**Abstract**

B cells develop in bone marrow and undergo antigen-induced activation and terminal differentiation in germinal centres of secondary lymphoid organs. Each B cell is a clone, which means that an individual B cell has a unique genetic code and produces only one type of antibody when stimulated by antigen, being able to multiply itself and originate several B cells with the same antigen specificity (clonal selection theory). However, their important role in adaptive immune responses is supported by the remarkable capacity of recognizing an unlimited array of antigens, due to mechanisms of antibody diversity, such as V(D)J recombination, class switching and somatic hypermutation. B cells can also function as antigen presenting cells that can activate T cells, improving the effectiveness of the immune response. Immune B cell tolerance surveillance through clonal deletion, anergy and receptor editing is also necessary to avoid pathological conditions, like autoimmune diseases. B cells can contribute to autoimmunity by autoantibody production, cytokine synthesis, antigen presentation, T cell activation and ectopic lymphogenesis.

**Key-Words:** B Cells; Bone Marrow; Antibody; Tolerance; Autoimmunity.

**Introduction**

The human body contains approximately $2 \times 10^{12}$ lymphocytes, of which 5-15% are B cells. B lymphocytes are small white blood cells (6-10 µm) with a dense nucleus and little cytoplasm that originate and mature in the bone marrow expressing a membrane-bound immunoglobulin. Discovered in the early 1960’s, these cells are important mediators of our immune system, more precisely the humoral response, by the production and secretion of proteins collectively called immunoglobulins (Ig) or antibodies. The main function of an antibody is to recognize a foreign antigen exhibited by an invading pathogen or at the surface of an altered cell, like a tumour cell, and facilitate the clearance and elimination of that antigen. Antibodies can eliminate pathogens by several mechanisms including...
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Opsonization, complement activation and direct lysis of bacteria and neutralization of viruses and toxins to prevent their entry into host cells. B cells can also function as antigen presenting cells (APCs) and interleukin (IL) producing cells that can further activate T cells and contribute to the development of an effective immune response.

Origin and B cell development

Bone marrow
B cells develop in the bone marrow in mammals, but in birds, where they first were studied, these cells develop and mature in a special organ called «Bursa of Fabricius» (hence, the designation «B»), situated in the cloaca of the animals.1,2 In humans, B cells are primarily produced in the fetal liver before birth and in the bone marrow afterwards. B cell development can be detected in human bone marrow from 20 weeks of gestation and continues throughout life.3 B cells arise from a lymphoid stem cell in the bone marrow and proceed through several maturation stages, during which they express different cell surface markers (Figure 1).4-7 The earliest distinctive B-lineage cell is the progenitor B cell (pro-B cell).8-11 Pro-B cells proliferate in the bone marrow, filling the extravascular spaces between large sinusoids in the shaft of a bone, and further differentiate into precursor B cells (pre-B cells).12,13 Human pre-B cells can be generally subdivided into large proliferating cells (designated pre-BI) and small postmitotic cells (designated pre-BII) on the basis of cell-cycle analysis.14-16 The bone marrow phase of B cell development culminates in the production of an immature B cell17 that is not fully functional. All B cell differentiation stages in the bone marrow do not require antigen, this corresponding to the antigen-independent phase of B cell development.

Periphery

Organization and structure of secondary lymphoid organs

LYMPH NODES
Lymph nodes (LNs) are specialized organs for trapping antigen from local tissues supplied by lymphatic vessels. LNs can be divided in three regions: the cortex, paracortex and medulla.18,19 During lymph node development, hematopoietic lymphoid progenitor cells interact with high endothelial venules (HEV) and mesenchymal cells. This interaction is mediated by adhesion molecules and leads to local release of chemoattractants, like CXCL13, that attract B cells into the nascent organ through HEV.20,22 These naïve B cells are then organized into follicles, which are spheroid aggregates located in the cortex of lymph nodes, surrounded by an outer T cell zone.23 In the presence of T cell help, B cells can proliferate and differentiate into GC B cells within follicles or short-lived plasma cells, generated outside follicles.

SPLEEN
The spleen is a large organ specialized in the destruction of old erythrocytes and trapping antigens in the circulating blood.24 The spleen is divided into two zones: the red pulp, mainly populated by macrophages and red blood cells, where old and defective erythrocytes are removed from the circulation; and the white pulp, that comprises B cell follicles, T cell zones (also designated periarteriolar lymphoid sheaths, or PALS) and a marginal zone, in the interface of the white and red pulps, that contains macrophages and B cells.19,25 In humans, the spleen has an additional region, the perifollicular zone, situated outside the marginal zone, that contains capillaries and blood-filled spaces that belong to the open splenic circulation.26 Lymphocytes are believed to enter the white pulp through...
the perifollicular zone. Circulating naïve B cells migrate to the spleen, where they reside until they are activated by antigens and start a GC reaction.

