Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Immunology

B-cell tolerance: mechanisms and implications

Antony Basten[1,2] and Pablo A Silveira[1,2]

Advances in our knowledge of the spectrum of B-cell activities combined with the remarkable clinical efficacy of B-cell inhibitors in autoimmunity and transplantation settings serve to re-emphasise the importance of tolerance to self and foreign antigens in the B-cell repertoire. In particular, new information is emerging about the molecular mechanisms involved in B-cell tolerance induction and identification of B-cell selective defects that contribute to the pathogenesis of autoimmune/inflammatory diseases.

Addresses
[1] Immunology Program, Garvan Institute of Medical Research, Darlinghurst, New South Wales, Australia
[2] St. Vincent's Clinical School, University of New South Wales, Sydney, New South Wales, Australia

Corresponding author: Basten, Antony (A.Basten@garvan.org.au)

Current Opinion in Immunology 2010, **22**:566–574

This review comes from a themed issue on
Immune tolerance
Edited by Herman Waldmann and Mark Greene

Available online 9th September 2010

0952-7915/$ – see front matter

DOI 10.1016/j.coi.2010.08.001

Introduction

It is essential that tolerance be imposed on the B-cell as well as the T-cell repertoires. The main reasons for this are: (a) the spectrum of functional activities performed by B-cells that include not only antibody production, but also antigen presentation and secretion of pro-inflammatory and anti-inflammatory cytokines [1]; (b) the fact that the B-cell repertoire is generated in two stages: in the first, V(D)J recombination occurs within the bone marrow (BM) to create the 'pre-immune repertoire', while the second involves somatic hypermutation (SMH) of immunoglobulin (Ig) variable region genes within germinal centres (GCs) following antigen stimulation and provision of co-stimulatory signals from T-cells and/or external pathogens (immune repertoire). In each case, the primary goal is to diversify the repertoire of B-cell specificities against foreign antigens, although the random nature of both V(D)J recombination and SMH inevitably leads to the appearance of cells expressing anti-self B-cell receptors (BCR) within both repertoires. Multiple overlapping mechanisms of tolerance at several checkpoints in B-cell differentiation have evolved to deal with the ever-present threat of autoimmunity posed by the generation of self-reactive B-cells. This range of mechanisms also applies when tolerance is acquired for foreign antigens on allografts or infectious agents [2,3••].

Decision between tolerance and immunity

For B-cells, the decision between tolerance and immunity can still be explained within the framework of the two-signal hypothesis of Bretscher and Cohn [4]. The factors that influence this decision can be divided into antigen or host (immune system) related categories (Table 1). Here we have focused on antigen structure, the role of host tissues and contributions made by regulatory cells, as these factors tend to be overlooked.

Conventional wisdom has it that *antigen structure* can determine the T-cell dependence of a B-cell response, but does not distinguish self from non-self. Two studies challenge this dogma. In the first, a highly immunogenic multimeric T-independent type 2 (TI-2) antigen, polyacrylamide, was 'decorated' with sialosides (terminal sugar motifs commonly expressed on glycoproteins of mammalian but not microbial cells) recognised by the inhibitory signalling molecules CD22 and Siglec-G on B-cells [5•]. This manoeuvre resulted in B-cell tolerance to subsequent challenge with the unmodified TI-2 antigen. The modified foreign antigen was therefore recognised as self. A similar interpretation may account for the link described between the degree of membrane sialylation of tumour cells (e.g. from B16F10 melanoma) and their metastatic potential [6]. In the second study, the attachment of opsonised complement components (C3dg) onto neo-self-antigens, resulting in co-ligation of BCR and CD21/35 complement receptors on B-cells, led to reversal of self-tolerance [7]. In this case, the self-antigen was recognised as foreign. Taken together, these studies demonstrate that an important function of B-cell co-receptors is to assist in distinguishing self-antigens from non-self-antigens by setting the threshold of reactivity to them (Table 1).

A potential role for *target tissue* factors in influencing the decision between tolerance and immunity requires emphasis. Matzinger has summarised this concept well in a recent review where she points out that tissues 'use all sorts of mechanisms to keep the cells and molecules of the immune system out until they need them and to control them when they arrive' [8•]. Independent evidence of a role for tissues in modulating tolerance comes from studying the genetics of autoimmune disease models, where two clusters of susceptibility genes are frequently identified, one controlling the level of reactivity of the immune system and the other encoding tissue susceptibility to autoimmune attack [9,10•]. These tissue

Table 1

Antigen and host-related factors leading to tolerance or immunity[a]

Factors	Tolerance	Immunity[b]
Antigen-related (signal 1)		
Structure	Increased sialylation	Lower sialylation
Concentration	Higher	Lower
Avidity	High	Intermediate
Duration of encounter	Chronic	Acute
Timing of encounter	Immature stage	Mature stage
Host-related (signal 2)[c]		
Intrinsic to immune system		
• Cytokines	TGF-β, IL10	IL-4, TNF
• Antibodies	IgG (via FcγRIIb)	IgM, IgG (via FcγRI or III)
• Regulatory cells	B_{reg} and T_{reg}	–
• Macrophages	TBM in GC	CD69+ in MZ and subcapsular sinus
• Complement	Guide B-cell to niches for negative selection	Anaphylatoxins in inflammation
• BCR signalling threshold	ITIM motifs (siglecs, e.g. CD22)	ITAM motifs (e.g. Lyn, CD19)
Extrinsic to immune system		
• Target tissue factors	No inflammation (no cell access)	Inflammation (cell access)
• External co-stimuli	Tumour-derived (e.g. TGF beta)	Pathogen-derived (e.g. toll like receptors)

[a] Reviewed in [2,5•,6,7,8•,25,29,53].
[b] Factors favouring immunity also contribute to breakdown in tolerance leading to autoimmunity and rejection of tolerated grafts.
[c] Host-related factors contribute to secondary mechanisms of tolerance.

