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Introductory Chapter: B-Cells

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1. Introduction

Etymologically, the “B” from B-cells, also referred to as B lymphocytes, stands from the name of bursa of Fabricius, a lymphoid organ found only in birds, as reported, in 1956, by Bruce Glick and Timothy Chang [1, 2], but not from the bone marrow as it has been believed.

B-cells represent about 5–15% of circulating blood lymphocytes and are responsible for the humoral immune response, as a critical component of adaptive immune system. Their roles are not limited only to the production of antigen-specific antibodies after antigen binding with high affinity *via* their membrane Ig but also to antigen presentation. This allows them to interact with cells involved in cell-mediated immunity and to produce cytokines [3] within immunological synapses (IS) [4, 5] that they create with both CD4⁺ T-cells [6] and CD8⁺ T-cells [7] (for review, see [8–10]).

The current chapter presents a brief overview on Igs and phases of B-cell ontogeny and B-lymphoid lineage markers. The end of the chapter summarizes the main types of diseases related to B-cell abnormalities.

2. Immunoglobulins: transmembrane and secreted B-cell receptors

2.1 Structure of Igs

2.1.1 Ig chains

The most common form of Igs in the blood has a heterodimeric structure, about approximately 150 kDa [11], with two antibody sites—paratopes—that bind to the epitope of a specific antigen, located in the fragment antigen-binding [F(ab)]. This structure is composed of two identical heavy (H) and two identical light (L) chains, these being either kappa (L κ) or lambda (L λ). The H and L chains are associated with each other by disulfide bridges (**Figure 1**).

2.1.2 Ig domains

Each chain of Igs is composed functionally of constant (H; C_H, L; C_L) and variable (H; V_H, L; V_L) domains. The constant region of H chain is composed of three (for IgG, IgA, IgD) or four (for IgM and IgE) constant domains, designated, respectively, C_H1, C_H2, C_H3, and C_H4. Except for IgM and IgE, the region between C_H1 and C_H2 domains is called the hinge “H” region, permitting flexibility in the chain [12], which is longer and more flexible in IgG3 than the other IgG subclasses [13]. Ig L chains are composed of two separate domains, each having an approximate molecular weight of 12 kDa [14]. The association of the variable domains of the H and L chains defines the site of attachment to the antigen (**Figure 1**).

Figure 1.

Molecular structure of a typical Ig molecule. Ig molecules have a symmetric structure that is stabilized by interchain disulfide bonds. The heavy chain determines the isotypes, i.e., the classes (IgM, IgG, IgA, IgD, IgE) and subclasses (IgG1, IgG2, IgG3, IgG4, IgA1, IgA2) of Igs. Panel (A) shows a simplified representation of an antibody molecule. Panel (B) illustrates a schematic representation of the four-chain composition and the separate domains comprising each chain. This representation is based on the X-ray crystallography of an IgG antibody. Three globular regions form a Y. The two antigen-binding sites are at the tip of the arms, which are attached to the trunk of the Y by a flexible hinge region [16]. Incubation of Igs with papain (papaya proteinase I), in the presence of a reducing agent, results in the production of two monovalent fab fragments (50 kDa each) and one intact fc fragment (about 50–70 kDa). Igs digestion by pepsin leads to the production of one $F(ab')_2$ fragment and numerous smaller peptide fragments of the fc portion. CDRs, complementarity-determining regions; C_H , heavy chain constant domain; CHO, carbohydrate; C_L , light chain constant domain; fab, fragment antigen binding; fc, fragment crystallizable; H, hinge region; Ig, immunoglobulin; V_H , heavy chain variable domain; V_L , light chain variable domain.

Constant domains have specify effector functions such as activation of complement or binding to FcRs [15].

Each Ig domain contains roughly 100–110 amino acids long [14, 17] and consists of a two-layer sandwich of seven to nine antiparallel beta-strands arranged in two beta-sheets/-barrels with a Greek topology [18].

