Dysfunctional B cells in systemic lupus erythematosus

Yves Renaudineau, Jacques-Olivier Pers, Boutahar Bendaoud, Christophe Jamin, Pierre Youinou*

Laboratory of Immunology, Brest University Medical School BP824, F29609 Brest cedex, France

Accepted 16 July 2004
Available online 14 October 2004

Abstract

The classical view of B cells in the biology of autoimmune responses to infectious and self-antigens (Ag) that they promote immunity primarily by producing antibodies (Ab) is far from being complete. Indeed, studies over the last decade suggest that B cells have extraordinarily diverse functions within the immune system other than Ab production, which could contribute to autoimmunity. They normally play a role in the development of lymphoid architecture, regulating dendritic cells (DC) and T cell subsets function through cytokine production, and in activation of T cells. Receptor editing is also important in B cells which aids in immunity to infection and, possibly, prevention of autoimmunity. Both abnormalities in the distribution of B cells subsets and clinical benefit response to B cell depletion in autoimmune diseases, including systemic lupus erythematosus (SLE), highlight their pivotal function. Transgenic (Tg) animal models have shown that sensitivity of B cells to B cell Ag receptor (BCR) cross-linking is correlated to autoimmunity. Indeed, negative signaling by CD5 and other molecules, such as CD22, in maintaining tolerance through recruitment of src-homology two domain-containing protein tyrosine phosphatase-1 (SHP-1) has also been documented. In fact, we have now reached a newer area whereby B cells returned as an important contributor to autoimmune disorders.

Keywords: B cells; Autoimmunity; B cell antigen receptor

Contents

1. Characteristics of B lymphocytes ........................................ 517
   1.1. B lymphocytes influence the formation of follicular DC networks and secondary lymphoid architecture. 517
   1.2. B lymphocytes coordinate T lymphocyte migration and differentiation in secondary lymphoid organs . 518
   1.3. B lymphocytes influence the pattern of immune responses through the production of cytokines in T cells and DC. 518
2. Overactivity of B cells in systemic diseases .............................. 519
Systemic lupus erythematosus (SLE) is remarkably diverse both in clinical presentation and associated immunological abnormalities, among which (Ab) to the self [1]. The disease affects different tissues and organs, the patients vary in age at onset, and the outcome is unpredictable from severe to relatively benign. Lupus is associated with different combinations of autoantibodies directed to a range of self-antigens, not only in different human patients, but also in different murine models of the disease. Further, with the widespread availability of targeted recombination technology, it has become evident that deficiency in any of a cascade of membrane and signaling molecules could lead to lupus-like syndromes. However, although the exact underlying immunological lesion in lupus autoimmunity remains unidentified, recent studies suggest that inherent B lymphocyte hyperactivity is likely to underpin lupus disease processes.

Human patients with SLE display such a multifaceted presentation that this disease has been portrayed as a mosaic of abnormalities [2]. Because of the extensive infiltration of T cells at sites of inflammation, including the kidneys and the skin, the IgG isotype of auto-Ab, and the presence of somatic mutation in the variable genes of these Ab, considerable research has long been focused on disturbances in the T lymphocyte compartment [3]. SLE was often described as a disease of defective T cell censorship, and while the role of B lymphocytes was acknowledged, this was restricted to the production of auto-Ab under instruction from self-reactive T lymphocytes. Extensive recent studies of B lymphocytes biology, however, have drawn a great deal of attention on the basis that, beyond their ability to synthesise and release Ab, these cells possess a variety of characteristics which may contribute to the development of monorgan-specific autoimmune diseases.

1. Characteristics of B lymphocytes

Intriguing new insights into the multiform influences of B lymphocytes on surrounding cells have been unravelled over the past decade. They are involved in shaping lymphoid white pulp architecture, including the formation of follicular dendritic cells (DC). They also coordinate T lymphocytes and influence the pattern of immune responses through the production of cytokines both in T cells and DCs (Fig. 1).