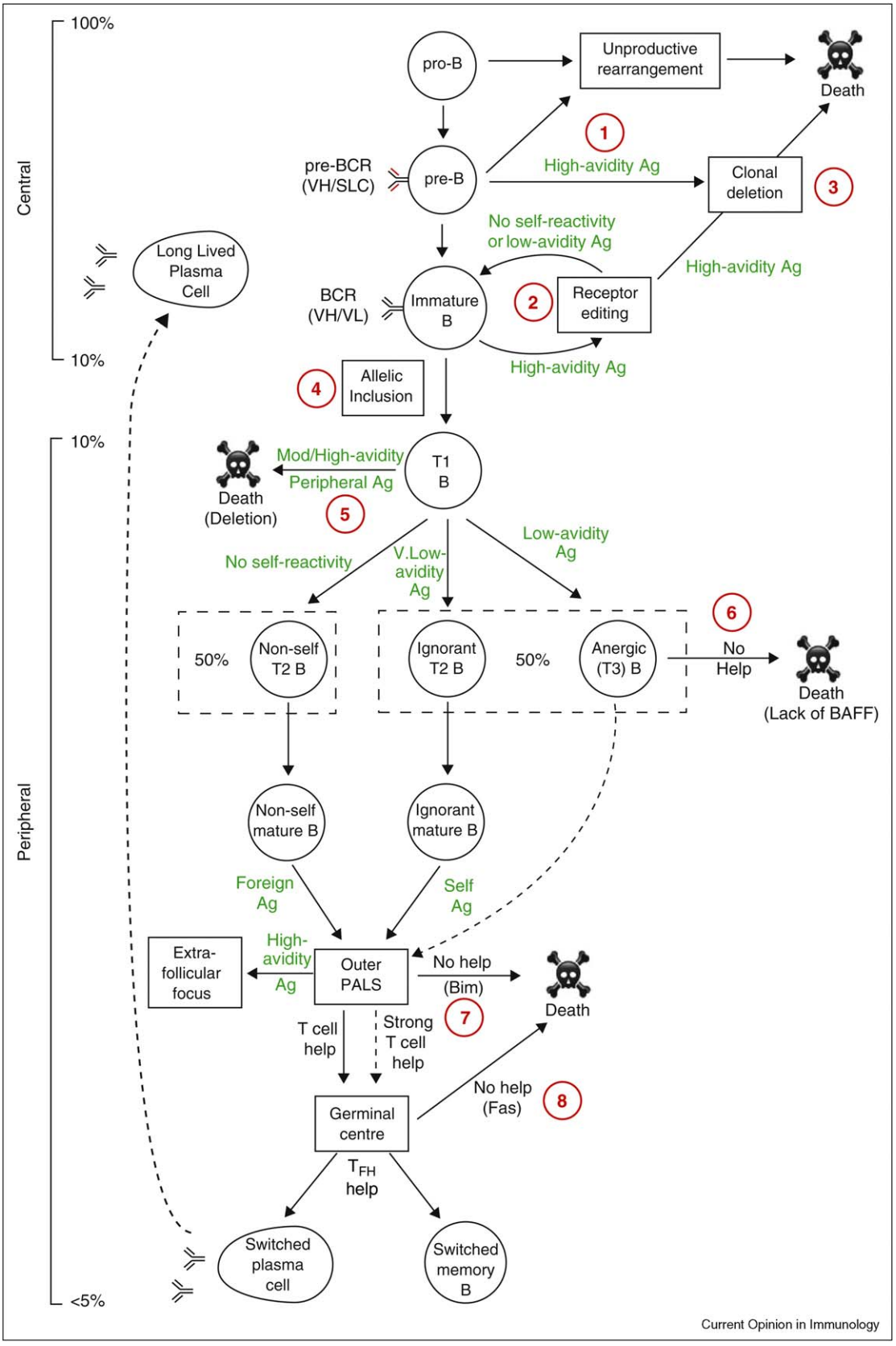
factors can operate directly on B-cells, as does the B-cell survival factor BAFF when produced, for example by synoviocytes in joints [11], or indirectly through their effects on other cells such as T-cells [9].

Another mechanism contributing to the decision between tolerance and immunity worth highlighting here is the alleged capacity of B-cells themselves to act as regulators rather than effectors. Although the first description of *regulatory B-cells* (B_{regs}) dates back many years [12], they have only just re-emerged in the form of a IL-10 secreting $CD5^{hi}CD1d^{hi}$ B-cell population in mice [13], and a comparable $CD19^{+}CD24^{hi}CD38^{hi}$ population in humans [14•], with potent 'tolerising' capabilities for both T-cell and B-cell mediated responses [15•]. In other studies, activated B-cells when acting as antigen presenting cells (APCs) have been shown to expand regulatory T-cells (T_{regs}) [16] or cause deletion or anergy of self-reactive T-cells [17,18]. The key issue posed by these observations is the relative importance of B-cells with regulatory versus effector functions in disease settings *in vivo*. For the most part, the remarkable efficacy of anti-CD20 antibodies (e.g. Rituximab) in eliminating B-cells with foreign and self-specificities without causing dramatic disturbances in regulation points to a predominant role for effector B-cells [19••]. On the contrary, the fact that prophylactic anti-CD20 mediated B-cell depletion has been shown to intensify experimental autoimmune encephalomyelitis (EAE) [20] and systemic lupus erythematosus (SLE) [21] in mice, as well as exacerbating ulcerative colitis and precipitating psoriasis, vasculitis and autoimmune cytopenia in some human patients [19••] is consistent with a suppressive role for B-cells in autoimmunity. In view of the different outcomes of anti-CD20 therapy, it is not surprising that the proportion of infiltrating B-cells found in human allografts has been associated both with improved graft survival [22] and with a greater risk of rejection [23] in different patient samples. Moreover, when interpreting the outcome of antibody therapy, it should be borne in mind that anti-CD20 like anti-TNF antibodies exert profound effects on the integrity of lymphoid tissue including GCs.

Mechanisms of tolerance

The requirement for multiple checkpoints to purge the B-cell repertoire of unwanted anti-self-specificities is now well accepted in both mice and humans (Figure 1) [3••,24••,25], although the concept continues to be refined, particularly with respect to the mechanisms underlying negative selection at the pre-B-cell [26••], transitional [27] and GC [28••] stages of differentiation. For convenience, the mechanisms of self-tolerance in the B-cell compartment can be divided into *de novo* (*primary*) *and secondary mechanisms*. The former operate throughout B-cell differentiation and contribute to shaping the repertoire, while the latter do not alter the repertoire but act in a fail-safe capacity in peripheral lymphoid organs by modulating the responsiveness of mature B-cells. Much of the work deciphering these mechanisms was performed in transgenic models involving self-antigens or neo-self-antigens [29]. However, similar conclusions can be drawn for acquired tolerance to foreign microorganisms and to MHC or blood group antigens involved in prevention of graft rejection [30,31]. With respect to microorganisms, they can affect B-cell responsiveness in several ways, for example by direct infection of B-cells in the case of EBV [32] and HIV [33] resulting in subversion of BCR signalling, or indirectly by inhibiting their antigen presenting capacity in the case of *Helicobacter pylori* [34].

