelines are the only clear document detailing the requirements for market approval of BS (European Medicines Agency 2005; Reichert et al. 2009) The EMEA guidelines advocate preclinical and clinical testing of BS to demonstrate safety and efficacy prior to market authorization, followed by tailored pharmacovigilance plans to monitor potential immunogenicity (European Medicines Agency 2005).

Table 1 General agreed standard definitions for conventional generic agents, biologic agents, and BS based on terminology used by the European Medicines Agency (EMEA)

Generic drug	Chemical and therapeutic equivalent of a low-molecular-weight drug whose patent has expired
Biologic agents	A medicinal product developed by means of one or more of the following biotechnology practices: rDNA, controlled gene expression, antibody methods
Biosimilar	A biological medicinal product referring to an existing one and submitted to regulatory authorities for marketing authorization by an independent application after the time of the protection of the data has expired for the original product

Moreover, European guideline states that in case the reference medicinal product has more than one indication, the efficacy and safety of a BS has to be justified or, if necessary, demonstrated separately for each of the claimed indications. However, the guideline brings up the possibility of "extrapolation" of data about efficacy and safety from trials designed for other indications, but only in specific circumstances, as was the case for the hematopoietic hormones erythropoietin and granulocyte-colony stimulating factor.

In the USA, the FDA has not yet issued a specific regulatory pathway (Dorner et al. 2013; FDA HR 3590-686 2010). The Biologics Price Competition and Innovation (BCPI) Act outlined a shortened approval process for "highly similar" biologic products, which enables a BS product to be evaluated against a single, already licensed, reference biologic therapy.

Data obtained from analytical and animal studies, and from at least one clinical trial conducted in patients with a disease for which the biological agent is licensed, will be required to demonstrate that a BS product is highly similar to the reference product. In February 2012, the FDA issued draft guidance for industry regarding implementation of the BPCI Act approval process for BS agents.

However, the draft guidance does not specify requirements for the size or duration of the required clinical trial, and the FDA has not yet indicated whether the trials will be intended to demonstrate non-inferiority, or to prove therapeutic equivalence with the BS agent.

The position of the American College of Rheumatology (ACR) has been also reported, stating that the ACR strongly believes that safe and effective treatments should be available to patients at the lowest possible cost. However, decisions about biosimilarity and interchangeability must be driven by scientifically sound evidence about safety and efficacy, as the highest priorities.

4 BSs and Rheumatic Disease: Clinical Efficacy and Safety

Although efficacy of a BS should be theoretically equivalent to the reference product, numerous factors may have an impact in this end-point. Product attributes related to manufacturing approach (including in-process controls and product

controls, impurities, aggregates, heterogeneity, fragments) are between them. Thus, even in cases where a well-established potency assay correlating with clinical efficacy is available, human data would likely be required for BS development. Data from physicochemical and biological characterization alone are not sufficient for a BS development, and data coming from clinical trial should be required to support similarity. The key question is, to what extent clinical trials are required for a BS? The goal of the clinical development program for the BS is to demonstrate no significant difference with the reference product. For that, equivalence trials of adequate sample size and, ideally, double-blinded should be conducted.

The authors have identified only one clinical trial published on BS (up to 2012). The search included MEDLINE, Current Contents, PubMed, and amplified using web-available search engine focusing on BSs version of etanercept (Gu et al. 2011). At the moment no clinical trial has been reported in B-cell targeting therapies.

4.1 Safety

As biologicals, BSs are structurally complex proteins with significant micro-heterogeneity, generated from genetically modified living cells, difficult to produce and purify. Manufacturing processes in terms of choice of cell type, production, purification, and formulation methods influence the quality, purity, biological parameters, and clinical activity of the final product, which in turn affects efficacy and safety. However, even if the BS products will have the same gene sequence, vector, host cell line, culture conditions, and purification methods as the reference protein, they can still differ substantially in some biological and clinical properties. Studies indeed have demonstrated differences in physical characteristics, activity, potency, safety, and isoform profile relative to BSs approved in other field apart from rheumatology (e.g., epoetin alfa) (Abraham and MacDonald 2012) It would be critical to identify significant differences of clinical relevance. The question still to be answered is when a BS is similar enough.

The major issues rose by the EMA about safety are immunogenicity and extrapolation of indications. When the safety of BS is being assessed, identical safety parameters that were used for the reference agent must be used in the development program. Currently, we lack of data about safety coming from well-designed clinical trial.

