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To B or not to B cells-mediate a healthy start to life

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Summary

Maternal immune responses during pregnancy are critical in programming the future health of a newborn. The maternal immune system is required to accommodate fetal immune tolerance as well as to provide a protective defence against infections for the immunocompromised mother and her baby during gestation and lactation. Natural immunity and antibody production by maternal B cells play a significant role in providing such immunoprotection. However, aberrations in the B cell compartment as a consequence of maternal autoimmunity can pose serious risks to both the mother and her baby. Despite their potential implication in shaping pregnancy outcomes, the role of B cells in human pregnancy has been poorly studied. This review focuses on the role of B cells and the implications of B cell depletion therapy in pregnancy. It highlights the evidence of an association between aberrant B cell compartment and obstetric conditions. It also alludes to the potential mechanisms that amplify these B cell aberrances and thereby contribute to exacerbation of some maternal autoimmune conditions and poor neonatal outcomes. Clinical and experimental evidence suggests strongly that maternal autoantibodies contribute directly to the pathologies of obstetric and neonatal conditions that have significant implications for the lifelong health of a newborn. The evidence for clinical benefit and safety of B cell depletion therapies in pregnancy is reviewed, and an argument is mounted for further clinical evaluation of B cell-targeted therapies in high-risk pregnancy, with an emphasis on improving neonatal outcomes and prevention of neonatal conditions such as congenital heart block and fetal/neonatal alloimmune thrombocytopenia.

Keywords: autoantibodies, autoimmune, B cell, pregnancy, rituximab

The implications of maternal B cells in pregnancy outcomes

An individual's lifetime health is critically programmed during the gestational period. During pregnancy, the maternal immune system is required not only to accommodate the allogeneic fetus but also to maintain protection against harmful infections in the otherwise immunocompromised mother and immuno-incompetent fetus [1]. The roles of innate and cell-mediated immunity, including natural killer, T helper type 1 or 2 (Th1/Th2) cells and regulatory T cells (T_{reg}) are well documented in pregnancy [2,3]. In contrast, there has been little focus on the role of B cells and antibody-mediated immunity. This is surprising, given the fundamental role of B cells as effectors and regulators of both innate and adaptive immune responses [4,5]. Maternal B cells also provide a vital source of antibody-mediated protective immunity for the mother and her baby during both pregnancy and lactation [6]. However, some maternal autoimmune conditions can be exacerbated during pregnancy and the production of deleterious autoantibodies by autoreactive maternal B cells can contribute directly to pregnancy complications that pose serious risks of morbidity and mortality to the mother and the fetus. *In-utero* exposure to these autoantibodies due to placental crossing can also result in permanent impairment to fetal development. These high-risk pregnancy conditions often result in poor outcomes such as preterm birth and low birth weight

that also increase significantly the predisposition of a newborn to developmental disability and chronic diseases later in life [7–10].

B cell depletion therapy has proven clinical benefits in the management of autoimmune conditions outside pregnancy. In this review, we will examine the available evidence of the possible contribution of B cells in shaping pregnancy outcomes and discuss the implication of B cell depletion in the clinical management of high-risk pregnancy.