Figure 1


100%
Central
10%
10%
Peripheral
<5%
pro-B
Unproductive rearrangement
Death
1
pre-BCR (VH/SLC)
pre-B
High-avidity Ag
Clonal deletion
3
No self-reactivity or low-avidity Ag
High-avidity Ag
Long Lived Plasma Cell
BCR (VH/VL)
Immature B
2
Receptor editing
High-avidity Ag
4
Allelic Inclusion
Mod/High-avidity Peripheral Ag
T1 B
Death (Deletion)
5
No self-reactivity
V.Low-avidity Ag
Low-avidity Ag
6
50%
Non-self T2 B
Ignorant T2 B
50%
Anergic (T3) B
No Help
Death (Lack of BAFF)
Non-self mature B
Ignorant mature B
Foreign Ag
Self Ag
Extra-follicular focus
High-avidity Ag
Outer PALS
No help (Bim)
Death
7
T cell help
Strong T cell help
No help (Fas)
8
Germinal centre
T_{FH} help
Switched plasma cell
Switched memory B
Current Opinion in Immunology

De novo mechanisms

The selection of *de novo* mechanisms is determined by the combination of antigen avidity (affinity and density) and signalling threshold of the BCR [25]. They operate at multiple checkpoints, centrally in BM (or foetal liver) and peripherally within secondary lymphoid tissue for foreign as well as self-antigens (Figure 1).

Central tolerance

Selection in the BM is mediated by *deletion* and *receptor editing* for B-cells with high to moderate avidity for self-antigens (Figure 1). Following the early loss of pro-B-cells through unsuccessful VDJ heavy-chain gene rearrangements, the remainder, that is those with successful rearrangements, develop into pre-B-cells expressing a surface antigen receptor composed of an Ig heavy chain (HC) combined with a surrogate light chain (SLC). The outcome of ligation of the pre-B-cell receptor was traditionally considered to be just a proliferative burst in positively selected clones. However, according to a recent study, negative selection may also take place at this stage in B-cell ontogeny as demonstrated by enhanced levels of antinuclear-antibodies in mice unable to produce pre-BCRs due to a knockout of the SLC [26••]. Nevertheless, it remains possible that the appearance of autoreactive clones in these mice could be due to reduced competition for survival factors (e.g. BAFF) and microenvironmental niches, since the absence of the SLC also resulted in diminished production of B-cells secondary to limited positive selection.

The situation is less controversial for immature B-cells that, once they have successfully rearranged heavy and light chains on their surface, become particularly susceptible to tolerance induction upon BCR engagement by cognate antigen [3••]. Until recently, the primary mechanism affecting high-avidity B-cells was thought to be deletion, while those recognising antigens with moderate avidity underwent receptor editing, a process involving reactivation of RAG genes and continued rearrangements of IgL (and less commonly, IgH) genes in an attempt to acquire a useful anti-foreign BCR specificity. Now, it is considered that receptor editing is the predominant mechanism of central tolerance, with clonal deletion serving as the default pathway for B-cells that retain their self-reactivity [35]. Curiously, about half of the immature B-cells undergoing receptor editing continue to express two or more different light (and occasionally heavy) chains [36]. This phenomenon, termed 'allelic inclusion', may not only explain the high incidence of poly-reactivity and self-reactivity early in B-cell development, but also how weakly self-reactive immature cells escape central tolerance through dilution of aberrant receptors and reach secondary lymphoid tissue [3••]. When the repertoire of developing B-cells was tracked in humans, a similar picture emerged with evidence of persistent self-reactivity and poly-reactivity among mature low-avidity B-cells in the periphery despite relatively efficient negative selection in the BM [24••]. The decision to undergo receptor editing in B-cells was thought to be the exclusive domain of BCR avidity; however, recent studies of patients with defects in MyD88, IRAK-4 and UNC-93B molecules point to an unpredicted role for pathways involving innate receptors (e.g. for IL-1 and TLRs) in regulating this mechanism of tolerance [37•].

No equivalent of the *AIRE* gene, which mediates expression of peripherally restricted antigens in the thymus, has been identified in BM, nor is it required given the fact that B-cells interact directly with antigen. Presumably, however, widely expressed cell associated self-antigens do exist at this site as indicated by a recent transgenic study showing that B-cells specific for the NCI domain on alpha3 type IV collagen, a target of anti-glomerular basement autoantibodies in Goodpasture's syndrome, normally undergo deletion and receptor editing in the BM [38••].

The information presented so far has been confined to conventional B2-cells. Do the same rules apply to B1-cells, given that they are also self-reactive and poly-reactive and, unlike B2-cells, are normally cycling? Although 'natural' autoantibodies from B1-cells tend to be of low-avidity and not highly pathogenic, support for some form of negative selection in B1-cells comes from the demonstration of greatly increased numbers in mice deficient in ITIM-containing molecules like CD22 and Siglec-G [39•] or overexpressing BAFF [40], where these cells play a direct

(Figure 1 Legend) Multiple checkpoints of B-cell tolerance. Most self-reactive B-cells (90%) are eliminated by central *de novo* tolerance mechanisms within the BM (1–4), while the remaining minority that escape into peripheral lymphoid organs are controlled by secondary as well as *de novo* mechanisms at these sites (5–8). (1) Pre-B-cells expressing strongly self-reactive Ig heavy chains (VH) paired with a surrogate light chain (SLC) undergo deletion. (2) Immature B-cells expressing strongly self-reactive Ig heavy and light chain (VL) combinations rearrange receptor genes (receptor editing), thereby reducing self-reactivity. (3) Receptor edited B-cells that remain strongly self-reactive undergo deletion. (4) Self-reactive BCRs are diluted on a proportion of receptor edited B-cells due to expression of a second Ig light (or sometimes heavy) chain (allelic inclusion). (5) T1 B-cells recognising peripheral self-antigen with moderate to high-avidity undergo Bim-dependent deletion in the spleen. (6) B-cells continually recognising self-antigen with low-avidity can no longer compete for limiting amounts of BAFF and withdraw into an unresponsive (anergic) state, in which they are short-lived, unless provided with strong T-cell help or other co-stimulatory signals (e.g. TLR). (7) B-cells recognising self-antigen with very low-avidity or which do not normally encounter a sequestered self-antigen (i.e. are ignorant) can mature along with non-self-reactive B-cells. However, once exposed to their cognate antigen in the absence of T-cell help, they undergo deletion in the outer PALS area of the spleen. (8) Similarly, in the absence of help from T_{FH}, B-cells undergoing SMH within GCs that become self-reactive undergo Fas-dependent death. Conversely, those receiving help survive and differentiate into antibody secreting plasma cells and memory B-cells.

role in induction of autoimmunity. Conversely, reduced numbers of B1-cells have been described in mice tolerised to xenoantigens that selectively interact with B1-cells [41]. The precise location of negative selection of B1-cells and B2-cells within BM remains elusive, although complement and other cells (e.g. macrophages and stromal cells) bearing complement receptors are likely to be important (Table 1).