Ig domains play well-defined roles, which depend on the location of each one. So the C_{H1} domain, located within the F(ab) region, interacts with the constant domain of L chains. The remaining C_H domains (C_{H2} - C_{H3} or C_{H2} - C_{H4}) comprise the Fc region, which defines the isotype, classes, and subclasses of the Ig. The C_{H2} (C_{H3} for IgM and IgE) domain allows an important role in mediating the effector functions, including interaction with FcRs and antibody stability thanks to the presence of N-linked glycan, which is conserved in mammalian IgGs at Asn297 as well as in homologous regions of other antibody isotypes [19]. The importance of N-glycosylation is well-known for IgGs, but little is known for other isotypes [20]. The C_{H3} domain allows dimerization and participates in the stabilization of the binding of the heavy chains to one another through interactions between the C_{H3} domains. For both IgM and IgA, the C_{H3} domains have short tailpieces to which the J-chain binds *via* disulfide bonds, whereas the secretory component is disulfide bonded to one of the C_{H2} domains of the dimer. C_{H3} domain of IgG binds to FcγR and of IgE to FcεRI and CD23 [15].

2.1.3 Framework and complementarity determining regions

Each Ig V domain contains three hypervariable regions, corresponding to the site of recognition of the antigen, thus forming the paratope (complementarity determining regions, CDR1, CDR2, and CDR3). CDRs separate four highly conserved segments with less variability, termed the framework regions and designated FR1, FR2, FR3, and FR4 [16] (Figure 2). As for the T-cell antigen receptor (TCR), the

Figure 2.
Subdivision of the variable region of the Ig molecule. The Ig V region contains seven amino acid regions, four of which are FRs and three of which are CDRs. The FRs are located on the tips of the Y-shaped molecule and act as a scaffold for the CDRs. Of note, this representation shows the physical location of the V_H region and the V_L region. N region between the V and D regions is called N1, and that between the D and heavy J regions is called N2. CDR, complementarity determining region; FR, framework region; C, invariant constant domain; V, variable domain; D, diversity domain; J, joining domain.

binding specificity of the antigen is therefore determined by the loops present at one end of V_L and V_H domains of Ig chains; the difference in specificity between antibodies is therefore related to these loops [21].

2.1.4 Isotypes and subisotypes of Igs

There are five isotypes (classes) of Igs that are structurally and functionally distinct, IgM, IgD, IgG, IgA, and IgE. The difference between the classes of Igs lies in the constant portion of the H chains: mu (μ) determines the IgM class, gamma (γ) determines the IgG class, alpha (α) determines the IgA class, delta (δ) determines the IgD class, and epsilon (ϵ) determines the IgE class. So the DNA encoding the constant part of an H chain contains several constant sequences. Thus, there are four subclasses of IgG in both humans (IgG1, IgG2, IgG3, IgG4) and mice (IgG1, IgG2a, IgG2b, and IgG3) and two subclasses of IgA (IgA1 and IgA2) in humans, which are unequally distributed in the body fluids [22].

Key Point 1 Main features of human Ig classes and subclasses			
mIg	sIg	Properties
Molecular formula	(μ ₂ κ ₂) ₅ or (μ ₂ λ ₂) ₅
		Subclasses	None
		MW	900–970 kDa
		Valency	10
		Structure	mIgM; monomer, sIgM: pentamer
		H chain type MW	μ 65–70 kDa

Key Point 1 Main features of human Ig classes and subclasses					
mIg	sIg	Properties			
		Serum concentration	0.5–2 mg/mL		
		<i>In vivo</i> serum half life	5 days		
		Relative serum abundance to total immunoglobulin	6–10%		
		Carbohydrate content by weight (glycosylation)	12%		
		Distribution	Mainly intravascular		
		Crosses placenta	None		
		Function	Primary antibody response		
		Classical complement pathway activation	+++
		Molecular formula	$\gamma_2\kappa_2$ or $\gamma_2\lambda_2$
				Subclasses	Yes (IgG1, IgG2, IgG3, IgG4)
MW	IgG1; 150 kDa, IgG2; 150 kDa, IgG3; 170 kDa, IgG4; 150 kDa				
Valency	2				
Structure	Monomer				
H chain type MW	γ_1 50 kDa, γ_2 50 kDa, γ_3 60 kDa, γ_4 50 kDa				
Serum concentration	Total IgG: 10–16 mg/mL (IgG1: 9 mg/mL; IgG2: 3 mg/mL; IgG3: 1 mg/mL; IgG4: 0.5 mg/mL)				
<i>In vivo</i> serum half life	IgG1: 23 days; IgG2: 23 days; IgG3: 8 days; IgG4: 23 days				
Relative serum abundance to total immunoglobulin	75–80%				
Carbohydrate content by weight (glycosylation)	3%				
Distribution	Intra- and extravascular				
Crosses placenta	IgG1: +; IgG2: +/-; IgG3: +; IgG4: +				
Function	Secondary antibody response				
Classical complement pathway activation	IgG1: +; IgG2: +/-; IgG3: ++; IgG4: none
Molecular formula	$(\alpha_2\kappa_2)_n$ or $(\alpha_2\lambda_2)_n$
		Subclasses	Yes (IgA1, IgA2) [in humans, serum IgA are mostly monomers, but a small amount of them is found in polymeric forms (pIgA), usually dimers, linked by disulfide bonds and by an additional, cysteine-rich polypeptide termed J chain (16 kDa). Serum monomeric IgA can also be found in most secretions. Mucosal secretory IgAs are mostly seen as a dimer with a secretory piece.]		