B cell subsets and their functions

B cells, while known primarily for antibody production, also act as antigen-presenting cells and regulators of the innate and adaptive immune systems [4,5]. The murine B cell compartment consists of two general populations, namely B1 and B2 cells. These cells have major differences in their phenotypes, anatomical location and functional characteristics [11,12]. In humans, the existence of a human B1 subset is still a contentious subject, and the distinctions between B1 and B2 cells remain undefined [12]. Nevertheless, both murine B1 and human B1-like cells have been characterized as B cell subsets that spontaneously secret large amounts of polyreactive natural antibody IgM against double-stranded DNA (dsDNA), phosphorylcholine (PC) and low-density lipoproteins [11–14]. In the mouse, B1 cells have been characterized by a pattern of surface markers of $B220^{low}$, immunoglobulin (Ig)M^{hi}, IgD^{low}, $CD5^{+/-}$, $CD43^{+}$ and $CD23^{-}$ expression, whereas B2 cells generally express $B220^{hi}$, $IgM^{hi/lo}$, IgD^{hi}, $CD43^{-}$ and $CD23^{+}$ markers but not CD5 markers, although B2 cells have been shown to express low levels of CD5 following activation *in vitro* and in some studies CD5 expression has been shown on anergic B2 cells [12,13]. In humans, CD5 expression has been described on both B1-like and activated B2 cells [12]. Recently, it has been suggested that the human B1-like cell population may include the circulating $CD5^{+/-}CD20^{+}CD27^{+}CD43^{+}IgM^{+}IgD^{+}$ B cell subset [14]. However, the definitive markers for the general human B1 cell population remain to be determined. B2 cells are known as conventional B cells, which make up the majority of the splenic B cell population. Unlike B1 cells, which appear in fetal liver tissue as early as mid-gestation and are regenerated by self-renewal processes in the peritoneal cavity, B2 cells emerge from bone marrow stem cells during the late neonatal period and their clones are selected by a stringent process of clonal deletion and expansion in the germinal centre of the spleen [12,13]. Murine and human B2 cells act mainly as effectors of adaptive immune responses by differentiating into the mature marginal zone and follicular B cell populations that then develop into antibody-secreting memory B cells and plasma cells. In contrast, B1 cells are considered as specialized B cells of innate immunity [12]. The murine B1 and human B1-like cells secrete mainly natural IgM antibodies that are often polyreactive and low affinity in nature. These natural antibodies, while autoreactive, mediate protective immune surveillance and maintenance of tissue homeostasis by facilitating clearance of dead cell bodies [12,13]. Conversely, antibodies produced by murine and human B2 cells are less likely to be autoreactive but are high in specificity and affinity due to their ability to undergo affinity maturation, somatic hypermutations and clonal selection via B cell receptor (BCR) activation [15]. Mature murine and human B2 cells can also undergo class-switch DNA recombination (CSR) to give rise to the production of IgA, IgE and IgG antibody subclasses [11,15]. Murine B1 cells are also generally more sensitive to BCR activation-induced apoptosis when subjected to affinity maturation [12]. Thus, they are often prevented from differentiating into autoreactive memory B cells or plasma cells capable of secreting high-affinity autoantibodies. However, murine B1 cells can migrate to spleen, where they differentiate into splenic marginal zone (MZ) B cell precursors that can undergo somatic hypermutation and isotype switching to give rise to antibody-secreting memory B cells and plasma cells [16]. In addition, B1 cells have the capacity to respond and migrate to distal sites of inflammation, where they act as phagocytic cells or as immune regulators through the secretion of cytokines [17–19].

B cell alterations in pregnancy

Alterations in B cell compartment during normal pregnancy

B cell subsets during pregnancy are poorly studied. B cell-deficient mice are not embryonic lethal and have normal litter sizes, suggesting that B cells are dispensable for normal murine pregnancy [20]. The treatment of mice and non-human primates with B cell-depleting agents also does not affect normal pregnancy [21–23]. During murine pregnancy, the formation of B cell precursors is suppressed selectively in the bone marrow [24]. This suppression occurs at the early stage of B lymphopoiesis and is driven by the pregnancy hormone oestrogen [24]. Maternal B cells that express autoantibodies specific for fetal antigens are also depleted during murine pregnancy, suggesting a mechanism of maternal–fetal immune tolerance [25–27]. However, oestrogen also has a positive effect on the survival of mature murine B cells [28], suggesting a compensatory effect of oestrogen at different stage of development to maintain a balance within the B cell compartment.

Similar changes in B cell compartment have been reported in a number of human pregnancy studies [29–36]. The absolute numbers and frequencies of circulating $CD5^{+}$ B cells are decreased in normal human pregnancy (Table 1), and can persist for up to 1 month postpartum [29,33, 37,38]. Normal pregnancy can induce loss of responsiveness of B cells to mitogens and infectious agents in both human and animal studies [39–41]. Thus, these studies

Table 1. Evidence of alterations in human B cell populations during normal pregnancy and obstetric complications.