1.1. B lymphocytes influence the formation of follicular DC networks and secondary lymphoid architecture

Several studies have reported the direct role for B cells in the DC development. Indeed, Cerny et al. [4] have reported that DCs are absent in the lymphoid organs of mice deprived of B cells. It has also been demonstrated that purified B cells obtained after exhaustive elimination of T cells are sufficient to induce DCs in SCID mice [5]. Using KO mice, the prominent and influential proinflammatory cytokines, such as tumour necrosis factor (TNF)-α and lymphotoxin (LT)-α and LT-β, have been shown to be involved in establishing and maintaining splenic architecture as well as disruption in LT-α and/or LT-β signaling (Table 1) [6–9]. In this animal models, defects in TNF/LT create disturbances in splenic architecture with absence of B cells within the follicles. The elegant studies by Gonzales et al. [10] are even more relevant to the role of B lymphocytes in that they showed that B cells encourage the appearance of DCs through the expression of membrane LT-α. These investigators observed that, following transfer of B cells from membrane LT-α+/+ but not LT-α−/−, the expression of LT-α and LT-β on B lymphocytes, but not on T lymphocytes, was critical for the formation of DC nettings. So far, a direct interaction
between B cells and DC precursor was essential for the DC function.

1.2. B lymphocytes coordinate T lymphocyte migration and differentiation in secondary lymphoid organs

Pathways of T lymphocyte activation and differentiation into effector T helper (Th)1 and Th2 cells have been the focus of intensive research over the last decade [11]. Thus, it is known that Th1 cells secrete interferon (IFN)-γ and interleukin (IL)-2, while Th2 cells produce IL-4, IL-5, and IL-6. This has brought about evidence that the T lymphocyte differentiation along these two pathways affects appropriate immunity to deal with infectious pathogens and also modulates susceptibility to autoimmune pathology. This multilineage growth factor induces the maturation of precursor T cells as well as the proliferation of B cells. In this respect, the role of different costimulatory molecules CD28-B7 [11] and CD40–CD40 Ligand (L) interactions [12], and more recently, the OX-40/OX-40L interaction have also been extensively investigated [13]. CD28 whose engagement by its ligands CD80 and CD86, constitutively expressed by mature DCs, is an absolute requisite for T-cell-dependent germinal centre (GC) formation, whereas this has a limited role in the T cell subset differentiation [14]. The CD40L, which is up-regulated following the initial stimulation by antigen (Ag) and the CD28–CD80/86 juncture, is suspected to stimulate DCs release of IL-12. This in turn influences the differentiation of effector T cells into Th1 cells [15]. Studies on the expression of chimiokines, such as CXCR5, and the migration of the activated T cells to GCs have also highlighted the importance of the bridge between OX-40 and OX-40L that is instigated in a CD28-dependent manner [16]. It appears that Ag-specific concerted action of B and T lymphocytes, such as the engagement of OX-40 on activated T cells by OX-40L on activated B cells, induces IL-4 synthesis, suppresses IFN-γ production, and results in the differentiation of Th2 lymphocytes and plasma cells under the influence of IL-4 [17].

1.3. B lymphocytes influence the pattern of immune responses through the production of cytokines in T cells and DC

Beyond the paradigm that, at least with respect to T cell-dependent responses, T lymphocytes maintain strict control over B lymphocytes, it is now

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TNF, lymphotoxin (LT), and TNF receptor transgenic mice</strong></td>
</tr>
<tr>
<td><strong>TNFα</strong></td>
</tr>
<tr>
<td><strong>LTα</strong></td>
</tr>
<tr>
<td><strong>LTβ</strong></td>
</tr>
<tr>
<td><strong>TNF-RI</strong></td>
</tr>
</tbody>
</table>
acknowledged that the latter cells have the capacity to solicit their own help from the former [18] and thus to produce a flurry of cytokines [19]. So far, B cells produce IL-10, IL-6, and TNFα, and they also acquire the ability to express IL-2, IFN-γ, IL-4, and IL-12 when stimulated with Ag in the presence of effector Th1 cells. Furthermore, it is even more important that naïve B cells differentiate themselves into polarized B cells with different cytokine profiles, following stimulation with Ag and polarized effector Th1 and Th2. These newly polarized B cells have been termed B effector (Be)1 and Be2, respectively [20], inasmuch as, once induced, they regulate the level of Th1 and Th2 cells. Consequently, Be1 cells, by virtue of their production of IFN-γ and presentation of specific Ag to T lymphocyte, promote the expansion of Th1 cells. In contrast, IL-4 produced by Be2 cells favor the development of Th2 cells. Therefore, Be1 and Be2 cells behave as classical Ag-presenting cells that regulate the profile of the immune response. The fact that Th1/Th2 and Be1/Be2 cells can cross-regulate the differentiation of naïve B and T cells leads to the possibility of a significant amplification of immune responses. Such positive feedback amplification, if out of control, could potentially lead to autoimmunity.