Key Point 1 Main features of human Ig classes and subclasses			
mIg	sIg	Properties
MW	Secretory tetramer IgA1 or IgA2: 600 kDa; serum monomer IgA1 or IgA2: 160 kDa
		Valency	2, 4, 6 or 8
		Structure	Monomers, dimers (more common), and trimers and tetramers (both are rare)
		H chain type MW	α 1 55 kDa, α 2 55 kDa
		Serum concentration	Total IgA : 1–4 mg/mL (IgA1: 3 mg/mL; IgA2 : 0.5 mg/mL)
		<i>In vivo</i> serum half life	IgA1: 6 days; IgA2: 6 days
		Secretion	Milk and secretions (tears, saliva, mucous, sweat, colostrum, and secretions from the genitourinary tract, gastrointestinal tract, prostate, and respiratory mucosa)
		Relative serum abundance to total immunoglobulin	13–15%
		Relative serum abundance to total IgA	IgA1; 85%, IgA2: 15% (higher in secretions)
		Carbohydrate content by weight (glycosylation)	10%
		Distribution	Intravascular and secretions
		Crosses placenta	None
		Function	Protects mucous membranes
		Classical complement pathway activation	None
Molecular formula	$\delta_2\kappa_2$ or $\delta_2\lambda_2$
		Subclasses	None
		MW	180 kDa
		Valency	2
		Structure	Monomers
		H chain type MW	δ 62–70 kDa
		Serum concentration	0–0.4 mg/mL
		<i>In vivo</i> serum half life	3 days
		Relative serum abundance to total immunoglobulin	0.2%
		Carbohydrate content by weight (glycosylation)	13%
		Distribution	Lymphocyte surface
		Crosses placenta	None
		Function	Somewhat unknown; while research have demonstrated its role in promoting immune defense related to inflammation and tissue damage by

Key Point 1 Main features of human Ig classes and subclasses				
mIg	slg	Properties
mIgE slgE			inducing the activation and infiltration of immune cells	
		Classical complement pathway activation	None	
		Molecular formula	$\epsilon_2\kappa_2$ or $\epsilon_2\lambda_2$	
		Subclasses	None	
		MW	190–200 kDa	
		Valency	2	
		Structure	Monomers	
		H chain type MW	ϵ 70–73 kDa	
		Serum concentration:	10–400 ng/mL	
		<i>In vivo</i> serum half life	2.5 days	
		Relative serum abundance to total immunoglobulin	0.002%	
		Carbohydrate content by weight (glycosylation)	12%	
		Distribution	Basophils and mast cells in saliva and nasal secretions	
		Crosses placenta	None	
		Function	Protects against parasites	
		Classical complement pathway activation	None	
mIg: membrane-bound immunoglobulin, slg: secreted immunoglobulin (serum or secretory Ig).				

2.2 Transmembrane B-cell receptor

B-cells are defined by the presence of membrane-bound Igs (mIg) that act as specific receptors for the appropriate antigen in mature B-cell but, also as an excellent marker of the B-cell line. The mIg constitutes, with other glycoprotein chains, the B-cell antigen receptor complex (BCR). These correspond to non-covalently associated transmembrane disulfide-linked heterodimer phosphoprotein Ig α /Ig β (CD79a and CD79b), which are encoded by *mb-1* and *B29* genes, and are structurally similar to CD3 γ , δ , and ϵ chains on T-cells and therefore involved in signal transduction of B-cell [23] (**Figure 3**). The intracytosolic portion size of the heterodimer allows it to initiate signal transduction, following the binding of the specific antigen to the mIg.