Peripheral tolerance

Only 10% of newly generated immature B-cells emerge from BM as transitional (T1 then T2) cells [2]. These migrate to the spleen where they may encounter peripheral self-antigens not present in BM. High-avidity interactions with these antigens lead to rapid Bim-dependent deletion of B-cells at the T1 stage (Figure 1) [25,42]. By contrast, low or very-low-avidity interactions result in the induction of *anergy* and *ignorance*, respectively [29]. Upon exposure to either self or foreign antigen, these cells, like mature naïve B-cells, relocate to the outer peri-arteriolar lymphoid sheath (PALS) of the T-cell zone in search of help, provided that receptor occupancy exceeds 25%, and the BCR signalling threshold (tonicity) is adequate [2]. In the absence of T-cell help or TLR dependent co-stimulatory signals, B-cells, irrespective of their specificity, die within 2–3 days; in effect undergoing delayed deletion [2].

The *anergic state* [43] is characterised by desensitisation of BCR signalling and its uncoupling from the NFκB pathway, resulting in decreased responsiveness, a failure of antigen presenting and antibody producing capabilities and an inability to compete for limiting amounts of the B-cell survival factor, BAFF [44]. Nevertheless, in the event that they are exposed within their shortened life-span to strongly cross-reactive antigens, excess BAFF or vigorous T-cell help, anergic B-cells survive negative selection and enter GCs where they undergo affinity maturation along with 'ignorant' cells of either foreign or self-specificities (Figure 1). Although the importance of this transient state of tolerance has been questioned, several lines of evidence point to a physiological role for it. First a subset of B-cells with unique marker profiles has been identified in the normal B-cell repertoire of mice ($CD93^+$ IgM^{lo} population termed An1) [45] and more recently in humans (unmutated IgM^- IgD^+ population termed B_{ND}) [46•] that display many of the functional and phenotypic characteristics of anergy defined in transgenic systems, with their specificity profile skewed towards self-reactivity. Secondly the introduction of several BCR transgenic systems onto autoimmune-prone mouse backgrounds has revealed that the failure of anergy is a common mechanism leading

Table 2

Examples of *de novo* defects in B-cell tolerance operating at multiple checkpoints in murine models of autoimmune disease

Mechanism	Mouse strains	Defect	Refs.
Central tolerance			
Deletion	NZM2410/NZW	Immature B-cells show decreased calcium flux and increased resistance to apoptosis following BCR cross-linking due to expression of the lupus associated Ly108.1 allelic variant.	[57••]
Receptor editing	MRL.*lpr*, NZB, NOD	BM immature B-cells exhibit low levels of additional IgL gene rearrangements and RAG reactivation indicative of decreased efficiency of receptor editing. Similar defects found in 30–55% of SLE and T1D patients (compared to 7% of healthy controls).	[55••]
Allelic inclusion	(NZB × NZW)F1	Allelic exclusion was strictly maintained in anti-DNA B-cells of (NZB × NZW)F1 mice. However, a large proportion of these clones maintained RNA expression of non-productively rearranged IgL chains, which may act to reduce anti-DNA BCR density on autoreactive B-cells.	[58]
Peripheral tolerance			
Deletion	NZB, NOD	T1 B-cells are resistant to apoptosis following IgM cross-linking due to increased expression of anti-apoptotic factor Bcl-2. Decreased generation of T1 B-cells or increased BAFF production also leads to impaired tolerance induction.	[59,60]
Anergy	NZM2410/NZW, NZB, MRL.*lpr*, (NZB × NZW)F1 and NOD	B-cell anergy induced by neo-self-antigen HEL or self-antigens including DNA, Sm antigen and rheumatoid factor in BCR transgenic mouse models on non-autoimmune-prone backgrounds is abrogated in mice on various autoimmune-prone genetic backgrounds.	[29,57••,59,61]
GC negative selection	MRL.*lpr*, NZM2410/NZW	Increased spontaneous generation of GCs in many autoimmune-prone strains correlated with onset of autoantibodies. In BCR transgenic mice specific for DNA and rheumatoid factor self-antigens, GCs persist to give rise to memory and autoantibody-forming cells in mice on autoimmune-prone, but not non-autoimmune-prone genetic backgrounds.	[28••,62,63]

to the activation of self-reactive B-cells [29,44]. Finally an increasing number of BCR-related intracellular events linked to the anergic state *per se* are being reported in mouse models [44,47•,48,49].

GCs play a pivotal role in regulating development of the 'immune' B-cell repertoire. Thus they represent a microenvironment in follicles where hypermutating B-cells are positively selected on the basis of affinity, but negatively selected against self-reactivity due to local competition for antigen presented on follicular dendritic cells and access to help provided at this site by T follicular helper (T_{FH}) cells [28••]. GC B-cells are characterised by a pro-apoptotic gene expression profile involving downregulation of Bcl-2 and upregulation of Bim family molecules, which means that these B-cells, like those in the outer PALS, are deleted following antigen exposure if they do not receive survival signals from T_{FH} cells. The other cell type of importance in GC is the tingible-body macrophage (TBM) that functions to remove the very large number of potentially immunogenic apoptotic bodies expressing nuclear antigens derived from apoptosing GC B-cells [50•]. Defects in T_{FH} and/or TBMs have been shown to predispose to autoimmunity, thereby confirming their importance in self/non-self discrimination within GCs [28••].

Secondary mechanisms

Secondary fail-safe mechanisms can be subdivided into B-cell extrinsic and intrinsic (Table 1) [2]. The former encompass microenvironmental niches wherein B-cell numbers are regulated, interactions with the complement pathway, the effects of soluble molecules (e.g. cytokines or anti-idiotypic antibodies) and negative T-cell influences (e.g. Tregs, lack of help, and/or direct killing by CD4 and CD8 T-cells) [51,52•]. B-cell intrinsic mechanisms, by contrast, include the various signalling pathways and receptors that mediate positive (e.g. CD19 and CD21/35) and negative (e.g. CD22 and FcγRIIB) influences responsible for regulating the threshold of BCR triggering and tonicity [29,53].