**Peyer’s Patches**

Peyer’s Patches (PPs) are organized lymphoid follicles similar to lymph nodes located in the mucosa and submucosa of the small intestine, especially the ileum. Since the lumen of the gastrointestinal tract constitutes a way of entry of potentially pathogenic microorganisms, PPs are fundamental for the establishment of the immune surveillance in the gut. Human PPs can be even centimetres big and have a large number of follicles.27 PPs contain specialized cells called M («microfold») cells, located in the intestinal epithelium over lymphoid follicles.28 The M cell basolateral membrane is deeply invaginated to form a large intraepithelial «pocket» containing B and T lymphocytes29 and occasional dendritic cells. M cells can internalize antigens from the lumen and transfer them efficiently to the underlying APCs that can further activate T cells. Activated T cells produce cytokines, such as TGF-β1, that induce B cells to differentiate into immunoglobulin A (IgA)-secreting plasma cells.30,31 IgA is the major antibody present in the intestinal secretions 32,33 and it constitutes the first line of defence against pathogenic microorganisms in mucosal surfaces by interfering with adhesion and invasion properties of microbes. Signals through chemokine receptors expressed by B cells, namely CXCR4, CXCR5 and CXCR7, contribute to B cell homing towards PPs,30 Moreover, CXCL13, expressed by HEVs in PPs, is required for B cell entry into these structures.21,22

**The Germinal Centre (GC) reaction**

When full maturation is achieved, signalled by the co-expression of IgM and IgD on the membrane, B cells leave the bone marrow. These naïve B cells, which have never encountered an antigen, circulate in the blood and lymphatic systems where they are carried to secondary lymphoid organs, where they reside. In the follicles of the secondary lymphoid organ, after encountering an antigen, mature B cells transform into large B-blasts that may follow two different pathways. Some proliferate and differentiate into short-living, IgM producing plasma cells, responsible for the early production of antibodies and, thus, first-line defence against the antigen. A minority of the B-blasts differentiate to form the follicle centre or germinal centre (GC).34,35 The non-antigen-triggered naïve B cells form the follicle mantle or mantle zone. The follicle, containing a GC and a mantle zone, is known as a secondary follicle. The inner part of the mantle zone of the B cell follicle is designated the lymphocytic corona and is mainly composed of mature, naïve B cells.36 The outer part of the mantle zone, called the marginal zone, is populated by different types of cells: macrophages, some T cells, granulocytes, plasma cells and marginal zone B cells.37 GC are the specialized sites for memory B cell generation, plasma cells differentiation and where affinity maturation of serum antibodies takes place.38,39 GC first appear at day 4-5 post-immunization, achieve their maximum cell number by day 10-11 and decline after about three weeks.34 B cells that form the GC are divided into centroblasts (large noncleaved cells) and centrocytes (small or large cleaved cells). Centroblasts are large, proliferating cells that lack surface immunoglobulin expression and accumulate at one pole of the GC, forming the dark zone (Figure 2). These cells can differentiate themselves into non-proliferating centrocytes that re-express surface immunoglobulin and accumulate at the opposite pole of the GC, known as the light zone.40 Activation of GC B cells is made by follicular dendritic cells (FDC), which have a pivotal role in promoting B cell proliferation and differentiation in GC.41,42 FDC not only are very efficient in trapping and retaining antigen-antibody complexes for long periods of time and present them to B cells, but also are involved

![Figure 2. V(D)J recombination process in light and heavy chains. During VDJ recombination, the gene segment rearrangements that occur in both light and heavy chains are possible due to specific enzymes, such as RAG proteins, that initiate the process. Further diversity is increased by nucleotide additions by Terminal deoxynucleotidyl transferase (TdT).](image-url)
in B cell recruitment to secondary lymphoid organs with consequent follicle formation and organization through the secretion of CXCL13, a B-lymphocyte chemoattractant. Positive selection of B cells in GC occurs through high-affinity binding to antigens trapped by FDC. These high-affinity B cells receive survival signals from both FDC and T cells located in the light zone, whereas low-affinity B cells non-triggered by antigens die by apoptosis. More than 90% of the GC B cells die as a result of apoptosis and are then phagocytosed and digested by the so-called tingible body macrophages. Only the GC B cells that are stimulated and able to synthesize high-affinity antibodies survive and may differentiate into long-lived plasma cells or memory B cells. Long-lived plasma cells are thought to reside mainly in the bone marrow and organs that are directly exposed to foreign antigens (gastrointestinal tract, lungs...), whereas memory cells reside in the follicle mantle or recirculate freely to survey for secondary antigen exposure. Memory B cells are long-lived and are responsible for the ability of the immune system to learn. During a second encounter with an antigen, memory B cells are able to recognize the antigen to which the organism was previously exposed and induce a more rapid and effective immune response. Since B cell activation and differentiation in the periphery require antigen, this stage comprises the antigen-dependent phase of B cell development. In the absence of antigen-induced activation, naïve B cells in the periphery have a short life span, dying within a few weeks by apoptosis.