This is particularly true for polyspecific Ig-bearing B cells, i.e., CD5 expressing B lymphocytes. For example, assessment of circulating Be1 and Be2 cells in SLE [21] has indicated elevated IL-10-producing CD5+ B cells. The precise mechanism for generating Be1 and Be2 cells remains, however, to be studied. In addition to the increased awareness of the role of B lymphocytes in affecting effector T lymphocyte differentiation and, by inference, their ability to induce Th1- or Th2-type cytokines, it has been demonstrated that B cells regulate the production of cytokines by DCs, as a result of their ability to produce IL-10. The latter cytokine, produced by B cells among other cells has indeed been shown to inhibit the production of IL-12 by DCs, which in turn induces IFN-γ production by Th1 cells and acts to suppress Th2 development as well as favor Th1-cell expansion. Hence, not only do B cells directly affect the differentiation of Th cells in an immune response, but they can also exert an effect on Th differentiation by DCs through an IL-10-dependent pathway.

2. Overactivity of B cells in systemic diseases

SLE is characterized by auto-Ab to a large assortment of nuclear Ags, including double-stranded DNA and widely expressed intracellular components, such as extractable nuclear Ags. Not long ago, evidence has been provided [22] that intracellular Ags positively select B cells to differentiate into IgM auto-Ab-secreting plasma cells. Consistent with this propensity to yield auto-Ab, B cells from patients with active SLE express activation markers, most notably CD154. Such a phenotype reflects an overwhelming activity of the GC and proceeds from CD40–CD154 interactions. Similar encounters take place within ectopic GCs located in the Hashimoto’s thyroid gland, the Sjögren’s syndrome (SS) salivary glands, and the rheumatoid synovium. There is also significant B lymphopenia in SLE, associated with disorders in the homeostasis of naïve, memory, and plasma B cells. Hence, B cells appear to be essential in the initiation of these disorders such that B-cell-deprived mice of the SLE-prone MRL/lpr strain do not exhibit activated T cells. Environmental factors, but also genetic traits, participate in the etiology of the B cell overactivity because production of IgG is also enhanced in the relatives of SLE patients.

3. Dysfunction of B cells in autoimmunity

The reasons for the selection of autoreactive B cells during development remain a subject of debate. This could be due to a defect in negative selection, resulting in aberrant survival. Altered longevity enables autoreactive B cells to escape the screening process before entry into secondary lymphoid organs (SLO) and within the GCs, due to excessive expression of Bcl-2, which is an antiapoptotic factor, or mutations in the gene of the death-inducing receptor Fas (Table 2).

3.1. Disturbances in peripheral blood B cell homeostasis

A handful of membrane markers have been useful in classifying developmental stages from naïve, Bm1, to memory B cells, Bm5 [23]. Based on the expression of such markers, the distribution of
Peripheral B cell subpopulations has been shown to be profoundly impaired in monorgan-specific autoimmune settings. For example, an excess of GC founder cells has been revealed in the circulation of patients with SLE [24]. In patients with primary SS [25], disturbances in the trafficking of mature B cells from SLO to target visceras is observed. These relate to proliferation of activated naïve GC founder cells and disappearance of memory B cells. Preferential accumulation of memory B cells in the SS-inflamed glands may explain their absence in the circulation. Still, the reasons for these biases are elusive.

The release of autoreactive B cells from deletion may, alternatively, be ascribed to external interferences [26]. One such signal comes from the B-cell-activating factor (BAFF) of the TNF family. This survival factor inhibits negative selection by auto-Ag via the BCR. As a result, transfection with the relevant cDNA induces the synthesis of membrane-bound and cell-free BAFF. Serum levels of this B-cell-specific cytokine have been found to be elevated in SLE, primary SS, and primary antiphospholipid Ab syndrome (PAPLS), and its overexpression claimed to be associated with an SLE-like condition in transgenic (Tg) mice and with primary SS as they age. At least three receptors have hitherto been identified. Two of them are shared by BAFF and a proliferation-inducing ligand (APRIL): the transmembrane activator and cyclophilin ligand interactor (TACI) and the B cell maturation Ag. The third receptor is termed BAFF-R, specific for BAFF and restricted to B cells. Once inserted into this third receptor, BAFF acts to rescue B cells from apoptosis. Given the increasing complexity of this system, it is understandable that loss of APRIL-binding TACI causes autoimmune glomerulonephritis, whereas BAFF costimulates B cells to secrete auto-Ab, depending on its ability to delay B cell apoptosis.