Breakdown of B-cell tolerance

This is mainly relevant to self-tolerance, although environmental factors like viruses can play a role in reversal of tolerance to foreign antigens like allografts [2]. Factors responsible for the failure in tolerance fall into the same two broad categories that control the decision between

Table 3

Recently described spontaneous autoimmune susceptibility genes contributing to breakdown of B-cell tolerance[a]

Gene	Mouse strain or human population	Associated diseases[b]	Tolerance phenotypes mediated by susceptibility alleles
Mice			
Fcgr2b	NZB/MRL/BXSB/NOD/ NZM2410/NZW	SLE	Reduced *Fcgr2* expression in GC B-cells leads to differentiation of self-reactive B-cells into plasma cells.
Ifi202	NZB	SLE	Increased expression reduces B-cell susceptibility to apoptosis.
Ly108	NZM2410/NZW	SLE	Dampens BCR signalling at immature/transitional stage impairing anergy, receptor editing and deletion.
Cr2	NZM2410/NZW	SLE	Impairment of C3d binding resulting in defective B-cell anergy and abnormal GC response.
Tlr7	BXSB	SLE	Increased expression due to gene duplication on Y Chromosome impairs B-cell tolerance to RNA-associated autoantigens.
Human			
PTPN22	Various	T1D/RA/GD/ SLE/MG	Dominant gain of inhibitory function mutation that impairs BCR (and TCR) signalling.
FCRL3	Asian	SLE/RA/GD	Increased expression dampens BCR signalling. Associated with increased autoantibody production.
FCGR2B	Asian/Caucasian	SLE/GP/ITP	Polymorphisms cause reduced induction of this inhibitory molecule on memory B-cells.
PDCD1	Asian/Caucasian/Hispanic	SLE	Decreased expression due to impaired binding of RUNX transcription factor to its enhancer. Functions as an inhibitor of BCR (and TCR) signalling.
BLK	Caucasian	SLE/APS	Decreased expression due to promoter polymorphism is thought to inhibit BCR signalling.
LYN	Caucasian (female)	SLE	Decreased expression due to intronic polymorphism is thought to result in hyper-responsiveness to BCR stimulation. Strong correlation with autoantibody production.
BANK1	Asian/Caucasian	SLE, SSc	Polymorphisms predicted to increase recruitment and activation of Lyn and IP3R resulting in sustained BCR signalling and B-cell hyperactivity.
CD40	Asian/Caucasian	RA, GD	Increased surface expression of CD40 on B-cells.

[a] Reviewed in [64–67].
[b] Abbreviations – APS: primary antiphospholipid antibody syndrome; GD: Grave's disease; GP: Goodpasture's disease; ITP: idiopathic thrombocytopenic purpura; MG: myasthenia gravis; RA: rheumatoid arthritis; SLE: systemic lupus erythematosus; SSc: systemic sclerosis; and T1D: type 1 diabetes.

tolerance and immunity, namely antigen and host (immune system) related (Table 1). From studies in a range of autoimmune-prone murine models expressing BCR transgenes of different avidities to self-antigens or neo-self-antigens, it has become apparent that any of the tolerance checkpoints shown in Figure 1 can be subject to failure depending on the genetic background of the host (Table 2). Similarly, when the frequency of self-reactive specificities was measured in the human B-cell repertoire, defects in tolerance were detected in patients with autoimmune diseases including SLE and rheumatoid arthritis (RA) at the late pre-B to immature and transitional to mature B-cell stages of differentiation, indicative of defects in both central and peripheral tolerance [54]. Several susceptibility genes that specifically contribute to B-cell tolerance defects in different autoimmune diseases are beginning to be identified in animal models as well as humans (Table 3). Not surprisingly, many of these appear to alter the BCR signalling pathway (e.g. *Ly108*, *Cr2*, *FCGR2B*, *PTPN22*, *FCRL3*, *BLK*, *LYN* and *BANK*). Nevertheless, what has also become evident from genetic studies is that full expression of clinical disease depends not only on genes causing a breakdown in B-cell tolerance *per se*, but also on abnormalities in other sets of genes operating along one or more distinct pathways such as those involved in mediating T-cell tolerance, apoptosis and target tissue inflammation. A good example is the development of experimental lupus, which requires defects in at least three pathways: (i) those causing loss of B-cell tolerance to nuclear antigens (e.g. *Ly108* or *PTPN22*); (ii) those mediating dysregulation of innate and adaptive immune systems (e.g. *Tlr7* and *FAS*); and (iii) those ultimately responsible for end organ damage rather than influencing the immune system *per se* (e.g. *Ifn-α* and *Icam1*) [10•].

Conclusions

The importance of tolerance in both B-cell and T-cell lineages is now well established. Both lineages are susceptible to deletion and anergy in primary and secondary lymphoid tissues; moreover B-cells with regulatory activity have rejoined their T-cell counterparts in suppressing inflammation at sites of disease including certain autoimmune conditions. What distinguishes central B-cell tolerance in particular is receptor editing that now appears to be more important than deletion in mediating negative selection in the BM. Perhaps not surprisingly defects in this mechanism of *de novo* tolerance are most clearly linked to susceptibility to B-cell-dependent autoimmune diseases like SLE [55••,56].

In the periphery, *de novo* and secondary (fail-safe) mechanisms of B-cell unresponsiveness continue to operate, again involving multiple checkpoints. These are mainly important for self-tolerance, given that a second wave of receptor diversification takes place in GC. Among these peripheral mechanisms is the still unresolved but intriguing role that B-cells play as APCs, where they have been shown to be capable of expanding T_{regs} and either switching off or on effector T-cells specific for foreign and self-antigens. Deciphering the conditions leading to these divergent roles will be essential for optimising the effectiveness of B-cell depleting drugs (e.g. anti-CD20) for the treatment of systemic autoimmune diseases (and, for that matter, graft rejection) in humans. Fortunately, however, peripheral B-cell tolerance is robust and clinical autoimmunity only occurs should genetic defects involving multiple distinct signalling pathways co-associate in the one individual.

Acknowledgements

We would like to thank the National Health and Medical Research Council of Australia for funding support and Drs Robert Brink and Tri Phan for their critical assessment of the manuscript.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Yanaba K, Bouaziz JD, Matsushita T, Magro CM, St Clair EW, Tedder TF: **B-lymphocyte contributions to human autoimmune disease**. *Immunol Rev* 2008, **223**:284-299.

2. Basten A, Brink R: **Tolerance and autoimmunity: B cells**. In *The Autoimmune Diseases,* fourth edn. Edited by Rose NR, Mackay IR.Academic Press; 2006:167-177.

3. •• von Boehmer H, Melchers F: **Checkpoints in lymphocyte development and autoimmune disease**. *Nat Immunol* 2010, **11**:14-20.
A concise and up to date review comparing recent advances and gaps in our knowledge of major checkpoints in B-cell versus T-cell differentiation.