B-1 and B-2 cell lineages
B cells comprise two main subsets, B-1 and B-2, a concept based on the discovery that a small proportion of B cells in mice expressed the T-cell marker CD5. B-1 cells are the CD5+ subpopulation, whereas B-2 cells represent the conventional B cell subpopulation. B-1 cells arise before B-2 cells, are long-lived, emerge early in development, during fetal life, are self-renewing and predominate in the follicular mantle zone, being minor populations in secondary tissues such as lymph nodes and spleen and are virtually absent in human adult bone marrow. B-1 cells can be subdivided in B-1a cells, which express CD5, and B-1b cells, that are CD5 negative. B-1a cells are largely responsible for the production of circulating immunoglobulin M (IgM) antibodies known as «natural antibodies». These low affinity antibodies are the first line of defence against bacterial and viral infections. The antibodies produced by B-1b cells are induced after antigenic exposure and are required for long-lasting protective immunity to pathogens. B-2 cells constitute the major group of B cells in humans and mice. They are CD5, surface and mRNA, negative cells. B-2 cells derive from resident bone marrow progenitors throughout life and undergo maturation primarily in the spleen. Although the developmental origin of B-1 cells has remained an unresolved issue, it was demonstrated that bone marrow CD45R0-CD19+ cells include a B-1 cell-specified progenitor, which supports the idea of the existence of distinct developmental pathways for B-1 cells. CD5 expression was identified in malignant human B cells and later shown to mark a minority of B lymphocytes in the normal blood. In fact, in humans, 5-30% of the circulating B cells are B-1a in adults; 4-6% are B-1b and 65-89% are B-2 cells. Nevertheless, a separate lineage for CD5+ B cells still awaits a demonstration in humans. Some lines of evidence suggest that in the human case CD5 might be an activation marker rather than a cell lineage feature, since there is a variety of stimuli capable of inducing the expression of CD5 on conventional B cells. It remains to be determined, nonetheless, the criteria that will define whether human B cell subsets are homologous to the mouse B-1a, B-1b and B-2 lineages.

Antibody production
Structure of an antibody
Antibodies, also called immunoglobulins, are the antigen-binding proteins present on the B cell membrane and secreted by plasma cells. An antibody molecule has a common structure of four peptide chains that consist of two identical light (L) chains and two identical heavy (H) chains, which are linked by disulfide bonds. The first 100-110 amino acids of the amino-terminal region of a light or heavy chain vary greatly among antibodies. These segments of highly variable sequence are called variable (V) regions: \( V_L \) in light chains and \( V_H \) in heavy chains. The carboxyl-terminal region of both light and heavy chains consists of relatively constant sequences, designated as constant (C) regions: \( C_L \) on the light chain and \( C_H \) on the heavy chain. Most of the differences among antibodies fall within areas of the V regions called Complementary-Determining Regions (CDRs) and these CDRs, on
both light and heavy chains, constitute the antigen binding site of the antibody molecule. There are two light chain types, kappa (κ) and lambda (λ). In humans, 60% of the light chains are kappa and 40% are lambda. A single antibody molecule contains only one light chain type, either κ or λ, never both. The heavy chain of a given antibody molecule determines the class of that antibody, or isotype. There are five different heavy-chain constant region sequences: μ, δ, γ, ε and α; hence, in humans, the five major classes of antibodies: IgM (μ), IgG (γ), IgA (α), IgD (δ) and IgE (ε), each with different structural and functional properties (Table I).

**Mechanisms of antibody diversity**

One of the most remarkable features of our immune system is its ability to respond to an apparently limitless array of foreign antigens. The major mechanisms that are responsible for the enormous diversity of antibodies (>10¹⁰ possibilities) produced by our immune system are random rearrangements in genes that codify the variable region (antigen binding domain) of immunoglobulins. Antibody diversity is achieved by V(D)J recombination, somatic hypermutation and class switching.

**V(D)J recombination**

Immunoglobulin light and heavy chains are encoded by three separate multigene families, situated on different chromosomes. In germ-line DNA, each of these multigene families contains several coding sequences, called gene segments, separated by noncoding regions. During B cell maturation, these gene segments are rearranged and brought together to form functional immunoglobulin genes. The κ and λ light-chain families contain V (for variable), J (for joining) and C (for constant) gene segments and the rearranged VJ segments encode the variable region of the light chains. The heavy-chain family contains V, D (for diversity), J and C gene segments and the rearranged VDJ gene segments encode the variable region of the heavy chain. In each gene family, C gene segments encode the constant region. V(D)J recombination is an ordered site-specific DNA recombination process that occurs in developing lymphocytes in the bone marrow initiated by two lymphocyte-restricted specific proteins RAG-1 and RAG-2, which together form an endonuclease responsible for DNA double-stranded breaks at recombination signal sequences (RSS). RAG proteins are expressed at high levels during ontogeny, but their expression diminishes in immature B cells and is usually absent in recirculating mature naïve B cells. In certain pathologies, however, like autoimmune diseases, RAG expression may be

