Review

B-cell: a logical target for treatment of rheumatoid arthritis

P. Youinou, C. Jamin, A. Saraux

Brest University Medical School, Brest, France.

Pierre Youinou, Christophe Jamin, Alain Saraux.

Please address correspondence to: Pierre Youinou, Laboratory of Immunology, Brest University Medical School Hospital, BP824, F29609 Brest Cedex, France.
E-mail: youinou@univ-brest.fr

Received on January 12, 2007; accepted in revised form on March 14, 2007.

Clin Exp Rheumatol 2007; 25: 318-328.

Key words: B-lymphocyte, autoimmune disease, dendritic cell, B-cell antigen receptor, CD5.

ABSTRACT

The interest for B-cells in rheumatoid arthritis (RA) is currently being revived. They are involved in the development and activation of lymphoid architecture by regulating dentritic cell and T-cell function through cytokine production. Receptor editing an revising are also essential in B-cells and aid in preventing autoimmunity. Abnormalities in the subset distribution and a default in any task assigned to the B-cells may favor autoimmunity. Beneficied responses to B-cell depletion in RA by anti-CD20 monoclonal antibody rituximab illustrate the importance of B-lymphocytes in the pathogenesis of this disease. A new avenue has thus been opened, whereby B-lymphocytes return as a significant contributor to autoimmune disorders.

Introduction

Rheumatoid arthritis (RA) is hallmarked by a number of humoral and cellular abnormalities. Based on the pronounced infiltration of T-cells of the synovial membrane (SM), the recognition by T-cell antigen (Ag) receptors of peptides presented by major histocompatibility complex (MHC) class II gene products, and their involvement in the tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ) and interleukin (IL)-1 production, T-cells have become a major research focus (1). As a consequence, B-lymphocytes were supplied a limited support as candidate causal agents, and their effects portrayed as the production of antibodies (Ab) to ubiquitous self Ag. A host of autoAb have subsequently been unveiled, of which some could well be pathogenic (2). The aberrant survival of these autoreactive B-cells was, therefore, assigned to a defect in the T-cell tolerance (3).

Over the past decade, research has focused on disturbances in the B-cell compartment, to such an extent that major advances have emerged in the parameters that influence the fate of autoreactive B-cells. Humoral abnormalities could simply derive from intrinsic hyperactivity of B-cells (4). Thus, B-cells are involved, not only in the efferent arm of the immune response, but also in its afferent arm. Characteristics suggestive of breakdown of normal Ab regulation, such as abnormal B-cell subset distribution and an array of autoAb, have been identified in the relatives of patients with RA (5) that might predispose them to full-blown diseases.

Delineation of more or less overlapping B-cell subsets has generated further complexity in this compartment. Our earliest concept that they are exclusively committed to the production of Ab has also been re-considered, based on recent insights into various other tasks than simply manufacturing autoAb. Any of them may be faulty, and the ensuing inadequacies facilitate the autoimmune process.

The last 20 years have witnessed numerous initially promising therapies for connective tissue diseases that proved disillusioning. Ongoing approaches, such as targeting B-cell subsets (6), are derived from a better awareness of the B-cell involvement as Ag-presenting cells (APC) and inflammatory cytokine producers (7). Not only did anti-CD20 monoclonal Ab (mAb) rituximab deplete B-cells efficiently, but the patients feel greatly improved by this therapy. Accumulating results are key in the understanding as to how the autoimmune process is initiated and perpetuated.

A – Heterogeneity of B-lymphocytes

I – Classification according to the expression of CD5

First described as a T-cell marker, and then as a marker of tumor B-cells, the transmembrane molecule CD5 was later shown to be expressed by a minor proportion of circulating normal B-

cells. CD5-expressing B-lymphocytes were endowed with the production of polyreactive autoAb (8), and the propensity for malignant accumulation (9). To come full circle, the latter cells can be elicited to release autoAb by phorbol ester (10).

The nomenclature distinguishes B1 from B2 lymphocytes (11). The first group refers to CD5+ B-cells, and the second to the conventional B-cells. It remains nonetheless uncertain whether B1 constitute a naïve population of cells. So have we proposed (12) that there may be different reasons for B-cells to express CD5, which depend on the co-receptor ligation on their surface. Put simply, some CD5-B-cells are innate, while others are acquired. Further, the B1 population has been subdivided into B1a and B1b subpopulations (13). The B1b sister population of cells lacks surface CD5, but shares all the other attributes of B1a cells, such as natural Ab production, and the expression of CD11b/CD18.

A great deal of effort has been put into trying to understand the relevance of CD5+B-cells to non organ-specific autoimmune diseases (14). Yet, some controversy remains over their lineage origins: the activation view implies that the CD5+ B-cells can be generated from CD5- B-cells (15), whereas the lineage paradigm posits the existence of separate progenitors for CD5+ and CD5- B-cells (16). B1-specified progenitor has just been found in the bone marrow (BM), but we cannot discount the possibility that the expression of CD5 may also be induced (17).

The distribution of B-cells into B1 and B2 subsets, as well as that of the B1 subset into B1a and B1b sub-subsets is regulated, at least in the mouse, by the MHC class 2 genes (18) and the level on interleukin (IL)-10 (19). It has also been shown that Epstein-Barr virus-stimulated CD5-non-expressing B-cells transcribe CD5 messenger RNA (mRNA) at levels comparable with those of their surface CD5-expressing counterparts (20).

Several groups, including ours, have established that the CD5+B-cell population may be expanded in patients (21-26) with RA, primary Sjögren's syndrome (pSS) and juvenile RA (26, 27). Interestingly, Anaya *et al*. (27) have shown that IL-10 influences autoimmune processes in pSS. Elevated levels of CD5+ B-cells are a distinctive feature of selected RA families, rather than a peculiarity of RA patients compared with their healthy relatives (5). The question as to whether these B1a cells are the source of autoAb has long been discussed. In support of the argument that autoAb production is confined to B1 cells, these were shown (8, 28, 29) to produce anti-double-stranded (ds) DNA Ab and rheumatoid factor (RF). These early claims were nonetheless denied by reports that CD5+B- cells produce low-affinity RF (30) and no high-affinity Ab to single-stranded DNA (31). These studies argue that broad polyreactivity is an inherent property of the Ab made by CD5+B-cells, and that these Ab may be what has been referred to as the natural Ab of the serum. Rather, increased numbers of CD5+B-cells might rather reflect defective regulation of B-cell function through CD5 itself.

II – Variations throughout the ontogenesis

B-cells originate in the BM, where they pass through a sequence of intermediate stages, while the B-cell Ag receptor (BCR) repertoire diversifies according to the ordered rearrangement of the gene segments V, D and J of the immunoglobulin (Ig) heavy chain locus, and those of the gene segments V and J of its light chain locus. Once their Ig genes have been productively rearranged, pro-B-cells progress to the pre-B cell stage.

These cells, referred to as immature B-lymphocytes, migrate to the periphery. Their course depends on BCR-derived signals, but we do not know whether this is tickled in all immature B-lymphocytes, or whether just a fraction (a selection?) of cells is involved. Following BCR-mediated maturation, they are pushed into leaving the BM and settling down in the secondary lymphoid organs (SLO). Massive death of B-cells has, however, never been observed in the BM, so that a handful of very immature B-cells may leave this primary lymphoid organ. The ensuing transitional B-cells have long been described in rodents (32), and recently identified in the peripheral blood (PB) of humans (33).

Cells that have just reached SLO become transitional B-lymphocytes type 1 (T1), T2 and T3 (34), and evolve step by step towards immune competence (35). These transitional B-cells mark the crucial link between BM immature and SLO mature B-cells. Only the cells enter the lymphoid follicles (LF), they generate germinal centers (GC) and recirculate (36). Non-circulating B-cells consist of marginal zone (MZ) B-cells (37). The first responsibe B-cell compartment consists, however of extrafollicular short-lived plasma cells (PC). They secrete IgM, but later isotype switching takes place and they also secrete IgG (38). Their differentiation is regulated (39) by a subset of dendritic cells (DC).