4. Bretscher P, Cohn M: **A theory of self-nonself discrimination**. *Science* 1970, **169**:1042-1049.

5. • Duong BH, Tian H, Ota T, Completo G, Han S, Vela JL, Ota M, Kubitz M, Bovin N, Paulson J *et al.*: **Decoration of T-independent antigen with ligands for CD22 and Siglec-G can suppress immunity and induce B cell tolerance in vivo**. *J Exp Med* 2010, **207**:173-187.
This paper makes the recently neglected point that alterations in antigen structure of native antigen can play a role in self/non-self recognition in the B-cell repertoire.

6. Chang WW, Yu CY, Lin TW, Wang PH, Tsai YC: **Soyasaponin I decreases the expression of alpha2,3-linked sialic acid on the cell surface and suppresses the metastatic potential of B16F10 melanoma cells**. *Biochem Biophys Res Commun* 2006, **341**:614-619.

7. Lyubchenko T, Dal Porto JM, Holers VM, Cambier JC: **Cutting edge: Complement (C3d)-linked antigens break B cell anergy**. *J Immunol* 2007, **179**:2695-2699.

8. • Matzinger P: **Friendly and dangerous signals: is the tissue in control?** *Nat Immunol* 2007, **8**:11-13.
This article stresses the fact that tissue factors can influence the decision between tolerance and immunity. Refs. [9] and [10•] provide examples of gene loci operating at the target tissue level.

9. Hill NJ, Hultcrantz M, Sarvetnick N, Flodstrom-Tullberg M: **The target tissue in autoimmunity—an influential niche**. *Eur J Immunol* 2007, **37**:589-597.

10. • Kanta H, Mohan C: **Three checkpoints in lupus development: central tolerance in adaptive immunity, peripheral amplification by innate immunity and end-organ inflammation**. *Genes Immun* 2009, **10**:390-396.
This paper provides a nice overview of the three categories of lupus susceptibility genes defined in murine models, the collective expression of which is required for the development of full blown clinical disease.

They act by contributing to (i) the breakdown of self-tolerance, (ii) amplification of autoimmunity and (iii) target organ destruction. This approach provides a useful framework for understanding the inheritance of SLE and other autoimmune diseases in humans.

11. Ohata J, Zvaifler NJ, Nishio M, Boyle DL, Kalled SL, Carson DA, Kipps TJ: **Fibroblast-like synoviocytes of mesenchymal origin express functional B cell-activating factor of the TNF family in response to proinflammatory cytokines**. *J Immunol* 2005, **174**:864-870.

12. Zembala M, Asherson GL, Noworolski J, Mayhew B: **Contact sensitivity to picryl chloride: the occurrence of B suppressor cells in the lymph nodes and spleen of immunized mice**. *Cell Immunol* 1976, **25**:266-278.

13. Yanaba K, Bouaziz JD, Haas KM, Poe JC, Fujimoto M, Tedder TF: **A regulatory B cell subset with a unique CD1dhiCD5+ phenotype controls T cell-dependent inflammatory responses**. *Immunity* 2008, **28**:639-650.

14. • Blair PA, Norena LY, Flores-Borja F, Rawlings DJ, Isenberg DA, Ehrenstein MR, Mauri C: **CD19(+)CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic Lupus Erythematosus patients**. *Immunity* 2010, **32**:129-140.
A human B-cell subset that is comparable to B10 cells in mice and has impaired function in systemic autoimmune disease. Whether these subsets have a unique molecular signature or display the same plasticity as CD4+ T-cells remains to be determined.

15. • DiLillo DJ, Matsushita T, Tedder TF: **B10 cells and regulatory B cells balance immune responses during inflammation, autoimmunity, and cancer**. *Ann N Y Acad Sci* 2010, **1183**:38-57.
Perhaps not surprisingly the phenotypically distinct subset of B-cells (B10 cells) appears to have predominantly regulatory activity mediated via IL-10. Such cells not only inhibit inflammation, but can also switch off unwanted T-cell responses as well, that is are tolerogenic in that sense.

16. Tu W, Lau YL, Zheng J, Liu Y, Chan PL, Mao H, Dionis K, Schneider P, Lewis DB: **Efficient generation of human alloantigen-specific CD4+ regulatory T cells from naive precursors by CD40-activated B cells**. *Blood* 2008, **112**:2554-2562.

17. Frommer F, Heinen TJ, Wunderlich FT, Yogev N, Buch T, Roers A, Bettelli E, Muller W, Anderton SM, Waisman A: **Tolerance without clonal expansion: self-antigen-expressing B cells program self-reactive T cells for future deletion**. *J Immunol* 2008, **181**:5748-5759.

18. Tretter T, Venigalla RK, Eckstein V, Saffrich R, Sertel S, Ho AD, Lorenz HM: **Induction of CD4+ T-cell anergy and apoptosis by activated human B cells**. *Blood* 2008, **112**:4555-4564.

19. •• Thaunat O, Morelon E, Defrance T: **Am''B''valent: anti-CD20 antibodies unravel the dual role of B cells in immunopathogenesis**. *Blood* 2010, **116**:515-521.
An example of the value of anti-CD20 antibodies, not just in a therapeutic setting, but as a probe for investigating the positive and negative functions of mature B-cells.

20. Matsushita T, Yanaba K, Bouaziz JD, Fujimoto M, Tedder TF: **Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression**. *J Clin Invest* 2008, **118**:3420-3430.

21. Haas KM, Watanabe R, Matsushita T, Nakashima H, Ishiura N, Okochi H, Fujimoto M, Tedder TF: **Protective and pathogenic roles for B cells during systemic autoimmunity in NZB/W F1 mice**. *J Immunol* 2010, **184**:4789-4800.

22. Turka LA, Lechler RI: **Towards the identification of biomarkers of transplantation tolerance**. *Nat Rev Immunol* 2009, **9**:521-526.

23. Zarkhin V, Kambham N, Li L, Kwok S, Hsieh SC, Salvatierra O, Sarwal MM: **Characterization of intra-graft B cells during renal allograft rejection**. *Kidney Int* 2008, **74**:664-673.

24. •• Wardemann H, Nussenzweig MC: **B-cell self-tolerance in humans**. *Adv Immunol* 2007, **95**:83-110.
A summary of almost a decade of elegant sequencing work on single human B-cells demonstrating the remarkably high frequency of poly-reactive and self-reactive cells throughout B-cell differentiation: hence the need for multiple tolerance checkpoints in primary and secondary lymphoid tissues.