Pregnancy conditions	B cell markers	Reported changes in percentages or absolute numbers	Ref.
Normal	$CD5^+$	Decreased†	[29,30]
	$CD19^+$	Decreased†	[31,32]
	$CD20^+$	Decreased†	[33]
	Ia^+/Ig^+	Decreased†	[34–36]
Post-partum	$CD19^+$	Decreased§	[33,37,38]
	$CD19^+CD5^+$	Decreased§	[29]
APS	$CD19^+CD27\text{-}IgD^+$	Increased‡	[42]
	$CD19^+CD5^+$	Increased‡	[43]
RSA	$CD19^+$	Increased‡	[44]
	$CD19^+CD5^+$	Increased‡	[45–47]
	$CD20^+CD5^+$	Increased‡	[48–50]
Pre-eclampsia	$CD19^+$	Increased‡	[51]
	$CD19^+CD27^+$	Increased‡	[52]
Preterm birth	$CD19^+$	Increased‡	[53–55]
IUGR	$CD19^+$	Increased‡	[56–58]
PIH	Ig^+	Increased‡	[59,60]

†Compared to healthy non-pregnant women. ‡Compared to gestational-match normal pregnant women. §Compared to normal gestation period. APS: anti-phospholipid syndrome; IUGR: intrauterine growth restriction; PIH: pregnancy-induced hypertension; RSA: recurrent spontaneous abortion; Ig: immunoglobulin.

suggest that the overall B cell compartment and its functions are suppressed partially during normal human pregnancy. The full biological significance of such suppression is unclear, but is believed to enable immune tolerance.

Aberrations in B cell compartment in adverse pregnancy

Aberrant B cell numbers and functions are associated with obstetric complications [42–59]. Earlier studies have shown that complicated pregnancies exhibit an abnormal increase in the frequencies or absolute numbers of circulating maternal B cells (Table 1). For instance, $CD5^+$ B cell counts are significantly higher in patients with anti-phospholipid syndrome (APS) and recurrent spontaneous abortion (RSA) groups than in healthy controls [43,45–50]. This B cell subset is also increased in placental tissues of RSA patients [50]. The absolute number and percentages of $CD19^+$ B cells are also increased in pregnancy complications [43,51–59], and a higher number of $CD19^+IgD^+$ B cell numbers are observed in APS mothers with associated risks of thrombotic events [42]. Increases in B cell activation markers and functions have also been reported in pre-eclampsia, intrauterine growth restriction (IUGR) and pregnancy-induced hypertension (PIH) cases in human studies [52,58,60,61]. Collectively, these studies present the evidence of an association between human pregnancy complications and an abnormal increase in B cell-activated functions and/or numbers.

Physiological factors of pregnancy may amplify B cell aberrations

It is not exactly clear what causes these anomalies in the B cell compartment of adverse pregnancies, and whether they simply represent an exacerbation of the pre-existing autoimmune conditions of the mother that is triggered by the physiological state of pregnancy. Under normal conditions, B lymphopoiesis is suppressed and autoreactive B cells are deleted during pregnancy to maintain maternal–fetal immune tolerance [25–27]. However, these normal regulatory mechanisms are impaired in autoimmunity leading to the expansion of autoreactive B cell subsets and deleterious autoantibody production. This notion is supported strongly by observations of an abnormally increased number of $CD19^+CD5^+$, mature $CD19^+CD27^+$ and $CD19^+IgD^+$ B cells in a number of obstetric conditions (Table 1). Indeed, these B cell subsets are well-known producers of autoantibodies such as rheumatoid factors, anti-thyroid, anti-ssDNA, anti-histone and anti-phospholipid autoantibodies [14,43,48,62–65]. In particular, the autoantibody-producing $CD19^+CD5^+$ B cell populations, which possibly include both human B1-like or activated B2 cells, are often expanded in autoimmune conditions such as APS, systemic lupus erythematosus (SLE) and primary Sjögren's syndrome [43,65,66], which are often exacerbated by pregnancy and linked strongly to risks of obstetric complications [9,10]. Thus, the strong link between $CD19^+CD5^+$ B cells and autoimmunity make them a prime candidate for further investigation in pregnancy conditions.