2.3 Mobility of mIgs, capping, and antigenic modulation

Igs are very mobile on the surface of B-cells. In addition, various specific ligands cause what is called capping, *i.e.*, a rapid redistribution of complexes on the surface of the cell, followed by internalization of the complexes or their release into the surrounding environment. This transient disappearance of the mIg receptor *via* its binding to specific ligands, like anti-immunoglobulin antibodies or antigens, is called antigenic modulation, which constitutes the initial signal for B-cell activation. The modulated membrane receptor may also be synthesized by the cell and returned to the surface when the B-cell is metabolically active [24, 25].

2.4 Transmembrane vs. secreted B-cell receptor

The only structural difference between transmembrane and secreted B-cell receptors (soluble immunoglobulins, sIgs) is that the C-terminal region of the heavy

Figure 3.
BCR complex structure. The BCR complex is composed of a mIg non-covalently bonded to a transmembrane disulfide-linked heterodimer phosphoprotein composed of CD79a (Igα)/CD79b (Igβ). CD, cluster of differentiation; mIg, membrane-bound immunoglobulin.

Figure 4.
Structural difference between the C-terminus of the H chain constant region of mIgM and sIgM. mIgM and sIgM differ simply by the COOH ends of the μ chain and therefore by mRNAs that differ at their 3' ends. Membrane-bound IgM (membrane μ) is slightly larger than that of secreted IgM (secreted μ) [27]. The numbers indicate the position of amino acids. N-CHO represents the position of glycosylation of asparagine (N, Asn) (N-glycosylation site). mIgM, membrane immunoglobulin M; sIgM, secreted immunoglobulin M.

chains contains a short hydrophobic stretch which spans the lipid bilayer of the membrane [26] (**Figure 4**).

slgs play a complementary role to that of T-cells. It needs to use other mechanisms for antigen removal. This implies that they bind other molecules, like complement molecules, or specialized receptors, called Fc receptors (FcRs, receptors of fragment crystallizable region), on the surface of effector cells that they activate, including phagocyte cells (**Figure 5**).

Antibodies allow B-cells to provide systemic protection of the host and immune surveillance through pathogen recognition and organization of immune reactions. Their expression varies according to the state of differentiation of B-cells. After activation, B-cells transform into plasma cells that secrete antibodies of the same specificity as their membrane BCR. Secreted antibodies are transported rapidly throughout the body by blood or lymph or secreted through the epithelia to protect the interface between the body and its environment. IgG antibodies also provide a mechanism by which acquired immunity can be transmitted from the mother to the fetus or infant, thus providing acquired immune protection during the critical period of early life. Nevertheless, some antibodies can bind to self-structures, referred to as autoreactive antibodies, and induce, under certain conditions, aberrant immune responses and tissue damage [28].

Figure 5. Human FcRs and their cell localization and immune functions. DCs, dendritic cells; FcRn, neonatal fc receptor; FcRs, receptors of fragment crystallizable region; FDCs, follicular dendritic cells; F₁₅₈, phenylalanine at position 158; GC, germinal center; H₁₃₁, histidine at position 131; ICs, antigen-antibody immune complexes; PlgR, polymeric immunoglobulin receptor; R₁₃₁, arginine at position 131; V₁₅₈, valine at position 158.

3. B-cell ontogeny

The differentiation of B-cells from hematopoietic stem cells into pro-B, then pre-B, then immature B-cells, and finally into mature B-cells is characterized by several events, including (i) modification of membrane differentiation markers; (ii) Ig gene rearrangement, which takes place at the pro-B and pre-B stages, allowing the expression of BCR; and (iii) negative clonal selection.