<table>
<thead>
<tr>
<th>Class of Antibody</th>
<th>Serum levels</th>
<th>Structure</th>
<th>Biological functions</th>
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<tbody>
<tr>
<td>IgM</td>
<td>5%</td>
<td>Monomer Pentamer</td>
<td>Membrane-bound immunoglobulin on the surface of immature and mature B cells, First antibody produced in a primary response to an antigen, Efficient in binding antigens with many repeating epitopes, such as viruses.</td>
</tr>
<tr>
<td>IgD</td>
<td>0.3%</td>
<td>Monomer</td>
<td>Membrane-bound immunoglobulin on the surface of mature B cells, No biological effector function known</td>
</tr>
<tr>
<td>IgA</td>
<td>7-15%</td>
<td>Monomer Dimer</td>
<td>Predominant antibody class in secretions (saliva, tears, breast milk) and mucosa, First line of defence against infection by microorganisms</td>
</tr>
<tr>
<td>IgG</td>
<td>85%</td>
<td>Monomer</td>
<td>Most abundant class with four isotypes - IgG1, IgG2, IgG3, IgG4, Crosses the placenta, Opsonization</td>
</tr>
<tr>
<td>IgE</td>
<td>0.02%</td>
<td>Monomer</td>
<td>Defence against parasite infections, Associated with hypersensitivity reactions (allergies), Found mainly in tissues</td>
</tr>
</tbody>
</table>
The importance of RAG for V(D)J recombination is supported with the finding that mutations in RAG genes abolish recombination activity and block differentiation of both B and T cells, leading to different forms of immune deficiency. The RSS, recognized by RAG in the initial event of the recombination process, are comprised of a highly conserved heptamer and nonamer sequences, separated by a relatively nonconserved spacer region of either 12 or 23 base pairs (bp). A gene segment with 12-bp spacer can only join with a segment flanked by 23-bp spacer (12-23 bp rule). During variable-region gene rearrangements the heavy-chain genes rearrange first, followed by light-chain rearrangements. The generation of a functional immunoglobulin heavy-chain gene requires two separate rearrangement events within the variable region. The first step of recombination is a D to J association. The resulting D-J segment then moves next to and joins a V segment to generate a VDJ unit that encodes the entire variable region. Light-chain DNA rearrangements occur by the joining of a functional V segment to a functional J-C segment (Figure 3). The combinatorial diversity generated by V(D)J recombination is further augmented by another enzyme, terminal deoxynucleotidyl transferase (TdT), that randomly adds up to 20 nucleotides, called N additions, to D-J and V-DJ junctions.

Somatic Hypermutation

Somatic Hypermutation (SHM) is a major mechanism for producing antibody diversity and increasing antibody affinity, which occurs in GC of secondary lymphoid organs. SHM is a process that introduces point mutations, mainly nucleotide substitutions, but also occasional deletions and duplications, at a very high rate into the DNA of heavy and light-chain variable-region genes, which may alter the specificity of the encoded antibodies. SHM introduces mutations at a rate of ~10⁻³ mutations per base pair per cell division, which is 10⁶-fold higher than the spontaneous mutation rate in somatic cells. As a result, following exposure to antigen, these B cells with higher-affinity receptors will be preferentially selected for survival. This differential selection is due to an increase in antibody affinity for an antigen, a process called affinity maturation. Thus, only the B cells with the best fitting receptors are selected for rapid proliferation and further maturation. SHM appears early in the phylogeny of vertebrate immune system and operates in all studied vertebrates. Although the mechanism of SHM remains unknown, excitement in this field was triggered by the discovery of a requirement for activation-induced cytidine deaminase (AID), which has homology with the RNA-editing cytidine deaminase APOBEC-1.

Class Switching

Class or isotype switching is a deletional DNA recombination process that occurs in mature B cells and consists in the replacement of an expressed heavy-chain constant-region gene (usually Cμ for IgM) with another one with a different biological function, allowing expression of IgG, IgA or IgE. The deleted sequence is excised in a form of circular DNA. In this way, the antigen binding specificity of the antibody remains unaltered, but its effector functions are changed. This process is mediated by a recombination event that deletes the DNA between repeated sequences (switch regions) located upstream of the constant-region genes involved. AID catalyzes the hydrolytic deamination of cytidine to uridine, either as a free nucleotide or in the context of RNA and participates in CSR. In fact, AID-deficient mice, generated by gene-targeted mutation, lack completely the ability to undergo CSR and only form antibodies belonging to IgM isotype. Moreover, in a group of immunodeficient patients with hyper-IgM syndrome, who had impaired CSR, the human gene encoding AID was mutated, so their B cells only produced IgM molecules. AID is expressed specifically in GC-centroblasts, but its exact role is unknown.
Importantly, it was demonstrated that AID deficiency also affects SHM in mice and humans, which supports the idea that AID is involved in both mechanisms, CSR and SHM.101