Immunogenicity means the ability of a protein antigen to elicit an immune response in a human or animal and the production of antibodies against the protein. BSs may stimulate the production of antibodies against any component of the BS. Several factors are known to affect a product's immunogenic potential. These can be related to the biopharmaceutical, the host or a combination of both. Immunogenicity can be induced by the active-drug substance, but more often results from manufacturing impurities originating from the producing cell line or media components. The presence of impurities in biological products, structural modifications as a result of the manufacturing process and/or suboptimal storage conditions can

increase the risk of immunogenicity (Dranitsaris et al. 2011). The antiproduct antibodies may bind to and thus attenuate or inactivate the BSs, also resulting in hypersensitivity reactions such as allergy or serum sickness, even anaphylaxis. The antibodies may also interfere or neutralize endogenous proteins, leading to unexpected effects, as happened in the cases of pure red cell aplasia induced by BS recombinant human erythropoietin (Gershon et al. 2002; Bennett et al. 2004; Schellekens 2005). Autoimmune processes appearing after the use of biological agents have been described since the initial studies of anti-tumor necrosis factor agents in patients with rheumatoid arthritis (Ramos-Casals et al. 2010). Since then, the number and diversity of autoimmune diseases triggered by these biological agents has increased in parallel with their increasing use, and cases of autoimmune diseases induced by other licensed biological agents have also been reported. With the advent of BSs we could assist to an emerging number of clinical and analytical autoimmune adverse events, even different from the one already reported from currently used biologicals, ranging from asymptomatic immunological alterations to life-threatening systemic autoimmune disease.

Thus post marketing surveillance and long-term follow-up will be mandatory. Emphasis should be put on developing well-designed pharmacovigilance programs following approval in order to identify rare and potentially serious events.

5 Biosimilars Targeting B-Cells

Rituximab (RTX) is a chimeric antibody that specifically recognizes the human CD20 molecule. The CD20 molecule is a non-glycosylated protein expressed mainly on the surface of B lymphocytes. In some pathogenic B cells, it shows an increased expression, thus becoming an attractive target for diagnosis and therapy. This antibody is indicated for the treatment of non-Hodgkin lymphomas and autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus and its use in these conditions has been described elsewhere in this book.

RTX is currently co-marketed by Biogen Idec and Genentech in the USA, by Hoffmann–La Roche in Canada and the European Union, and by Chugai Pharmaceuticals and Zenyaku Kogyo in Japan.

BS versions of RTX are being developed. In April 2007, Dr. Reddy's Laboratories Ltd. (Hyderabad, India) launched Reditux, a intended copy of RTX, for the treatment of non-Hodgkin's lymphoma and, later, as Reditux-RA for the treatment of rheumatoid arthritis. Other manufacturers are conducting some phase I and phase II clinical trials or have BS versions of RTX in their pipelines (Table 2). Teva Pharmaceutical Industries Ltd. (Petach Tikva, Israel), Sandoz (Basel, Switzerland), Pfizer (US), and Celltrion (South Korea) are conducting a phase I/II trial comparing the pharmacokinetics of their BS anti-CD20 moABS with RTX in patients with active rheumatoid arthritis. However, in October 2012 Teva suspended plans for a phase III trials on its RTX BS.

Table 2 Current rituximab-biosimilars clinical trials in EU and US

Producer	Molecule	Phase
Boehringuer	BI695500	Phase IV
Pfizer	PF-05280	Phase I/II
Sandoz	GP-2013	Phase I/II
Celtrion	CT-P10	Phase I
Merck & Co	MK-8808	Phase I

Other B-cell targeting therapies, such as ocrelizumab (a 90–95 % humanized B cell-depleting agent) and ofatumumab (HuMax-CD20, a fully human B cell-depleting agent), have been more recently developed and their patent and regulatory data protection periods are still far from expire, reducing at the moment the potential interest from the BSs developers.

6 Biosimilar as a Strategy to Provide Economically Affordable Treatments?

Biologics are a successful class of therapeutic agents, but many treatments remain costly, which may limit their use.

The potential for cost savings has to be seriously taken into account if BS versions of biological therapies become available. Nevertheless, savings cannot be expected to be in the same order of magnitude as in the case of generics, due to high manufacturing costs, the need to perform nonclinical and clinical studies, and an appropriate pharmacovigilance program. As an example, in the UK the list prices of four BS (Omnitrope®, Binocrit, Retacrit, and Ratiograstim®) compared with their respective innovator products are about 10–25 % less. To thwart competition between different manufactures, the cost of BSs may also be reduced.

7 Conclusion

Major concerns must be addressed before a rheumatologist can routinely substitute a biologic agent by a BS. BS must undergo the required comparability qualification in accordance with scientific principles endorsed by authorities, such as EMA or FDA. Albeit savings in costs are hoped, physicians prescribing BS must be aware of any developments concerning BSs mainly in terms of safety and efficacy, and be vigilant in their use.

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