For B-cells to function properly, their precursors have to move from the outer T-cell area to their appropriate locations. Three non-mutually exclusive models account for this anatomical distribution (Fig. 1). In the first, localization of B-cells in the MZ or the GC is determined by the strength of the BCR-derived signals (40), and modulated by the notch receptors (41). Coupled with their Jagged-1, Jagged-2 and delta ligands, these receptors determine the fate of B-cells (42). In the second model, the high load of mutations in MZ B-cells (43), in the absence of Bcl-6 and activation-induced cytidine deaminase, which are essential for somatic hypermutation, suggests that these cells have undergone their mutations elsewhere, possibly in the GC (44). Analysis of mutations in Ig heavy chain variable region genes of MZ B-cells discovers a reservoir of memory B-cells (45), but the concern of other sites for diversification of memory B-cells has never been fully addressed (46). Although the PB B-cell compartment displays a large proposition of IgM+IgD+CD27+ memory B-cells carrying a mutated Ig receptor (47), there is no explanation as to where this population has been prediversified (46). Consistent with this view is that RF response may occur *via*

somatic hypermutation outside the GC (48). Therefore, these RF derive from short-lived PC, and it is not surprising that serum levels of RF fall by 1-2 months after rituximab in RA patients (49). The third and last model for transitional B-cell allocation to the MZ and the GC rests on the capacity of sphingosine 1-phosphate (S1P) to overcome the recruiting activity of CX chemokine ligand (CL) 13 for CX chemokine recptor 5- expressing B-cells to the GC, and thereby retains them in the MZ (50). Naïve B-cells should be driven to LF by CXCL13, but a more prominent effect of SIP than CXCL13 is supported by the finding that S1P-receptor agonist FTY720-phosphate displaces B-cells from the MZ to the GC (51).

III – Maturation of B-cells in the germinal centers

Entry into the LF depends on specific chemokines, but maintenance signals for B-cells are also delivered by the BCR. The importance of the underlying competition is supported by engagement of the BCR by an autoAg that may occur in the rheumatoid synovium, be it IgG (52) or citrullinated peptides (53). Some B-cells undergo apoptosis, while others are rescued by T-cells. The capacity of the latter T-cells to promote B-cell proliferation seems to be caused by the insertion of CD154 into CD40 (54).

In patients with RA, the SM of the affected joints is colonized by lymphoid cells which may get organized into lymphoid aggregates resembling GC (Fig. 2). In a series of 64 SM biopsies, LF with GC were found in 23% of those patients with RA (55). Substantial evidence has been provided that this process is Ag-driven (56), so that the rheumatoid lesion functions like a SLO. As formulated by others (57), ectopic GC take autoimmunity into the fast line.

Newly formed GC represent oligoclonal B-cell populations (58). On average, each mature GC is derived from only 1-3 B-cell clones. Further analysis of these B-cells has identified a battery of surface markers. In particular, IgD and CD38 have been useful in classifying stepwise stages (59). Thus, seven mature B (Bm) cell subpopulations

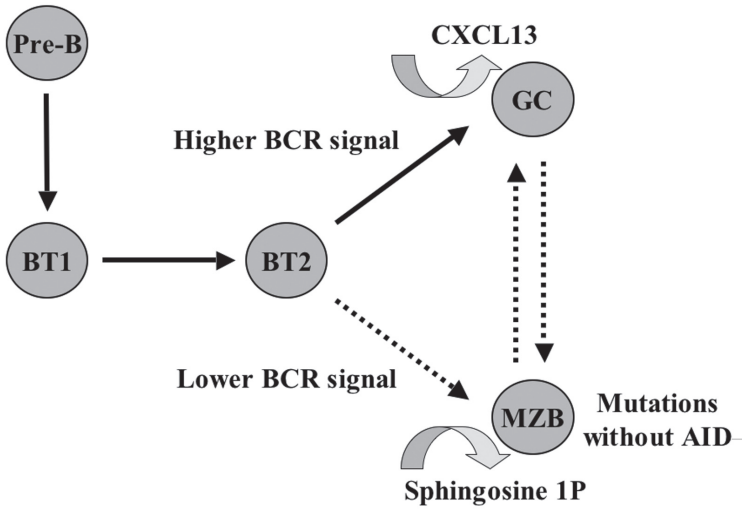

Fig. 1. Transitional B-cells (BT) give rise to germinal center (GC) and marginal zone (MZ) B-cells, depending on the signal from the B-cell antigen receptor (BCR). Some MZ B-cells might have undergone mutations, not in the MZ, where the activation induced deaminase (AID) is not expressed, but in the GC. Conversely, some B-cells may be retained in the MZ by sphingosine 1P that resists CXCL13.

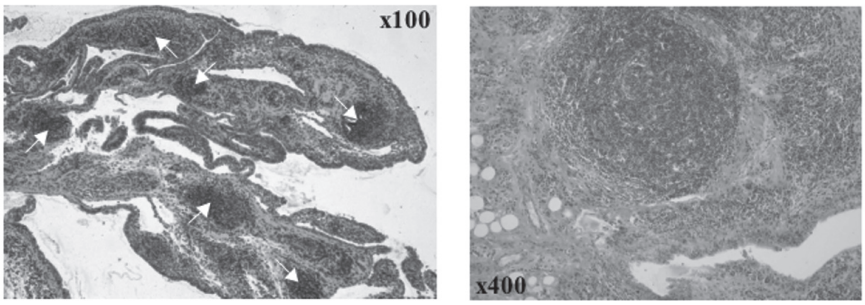

Fig. 2. A number of germinal centers (arrows) are seen in this rheumatoid synovium.

differ with respect to the expression of these two markers (Table I). Naïve (IgD+ CD38-) Bm1, once activated as (IgD+ CD38+) Bm2, differentiate into GC founder (IgD+ CD38++) Bm2'. A handful of Ab have been produced by early PC outside the GC. The Ag may thus by held in the form of immune complexes (IC) on the surface of follicular DC, and affinity maturation take place within the GC. This results in a massive expansion of centroblasts Bm3, and a selection, of centrocytes (IgD- CD38++) Bm4. They terminate as (IgD- CD38+) early Bm5 and (IgD- CD38-) mature Bm5 cells. In the latter subset, memory B-cells express CD27 and PC do not. No PC express CD20, but there appears to be two types of long-lived PC: one is activated and recruited to the BM, the other is resting and located in specialized microanatomical niches of secondary lymphoid organs. If anti-tetanus toxoid Ab derive

Table I – Mature B (Bm) lymphocyte subsets within secondary lymphoid organs.

Stepwise stage	IgD	CD38	CD23	CD27
Bm1 (naïve)	+	-	-	-
Bm2 (activated)	+	+	+	-
Bm2' (germinal center founder)	+	++	+	-
Bm3 (centroblast)	-	++	-	-
Bm4 (centrocyte)	-	++	-	-
Early Bm5 (antibody or memory)	-	+	-	-
Bm5 (memory)	-	-	-	+

Table II – B-lymphocytes are not dispensable for the development of lupus glomerulonephritis in the mouse, but autoantibodies (Ab) are. These cells produce Ab and autoAb, but exert other functions.

- Absence of T-cell infiltrates in MRL *lpr/lpr* mice homologous for the deletion JH/JH.
- The IgM MRL/MpJ-Fas^{lpr} mice have a transgene encoding surface Ig, but not permitting its secretion. They develop nephritis characterized by T-lymphocyte infiltration.

Table III – Immunobiologic functions of B-cells, in addition to antibody production.

- Shaping of the splenic architecture: dendritic cells and T-lymphocytes.
- Antigen presentation (in particular CD5- expressing and rheumatoid factor-making B-cells).
- Production of cytokines by B effector (Be)1 and Be2 to trigger polarization of naïve T-lymphocytes into T helper (Th)1 or Th2, respectively.
- Suppression of regulatory T-cells by regulatory B-cells.

from such long-lived PC, one may predict that, unlike RF, they will not be impaired by rituximab (49).

Thus, it is relevant that short-lived plasmablasts dominate the early spontaneous RF response (60), whereas the long-lived PC pool can serve a role in natural immunity (61). Of note, pathologic RF can be tolerized by competition with natural RF (62).

This Bm1 through Bm5 sequence keeps being generated, but leaks fractions of each B-cell subset into the PB. Their distribution is skewed in autoimmune patients: characteristic signatures have recently been described. Staining of circulating B-cells disclosed increased frequencies of GC founder cells (63), and reduced numbers of memory B-cells in pSS (64). Active systemic lupus erythematosus (SLE) is, in contrast, characterized by an increase in circulating plasmoblasts (65). No significant differences in B-cell subpopulations were seen in RA patients compared with healthy donors (66, and our unpublished results). However, CD20+ CD38- B-lymphocytes with defective proliferative responsiveness exist (67) in RA synovial fluid. Although considered to be Ig-producing effector B-cells, their significance is unclear.

B – Various commitments of B-cells

B-cells are critically required for the development of autoimmune conditions. This interest has been sparked off by Reininger *et al*. who demonstrated that long-term *in vitro* proliferating fetal liver pre-B-cell lines derived from autoimmune-prone mice, but not control mice, differentiate in severe combined immunodeficient (SCID) mice to produce autoAb (68).