The underlying mechanisms that exacerbate autoimmunity during pregnancy are still unclear. Evidence from both animal models and human studies suggest that the elevated female sex hormone levels and a Th2-biased immunological state in pregnancy play a major role in promoting the expansion of autoreactive B cells. In mouse models of human SLE, both oestrogen and prolactin can exacerbate and accelerate autoimmune conditions by exerting a positive influence on the survival, proliferation, maturation and autoantibody production of the mature B cell population [28,67–70]. Such findings from animal models strongly reflect the evidence in human clinical studies where female populations have a significantly higher ratio of autoantibody-mediated autoimmune conditions (including SLE, APS, Grave's disease, myasthenia gravis, scleroderma and Sjögren's syndrome) than males, and these conditions are often exacerbated during pregnancy, where elevated levels of the female sex hormones occur [70]. The Th2-biased state of pregnancy, which is influenced positively by high levels of oestrogen during pregnancy, is also well known to promote B cell proliferation, activation and

antibody production in experimental animal models [70]. Evidence from animal studies and human B cell models show that the expansion and activation of autoreactive B cells can be amplified by mutual positive regulatory feedback loops between the oestrogen-receptor alpha (ER-α) pathway and other autoimmune-promoting cytokines such as interferon (IFN)-α and B cell-activating factor (BAFF) to promote survival, maturation and expansion of autoreactive B cells [71,72]. Data from animal models, in conjunction with evidence from human studies, suggest that these co-operative signalling pathways can also promote the antibody class-switching of polyreactive natural antibody IgM to a more pathogenic IgG autoantibody production by B1 cells [13,70–74]. The positive feedback loop and the production of IFN-α and BAFF may be activated and amplified through the innate pathways mediated by endogenous ligands and Toll-like receptors (TLRs) on B cells, monocytes and dendritic cells. Such endogenous ligands may consist of self-antigens, including lipoproteins, glycoprotein, single-stranded RNA (ssRNA) and dsDNA materials that are generated as a by-product from placental tissue-shedding during pregnancy. These endogenous ligands also provide a readily available source of autoantigens for the positive selection and activation of autoreactive B cell clones through BCR signals as well as the activation of TLR-mediated innate responses that contribute further to the exacerbation of the maternal autoimmunity and expansion of pathogenic autoantibody production.

The implications of maternal antibodies in pregnancy outcomes

Evidence from epidemiological, clinical and experimental studies has established that autoantibodies produced by maternal B cells contribute directly to adverse pregnancy outcomes [9,10]. The transplacental transfer of maternal autoantibodies is implicated as a causative factor in a number of obstetric and neonatal conditions (Table 2). Such maternal immunological imprinting and *in-utero* exposure of the fetus resulting in adverse pregnancy outcomes are best exemplified in pregnancies with autoimmune conditions such as APS, SLE, myasthenia gravis and primary Sjögren's syndrome.

APS

Patients with APS often have anti-phospholipid autoantibodies that are reactive against phospholipid proteins, such as β2-glycoprotein, cardiolipin, tissue plasminogen activator, thrombin, protein C and platelet antigens. The pathogenicity of anti-phospholipid autoantibodies is often associated with IgG classes and they target proteins that are involved in thrombosis, platelet and complement pathway activation, monocyte and endothelial cell functions [75]. These autoantibodies can be either agonistic or antagonistic

Table 2. Autoimmune conditions and adverse risks for mothers and neonates.

Maternal autoimmune conditions	Risks of maternal and obstetric complications	Causative maternal autoimmune factors	Risks for fetus and neonate
SLE	Exacerbation, anaemia, pre-eclampsia, thrombosis, haemorrhage, hypertension, kidney failure	Autoantibodies, placental transfer of maternal autoantibodies	Fetal loss, still birth, preterm birth, low birth weight, developmental and neurological abnormalities, CHB
APS	Exacerbation, anaemia, pre-eclampsia, thrombosis, haemorrhage, hypertension, kidney failure	aPL autoantibodies	Fetal loss, preterm birth, low birth weight
T1DM	Pre-eclampsia, hypertension, hyperglycaemia, exacerbation of metabolic aberrations	Anti-insulin, islet, GAD	Fetal loss, stillbirth, preterm birth, congenital malformation, increased birth weight
Haemophilia A	Antenatal or postpartum haemorrhages	Anti-Factor VIII or IX	Acquired haemophilia A
Primary Sjögren's syndrome	Stable disease	Placental transfer of maternal anti-Ro/SSA and La/SSB autoantibodies	CHB, NLS, low birth weight
Graves' disease	Exacerbation	Placental transfer of maternal anti-TSHR autoantibodies	Neonatal Graves' disease, NMG
Systemic sclerosis	Exacerbation	Unknown	Fetal loss, preterm birth, low birth weight
ITP	Haemorrhage	Anti-platelet, placental transfer of maternal IgG autoantibodies	FNAIT, intracranial haemorrhage, fetal loss, low birth weight
Myasthenia Gravis	Exacerbation, difficult labour	Placental transfer of maternal anti-AChR autoantibodies	NMG, AMC, fetal loss, stillbirth