**B cell activation**

**B cell receptor (BCR) and B cell co-receptor**

Signals propagated through the BCR are vital for the development and survival of B lymphocytes in both the bone marrow and the periphery. The BCR of mature B cells is a multiprotein structure containing an antigen-binding membrane immunoglobulin, noncovalently associated with signal transducing elements Ig-α (CD79a) and Ig-β (CD79b), that form an heterodimer, localized mostly or wholly within the cell.102-103 The cytoplasmic tails of both Ig-α and Ig-β contain an 18-residue motif termed the immunoreceptor tyrosine-based activation motif (ITAM).104 BCR signalling is initiated upon binding of antigen to membrane immunoglobulin, which induces a subsequent phosphorylation of ITAMs of Ig-α and Ig-β that, in turn, transduce the stimulus into an effective intracellular signal.104-106 The activating signals transmitted through the BCR during B cell stimulation can also be amplified by a B cell co-receptor. The B cell co-receptor is a complex of three proteins: CD19, CR2 (CD21) and CD81.107-109 CD19 is a member of the immunoglobulin superfamily; CR2 is a receptor for C3d, a breakdown product of the complement system, which is an important effector mechanism for destroying invaders; and CD81 is another transmembrane protein, also designated as a target for anti-proliferative antigen-1 (TAPA-1). Following antigen cross linking of the BCR and activation of B cell co-receptors, a cascade of phosphorylations is initiated by protein tyrosine kinases (PTKs)102,110-113 that culminate in the activation of transcription factors114 and the release of intracellular Ca2+.113,115 that leads to B cell activation. In addition to the stimulatory co-receptor, there are also inhibitory receptors expressed on B cells. Each inhibitory receptor contains one or more immunoreceptor tyrosine-based inhibitory motifs (ITIMs) within its cytoplasmic domain, essential for generation and transduction of inhibitory signals. The surface molecules CD22 and FcγRIIb are examples of inhibitory B cell surface receptors. CD22 is a member of the immunoglobulin gene superfamily that is first expressed in the cytoplasm of pro-B and pre-B cells and, later, is expressed on the surface of mature B cells. CD22 functions as a B cell inhibitor,116,117 although there are studies that indicate that it has a dual role in B cell activation.118 FcγRIIb is also a member of the immunoglobulin superfamily and it is a low-affinity receptor for the Fc portion of IgG.119 FcγRIIb is exclusively expressed on B cells and has the potential to terminate B cell signal transduction. Coligation of FcγRIIb to the BCR leads to tyrosine phosphorylation of the ITIM by the tyrosine kinase Lyn, recruitment of phosphatase SHIP and inhibition of Ca2+ influx and proliferation.120

**B cell – T cell interactions**

For an efficient activation of B cells by soluble protein antigens, an involvement of T cells is required. Binding of an antigen to the BCR does not, by itself, induce an effective competent signal without additional interactions between numerous receptor-ligand pairs on the two cell types. Antigen capture by membrane immunoglobulin on B cells initiates signalling through the BCR (signal 1) that leads to an up-regulation of the expression of class II Major Histocompatibility Complex (MHC) molecules121-125 and costimulatory B7 molecules.123-125 Antigen is internalized, processed and degraded to peptides that are then presented to the cell surface as MHC-peptide complexes.126-127 T cells can recognize these MHC-peptide complexes on B cell membrane. Both T and B cells interact to form a T-B conjugate that ultimately leads to T cell-dependent B cell activation.23,125-128 This interaction stimulates the expression of CD40-L (CD154) on the T cells, which is the ligand for CD40, expressed on B cells.129,130 CD40, expressed on B-lineage cells (pro-B through plasma cells), belongs to the tumour necrosis factor (TNF) family of cell-surface proteins and soluble cytokines that regulate cell proliferation. CD40L, expressed on T cells, belongs to the TNF receptor (TNFR) family. Interaction of CD40-L with CD40 on the B cell delivers a signal (signal 2)131-133 to the B cell that, in concert with the signal generated by the BCR crosslinkage, induces the expression of costimulatory molecules of the B7 family.134,135 The B7 costimulatory family has two ligands, B7-1 (CD80) and B7-2 (CD86) that can activate or inhibit a T-cell response, depending on the membrane molecule on T cell with which they interact, CD28 or CTLA-4.136,137 CD28 is expressed by both resting and activated T cells and is the predominant receptor for B7 on resting T cells.
CTLA-4 is expressed only on activated T cells. Upon binding of a B7-costimulatory molecule with CD28, a positive costimulatory signal is generated and the T cell is activated, while signalling through CTLA-4 inhibits the response of the T cell. So, for an efficient T-cell-dependent B cell activation a B7-CD28 interaction has to occur. The Inducible Costimulator Molecule (ICOS), a member of the CD28 family of costimulatory molecules expressed by T cells, is also a key regulator of humoral immunity and B cell homeostasis and functions. ICOS binds to a B7-like molecule, B7RP-1, expressed constitutively on B cells. A major role for the ICOS/B7RP-1 pathway in the T cell-dependent B cell responses is supported by studies with both ICOS-/- and B7RP-1-/- mice that have decreased levels of serum antibodies and defective GC formation. Once activated, B cells begin to express membrane receptors for various cytokines produced by the interacting T cell. The signals produced by these cytokine-receptor interactions induce a number of intracellular signalling pathways that ultimately result in changes in gene expression that support B cell proliferation and can induce differentiation into plasma and memory cells, class switching and affinity maturation. Also, it has been demonstrated that activated B cells by sequential BCR and CD40 stimulation proliferate and secrete TNF-α, lymphotoxin and IL-6, cytokines that can act not only as autocrine growth and differentiation factors, but also serve to amplify the ongoing immune response.