### 3.2. Altered signaling of B lymphocytes

The B cell Ag receptor (BCR) is a multiprotein complex [27]. In the vicinity of this complex, a number of surface molecules, most notably CD19 [28] and CD22 [29], play a role in maintenance of tolerance. On the one hand, CD19 is physically coupled and functionally associated with surface Ig. This coreceptor amplifies the BCR transduction through several mechanisms; among which is the increase of the protein tyrosine kinase activity. B lymphocytes from CD19-defective mice become indeed hyporesponsive to transmembrane signals, whereas those from mice that overexpress this molecule turn out to be hyperresponsive. It is of great interest that modest changes in its expression or function may shift the balance between tolerance and immunity to autoimmunity, as established in systemic sclerosis [30]. On the other hand, CD22 acts to dampen signals generated through the BCR, so that its deficiency correlates with the development of auto-Ab. Such abnormalities could generate dysfunction of the BCR-associated lipid rafts (LR) of B cells in autoimmune states. These membrane microdomains act as platforms for signaling and trafficking from the BCR [31]. Owing to the prolonged residency of the BCR into LRs, CD19 retards its internalization, a function that may contribute to enhance the B lymphocyte response in autoimmunity [32]. Using confocal microscopy, we have observed (d’Arbonneau et al., unpublished data) that the delivery of the BCRs from the LRs was delayed in CD19bright than in CD19dim B cells from autoimmune patients.

### 3.3. CD5+ B cells revisited

It does not appear that CD5+ B cells have the exclusive rights to the production of pathogenic auto-Ab [33]. Rather, increased numbers of CD5+ B cells might reflect defective regulation of B cell function through the CD5 molecule itself. There is

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Genetic events causing autoimmunity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BCR signaling</strong></td>
<td></td>
</tr>
<tr>
<td>CD19</td>
<td>Overexpression</td>
</tr>
<tr>
<td>CD22 Knock out</td>
<td></td>
</tr>
<tr>
<td>Lyn Knock out</td>
<td></td>
</tr>
<tr>
<td>Tyrosin phosphatase SHP-1 Knock out</td>
<td></td>
</tr>
<tr>
<td><strong>Survival factors</strong></td>
<td></td>
</tr>
<tr>
<td>BAFF Overexpression</td>
<td></td>
</tr>
<tr>
<td><strong>Apoptosis</strong></td>
<td></td>
</tr>
<tr>
<td>Fas Knock out</td>
<td></td>
</tr>
<tr>
<td>Bcl-2 Overexpression</td>
<td></td>
</tr>
</tbody>
</table>
now a growing body of evidence that it is essential in modulating signals downstream of the BCR. We have shown that ligation of CD5 or IgM on tonsillar B but not blood T cells resulted in apoptosis. In addition, anti-CD5 Ab maintained the proliferation of tonsillar B cells, provided these had been preactivated with anti-IgM and IL-2. This result was in contrast to cord blood CD5+ B cells which did not apoptose in response to anti-CD5 (Pers and Youinou, unpublished results) but reflected the fact that the cord blood B cells were continuously exposed to auto-Ag in vivo. CD5 has been demonstrated to be associated both physically and functionally with the BCR, and it is important to note that the src-homology two domain-containing protein tyrosine phosphatase-1 (SHP-1) is constitutively linked with the transducing Igα/Igβ chains of the BCR through the immunoregulatory tyrosine-based inhibitory motif of CD5. It has thus been suggested that such an interaction with CD5 sequesters the SHP-1 and limits its role with important molecules in positive signaling through the BCR. Furthermore, the role of CD5 in the maintenance of clonal anergy has recently been addressed by elegant experiments [34] using the hen egg lysozyme (HEL)-Ig Tg mouse. In this model, mice Tg for HEL-Ig and the membrane-bound form of the self Ag HEL mount apoptosis of HEL-specific B cells, while those Tg for HEL-Ig and the soluble form of HEL initiate anergy via the SHP-1. Breeding of the latter Tg mice onto a CD5−/− background results in loss of tolerance. These data indicate that the presence of CD5 raises the threshold required for activation of self-reactive B cells and thereby determines their ultimate fate. Consistent with this novel role for CD5 is another recent model in which CD5−/− spleen cells from mice made Tg for anti-RNP Ab (a common auto-Ab in SLE and other connective tissue diseases) were injected into irradiated naïve mice. They migrated to the peritoneal cavity (the site where most of the CD5+ B cells are found) and began to express CD5 which prevented their production of auto-Ab.