AChR: acetylcholine receptor; AMC: arthrogryposis multiplex congenita; APS: anti-phospholipid syndrome; CHB: congenital heart block; ITP: idiopathic thrombocytopenic purpura; FNAIT: fetal/neonatal autoimmune thrombocytopenia; NLS: neonatal lupus syndrome; NMG: neonatal myasthenia gravis; TSHR: thyroid-stimulating hormone receptor; SLE: systemic lupus erythematous; T1DM: type 1 diabetes mellitus.

in nature. They contribute to the pathologies of APS by promoting thrombotic events, impairing endothelial cell function and provoking overt inflammatory responses in the maternal circulation and placental tissues. This may lead to vasoconstriction, impaired endothelial function and placental dysfunction that restrict blood supply to the placenta and result in placental ischaemia and/or hypertensive disorders. Such a cascade of events can lead to a range of poor pregnancy outcomes such as RSA, IUGR, pre-eclampsia or stillbirth. Mild to moderate thrombocytopenia is common in APS, and this can worsen in pregnancy [9]. The causes of APS-associated thrombocytopenia are poorly understood: unlike immune thrombocytopenia (ITP), specific antibodies against the major platelet adhesion receptors (GPIIb-IIIa or GPIb-V-IX) are uncommon.

SLE

Pregnant women with SLE carry not only a risk of maternal and fetal morbidity, but also risks of long-term disability to the newborn. The immunopathologies of SLE pregnancy display several features of those seen in APS. Thus, it is not surprising that SLE pregnancy shares many of the adverse risks and poor outcomes of APS, such as maternal morbidity, IUGR, pre-eclampsia, stillbirth or preterm birth [9]. In addition, the autoimmune conditions of SLE and APS are often exacerbated during pregnancy and contribute further to the disease burden and dysfunction of the maternal circulation and renal system. The deposition of anti-nuclear proteins, anti-dsDNA, anti-basement membrane autoantibodies and autoreactive antibodies in kidney glomeruli can cause nephritis that results in further damage to the already compromised kidney function. This, in turn, exacerbates the hallmark signs of pre-eclampsia, such as hypertension and proteinuria. In addition, neonates of mothers with SLE or primary Sjögren's syndrome are at risk of developing neonatal lupus syndrome and congenital heart block [9,10]. These neonatal conditions often occur in mothers who are seropositive for anti-Ro/SSA and/or anti-La/SSB autoantibodies. Although the risk of congenital heart block in a fetus from a seropositive mother is only 3–5%, suggesting that other co-factors may be involved, and the risk of recurrence in the same mother increases to 15–20%, clinical and experimental studies have demonstrated the causative role of the placental transfers of maternal IgG autoantibodies specific for Ro/SSA and /or La/SSB in the pathogenesis of these neonatal conditions [76,77]. In addition, children who are born to APS or SLE mothers have a significantly higher risk of developmental and neurological abnormality, with an increased rate of learning disabilities [78,79].

Myasthenia gravis

The disease is exacerbated more frequently during the first trimester of pregnancy and is believed to be due to the effects of oestrogen on Th1 and autoreactive B cells [67,80]. Mothers with myasthenia gravis have autoantibodies that are specific for maternal acetylcholine receptor (AChR) at the neuromuscular junction. These autoantibodies target fetal AChR preferentially. The placental transfer of these autoantibodies results in a severe developmental abnormality that causes arthrogryposis multiplex congenita. This condition causes joint contracture in the fetus, resulting in a lack of movement *in utero* and, in severe cases, leading to a high risk of fetal death or stillbirth [67,81].