Efforts to identify how these autoAb contribute to autoimmunity have reached conclusions difficult to incorporate into a unifying model. For example, nephritis does not occur in genetically-modified MRL *lpr*/*lpr* mice (Table II) that lack B-lymphocytes (69). Spontaneous T-cell activation is inhibited in B-deficient mice, and there is no lymphocytic infiltration in the skin and the kidneys. These results prompt a re-evaluation of the idea that systemic autoimmune disease is strictly due to IC. Further experiments reveal that mice that have B-cells with membrane but not secreted Ig develop nephritis (70). These results imply that the part of B-cells is essential in the play, but, above all, that their role in nephritis may not be through autoAb. Aside from their role as precursors of autoAb-secreting cells, these cells have indeed been endowed with various tasks within the immune system.

I – Influence of B-lymphocytes on neighboring cells

Both TNF-α and lymphotoxins (LT-α and –β) are involved in initiating and maintaining normal splenic architecture, since LT-α, LT-β, TNF-α receptor-I and TNF-α knockout (KO) mice have abnormal splenic architecture (71). The same holds true for those mice with LT-α- and/or LT-β- disrupted signaling. The sequential role of LT and B-cells in the development of LF has been extensively studied (72). The transfer of lymphocytes into SCID mice induces a series of histological changes in the spleen, including the appearance of mature follicular DC. LT favors the differentiationn of the white pulp to create sites for lymphocyte segregation, and then drives the maturation of DC and the organization of B-cell LF. Intriguingly, DC networks, which are compiscuous components of LF, are lacking in TNF- or LT-KO mice.

Owing to the impulse that T-lymphocyte differentiation offers to autoimmune susceptibility, their activation pathways into effector T helper (Th) 1 and Th2 cells have long been the focus of feverish research. There appears that Ag-specific interaction of B- and T-lymphocytes, along with the engagement of OX40 receptor on activated T-cells by OX40 ligand on stimulated B-cells, induces IL-4 production, restrains IFN-γ synthesis and favors the differentiation of Th2 and PC under the influence of IL-4 (73).

II – B-lymphocytes as Ag-presenting cells

All B-cells have the ability to act as APC, and this function is not differentially regulated in defined B-cell subpopulations (74). A given surface Ig takes up the Ag, and the resulting IC is internalized by the B-cell and processed (Fig. 3). The MHC-peptide complex is then presented by this B-cell to a T-cell nearby, or to another B-cell (75). B1a-cells are particularly suitable to the membrane expression of multispecific anti-self Ig, and therein appropriate to stretch out self components to other B1, B2 or T-lymphocytes (76). Splenic B-1a cells do behave as potent APC in that they induce two-fold greater levels of lymph mode T-lymphocyte IFN-γ release than B2 cells in certain lupus mice (77). RF B-cells can also present any Ag by taking up cognate IgG-containing IC (78).

The innovative concept that T-cell activation is B-cell-dependent in RA has recently been launched (79). Supporting this view, SM that are infiltrated by T-cells, macrophages and DC, but lack B-cells, cannot be activated by transferred T-cells, leaving with the possibility that B-cells provide a critical function in T-cell activation. This is consistent with a direct pathogenic role for

T-cells activated by Ag-specific B-cells (80), as established in a murine model of autoimmune arthritis. Both autoAb and autoreactive T-cells are required to cause severe arthritis, indicating that at least two B-cell-mediated effector pathways contribute synergistically to the damages. In fact, Ag presentation involves B-lymphocyte synapses, extracting intact Ag from APC, and aiding in the generation of peptide-MHC complexes for T-lymphocyte synapse formation (81).

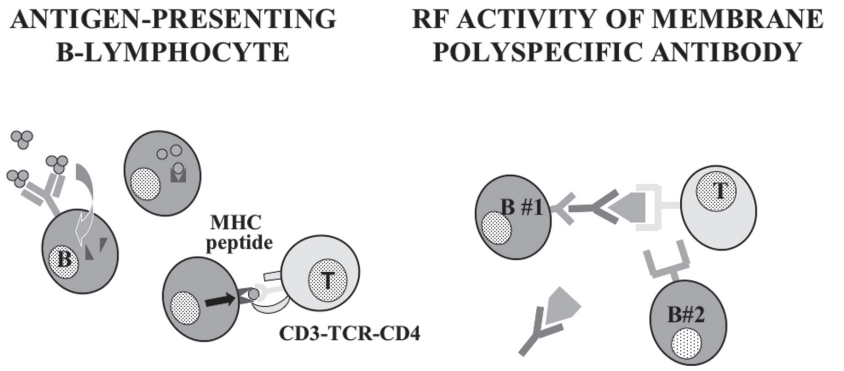

Fig. 3. B-cells serve as antigen (Ag)-presenting cells, either (left) by taking-up, internalizing, processing and exposing residual peptides, or (right) by binding IgG-containing immune complexes irrespective of the Ag through membrane rheumatoid factor.

III – Polarized cytokine production by B-cells

Cytokines are essential in disease activity. IL-6 which stimulates the final stages of B-lymphocyte maturation is an appealing candidate. This is abundantly produced in autoimmune states. Given the constitutive expression of IL-6 receptors (82), part of dysregulation of B-cell activity might be T-cell independent. B-cell-derived IL-6 would thus be involved in autocrine loops. Another candidate is IL-10 (83), as substantiated by six experiments: 1)- B1a cells are the main source of B-cell-derived IL-10; 2)- continuous treatment of normal mice with anti-IL-10 Ab depletes B1, but not B2 cells; 3)- antisense oligodesoxynucleotides specific for IL-10 mRNA inhibit the growth of murine leukemic B1 cells *in vitro*; 4)- IL-10 is involved in autoreactive B-cell hyperactivity and elevated in the serum of SLE patients; 5)- IL-10 agonist administration to patients is beneficial to refractory SLE; 6)- and we have, ourselves (19), sorted B1a, B1b and B2-lymphocytes from normal and IL-10-KO mice, and identified IL-10 mRNA by reverse-transcriptase (RT) polymerase-chain reaction (PCR) in B1a cells.

The paradigm of the Th1 and Th2 pathways has been extensively analyzed. They produce distinct spectra of cytokines compatible with the kind of response required by a given Ag. Th1 cells secrete IFN-γ and IL-2, enhance further polarization of Th1 cells, and inhibit Th2 cells. The other way round Th2 cells produce Il-4, Il-5 and IL-6, work upon activation and maturation of B-lymphocytes, enhance further polarization of Th2, and inhibit Th1 cells.

B-cells produce cytokines, such as IL-10, IL-6 and TNF-α (84). This is restricted to activated B-cells. They also acquire the ability to express IL-2, IFN-γ, IL-4 and IL-12 when stimulated in the presence of Th1 cells. An exciting novel issue is that naïve B cells differentiate into B-cells with cytokine profiles similar to Th1 and Th2 cells, following stimulation with Ag and polarized Th1 or Th2, respectively. The Th1-like B-cells have been dubbed B effectors (Be)1, and the Th2-like B-cells Be2 (85). Once generated, these B-lymphocytes impart functional capacities to the T-lymphocytes. For example, the development of IL-4-producing B-cells is controlled by IL-4, IL-4 receptors and Th2 cells (86). Thus, Be1 cells promote the expansion of Th1 cells, while IL-4 released by Be2 cells encourages Th2 cell development. In brief, Be1 and Be2 cells behave as classical APC that regulate the profile of the response, for example within the salivary glands (SG) of patients with pSS (87). Noteworthy is that B-cell-derived cytokines play pathologic as well as protective roles in immune responses to autoAg (88).

IV – Regulatory B-cells

An immunoregulatory B-cell subset that plays a role in immune regulation resulting in complete recovery from acute experimental autoimmune encephalomyelitis was first reported by Janeway *et al*. (89). A key role was then ascribed to IL-10 in dampening down autoimmunity (90). A B regulatory subset that is capable of producing TGF-β has also been identified (91). A number of pathways have since been describe for regulatory B-cells (92). Ironically, some of these B-cells might serve as regulators for regulatory T-cells (93).

C – B-cell tolerance breakdown

I – The B-cell Ag receptor

(i) – The Ag binds to the B cell Ag receptor

Survival and selection of B-lymphocytes are determined by their BCR specificity. Transduction of the ensuing signal is tuned by a dozen of co-receptors (94). Activation is favored by protein tyrosine kinases (PTK), and hindered by protein tyrosine phosphatases (PTP).

The BCR consists of a binding subunit, the membrane Ig, and a signaling subunit with Ig-α and Ig-β proteins (95). Their ectodomains are both necessary for the BCR to proceed from the endoplasmic reticulum to the cell surface, and their intra-cytoplasmic tails contain immunoreceptor tyrosine-based activation motifs (ITAM) which constitute as many docking sites for PTK. High-affinity interactions between autoAg and membrane-bound Ab result in apoptosis of immature B-cells. The ensuing crucial process of affinity maturation for exoAg takes place in the GC (96): centroblasts undergo hypermutations, and centrocytes emerge : either low-affinity cells that endure apoptosis, or high-affinity cells that change into Ab-forming cells.