3.1 B-cell ontogenesis and maturation

B-cell ontogenesis occurs in the fetal liver, then in the bone marrow, and continues throughout life. It starts from a hematopoietic stem cell and leads to the development of a so-called “immature” B-cell with the same and unique antigenic specificity. The immature B-cell migrates to the peripheral lymphoid organs, where the different stages of maturation will take place, leading to the Ig-producing plasma cells and memory B-cells [24]. Ultra-complex regulatory mechanisms are involved during all stages of B-cell development and lead to the generation of B-cell repertoire with a vast diversity of antigen recognition capacity.

3.1.1 Lymphoid progenitor cells and cell fate decisions

Knowing that B-cells, T-cells, and natural killer (NK) cells all develop from the early lymphoid progenitors that originate from totipotent hematopoietic stem cells, cell fate results from several lineage choices. The B-cell progenitors continue to develop in the bone marrow [21].

3.1.2 Positive and negative selection and B-cell receptor reformatting

During their development, B-cell and T-cell undergo a dual process of positive and negative selection in which cells that react with high affinity against self-antigens are eliminated because they constitute a significant danger of triggering autoimmune responses.

In the case of B-cells, outside the negative selection mechanisms, cell survival depends essentially on their ability to compete for survival factors such as the B-cell activating factor (BAFF, also known as B lymphocyte stimulator (BLyS), a subset of the tumor necrosis factor (TNF) tumor necrosis factor ligand superfamily member 13B), which is present in the circulation and produced by resident cells within secondary lymphoid organs [24]. Positive selection mainly corresponds to BCR functionality test and depends on a moderate response to the self-antigen, which stimulates cell maturation and survival. When self-reactivity exceeds a certain level, a process so-called *receptor reformatting* is triggered by inducing a new cycle of gene rearrangements, principally in the IG L chain loci (see Chapter 2). If the receptor reformatting fails, or if the developing B-cell leaves the bone marrow and encounters the self-antigen soon after it arrives in the spleen, this cell will be eliminated [21].

3.1.3 Immature B-cell generation

The differentiation of hematopoietic stem cell (HSC) into immature B-cell passes through four successive steps, which could be identified by the presence of certain markers, corresponding to the different stages of rearrangement of Ig genes:

- a. **Early pro-B-cell.** These cells appear before the start of rearrangements. They are identified by CD45R, major histocompatibility complex class II (MHC II), and CD19 and CD38 markers.
- b. **Late pro-B-cell.** Variable (V), diversity (D), and joining (J) recombinations for Ig H chain gene segments and receptor expression for interleukin-7 (IL-7R) are induced at this stage.
- c. **Pre-B-cell.** There is contact between adhesion molecules and, in particular, vascular cell adhesion molecule 1 (VCAM-1/CD106), on the surface of the stromal cells and its ligand, very late antigen-4 (VLA-4 or $\alpha 4\beta 1$ integrin, a dimer composed of CD49d ($\alpha 4$) and CD29 ($\beta 1$)), on the surface of the pre-B-cells. At this point, intact μ H chains are produced and exported to the cell surface in association with proteins that simulate light chains: $\lambda 5$, which is similar to a C λ domain, and VpreB (CD179a), which has an Ig V domain-like structure. The μ chains associated to $\lambda 5$ and VpreB form a complex which, together with Ig α and Ig β proteins, can be expressed at the cell surface and transduce signals that inhibit other rearrangements of genes encoding the H chains but stimulate the genes encoding L chains and cell proliferation. Moreover, expression of the CD20 marker can be detected at this stage.
- d. **Immature B-cell.** Thanks to a productive Ig L chain genes' rearrangement, these cells express IgM on their surface, which triggers a phenomenon termed *feedback inhibition*, which blocks any new rearrangement of the genes encoding the L chains, thus allowing the expression of a single and unique Ig specificity by each B-cell clone. At this point, the cells express the CD21 marker.

These stages are strictly dependent on the presence, in the fetal liver and bone marrow, of nonlymphoid stromal cells that come into contact with the B-cell precursors and provide the input of soluble factors in the cell microenvironment that are essential for differentiation, such as stem cell factor (SCF) and interleukin 7 (IL-7). It should be noted, moreover, that the probability of reaching productive/efficient rearrangements of Ig genes encoding the H and L chains and obtaining an intact Ig expressed on the cell surface (mIg/BCR) is low. So most of the time there are nonproductive rearrangements, which leads to the deletion of the resulting B-cells. In addition, the differentiation of B-cells also depends, according to the cell development stage, on the presence of intracellular enzymes. Thus, the enzymes encoded by the recombination-activating genes (RAGs), RAG-1 and RAG-2, are active in early and late pro-B-cells and in pre-B-cells. Finally, the activity of the enzyme terminal deoxynucleotidyl transferase (TdT), which is involved in the addition of N-nucleotides, stops at the pre-B-cell stage.