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

26. •• Keenan RA, De Riva A, Corleis B, Hepburn L, Licence S, Winkler TH, Martensson IL: **Censoring of autoreactive B cell development by the pre-B cell receptor**. *Science* 2008, **321**:696-699.
Dogma has it that B-cells become susceptible to negative selection at the immature stage of development. In this study, however, mice unable to produce a pre-B-cell receptor due to the knockout of the SLC were found to have augmented levels of self-reactive B-cells capable of producing autoantibodies in the periphery. These observations point to the presence of an additional checkpoint at the pre-B-cell stage of differentiation where the self-reactivity of a newly rearranged Ig heavy chain can be scrutinised before it is paired with a *bona fide* light chain.

27. Henderson RB, Grys K, Vehlow A, de Bettignies C, Zachacz A, Henley T, Turner M, Batista F, Tybulewicz VL: **A novel Rac-dependent checkpoint in B cell development controls entry into the splenic white pulp and cell survival**. *J Exp Med* 2010, **207**:837-853.

28. •• Vinuesa CG, Sanz I, Cook MC: **Dysregulation of germinal centres in autoimmune disease**. *Nat Rev Immunol* 2009, **9**:845-857.
A thoughtful overview of the GC as the major site of peripheral positive and negative selection in the B-cell repertoire. The article also compares post-GC antibody responses with those derived from extrafollicular foci as well as discussing the controversial issue of ectopic GCs.

29. Shlomchik MJ: **Sites and stages of autoreactive B cell activation and regulation**. *Immunity* 2008, **28**:18-28.

30. Fan X, Ang A, Pollock-Barziv SM, Dipchand AI, Ruiz P, Wilson G, Platt JL, West LJ: **Donor-specific B-cell tolerance after ABO-incompatible infant heart transplantation**. *Nat Med* 2004, **10**:1227-1233.

31. Urschel S, Campbell PM, Meyer SR, Larsen IM, Nuebel J, Birnbaum J, Netz H, Tinckam K, Kauke T, Derkatz K *et al.*: **Absence of donor-specific anti-HLA antibodies after ABO-incompatible heart transplantation in infancy: altered immunity or age?** *Am J Transplant* 2010, **10**:149-156.

32. Hasler P, Zouali M: **Subversion of B lymphocyte signaling by infectious agents**. *Genes Immun* 2003, **4**:95-103.

33. Rudnicka D, Schwartz O: **Intrusive HIV-1-infected cells**. *Nat Immunol* 2009, **10**:933-934.

34. Baldari CT, Lanzavecchia A, Telford JL: **Immune subversion by *Helicobacter pylori***. *Trends Immunol* 2005, **26**:199-207.

35. Halverson R, Torres RM, Pelanda R: **Receptor editing is the main mechanism of B cell tolerance toward membrane antigens**. *Nat Immunol* 2004, **5**:645-650.

36. Liu S, Velez MG, Humann J, Rowland S, Conrad FJ, Halverson R, Torres RM, Pelanda R: **Receptor editing can lead to allelic inclusion and development of B cells that retain antibodies reacting with high avidity autoantigens**. *J Immunol* 2005, **175**:5067-5076.

37. • Isnardi I, Ng YS, Srdanovic I, Motaghedi R, Rudchenko S, von Bernuth H, Zhang SY, Puel A, Jouanguy E, Picard C *et al.*: **IRAK-4- and MyD88-dependent pathways are essential for the removal of developing autoreactive B cells in humans**. *Immunity* 2008, **29**:746-757.
This paper shows that patients with defects in critical TLR signalling molecules (IRAK-4, MyD88, and UNC-93B) have abnormalities in central and peripheral mechanisms that normally prevent accumulation of self-reactive B-cells, thus revealing an unexpected role for TLR in the induction of B-cell self-tolerance.

38. •• Zhang Y, Su SC, Hecox DB, Brady GF, Mackin KM, Clark AG, Foster MH: **Central tolerance regulates B cells reactive with Goodpasture antigen alpha3(IV)NC1 collagen**. *J Immunol* 2008, **181**:6092-6100.
Little is known about self-antigens in the BM, nor where antigen-dependent negative selection takes place at that site. This paper describes an example of such an antigen, which is the target of pathogenic autoantibodies in Goodpasture's syndrome, but as shown in a transgenic model, normally induces a tolerogenic response via receptor editing. In patients, the autoimmune response may be initiated by exposure to cross-reactive epitopes on a foreign antigen (e.g. microorganism) since the self-epitope is cryptic.

39. Jellusova J, Wellmann U, Amann K, Winkler TH, Nitschke L: **CD22**
• **x Siglec-G double-deficient mice have massively increased B1 cell numbers and develop systemic autoimmunity**. *J Immunol* 2010, **184**:3618-3627.

Most models of B-cell tolerance deal with conventional B2-cells. However, B1-cells are also subject to inhibitory effects of ITIM-containing siglecs (Ref. [5•]) and tolerance induction to xenoantigens like Gala1,3Gal (Ref. [41]).

40. Fletcher CA, Sutherland AP, Groom JR, Batten ML, Ng LG, Gommerman J, Mackay F: **Development of nephritis but not sialadenitis in autoimmune-prone BAFF transgenic mice lacking marginal zone B cells**. *Eur J Immunol* 2006, **36**:2504-2514.

41. Sykes M: **Immune tolerance: mechanisms and application in clinical transplantation**. *J Intern Med* 2007, **262**:288-310.

42. Enders A, Bouillet P, Puthalakath H, Xu Y, Tarlinton DM, Strasser A: **Loss of the pro-apoptotic BH3-only Bcl-2 family member Bim inhibits BCR stimulation-induced apoptosis and deletion of autoreactive B cells**. *J Exp Med* 2003, **198**:1119-1126.

43. Nossal GJ, Pike BL: **Clonal anergy: persistence in tolerant mice of antigen-binding B lymphocytes incapable of responding to antigen or mitogen**. *Proc Natl Acad Sci U S A* 1980, **77**:1602-1606.

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

45. Merrell KT, Benschop RJ, Gauld SB, Aviszus K, Decote-Ricardo D, Wysocki LJ, Cambier JC: **Identification of anergic B cells within a wild-type repertoire**. *Immunity* 2006, **25**:953-962.

46. Duty JA, Szodoray P, Zheng NY, Koelsch KA, Zhang Q,
• Swiatkowski M, Mathias M, Garman L, Helms C, Nakken B *et al.*: **Functional anergy in a subpopulation of naive B cells from healthy humans that express autoreactive immunoglobulin receptors**. *J Exp Med* 2009, **206**:139-151.