B cells and Toll-like receptors

Toll-like receptors (TLRs) are pattern recognition receptors that sense molecular patterns specific of invading pathogens. Eleven TLRs have been identified so far, although in humans TLR11 is thought to be non-functional. Recent evidence suggest that the generation of T-dependent B cell responses requires the activation of TLRs in B cells. The regulated expression of selected TLRs in B cells may play an important role in linking innate to adaptive immune responses. Human B lymphocytes express a distinct TLR expression profile in which TLR9 and TLR10 predominate. In naïve B cells most TLRs (TLR1-2 and TLR6-10) are expressed at low to undetectable levels, but the expression of TLR9 and TLR10 is rapidly induced following BCR triggering. In contrast, memory B cells express several TLRs at constitutively high levels. Unmethylated CG-dinucleotides with certain sequence contexts (CpG DNA), which function as TLR9 ligand, are recognized by our immune system as foreign DNA (bacterial or viral) and it has been demonstrated that CpG DNA motifs can trigger proliferation and activation of primary human B cells. In fact, CpG DNA increases B cell expression of costimulatory molecules (CD80, CD86) and enhances the expression of both class I and class II MHC proteins. Furthermore, memory B cells proliferate and differentiate to immunoglobulin secreting cells in response to CpG DNA, while naïve B cells only do so if simultaneously triggered through the BCR. So, in human B cells TLRs play distinct functions in primary responses and immunologic memory: on the one hand, the BCR induced expression of TLRs in naïve B cells prevents polyclonal activation in a primary response, because it restricts stimulation to antigen-specific B cells and, on the other hand, the constitutive expression of TLRs in memory B cells allows polyclonal activation of the entire memory pool, thus sustaining serologic memory. Importantly, B cell activation by TLRs occurs when the signal is delivered to B cells by a variety of microbial products (TLR agonists) acting directly on the TLRs expressed on B cells or indirectly through activation of dendritic cells that release cytokines, namely IL-6 and IL-12, that sustain B cell activation. Besides the effect on B cell proliferation and plasma cell differentiation, it has been suggested that TLR stimulation is also required for induction of CSR, a fact supported by the up-regulation in the mRNA levels of AID on B cells stimulated by BCR, T cell help and CpG simultaneously.

B cells and antigens

T cell independent (TI) and T cell dependent (TD) antigens

After generation and migration of B cells from the bone marrow, activation, proliferation and differentiation occur in the periphery in a process that requires antigen. An antigen can be classified as T cell independent (TI) or T cell dependent (TD). TI antigens do not require T cell help to induce an immune response and do not produce immunologic memory. These antigens are generally polysaccharides and, as a result, cannot be presented to T cells via MHC molecules. They can be divided into two classes, TI-1 and TI-2. TI-1 antigens, such as bacterial lipopolysaccharide
(LPS),\textsuperscript{158} are potent B cell mitogens that probably act through the activation of the Toll-like receptors,\textsuperscript{159} which leads to a non-specific polyclonal activation of B cells, that is, they are able to activate B cells regardless of their antigenic specificity.\textsuperscript{160} In contrast, TI-2 antigens consist of large molecular weight, repetitive structures, such as capsular polysaccharides from bacteria and, unlike TI-1 antigens, they do not function as B cell mitogens. These antigens are resistant to degradation \textit{in vivo} and are poorly internalized by B cells. TD antigens consist of proteins or peptides that require immune stimulation from helper T cells to elicit an immune response. Such antigens are presented to T cells in the context of MHC molecules expressed on B cells and other antigen presenting cells, following pathogen exposure.\textsuperscript{23} The subsequent activation of T cells induces cytokine production that can further stimulate B cell responses. Importantly, TD antigens are more effective at inducing a lasting immune response, since they lead to the production of high affinity antibodies of multiple isotypes and memory B cells.