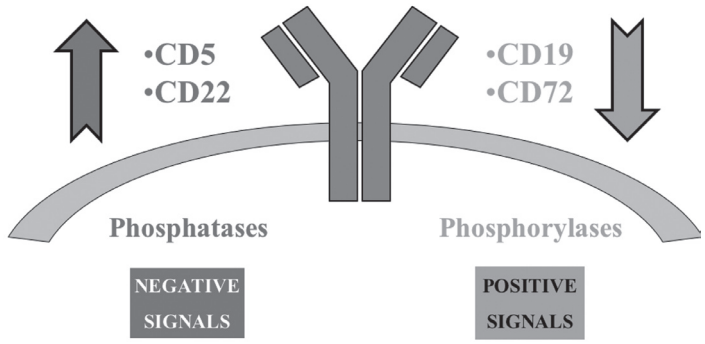

Fig. 4. Modulation of B-lymphocyte antigen receptor signal transduction : the threshold is augmented by phosphatases, and diminished by phosphorylases.

(ii) – The transduction machinery
Activation with Ag switches on a number of PTK (97), including *Lyn* and *Btk* (which are *src*-family members), and *syk*. Downstream, the phosphatidylinositol-3 kinase and serine/threonine kinase *Akt* transduction pathway possesses the ability to prevent apoptosis. This promotes cell survival by activating protein kinases C (PKC), and implicating the three families of mitogen-activated kinases (MAP): p38, the C-Jun NH2-terminal kinase families, and the extracellular-regulated kinase family. Following activation, phospholipase Cγ engenders diacylglycerol and inositol-1, 4, 5-triphosphate from membrane phosphatidylinositol-4, 5 biphosphate. These are required for activation of PKC and release of calcium, respectively. MAP kinases are then activated by PKC which has just been tyrosine-phosphorylated.

(iii) – Involvement in the tolerance
Contrasting advances have been described regarding the positive selection of mature B-lymphocytes by autoAg (98). There is, however, compelling evidence that recruitment of mature short-lived B-cells into the recirculating pool is dependent on BCR engagement by such autoAg (99). Additional mechanisms of self-tolerance in the periphery have led to silence mature B-cells that have dodged central tolerance. Interestingly, by downregulating BCR surface expression, leukemic B-lymphocytes (100, 101) still generate constitutive BCR-mediated survival signals, whilst their level of signaling required for apoptosis cannot be achieved. Molecular aberrations include signaling defects (102) and abnormal apoptosis (103) of autoreactive lymphocytes.

II – Coordinated and intricate mechanisms of self-tolerance
(i) – Regulation of signaling thresholds
Despite the profusion of mechanisms involved to achieve tolerance, this is not foolproof at all. Throughout the course of a response, co-receptors lift or lower the BCR threshold by modulating the activation of molecules required for transmitting a signal (Fig. 4). The machinery is raised by CD19 and CD21, and dampened down by CD22, CD72 and CD5 (98). In the context of autoimmune diseases, it is interesting that ligation of CD5 results in apoptosis of resting B-cells, but not resting T-cells (104), while anti-CD5 extends the proliferative response of activated B1a cells (105).
CD32 which is one of the receptors for the Fcγ part of Ig is also involved in blocking B-cell responses. CD22 is another potent role as a regulator of B-cell life span, but, despite this property, we found that the CD22 co-receptor was expressed at normal levels in B-lymphocytes from autoimmune patients (106). All these glycoproteins harbor ITAM and immunoregulatory tyrosine-inhibiting motifs, which recruite PTP in order to reverse the effects of PTK, *viz* SH2-containing PTP (SHP)-1 for CD22, CD72 and CD5, and SH2-containing inositol polyphosphate 5-phosphatase (SHIP) for CD32. *Lyn* is required to phosphorylate CD22 and to link SHP-1 to the CD22/BCR complex and/or to the CD5/BCR complex.
Cell surface CD19 is also critical for the regulation of intrinsic and BCR-induced signaling thresholds. High density of CD19 generates spontaneous autoAb, such as RF and anti-dsDNA Ab in a murine genetic background not associated with autoimmunity (107). Modest increases in the expression or the effect of this receptor are sufficient to shift the balance between tolerance and immunity to auto-immunity. For example, B-cells from patients with systemic sclerosis overexpress CD19 by as few as 20% (108). The level of CD19 expression was also increased in B-cells from patients with pSS (63) compared with that in normal controls, particularly in Bm2 and Bm2' cells.

(ii) – Anergy in self-reactive B-cells
Constant tickling of BCR by self Ag transmits tolerogenic signals by activation feedback processes that turn the cell anergic. Again, tolerance is governed by BCR signaling thresholds. Hippen *et al.* (109) have elegantly demonstrated in double-transgenic (Tg) mice that CD5 contributes to maintain tolerance in anergic B-cells: those B-lymphocytes of mice Tg for anti-hen egg lysozyme (HEL) Ab and membrane HEL Ag undergo apoptosis based on their robust activation. They rather become anergic if the mice are Tg for soluble HEL, but this tolerance is broken if this model for B-cell anergy is bred onto the CD5-KO background.

(iii) – Secondary changes in the BCR specificity
The role of self Ag to negative selection in the bone marrow has recently been established. Yet, it has been difficult to test whether self Ag also plays a role in the positive selection of B-cells. By altering the specificity of their BCR, auto-reactive B-cells may initiate new Ig rearrangements (110). The system can thus get rid of self-reactive BCR generated by the recombination process, by classical deletion and sustained recombination. Immature B-cells that undergo this receptor editing transcribe recombinase-activating genes, RAG1 and RAG2, and synthesize the recombination signal sequence-specific endonucleases that activate V(D)J re-organizations. The genes are then extinguished by a feedback mechanism

initiated by the BCR (111). It is interesting that the expression of RAG in B-cells that expand in the SM of RA patients results in aberrant rearrangement (112). Furthermore, Devauchelle et al have recently shown that fibroblast-like synoviocytes from RA patients, but not from osteoarthritis patients, induce reexpression of RAG by B-cells from normal PB (113).

Edition and revision of the BCR might be associated with the strength of signal through the BCR, and thereby to the expression of its receptors, as is the case for CD5. By using single-cell sorting and RT-PCR ligation-mediated PCR, we have shown (114) that activated mature CD5+ human tonsil B-lymphocytes express mRNA for both RAG genes, assemble the proteins, and display DNA cleavage resulting from their activity. Dual immunofluorescence staining detected both enzymes in one-third of CD5+ Bm2 lymphocytes. To further elucidate the link between CD5 expression and RAG transcription, CD5-non-expressing Bm1 and Bm2 lymphocytes were sorted and stimulated with anti-IgM and CD40 ligand. A CD5+ B-cell population was generated anew, and RAG1 and RAG2 co-expressed. Mechanisms contributing to the maintenance of tolerance in anergic CD5+B-cells may thus include the control of V(D)J recombination. One may speculate that control of CD5 expression and/or receptor revision before the cells enter the GC may be inadequate in RA, and lead to the emergence of autoAb-secreting cells.

III – Tuning the response of the BCR

(i) – The lipid raft model

Following its engagement, the BCR partitions into microdomains of the cell membrane (115) which are enriched in cholesterol and ganglioside M1 and designated lipid rafts (LR). They can be visualized by Alexa Fluor 594-conjugated cholera toxin B that binds to ganglioside M1, and the BCR by fluorescein-conjugated anti-IgM (Fig. 5).

One mechanism by which CD19 and CD32 may modulate this process is to prolong their residency in the LR, or to block the signal. CD19 delayes the exclusion of the BCR from the LR (116), whereas CD32 recruits SHIP (117). Supporting this view, LR composition is altered in RA and SLE (118), the BCR resides longer in the LR of pSS patients than in controls (63), and entry of the BCR into these signaling platforms is inhibited in tolerant B-cells (119). Interestingly, store-operated cation entry in LR is mediated by CD20 (120), and cross-linking of CD20 by rituximab induces its rapid distribution into the LR (121). Clearly, a greater understanding of CD20 biology will allow its more efficient exploitation as a therapeutic target.