Furthermore, we have recently identified a novel exon 1 for the CD5 gene [35]. This is exclusively transcribed in B lymphocytes. Intriguingly, its existence is due to a defective human endogenous retrovirus. Our data provides attractive evidence for a reciprocal expression of this alternative exon 1, designated exon 1B, with the conventional exon 1, referred to as exon 1A. Exon 1B-type transcripts are translated into a truncated variant of the CD5 molecule devoid of leader peptide. Consequently, whereas exon 1A promotes expression of membrane CD5 protein in T and a subset of B cells, exon 1B tends to reduce CD5 protein expression in other B cells and therefore, possibly, to prevent the signaling functions of CD5, the translocation of SHP-1, and thereby the production of Ab against infectious agents and auto-Ab, e.g., anti-DNA Ab. This balance between the two exons 1 might be important in the regulation of membrane expression of CD5. By raising the threshold of the BCR for the response, CD5 should trigger the expression of recombination activating genes [36] and thereby facilitate the revision of variable genes which has been shown to be instrumental in the prevention of autoimmune states.

To conclude, interest revives B lymphocytes as contributors to the cause of SLE. Although there is still a long way before setting up a unifying model for how such a B cell hyperactivity leads to the disease, we are rather close to understand the way these lymphocytes operate in autoimmunity. Paradoxically, in the light of recent findings of the modulation of B cell signaling by CD5, this and companion molecules play a crucial role in preventing autoimmunity instead of inducing auto-Ab-related diseases. Evidence is therefore accumulating that aberrations of the transduction through CD5 do exist. They could lead to autoimmune disorders. Hence, the present views on the potential functions of CD5+ B cells in autoimmunity are quite different from the previous and rather naïve interpretation that the increased levels of CD5+ B cells represented a direct source of auto-Ab leading to pathogenesis. Finally, B cell tolerance entails administering synthetic mimotope peptides, and it has become likely that B cell ablative treatments of PAPS will be completed in the next few years. Thus, interest revives B lymphocytes as contributors to the cause of autoimmune states. Although there is still a long way to go in setting up a unifying model for how such a hyperactivity leads to SLE, it has become most likely that B cell ablative treatments will be launched in the next few years.
Take-home messages

- B cells play a critical role in regulating T cells and dendritic cells (DC).
- Systemic lupus erythematosus (SLE) is associated with B cell hyperactivity and with abnormal expression of B-cell-activating factor (BAFF), interleukin (IL)-10 and IL-6.
- Lupus like syndromes are observed in mice rendered deficient for genes controlling the B cell receptor (BCR) signalling pathway.
- BCR-associated molecules in patients with SLE are expressed at abnormal levels and contribute to the disruption of self-tolerance.
- CD5 B cell subset appears to play a key role in monorgan-specific autoimmune diseases.

References

[36] Hillion S, Youinou P, Jasmin C. Expression of recombinating activating genes in peripheral B cells outside germinal centers is regulated by CD5. Submitted for publication.

The World of Autoimmunity: Literature Synopsis

**Autoantibody-mediated neuronal cell signaling in Sydenham chorea**
Rheumatic fever is an example for the association of autoimmunity with infectious agents due to molecular mimicry between host and pathogen. The pathogenesis of Sydenham chorea, the major neurological manifestation of acute rheumatic fever, is not understood; Kirvan et al. (Nat Med 2003;9:914) report on monoclonal antibodies with specificity for mammalian lysoganglioside and n-acetyl-beta-D-glucosamine, the dominant epitope of the group A streptococcal carbohydrate in Sydenham chorea. These antibodies targeted the surface of human neuronal cells, with specific induction of calcium/calmodulin-dependent protein kinase II activity, by both monoclonal antibody and sera from active chorea. Moreover, sera from other streptococcal disease and convalescent sera did not activate the kinase. These results support an autoantibody-mediated neuronal cell signaling in the pathogenesis of Sydenham chorea and shed more light on the pathogenesis of rheumatic fever.

**SH2D1A regulates T cell-dependent humoral autoimmunity**
Hron JD et al. (J Exp Med 2004;200:261) report that deficiency in SH2D1A protects mice from an experimental model of lupus, including the development of hypergammaglobulinemia, autoantibodies including anti-double stranded DNA, and renal disease. The signaling lymphocytic activation molecule (SLAM)/CD150 family includes a family of chromosome 1-encoded cell surface molecules with costimulatory functions mediated in part by the adaptor protein SH2D1A (SLAM-associated protein, SAP). Nonetheless, this protection from experimental lupus did not reflect grossly defective T or B cell function per se; however, T-dependent antibody responses were impaired in SH2D1A-deficient mice, reflecting defective germinal center formation. Thus the SLAM-SH2D1A system has an important role in the regulation of T-dependent humoral immune responses. The authors speculate that members of the CD150-SH2D1A family can be targets for therapy of antibody-mediated autoimmune and allergic diseases.