**B cells as antigen presenting cells (APCs)**

Binding of antigen to BCR leads to antigen internalization and presentation to T cells, a critical process in the initiation of the humoral immune response.\textsuperscript{161,162} B cells can internalize and present antigen that has been encountered in soluble form, as particles or when tethered to a non-internalizable surface.\textsuperscript{163} The dependence of the efficiency of presentation differs for these three forms of antigen and it relies on antigen-BCR affinity.\textsuperscript{164} After encountering antigen, an APC can internalize it by three means: phagocytosis, fluid-phase pinocytosis and receptor-mediated endocytosis. B cells have the ability to present antigen efficiently, since they can find T cells in secondary lymphoid organs shortly after antigen entrance and BCR-mediated endocytosis allows them to concentrate small amounts of specific antigen.\textsuperscript{165-167} In fact, BCR affinity is directly proportional to the capacity of B cells to present antigen to CD4\textsuperscript{+} T cells. It has been demonstrated that cells with a BCR of low affinity required ten times more antigen to induce CD4\textsuperscript{+} T cell proliferation than cells with a BCR of very high affinity, while presentation after uptake by fluid phase pinocytosis needed concentrations about 5000 times higher.\textsuperscript{168} So, the high affinity of the BCR for its specific antigen allows its efficient internalization even in the presence of small amounts of antigen.\textsuperscript{166} Also, BCR signalling up-regulates MHC-II expression and, consequently, the generation of peptide-MHC II complexes,\textsuperscript{127,168} which enhances the efficiency of antigen presentation. Given the complexity of immune responses and the diversity of antigens relevant to human disease, B cells should participate as APC in many situations.

**Immune B cell tolerance**

Upon encountering an antigen, the immune system can either develop an immune response or enter a state of unresponsiveness called tolerance. The development of immunity or tolerance must be carefully regulated since an inappropriate response, whether it is immunity to self-antigens (autoimmunity) or tolerance to a potential pathogen, can have serious and possibly life-threatening consequences. Every time an antigen is introduced in the human body, important regulatory decisions determine the branch of the immune system (humoral or cell-mediated immunity) to be activated, the intensity of the response and its duration. The mechanisms that play a role in the induction of B cell tolerance are anergy, clonal deletion and receptor-editing.

**Clonal Deletion and Anergy**

During B cell development in the bone marrow, 50-75% of B cells produced are autoreactive and must be silenced.\textsuperscript{169} Tolerance is achieved by the clonal deletion (apoptosis) of self-reactive lymphocytes expressing receptors with high avidity for self.\textsuperscript{170,171} Another mechanism that is responsible for silencing a significant proportion of autoreactive B cells that arise in the bone marrow or that circulate in the periphery is anergy.\textsuperscript{172-174} A cell is said to be anergic if it gives no activation response when exposed to an antigen that binds to its receptor. The decision of which silencing mechanisms are used depends upon receptor affinity and autoantigen avidity. Higher avidity favors clonal deletion and, hence, cell death. Low avidity interactions invoke anergy.

**Receptor Editing**

In the bone marrow, newly formed B cells expressing autoreactive receptors can either be deleted by apoptosis, or escape cell death by modifying their antigen receptors by receptor editing.\textsuperscript{175} Receptor editing is a process that alters antigen receptors by
allowing secondary V(D)J rearrangements (usually on light chains), that change the receptor specificity.\textsuperscript{176,177} So, signalling through an autoreactive antigen receptor promotes further receptor-gene rearrangements, which can destroy the receptor gene that confers autoreactivity, thereby eliminating the autoreactivity of the cell without eliminating the cell itself.\textsuperscript{178,179} This process is the dominant tolerance mechanism for developing B cells and it occurs at the immature B cell stage, in the bone marrow.\textsuperscript{177} If a B cell with a forbidden receptor fails to edit to a less self-reactive receptor, cell death occurs within 1-2 days, either in the bone marrow or shortly after arriving in the spleen.\textsuperscript{180} Importantly, about 25\% of the light chains found on the surface of developing B cells in vivo are produced by receptor editing,\textsuperscript{181} a fact that can emphasize the important contribution of this process to the normal antibody repertoire.