(ii) – Cytokinic survival factors

B-cells are also controlled by survival factors. The most potent of these recently-described cytokines are the B-cell activating factor of the TNF family (BAFF) and a proliferation-inducing ligand (APRIL). BAFF rescues B-lymphocytes from apoptosis, increases the number of MZ B-cells and promotes autoAb production (122). Furthermore, patients with RA harbor elevated levels of BAFF (123, and Ferraccioli *et al*. unpublished results) and APRIL (124). This cytokine is produced at sites of inflammation, such as arthritic joints of RA patients (125) and SG of pSS patients (126). Interestingly, it has been established that B-cells infiltrating the SG of pSS patients express BAFF (127), as shown using *in situ* hybridi-

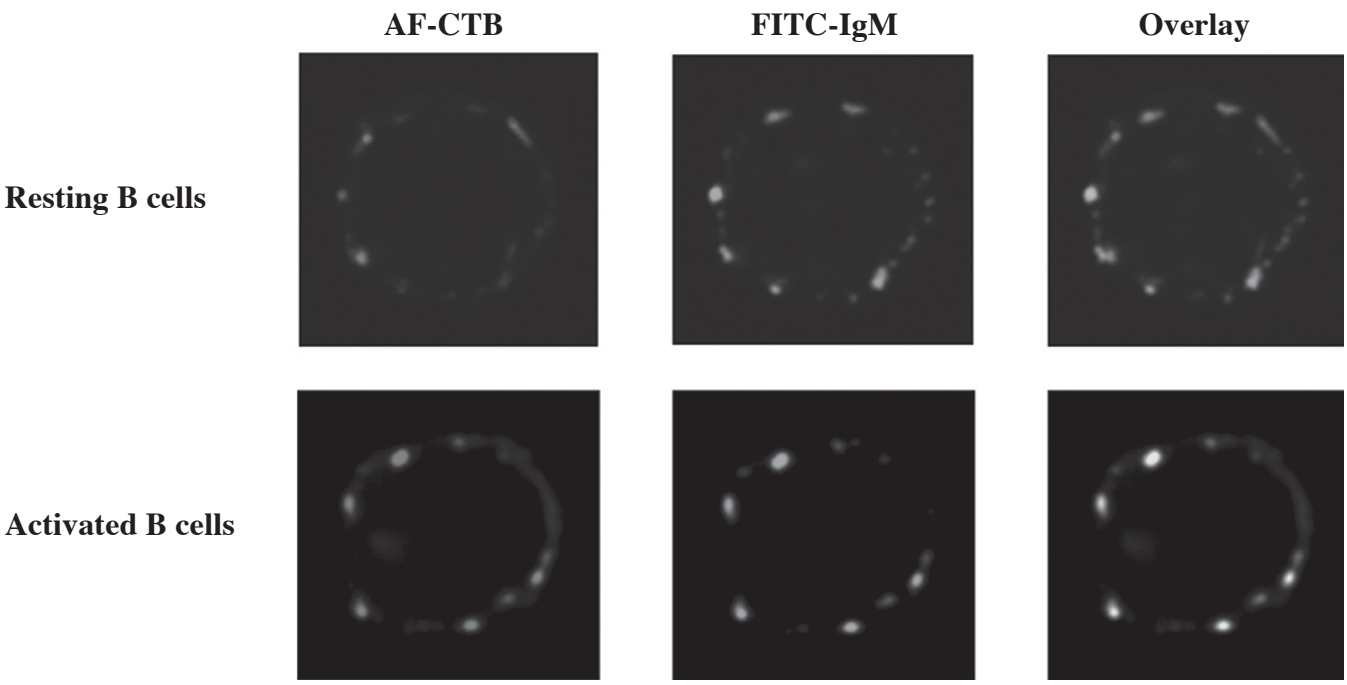

Fig. 5. Involvement of the lipid rafts in signaling through surface IgM cross-linking of B-cells. The cells were incubated with Alexa-Fluor (AF)-conjugated cholera toxin B and Fluorescein isothyocyanate (FITC)-conjugated anti-IgM antibody. When the cells were activated, overlay of red (AF) and green (FITC) was seen yellow.

zation, real-time RT-PCR of cultured cells and RT-PCR of sorted single-cell B-lymphocytes eluted from the SG. Three cognate receptors have been uncovered for this factor: B cell maturation Ag (BCMA), transmembrane activator and Ca^{2+} modulator and cyclophilin ligand receptor (TACI) and the third BAFF receptor (BR3). BAFF is a positive regulator provided its binds to BR3, but it is essential that its overexpression benefits exclusively self-reactive B-cells, and facilitates their migration into otherwise forbidden microenvironments, such as the SG in pSS and the SM in RA (128). In contrast, BCMA is dispensable for B-cell activation, and TACI inhibits this process.

(iii) – Genetic regulation of CD5 expression

We have recently described (129) a novel regulatory motif upstream of the non-coding region of the *cd*5 gene: this was designated E1B, and the known exon 1 was renamed E1A. The novel exon 1 lacks in the mouse, because it originates from a human endogenous retrovirus at a time interval between the divergence of New World and old World monkeys (130). The E1B-containing transcripts encode a truncated protein which is devoid of leader peptide and, therefore, retained intracellularly. It follows that the amount of E1A-containing transcripts is down-regulated, the protein membrane expression diminished, and translocation of SHP-1 prevented. Studies of transfected cells may hint to closer ties between the gene and the synthesis of the CD5 molecule. In this setting, the strength of the BCR-mediated signaling might lead to the expansion of autoreactive B-lymphocytes.

Finally, autoimmune diseases can be divided into three families: B-cell-dominant, T-cell-dominant and combinational types. A number of B-lymphocyte irregularities have to be incorporated into the pathophysiological schemes. Fundamental discoveries about B-cell pathology (131) are currently in the process of being translated into the clinic. Enthusiasm was recently bolstered by trials with rituximab (131). It may thus be safely assumed that B-lymphocyte depletion from the pre-B to the mature B-cell stages by use of mAb (7,132) has a key role to play in the treatment of RA.

Acknowledgements

The secretarial assistance of Simone Forest and Cindy Séné was greatly appreciated.