Other antibody-mediated conditions

Autoimmune diseases are not the only source of pathogenic autoantibodies that pose significant risks of maternal and neonatal complications. Women who are asymptomatic of autoimmune disease but seropositive for autoantibodies such as anti-nuclear proteins, anti-dsDNA and anti-thyroid antibodies also carry a similar risk of obstetric complications such as IUGR and pre-eclampsia [9,10]. The presence of anti-fetal human leucocyte antigen (HLA) antibodies in the maternal circulation is associated significantly with risk of preterm placental abruption [82]. The agonistic autoantibodies against the angiotensin receptor from pre-eclampsia mothers can directly induce hypertension and proteinuria in pregnant mice, suggesting their contribution to the pathologies of human pregnancy conditions [83,84]. Transplacental transfer of inhibitory antibodies against factor VIII from a haemophilic mother can cause life-threatening acquired haemophilia or the fetal/neonatal alloimmune thrombocytopenia (NFAIT) condition in her baby [85]. Children from healthy mothers who are seropositive for maternal antibodies reactive to fetal brain proteins have a higher incidence of autism [86,87]. Injection of pooled maternal antibodies from mothers with autistic children into pregnant mice or non-human primates cause neurodevelopmental and neurobehavioural abnormalities similar to those of an autistic child in their progenies, and thereby demonstrate directly a pathogenic role of *in-utero* exposure to maternal antibodies in human autism [88,89].

Protective immunity during gestation and lactation

Not all exposure to maternal antibodies is detrimental to the health of the baby. In fact, there is a wealth of evidence from animal and human studies demonstrating the benefits of *in-utero* exposure to maternal antibodies and in early infancy by providing protective immunity against infection and reducing the risks of developing certain allergies and autoimmune immune conditions [90–92]. Contrary to animal models, children exposed to anti-islet autoantibodies from mothers with type 1 diabetes mellitus (T1DM) during pregnancy have a marginally reduced incidence of developing anti-islet autoantibodies and T1DM later in life

[93,94]. Placental and breast-feeding transfer of maternal antibodies provides vital protective immunity for neonates during the first 6 months of life, where infants are immunologically defenceless against deadly pathogens such as tetanus, measles, pertussis and influenza [95–98]. In murine models, postpartum transfer of immunoglobulin through breast feeding prevents neonatal death and growth retardation of pups [21]. Interestingly, maternal antibodies can transfer protective immunity, yet can also suppress vaccination responses in early infants [99]. Breast milk antibodies can either inhibit or facilitate transmission of the human immunodeficiency virus (HIV) to infants [100]. Taken together, these studies demonstrate clearly that exposure to maternal antibodies can carry some potential clinical benefits as well as burdens on pregnancy and the health outcome of a newborn.

Clinical evidence of B cell depletion in pregnancy

B cell depletion therapy with rituximab (Genentech, San Francisco, CA, USA), a chimeric monoclonal antibody directed against B cells surface antigen CD20, has been used successfully to treat B cell malignancies and a number of autoimmune conditions. Rituximab is combined routinely with chemotherapy in the treatment of high-grade lymphomas, and used as a single agent to prolong remissions in low-grade lymphoma. Rituximab has been used as a single agent to treat severe antibody-mediated conditions, and also combined with immunosuppressive agents, such as cyclophosphamide, corticosteroids and plasmapheresis. The clinical benefits of rituximab result from severe and sustained depletion of the B cells that leads to a reduction in serum levels of some autoantibodies and suppression of generic T cell responses [101].

B cell depletion therapy has shown promising benefits in the clinical management of high-risk pregnancies. Early evidence of the clinical benefits of rituximab in high-risk pregnancy has been demonstrated in non-Hodgkin lymphoma (NHL) to maintain aggressive B cell lymphomas in remission until delivery [102]. Since then, there have been more reports of rituximab in the clinical management of B cell lymphoma and autoimmune conditions in high-risk pregnancies (Table 3). Currently, there have been 21 known reported uses of rituximab in the clinical management of high-risk cases of established pregnancies that involve Burkitt's lymphoma, NHL, diffuse large cell B lymphomas, autoimmune haemolytic anaemia, thrombotic thrombocytopenic purpura (TTP) and ITP [102–112]. Gestational exposure to rituximab has been reported in all three trimesters [112]. Of the 21 known reported cases of antenatal rituximab, all but three cases were administered during the second or third trimesters [112]. In the majority of cases, maternal autoimmune conditions were managed successfully during pregnancy with reports of the reduction of risk of maternal morbidity and mortality.

