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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