References

1. BENNETT SR, FALTA MT, BILL J, KOTZIN BL: Antigen-specific T-cells in rheumatoid arthritis. *Curr Rheumatol Rep* 2003; 5: 255-63.
2. STUART JM, CREMER MA, TOWNES AS, KANG AH: Type II collagen-induced arthritis in rats. Passive transfer with serum and evidence that IgG anticollagen antibodies can cause arthritis. *J Exp Med* 1982; 155: 1-16.
3. WEYAND CM, GORONZY JJ: T-cell responses in rheumatoid arthritis: systemic abnormalities-local disease. Curr Opin Rheumatol 1999; 11: 210-7.
4. DAVIDSON A, DIAMOND B: Autoimmune diseases. *N Engl J Med* 2001; 345: 340-50.
5. YOUINOU P, LE GOFF P, MERDRIGNAC G *et al.*: The relation between CD5-B-lymphocytes and serologic abnormalities in rheumatoid arthritis patients and their relatives. *Arthritis Rheum* 1990; 33: 339-48.
6. LOONEY RJ: Treating human autoimmune disease by depleting B-cells. *Ann Rheum Dis* 2002; 61: 863-6.
7. EDWARDS JC, SZCZEPANSKI L, SZECHINSKI J *et al.*: Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. *New Engl J Med* 2004; 350: 2572-81.
8. LYDYARD PM, MACKENZIE LE, YOUINOU P, DEANE M, JEFFERIS R, MAGEED RA: Specificity and idiotype expression of IgM produced by CD5+ and CD5- cord blood B-cells. *Ann NY Acad Sci* 1992; 651: 527-35.
9. YOUINOU P, MACKENZIE L, LAMOUR A, MAGEED RA, LYDYARD PM: Human CD5-positive B-cells in lymphoid malignancy and connective tissue disease. *Eur J Clin Invest* 1993; 23: 139-50.
10. BRÖKER BM, KLAJMAN A, YOUINOU P *et al.:* Chronic lymphocytic leukemia cells secrete multispecific autoantibodies. *J Autoimmun* 1988; 1: 469-81.
11. KANTOR A: A new nomenclature for B-cells. *Immunol Today* 1991; 12: 388-91.
12. YOUINOU P, JAMIN C, LYDYARD PM: CD5 expression in human B-cell populations. *Immunol Today* 1999; 20: 312-6.
13. KASSIAN MT, IKEMATSU H, CASALI P: Identification and analysis of a novel surface CD5-B-lymphocyte subset producing natural antibodies. *J Immunol* 1992; 148: 2690-702.
14. YOUINOU P, LYDYARD PM: CD5+B-cells in nonorgan-specific autoimmune diseases: a fresh look. *Lupus* 2001; 10: 523-5.
15. WORTIS HH, BERLAND R: Cutting edge commentary: origins of B-1 cells. *J Immunol* 2001; 166: 2163-6.
16. HERZENBERG LA: B-1 cells: the lineage question revisited. *Immunol Rev* 2000; 175: 9-19.
17. MONTECINO-RODRIGUEZ E, LEATHERS H, DORSHKIND K: Identification of a B-1 B-cell-specified progenitor. *Nat Immunol* 2006; 7: 293-301.
18. PERS JO, JAMIN C, LYDYARD PM, CHARREIRE J, YOUINOU P: The H2 haplotype regulates the distribution of B-cells into B1a, B1b and B2 subsets. *Immunogenetics* 2002; 54: 208-11.
19. PERS JO, JAMIN C, YOUINOU P, CHARREIRE J: Role of IL-10 in the distribution of B-cell subsets in the mouse B1 cell population. *Eur Cytokine Netw* 2003; 14: 178-85.
20. PAAVONEN T, QUARTEY-PAPAFIO R, DELVES PJ *et al.*: CD5 mRNA expression and autoantibody production in early human B-cells immortalized by EBV. *Scand J Immunol* 1990; 31: 369-74.
21. PLATER-ZYBERK C, MAINI RN, LAM K, KENNEDY TD, JANOSSY G: A rheumatoid arthritis B-cell subset expresses a phenotype similar to that in chronic lymphocytic leukemia. *Arthritis Rheum* 1985; 28: 971-6.
22. YOUINOU P, MACKENZIE LE, JOUQUAN J, LE GOFF P, LYDYARD PM: CD5-positive B-cells in patients with rheumatoid arthritis: phorbol ester-mediated enhancement of detention. *Ann Rheum Dis* 1987; 46: 37-9.
23. DAUPHINÉE M, TOVAR Z, TALAL N: B-cells expressing CD5 are increased in Sjögren's syndrome. *Arthritis Rheum* 1988; 31:642-7.
24. YOUINOU P, MACKENZIE L, LE MASSON G *et al:* CD5-expressing B-lymphocytes in the blood and salivary glands of patients with primary Sjögren's syndrome. *J Autoimmun* 1988; 1: 185-94.
25. MARTINI A, MASSA M, DE BENEDETTI F, VIOLA S, NEIROTTI G, BURGIO RG: CD5 positive B-lymphocytes in seronegative juvenile arthritis. *J Rheumatol* 1990; 17: 932-5.
26. JARVIS JN, KAPLAN J, FINE N: Increase in CD5+ B-cells in juvenile rheumatoid arthritis. Relationship to IgM rheumatoid factor expression and disease activity. *Arthritis Rheum* 1992; 35:204-7.
27. ANAYA JM, CORREA PA, HERRERA M, ESKDALE J, GALLAGHES G: Interleukin 10 (IL-10) influences autoimmune response in primary Sjögren's syndrome and is linked to IL-10 gene polymorphism. *J Rheumatol* 2002; 29: 1874-6.
28. CASALI P, BURASTERO SE, NAKAMURA M, INGHIRAMI G, NOTKINS AL: Human lymphocytes making rheumatoid factor and antibody to ssDNA belong to Leu-1+ B-cell subset. *Science* 1987; 236: 77-81.
29. HARDY RR, HAYAKAWA K, SHIMIZU M, YAMASAKI K, KISHIMOTO T: Rheumatoid factor secretion from human Leu-1+ B-cells. *Science* 1987; 236: 81-3.
30. NAKAMURA M, BURASTERO SE, NOTKINS AL, CASAL P: Human monoclonal rheumatoid factor-like antibodies from CD5 (Leu-1)+ B cells are polyreactive. *J Immunol* 1988; 140: 4180-6.
31. CASALI P, BURASTERO SE, BALOW JE, NOTKINS AL: High-affinity antibodies to ssDNA are produced by CD-B cells in systemic lupus erythematosus patients. *J Immunol* 1989; 143: 3476-83.

32. CARSETTI R, KOHLER G, LAMERS MC: Transitional B-cells are the target of negative selection in the B-cell compartment. *J Exp Med* 1995; 181: 2129-40.
33. SIMS GP, ETTINGER R, SHIROTA Y, YARBORO CH, ILLEI GG, LIPSKY PE: Identification and characterization of circulating human transitional B-cells. *Blood* 2005; 105: 4390-8.
34. ALLMAN D, LINDSLEY RC, DEMUTH W, RUDD K, SHINTON SA, HARDY RR: Resolution of three nonproliferative immature splenic B-cell subsets reveals multiple selection points during peripheral B-cell maturation. *J Immunol* 2001; 167: 6834-40.
35. CHUNG JB, SILVERMAN M, MONROE JG: Transitional B-cells: step by step towards immune competence. *Trends Immunol* 2003; 24:343-9.
36. MARTIN F, KEARNEY JF: Marginal-zone B-cells. *Nat Rev Immunol* 2002; 2: 323-35.
37. CAMACHO SA, KOSCO-VILBOIS MH, BEREK C: The dynamic structure of the germinal center. *Immunol Today* 1998; 19: 511-4.
38. JACOB J, KASSIR R, KELSOE G: In situ studies of the primary immune response to (4-hydroxy-3-nitrophenyl)acetyl. I. The architecture and dynamics of responding cell populations. *J Exp Med* 1991; 173: 1165-75.
39. GARCIA DE VINUESA C, GULBRANSON-JUDGE A, KHAN M *et al.:* Dendritic cells associated with plasmablast survival. *Eur J Immunol* 1999; 29: 3712-21.
40. CASOLA S, OTIPOBY KL, ALIMZHANOV M *et al*.: B-cell receptor signal strength determines B-cell fate. *Nat Immunol* 2004; 5: 317-27.
41. SAITO T, CHIBA S, ICHIKAWA M *et al.*: Notch2 is preferentially expressed in mature B-cells and indispensable for marginal zone B lineage development. *Immunity* 2003; 18: 675-85.
42. ARTAVANIS-TSAKONAS S, RAND MD, LAKE RJ: Notch signaling: cell fate control and signal integration in development. *Science* 1999; 284: 770-6.
43. WELLER S, FAILI A, GARCIA C *et al*.: CD40-CD40L independent Ig gene hypermutation suggests a second B-cell diversification pathway in humans. *Proc Natl Acad Sci U S A* 2001; 98: 1166-70
44. WILLENBROCK K, JUNGNICKEL B, HANSMANN ML, KUPPERS R: Human splenic marginal zone B-cells lack expression of activation-induced cytidine deaminase. *Eur J Immunol* 2005; 35:3002-7.
45. DUNN-WALTERS DK, ISAACSON PG, SPENCER J: Analysis of mutations in immunoglobulin heavy chain variable region genes of microdissected marginal zone (MGZ) B-cells suggests that the MGZ of human spleen is a reservoir of memory B-cells. *J Exp Med* 1995; 182: 559-66.
46. WELLER S, REYNAUD CA, WEILL JC: Splenic marginal zone B-cells in humans: where do they mutate their Ig receptor? *Eur J Immunol* 2005; 35: 2789-92.
47. WELLER S, BRAUN MC, TAN BK *et al*.: Human blood IgM "memory" B-cells are circulating splenic marginal zone B-cells harboring a prediversified immunoglobulin repertoire. *Blood* 2004; 104: 3647-54.
48. WILLIAM J, EULER C, CHRISTENSEN S, SHLOMCHIK MJ: Evolution of autoantibody responses via somatic hypermutation outside the germinal centers. *Science* 2002; 297: 2066-70.
49. CAMBRIDGE G, STOHL W, LEANDRO MJ, MIGONE TS, HILBERT DM, EDWARDS JC: Circulating levels of B lymphocyte stimulator in patients with rheumatoid arthritis following rituximab treatment: relationships with B cell depletion, circulating antibodies, and clinical relapse. *Arthritis Rheum* 2006; 54: 723-32.
50. CINAMON G, MATLOUBIAN M, LESNESKI MJ *et al*.: Sphingosine 1-phosphate receptor 1 promotes B-cell localization in the splenic marginal zone. *Nat Immunol* 2004; 5: 713-20.
51. VORA KA, NICHOLS E, PORTER G *et al*.: Sphingosine 1-phosphate receptor agonist FTY720-phosphate causes marginal zone B-cell displacement. *J Leukoc Biol* 2005; 78: 471-80.
52. YOUINOU PY, MORROW JW, LETTIN AW, LYDYARD PM, ROITT IM: Specificity of plasma cells in the rheumatoid synovium. I. Immunoglobulin class of antiglobulin-producing cells. *Scand J Immunol* 1984; 20: 307-15
53. BAETEN D, PEENE I, UNION A *et al*.: Specific presence of intracellular citrullinated proteins in rheumatoid arthritis synovium: relevance to antifilaggrin autoantibodies. *Arthritis Rheum* 2001; 44: 2255-62.
54. GRAMMER AC, LIPSKY PE: CD154-CD40 interactions mediate differentiation to plasma cells in healthy individuals and persons with systemic lupus erythematosus. *Arthritis Rheum* 2002; 46: 1417-29.
55. TAKEMURA S, BRAUN A, CROWSON C *et al*.: Lymphoid neogenesis in rheumatoid synovitis. *J Immunol* 2001; 167: 1072-80.
56. SCHRODER AE, GREINER A, SEYFERT C, BEREK C: Differentiation of B-cells in the nonlymphoid tissue of the synovial membrane of patients with rheumatoid arthritis. *Proc Natl Acad Sci U S A* 1996; 93: 221-5.
57. WEYAND CM, KURTIN PJ, GORONZY JJ: Ectopic lymphoid organogenesis: a fast track for autoimmunity. *Am J Pathol* 2001; 159: 787-93.
58. KELSOE G: Life and death in germinal centers (redux). *Immunity* 1996; 4:107-11.
59. LIU YJ, ARPINC C: Germinal center development. *Immunol Rev* 1997; 156: 111-26.
60. WILLIAM J, EULER C, SHLOMCHIK MJ: Short-lived plasmablasts dominate the early spontaneous rheumatoid factor response: differentiation pathways, hypermutating cell types, and affinity maturation outside the germinal center. *J Immunol* 2005; 174: 6879-87
61. MANZ RA, RADBRUCH A: Plasma cells for a lifetime? *Eur J Immunol* 2002; 32: 923-7.
62. STEWART JJ, AGOSTO H, LITWIN S *et al*.: A solution to the rheumatoid factor paradox: pathologic rheumatoid factors can be tolerized by competition with natural rheumatoid factors. *J Immunol* 1997; 159: 1728-38.
63. D'ARBONNEAU F, PERS JO, DEVAUCHELLE V, PENNEC Y, SARAUX A, YOUINOU P: BAFF-induced changes in B-cell antigen receptor-containing lipid rafts in Sjögren's syndrome. *Arthritis Rheum* 2006; 54: 115-26.
64. BOHNKORST JØ, BJØRGAN MB, THOEN JE, NATVIG JB, THOMPSON KM: Bm1-Bm5 classification of peripheral blood B-cells reveals circulating germinal center founder cells in healthy individuals and disturbance in the B-cell subpopulations in patients with primary Sjögren's syndrome. *J Immunol* 2001; 167: 3610-8.
65. ODENDAHL M, JACOBI A, HANSEN A *et al*.: Disturbed peripheral B-lymphocyte homeostasis in systemic lupus erythematosus. *J Immunol* 2000; 165: 5970-5979.
66. POTTER KN, MOCKRIDGE CI, RAHMAN A *et al*.: Disturbances in peripheral blood B-cell subpopulations in autoimmune patients. *Lupus* 2002; 11: 872-7.
67. REPARON-SCHUIJT C, VAN ESCH WEJ, VAN KOOTEN C *et al*.: Presence of a population of CD20+ CD38- lymphocytes with defective proliferative responsiveness in the synovial compartment of patients with rheumatoid arthritis. *Arthritis Rheum* 2001; 44: 2029-37.
68. REININGER L, WINKLER TH, KALBERER CP, JOURDAIN M, MELCHERS F, ROLINK AG: Intrinsic B-cell defects in NZB and NZW mice contribute to systemic lupus in (NZBXNZW) F1 mice. *J Exp Med* 1996; 184: 853-61.
69. CHAN OTM, HANNUM LG, HABERMAN AM, MADAIO MP, SHLOMCHIK MJ: A novel mouse with B-cells but lacking serum antibody reveals an antibody-dependent role for B-cells in murine lupus. *J Exp Med* 1999; 189: 1639-48.
70. CHAN O, SHLOMCHIK MJ: A new role for B-cells in systemic autoimmunity: B-cells promote spontaneous T cell activation in MRL-*lpr*/*lpr* mice. *J Immunol* 1998; 160: 51-9.
71. PASPARAKIS M, ALEXOPOULOU L, EPISKOPOU V, KOLLIAS G: Immune and inflammatory responses in TNF-α-deficient mice: a critical recruitement for TNF-α in the formation of primary B-cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. *J Exp Med* 1998; 184: 1397-411.
72. GONZALEZ M, MACKAY F, BROWNING JL, KOSCO-VILBOIS MH, NOELLE RJ: The sequential role of lymphotoxin and B-cells in the development of splenic follicles. *J Exp Med* 1998; 187: 997-1007.
73. FLYNN S, TOELLNER KM, RAYKUNDALIA C, GOODALL M, LANE P: CD4 T-cell cytokine differentiation: the B-cell activation molecule, OX40 ligand, instructs CD4 T-cells to express interleukin-4 and upregulates expression of the chemokine receptor Bbr-1. *J Exp Med* 1998; 188: 297-304.
74. CHESNUT RW, GREY HM: Studies on the capacity of B cells to serve as antigen-presenting cells. *J Immunol* 1981; 126: 1075-9.
75. SCHULTZ KR, KLARNET JP, GIENI RS, HAYGLASS KT, GREENBERG PD: The role of B-cells for *in vivo* T-cell response to a Friend virus-induced leukemia. *Science* 1990; 249: 921-3.