Table 3. Reported cases and outcomes of rtuximab treatment in established pregnancy.

		Time of	Pregnancy outcomes				
Maternal conditions	Treatment	treatment	Outcomes	Terms (weeks)	Birth weights	Neonatal complications	Ref.
DLCBL	Rituximab + CHOP	21 weeks	Healthy	35	n.a.	Low B cell counts	[102]
Follicular B cell lymphoma	Rituximab	1st trimester	Healthy	40	3·6 kg	n.r.	[103]
AIHA	Rituximab	7–10 weeks	Healthy	38	3·1 kg	n.r.	[104]
DLCBL	Rituximab + CHOP	15 weeks	Healthy	33	Normal range	Low B cell counts	[105]
Burkitt lymphoma	Rituximab + CHOP	16 weeks	Healthy	41	3·8 kg	High level of rituximab at birth	[106]
TTP	Rituximab	27 weeks	Premature	31	1·04 kg	Intensive care but normal development	[107]
ITP	Rituximab + steroid	30 weeks	Healthy	38	3·8 kg	Low B cell counts	[108]
NHL	Rituximab + CHOP	18 weeks	Healthy	33	2·5 kg	Low B cell counts	[109]
ITP	Rituximab	26 weeks	Healthy	n.s.	n.s.	Low B cell counts	[110]
Atopic dermatitis	Rituximab	1st trimester	Healthy Twins	36	n.a.	n.r.	[111]

AIHA: autoimmune haemolytic anaemia; CHOP: cyclophosphamide, hyroxydaurubicin (doxorubicin), oncovin (vinscristine), and prednisone; DLBL: diffuse large cell B lymphoma; ITP: idiopathic thrombocytopenic purpura; NHL: non-Hodgkin's lymphoma; TTP: thrombotic thrombocytopenic purpura; weeks, weeks of gestation; n.a.: data not available; n.r.: none reported.

The initial concern of B cell depletion is the potential for adverse effects on pregnancy outcomes due to a severe and sustained suppression of B cell numbers that may compromise the immunological defence of the mother and disrupt the finely balanced immunological state of pregnancy, resulting in unforeseeable consequences on pregnancy. However, accumulated data from the number of reports so far have eased this concern. Although the numbers of reported cases are still limited, the pregnancy outcomes for neonates exposed to rituximab during gestation have been encouraging [112]. There have been no reports of fetal losses, congenital malformations or serious infection. The majority of newborns in published case studies were reported to be healthy and normal (Table 3). Of the 21 known reported cases of antenatal rituximab, 15 babies were delivered with normal birth weight and at full term, with the remaining cases being delivered at between 31 and 35 weeks [112]. There is still little information on the effect of the timing of gestational exposure to rituximab on the newborn's immune system. There are three reported cases of placental transfer of antenatal rituximab, including one case that was received as early as week 16 [106], which were detected in cord or neonatal blood at birth [112]. The placental transfer of rituximab can therefore lead to depletion of neonatal B cells and may also explain the low neonatal B cell counts in several reported cases [102,105,108–110]. Of the 21 cases of antenatal rituximab, there are 11 reported cases of neonatal cytopenias that include B cell depletion, low white blood cells, neutropenia, lymphopenia, thrombocytopenia and anaemia [102,105–107,112]. Most cytopenia cases appeared to be transient and recovered spontaneously within 12–16 weeks in follow-up studies [105–107,112]. Despite the high incidence of haematological disturbance and significant reduction in B cell counts in neonates, there has been no report of infections associated with these cytopenia cases. All babies developed normally with an intact vaccine response [112].