In Ref. [45], the description of an anergic B-cell subset (An1) in the normal murine B-cell repertoire provided support for a physiological role for this mechanism of tolerance. In this paper a similar human B-cell subset, which is also IgM^{lo} (B_{ND}), is described and shown to be functionally defective in some patients with systemic autoimmunity.

47. Browne CD, Del Nagro CJ, Cato MH, Dengler HS, Rickert RC:
• **Suppression of phosphatidylinositol 3,4,5-trisphosphate production is a key determinant of B cell anergy**. *Immunity* 2009, **31**:749-760.

In this study, a novel molecular mechanism underlying the induction and maintenance of anergy in B-cells is described consisting of a decrease in their capacity to produce the lipid product $PI(3,4,5)P_3$ as a result of augmented expression of the PI3K inhibitor, PTEN.

48. Kitaura Y, Jang IK, Wang Y, Han YC, Inazu T, Cadera EJ, Schlissel M, Hardy RR, Gu H: **Control of the B cell-intrinsic tolerance programs by ubiquitin ligases Cbl and Cbl-b**. *Immunity* 2007, **26**:567-578.

49. O'Neill SK, Veselits ML, Zhang M, Labno C, Cao Y, Finnegan A, Uccellini M, Alegre ML, Cambier JC, Clark MR: **Endocytic sequestration of the B cell antigen receptor and toll-like receptor 9 in anergic cells**. *Proc Natl Acad Sci U S A* 2009, **106**:6262-6267.

50. Kranich J, Krautler NJ, Heinen E, Polymenidou M, Bridel C,
• Schildknecht A, Huber C, Kosco-Vilbois MH, Zinkernagel R, Miele G *et al.*: **Follicular dendritic cells control engulfment of apoptotic bodies by secreting Mfge8**. *J Exp Med* 2008, **205**:1293-1302.

The mechanism whereby apoptotic bodies generated in large numbers in GCs are removed has been unclear. This paper shows that the tingible-body macrophage plays a key role by secreting milk fat globule-EGF factor 8 protein that binds to phosphotidylserine exposed on the surface of these bodies, promoting their phagocytosis.

51. Zhao DM, Thornton AM, DiPaolo RJ, Shevach EM: **Activated CD4+CD25+ T cells selectively kill B lymphocytes**. *Blood* 2006, **107**:3925-3932.

52. Ludwig-Portugall I, Hamilton-Williams EE, Gottschalk C, Kurts C:
• **Cutting edge: CD25+ regulatory T cells prevent expansion and induce apoptosis of B cells specific for tissue autoantigens**. *J Immunol* 2008, **181**:4447-4451.

This is one of a series of recent articles illustrating how B-cells, when acting as APCs can influence overall immune responsiveness in the host by inhibiting T-cell function directly or indirectly (e.g. via expansion of Tregs). Such papers serve to remind us that interactions between B-cells and T-cells are two-way processes.

53. Healy JI, Goodnow CC: **Positive versus negative signaling by lymphocyte antigen receptors**. *Annu Rev Immunol* 1998, **16**:645-670.

54. Meffre E, Wardemann H: **B-cell tolerance checkpoints in health and autoimmunity**. *Curr Opin Immunol* 2008, **20**:632-638.

55. Panigrahi AK, Goodman NG, Eisenberg RA, Rickels MR, Naji A,
•• Luning Prak ET: **RS rearrangement frequency as a marker of receptor editing in lupus and type 1 diabetes**. *J Exp Med* 2008, **205**:2985-2994.

This paper reveals a deficiency in the extent of receptor editing in polyclonal B-cells derived from MRL and NOD mouse models of SLE and T1D, respectively, which is mirrored in a significant proportion of patients developing these autoimmune diseases.

56. Zouali M: **Receptor editing and receptor revision in rheumatic autoimmune diseases**. *Trends Immunol* 2008, **29**:103-109.

57. Kumar KR, Li L, Yan M, Bhaskarabhatla M, Mobley AB, Nguyen C,
•• Mooney JM, Schatzle JD, Wakeland EK, Mohan C: **Regulation of B cell tolerance by the lupus susceptibility gene Ly108**. *Science* 2006, **312**:1665-1669.

One of the first studies to identify a non-MHC lupus susceptibility gene specifically affecting B-cell tolerance, *Ly108*. The susceptibility allele encodes a version of Ly108 that dampens BCR signalling in immature B-cells, with the result being that their capacity to undergo deletion, receptor editing and anergy is impaired. This may provide important insights into how human SLE susceptibility genes such as *PTPN22*, which also inhibits BCR signalling, may contribute to the development of autoimmunity.

58. Makdasi E, Fischel R, Kat I, Eilat D: **Autoreactive anti-DNA transgenic B cells in lupus-prone New Zealand black/New Zealand white mice show near perfect L chain allelic exclusion**. *J Immunol* 2009, **182**:6143-6148.

59. Cox SL, Silveira PA: **Emerging roles for B lymphocytes in Type 1 diabetes**. *Expert Rev Clin Immunol* 2009, **5**:311-324.

60. Roy V, Chang NH, Cai Y, Bonventi G, Wither J: **Aberrant IgM signaling promotes survival of transitional T1 B cells and prevents tolerance induction in lupus-prone New Zealand black mice**. *J Immunol* 2005, **175**:7363-7371.

61. Xu Z, Duan B, Morel L: **Genetics of autoreactive B cells**. *Front Biosci* 2007, **12**:1707-1721.

62. Vuyyuru R, Mohan C, Manser T, Rahman ZS: **The lupus susceptibility locus Sle1 breaches peripheral B cell tolerance at the antibody-forming cell and germinal center checkpoints**. *J Immunol* 2009, **183**:5716-5727.

63. William J, Euler C, Primarolo N, Shlomchik MJ: **B cell tolerance checkpoints that restrict pathways of antigen-driven differentiation**. *J Immunol* 2006, **176**:2142-2151.

64. Kono DH, Theofilopoulos AN: **Genetics of SLE in mice**. *Springer Semin Immunopathol* 2006, **28**:83-96.

65. Gregersen PK, Olsson LM: **Recent advances in the genetics of autoimmune disease**. *Annu Rev Immunol* 2009, **27**:363-391.

66. Moser KL, Kelly JA, Lessard CJ, Harley JB: **Recent insights into the genetic basis of systemic lupus erythematosus**. *Genes Immun* 2009, **10**:373-379.

67. Chistiakov DA, Chistiakov AP: **Is FCRL3 a new general autoimmunity gene?** *Hum Immunol* 2007, **68**:375-383.