76. JANEWAY CA JR, RON J, KATZ ME: The B-cell is the initiating antigen-presenting cell in peripheral lymph nodes. *J Immunol* 1987; 138: 1051-5.
77. MOHAN C, MOREL L, YANG P, WAKELAND EK: Accumulation of splenic B-1a cell with potent antigen-presenting capability in NZM2410 lupus-prone mice. *Arthritis Rheum* 1998; 41: 1652-62.
78. ROOSNEK E, LANZAVECCHIA A: Efficient and selective presentation of antigen-antibody complexes by rheumatoid factor B-cells. *J Exp Med* 1991; 173: 487-9.
79. TAKEMURA S, KLIMIUK PA, BRAUN A, CORONZY JJ, WEYAND CM: T-cell activation in rheumatoid synovium in B-cell-dependent. *J Immunol* 2001; 167: 4710-8.
80. O'NEILL SK, SHLOMCHIK MJ, GLANT TT, CAO Y, DOODES PD, FINNEGAN A: Antigen-specific B-cells are required as APCs and autoantibody-producing cells for induction of severe autoimmune arthritis. *J Immunol* 2005; 174: 3781-8
81. DUSTIN ML, DUSTIN LB: The immunological relay race: B-cells take antigen by synapse. *Nat Immunol* 2001; 2: 480-2.
82. NAGAFUCHI H, SUZUKI N, MIZUSHIMA Y, SAKANE T: Constitutive expression of IL-6 receptors and their role in the excessive B-cell function in patients with systemic lupus erythematosus. *J Immunol* 1993; 151: 6525-34.
83. MOORE KW, DE WAAL MALEFYT R, COFFMAN RL, O'GARRA A: Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 2001; 19: 683-765.
84. PISTOIA V: Production of cytokines by human B-cells in health and disease. *Immunol Today* 1997; 18: 343-50.
85. HARRIS DP, HAYNES L, SAYLES PC *et al.:* Reciprocal regulation of polarized cytokine production by effector B and T-cells. *Nat Immunol* 2000; 1: 475-82.
86. HARRIS DP, GOODRICH S, MOHRS K, MOHRS M, LUND FE: Cutting edge: the development of IL-4-producing B-cells (B effector 2 cells) is controlled by IL-4, IL-4 receptor alpha, and Th2 cells. *J Immunol* 2005; 175: 7103-7.
87. YOUINOU P, DARIDON C, SARAUX A, DEVAUCHELLE V, PERS JO: Is B-cell the conductor of the lymphocyte orchestra in the salivary glands of patients with primary Sjögren's syndrome? *Clin Exp Rheumatol* 2006; 24: 491-2.
88. LUND FE, GARVY BA, RANDALL TD, HARRIS DP: Regulatory roles for cytokine-producing B-cells in infection and autoimmune disease. *Curr Dir Autoimmun* 2005; 8: 25-54.
89. WOLF SD, DITTEL BN, HARDARDOTTIR F, JANEWAY CA JR: Experimental autoimmune encephalomyelitis induction in genetically B-cell-deficient mice. *J Exp Med* 1996; 184: 2271-8.
90. FILLATREAU S, SWEENIE CH, MCGEACHY MJ, GRAY D, ANDERTON SM: B-cells regulate autoimmunity by provision of IL-10. *Nat Immunol* 2002; 3: 944-50.
91. PAREKH VV, PRASAD DV, BANERJEE PP, JOSHI BN, KUMAR A, MISHRA GC: B-cells activated by lipopolysaccharide, but not by anti-Ig and anti-CD40 antibody, induce anergy in CD8+ T-cells: role of TGF-beta 1. *J Immunol* 2003; 170: 5897-911.
92. MIZOGUCHI A, BHAN AK: A case for regulatory B-cells. *J Immunol* 2006; 176: 705-10.
93. LAN RY, ANSARI AA, LIAN ZX, GERSHWIN ME: Regulatory T cells: development, function and role in autoimmunity. *Autoimmun Rev* 2005; 4: 351-63.
94. JUSTEMENT LB: Signal transduction via the B-cell antigen receptor: the role of protein tyrosine kinases and protein tyrosine phosphatases. *Curr Tp Microbiol Immunol* 2000; 245: 1-51.
95. MTSUCHI L, GOLD MR: New views of BCR structure and organization. *Curr Opin Immunol* 2001; 13: 270-7.
96. HEALY JI, GOODNOW CC: Positive versus negative signalling by lymphocyte antigen receptors. *Annu Rev Immunol* 1998; 16: 645-70.
97. CRAXTON A, OTIPOBY KL, JIANG A, CLARK EA: Signal transduction pathways that regulate the fate of B-lymphocytes. *Adv Immunol* 1999; 73: 79-152.
98. HEALY JI, GOODNOW CC: Positive versus negative signaling by lymphocyte antigen receptors. *Annu Rev Immunol* 1998; 16: 645-70.
99. LAM KP, HÜHN R, RAJEWSKY R: *In vivo* ablation of surface immunoglobulin on mature B-cells by inducible gene targeting results in rapid cell death. *Cell* 1997; 90: 1073-83.
100. RENAUDINEAU Y, NÉDELLEC S, BERTHOU C, LYDYARD PM, YOUINOU P, PERS JO: Role of B-cell antigen receptor associated molecules and lipid rafts in CD5-induced apoptosis of B CLL cells. *Leukemia* 2005; 19: 223-9.
101. NÉDELLEC S, RENAUDINEAU Y, BERTHOU C *et al.:* B-cell response surface IgM cross-linking identifies different prognostic groups of B-chronic lymphocytic leukemia patients. *J Immunol* 2005; 174: 3749-56.
102. LIOSSIS SMC, KOVACS B, DENNIS G, KAMMER GC: B-cells from patients with systemic lupus erythematosus display abnormal antigen receptor-mediated early transduction events. *J Clin Invest* 1996; 98: 2549-57.
103. CASCIOLA-ROSEN LA, ANHALT G, ROSEN A: Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J Exp Med* 1994; 179: 1317-30.
104. PERS JO, JAMIN C, LE CORRE R, LYDYARD PM, YOUINOU P: Ligation of CD5 on resting B-cells, but not on resting T-cells, results in apoptosis. *Eur J Immunol* 1998; 28: 4170-6.
105. JAMIN C, LE CORRE R, LYDYARD PM, YOUINOU P: Anti-CD5 extends the proliferative response of human CD5+ B-cells activated with anti-IgM and interleukin-2. *Eur J Immunol* 1996; 26: 57-62.
106. HUCK S, LE CORRE R, YOUINOU P, ZOUALI M: Expression of B-cell receptor-associated signaling molecules in human lupus. *Autoimmunity* 2001; 33: 213-24.
107. INAOKI M, SATO S, WEINTRAUB BC, GOODNOW CC, TEDDER TF: CD19-regula-ted signaling thresholds control peripheral tolerance and autoantibody production in B-lymphocytes. *J Exp Med* 1997; 186: 1923-31.
108. SATO S, HASEGAWA M, FUJIMOTO M, TEDDER TF, TAKEHARA K: Quantitative genetic variation in CD19 expression correlates with autoimmunity. *Immunol* 2000; 165: 6635-43.
109. HIPPEN KL, TZE LE, BEHRENS TW: CD5 maintains tolerance in anergic B-cells. *J Exp Med* 2000; 191: 883-9.
110. TIEGS SL, RUSSEL DM, NEMAZEE D: Receptor editing in self-reactive bone marrow B-cells. *J Exp Med* 1993; 177: 1009-20.
111. MEFFRE E, PAPAVASILIOU F, COHEN P *et al.*: Antigen receptor engagement turns off the V(D)J recombination machinery in human tonsil B-cells. *J Exp Med* 1998; 188: 765-72.
112. ITOH K, MEFFRE E, ALBESIANO E *et al.*: Immunoglobulin heavy chain variable region gene replacement as a mechanism for receptor revision in rheumatoid arthritis synovial tissue B-lymphocytes. *J Exp Med* 2000; 192: 1151-64.
113. ROCHAS C, JAMIN C, HILLION S *et al.*: Fibroblast-like synoviocytes from synovial tissue of rheumatoid arthritis induce autoreactivity in B-cells. *Arthritis Rheum* 2006; 54 (Suppl. 9): S509.
114. HILLION S, SARAUX A, YOUINOU P, JAMIN C: Expression of RAGs in peripheral B-cells outside germinal centers is associated with the expression of CD5. *J Immunol* 2005; 174: 5553-61.
115. PIERCE SK: Lipid rafts and B-cell activation. *Nat Rev Immunol* 2002; 2: 96-105.
116. CHERUKURI A, CHENG PC, SOHN HW, PIERCE SK: The CD19/CD21 complex functions to prolong B-cell antigen receptor signaling from lipid rafts. *Immunity* 2001; 14: 169-79.
117. AMAN MJ, TOSELLO-TRAMPONT AC, RAVICHANDRAN K: Fcγ RII B1/SHIP-mediated inhibitory signaling in B-cells involves lipid rafts. *J Biol Chem* 2001; 276: 46371-8.
118. FLORES-BORJA F, KABOURIDIS PS, JURY EC, ISENBERG DA, MAGEED RA: Altered lipid raft-associated proximal signaling and translocation of CD45 tyrosine phosphatase in B lymphocytes from patients with systemic lupus erythematosus. *Arthritis Rheum* 2006; 56: 291-302.
119. WEINTRAUB BC, JUN JE, BISHOP AC, SHOKAT KM, THOMAS ML, GOODNOW CC: Entry of B cell receptor into signaling domains is inhibited in tolerant B cells. *J Exp Med* 2000; 191:1443-8.
120. LI H, AYER LM, LYTTON J, DEANS JP: Store-operated cation entry mediated by CD20 in membrane rafts. *J Biol Chem* 2003; 278: 42427-34.
121. JANAS E, PRIEST R, MALHOTRA R: Functional role of lipid rafts in CD20 activity? *Biochem Soc Symp* 2005; 72: 165-75.
122. MACKAY F, BROWNING JL: BAFF: a fundamental survival factor for B-cells. *Nat Rev Immunol* 2002; 2: 465-75.
123. PERS JO, DARIDON C, DEVAUCHELLE V *et al.:* BAFF overexpression is associated with autoantibody production in autoimmune diseases. *Ann N Y Acad Sci* 2005; 1050: 34-9.
124. SEYLER TM, PARK YW, TAKEMURA S *et al.*:

BLyS and APRIL in rheumatoid arthritis. *J Clin Invest* 2005; 115: 3083-92.
125. TAN SM, XU D, ROSCHKE V, PERRY JW *et al*.: Local production of B-lymphocyte stimulator protein and APRIL in arthritic joints of patients with inflammatory arthritis. *Arthritis Rheum* 2003; 48: 982-92.
126. SZODARAY P, JELLESTAD S, OHLSSON TEAGUE M, JONSSON R: Attenuated apoptosis of B-cell activating factor-expressing cells in primary Sjögren's syndrome. *Lab Invest* 2003; 83: 357-65.
127. DARIDON C, DEVAUCHELLE V, HUTIN P *et al*.: Aberrant expression of BAFF by B-lymphocytes infiltrating the salivary glands of patients with primary Sjögren's syndrome. *Arthritis Rheum* 2007 in press.
128. DARIDON C, YOUINOU P, PERS JO: BAFF, APRIL, TWE-PRIL: who's who? *Autoimmun Rev*, 2007 in press.
129. RENAUDINEAU Y, HILLION S, SARAUX A, MAGEED RA, YOUINOU P: An alternative exon 1 of the CD5 gene regulates CD5 expression in human B-lymphocytes. *Blood* 2005; 106: 2781-9.
130. RENAUDINEAU Y, VALLET S, LE DANTEC C, HILLION S, SARAUX A, YOUINOU P: Characterization of the human CD5 endogenous retrovirus-E in B lymphocytes. *Genes Immun* 2005; 6: 663-71.
131. VINUESA CG, COOK MC: The molecular basis of lymphoid architecture and B cell responses: implications for immunodeficiency and immunopathology. *Curr Mol Med* 2001; 1: 689-725.
132. EDWARDS JC, CAMBRIDGE G: B-cell targeting in rheumatoid arthritis and other autoimmune diseases. *Nat Rev Immunol* 2006; 6: 394-403.