B cells and Autoimmunity

Autoimmune diseases result from disorders in the immune system that erroneously recognizes self antigens. Autoreactive B cells can arise in the bone marrow or in the periphery and, if not properly inhibited or eliminated, autoimmune diseases may develop. In fact, the pathogenic roles of B cells in autoimmunity can be due to several mechanisms that include autoantibody production and immune complex formation, cytokine synthesis, chemokine-mediated functions, antigen presentation, T cell activation and ectopic lymphogenesis. These effects are part of the process of antibody formation and their exact roles in human autoimmunity are in fact unclear. Nevertheless, there is a strong correlation between autoimmune diseases and autoantibody production: in Rheumatoid Arthritis (RA), rheumatoid factors (RF) against the Fc portion of Ig are produced and form immune complexes that are deposited in the joints;\textsuperscript{182,183} Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease with autoantibodies directed against a vast array of tissue antigens;\textsuperscript{184,185} Myasthenia gravis is an autoimmune disease that ultimately leads to a progressive weakening of the skeletal muscles due to autoantibodies that bind the acetylcholine receptors;\textsuperscript{186} or Idiopathic Thrombocytopenia Purpura (ITP), that results from the production of autoantibodies against platelets.\textsuperscript{187,188} Evidence indicating an important role for B cells in directing T cell function in autoimmunity is provided by experiments involving CD40/CD40L blockade. In fact, it has been demonstrated that the use of anti-CD40L monoclonal antibodies is beneficial in several mouse models of SLE, inflammatory arthritis and colitis.\textsuperscript{189,190} Importantly, it is necessary to realize that a careful extrapolation to the human case has always to be considered, since the aetiology of experimental autoreactivity in animal models is not likely to be the same which applies in spontaneous autoimmunity in humans. B cells also secrete cytokines and chemokines, including IL-16, MIP1\(\alpha\) and MIP1\(\beta\), that can modulate DC migration and function.\textsuperscript{191,192} Lymphoid-like follicles have also been described in several autoimmune diseases, like the synovium in RA patients,\textsuperscript{193,194} inflamed salivary glands in Sjögren’s Syndrome,\textsuperscript{43,195} the ventricular-meningeal compartments in Multiple Sclerosis (MS),\textsuperscript{196,197} thyroid lobes in autoimmune thyroiditis\textsuperscript{198,199} and kidneys in lupus nephritis patients.\textsuperscript{200} Within these structures, B cells are fundamental for the generation of inflammatory signals and for activating T cells and DC, contributing to inflammatory disease. For instance, lymphotoxin \(\alpha/\beta\), which is expressed by B cells, is essential for the differentiation of FDC in secondary lymphoid organs and the development of effective lymphoid architecture.\textsuperscript{201} Defects in B cell signalling can also be responsible for autoimmune conditions. For example, deficiency in CD22 is sufficient to predispose to development of high-affinity autoantibodies.\textsuperscript{202} Moreover, over-expression of CD19 correlates with autoimmunity by the induction of hyperresponsive B cells.\textsuperscript{203,204} Furthermore, alterations in the expression of genes that regulate B cell survival can lead to the development of autoimmunity. MRL mice homozygous for mutations in the Fas gene, a death-inducing receptor required for normal regulation of B cell lifespan, develop a spectrum of autoreactivity resembling that found in human SLE and other autoimmune diseases.\textsuperscript{205,206} BAFF, also known as BlyS, TALL-1, THANK or zTNF4, is a member of the TNF family of cytokines produced by DC, monocytes and macrophages that has a fundamental role in B cell growth, differentiation and survival. Mice transgenic for BAFF have SLE-like disease, with anti-DNA antibodies, elevated serum IgM, vasculitis and glomerulonephritis.\textsuperscript{207,208} Importantly, several of the autoimmune diseases might result from the deficient B cell tolerance associated, in part, with the over-production of BAFF that might lead to inap-
propriate survival of autoreactive B cells. In fact, elevated BAFF levels can be found in the sera of patients with autoimmune diseases, particularly those with SLE, RA, Sjögren’s syndrome, myasthenia gravis and ITP. Having in mind the role of B cells in autoimmunity, the development of B cell depletion therapies is of great importance in order to stop or attenuate the disease. The introduction of B cell depletion therapy in RA patients using Rituximab (RTX), a monoclonal antibody directed to CD20, has confirmed and reinforced the importance of these cells in established RA. In fact, following B cell depletion in patients with RA, there is clinical and serological improvement which parallels a decrease in RF levels. Moreover, RTX therapy was also beneficial in the treatment of ITP and SLE. Furthermore, the efficacy of B cell depletion therapy was also observed in animal models: B cell deprived MRL/lpr mice not only lack the immune deposit manifestations of nephritis, but also have no cellular infiltrates in the kidney or vasculitis, which implies an essential role for B cells in this animal model of lupus. However, in spite of the benefits that B cell depletion therapies may have in patients suffering with an autoimmune condition, clinical relapses are observed. This might indicate that new and more effective methods for treating autoimmune diseases are needed and further research in this area is fundamental and necessary.

Conclusions

The life of a B cell is a complex process that has to be tightly regulated. During B cell development in the bone marrow and later in the periphery in secondary lymphoid organs, immune B cell tolerance mechanisms are fundamental in order to avoid pathologies like autoimmune diseases that, if not properly diagnosed and treated, can have life-threatening consequences. In fact, B cell signalling and activation, B-T cell interactions and processes to generate antibody diversity can be responsible for the development of abnormal immune conditions when not efficiently regulated and monitored. So, research areas that concern B cell targeted therapies are of great importance to stop or, at least, to attenuate the effects of diseases with a B cell origin, such as autoimmune diseases, or B cell lymphomas. Nevertheless, B cells do play an essential role in our immune system, most notably for the astonishing capacity to produce antibodies to an apparently limitless array of antigens and by interacting with T cells, thus enabling a more effective immune response.

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Correspondence to:
João Eurico Fonseca
Unidade de Investigação em Reumatologia
Instituto de Medicina Molecular
Faculdade de Medicina da Universidade de Lisboa
Edifício Egas Moniz
Av. Professor Egas Moniz
1649-028 Lisboa, Portugal
E-mail: jefonseca@netcabo.pt

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