Despite the possible clinical benefits of rituximab in high-risk pregnancy, exposure to rituximab during pregnancy is not recommended, except in the case of life-threatening refractory diseases, because of the very limited data available on safety and efficacy [113]. From the limited data available, confounding factors such as concomitant exposure to other medications in reported cases also make it difficult to make a sound interpretation and recommendation on the efficacy and safety of rituximab in pregnancy [112]. Adverse drug infusion reactions and severe infections remain a concern with the general prescription of rituximab. Although the incidence is rare and dependent on the types of diseases being treated, B cell depletion with rituximab has a known risk of reactivating latent John Cunningham (JC) viral infection in treated patients, leading to the potentially fatal condition of progressive multi-focal leucoencephalopathy (PML) [114]. Sustained suppression of the B cell compartment can lead to impairment of T cell responses, resulting in a prolonged immunosuppressive state with an increased risk of vertical transmission of cytomegalovirus (CMV) infection from mother to fetus [112]. Pan-specific depletion of B cells can deplete autoantibodies as well as protective natural antibodies and regulatory B cell subsets [5]. Therefore, it is clear that carefully planned clinical trials are needed to evaluate the full benefits and harms of rituximab in pregnancy before it can be recommended for wider use in pregnancy.

Conclusion and future perspectives

The evidence presented in this review has clearly highlighted the important role of B cells in shaping pregnancy outcomes that have implications for long-term human health. Despite this, there are still limited data detailing the changes in the human B cell compartment, and the role of B cell subsets in pregnancy outcomes is poorly studied. This is due to the limited number of B cell markers used in earlier studies to describe changes in B cell subsets during pregnancy. Recent advances in B cell biology indicate clearly that these markers alone are not adequate in describing their full functions in human pregnancy. Further efforts should be dedicated to delineate the contribution of these B cell subsets in the maintenance of a healthy pregnancy as well as their roles in pregnancy complications.

In light of the potential benefits of rituximab in depleting autoreactive B cells and the emerging safety profile of rituximab in pregnancy, it is anticipated that B cell depletion therapies will eventually be trialled in obstetric complications that involve autoantibodies such as APS, SLE or ITP. It is reasonable to expect that rituximab will make some advances in the treatment of refractory conditions in pregnancy and provide a viable option that spares the use of high doses of chemotherapeutics and steroids in high-risk pregnancy to reduce risk of fetal toxicity [115], and thereby allows the pregnancy a better chance to develop to full term. Future pilot studies into the safety and efficacy of rituximab in pregnant patient cohorts are needed to provide a rational basis for larger studies. Although B cell depletion has demonstrated clinical benefits for maternal conditions in high-risk pregnancies, its potential benefits and risks for neonatal outcomes have not yet been investigated fully. It remains to be determined whether or not B cell depletion can improve neonatal outcomes on preterm birth, low birth weights, congenital malformations and their associated long-term health consequences. The potential benefit of B cell depletion therapy on neonatal outcomes will probably depend upon whether it can deplete autoreactive B cells and suppress placental transfer of maternal autoantibodies such as anti-platelet antibodies in maternal ITP or anti-Ro/SSA and/or anti-La/SSB autoantibodies, as well as co-culprit factors such as inflammation or other autoantibodies, in SLE and primary Sjögren's syndrome. This effect will depend probably on the properties of B cell-depleting

agents and the susceptibility of autoreactive B cell clones to the immunomodulatory activities of these agents. Equally important is the timing of the administration of B cell-depleting agents, whereby it can deplete the pool of autoreactive B cells early enough before these cells develop into plasma or memory B cells which are capable of producing high levels of pathogenic autoantibodies of IgG classes that can cross the placental barrier in sufficient quantities to reach a threshold that can cause damage to the fetal tissues. Such effects may have a novel clinical application in preventing life-threatening conditions such as NFAIT or congenital malformations such as congenital heart block, a long-term condition that is currently unpreventable. The development of new B cell-targeted therapies may also improve the specificity of depletion of autoreactive B cells while sparing the beneficial regulatory B cell subsets and the protective natural antibody responses to maximize the benefits and minimize the risks of sustained suppression of the B cell compartment [116–118]. Therefore, lessons from future clinical studies and new developments in B cell-targeted therapies are important and necessary to give the newborn of a high-risk pregnancy a better chance at a healthy start to life.

Review criteria

Our literature review was performed by searching in MEDLINE and PubMed database using search terms 'Autoimmune', 'B cell', 'B-cell depletion', 'Pregnancy' and 'Rituximab'. All included articles were in English-language, full-text papers published between 1975 and May 2012. We also searched reference list of these articles.

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Disclosure

The authors declare no conflicts of interest.

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