Key Point 2|Stromal cells

- Stromal cells constitute a support tissue of an organ, *i.e.*, a connective tissue cell that allows the multiplication of hematopoietic stem cells (HSCs) and their differentiation by creating a microenvironment adapted to the growth and differentiation of HSCs. They are also able to differentiate into many cell types.
- It has recently been suggested that stromal cells play a major role, not only in the functional regulation of many tissues and organs, but also more particularly in the immune responses. Of note, non-hematopoietic stromal cells play a key role in the development and function of the immune

system, but, paradoxically, they can also promote the persistence of many cancers and various immune-mediated diseases. The main non-hematopoietic stromal cells involved in immunity include fibroblasts, myofibroblasts, endothelial cells, pericytes, smooth muscle cells and mesenchymal stromal cells.

- Bone marrow stromal stem cells (BMSC) are almost all of mesenchymal origin and, therefore, are also known as skeletal or mesenchymal stem cells. They influence the microenvironment surrounding B-cell precursors, and thus exert local effects on their development through cytokines and chemokines.

HSCs are multipotent cells; *i.e.*, they are able to differentiate into each of the types of blood cells. After each division into two cells, one of them maintains the HSCs stock and the other one differentiates into one of three types of blood cell, *i.e.*, leukocyte, red blood cell, or platelet.

3.1.4 Transition from immature B-cell to mature B-cell

The immature B-cell leaves the bone marrow parenchyma and passes through an endothelial barrier and enters the blood sinusoids, where they are retained, before finally being released into the peripheral blood [29], which allows them to migrate to the spleen where they complete their development [30]. The transition from the immature B-cell to the mature B-cell takes place in a few days and leads, via alternative splicing of long primary mRNA transcripts from the IG heavy (IGH) locus [31], to the coexpression on naïve mature B-cells of membrane IgM and IgD that share the same antigenic specificity. Only about 5% of immature B-cells will sustainably give rise to peripheral B-cells; however, most newly formed ones disappear within a few days.

3.1.4.1 Tonic BCR signal and notch activation

A so-called BCR-mediated positive signaling *tonic signal* and activation of Notch pathway, more precisely of Notch2, which is induced following the interaction with delta-like canonical Notch ligand 1 (DLL-1) expressed on splenic endothelial niches and required for marginal zone (MZ) B-cell development [32], allow the triggering of a first differentiation by a lineage cell choice that does not involve the antigenic specificity.

3.1.4.2 Intensity of tonic signal and differentiation of lymphoid follicular B-cells or MZ B-cells

- Low tonic signal and Notch2 commitment.** These will lead to differentiation into MZ B-cells. The latter forms a ring around the follicle and has a more complex structure in humans, also surrounded by a perifollicular zone [PFZ] that surrounds the red pulp. It is a macrophage-rich area (MZ macrophages [MZMs] and metallophilic macrophages [MMMs]) that will efficiently retain antigens from the bloodstream. In mice, the MZ B-cells are CD21⁺⁺CD23⁻CD11⁺⁺CD11c⁺⁺, IgM⁺⁺IgD^{+/-}. In humans, it is not yet known whether MZ B-cells are derived from the germinal center (CG), by an early exit at the IgM stage, or are produced by a lineage choice during early B-cell differentiation.
- Strong tonic signal, without Notch2 commitment.** These lead to differentiation of lymphoid follicular B-cells. Recall that these are structured by specialized stromal cells and follicular dendritic cells (FDCs), which play an important role in immune response by their ability to retain

antigen–antibody complexes as well as in the selection of memory B-cells during GC reactions (GCRs) [33]. They contain naive B-cells, and adjacent to which are T-cell zones that form the periarteriolar lymphoid sheath (PALS). Phenotypically, they are IgM+/-IgD++, CD21+/-, and CD23+, in mice.

3.2 Phases of B-cell ontogeny: antigen-dependent and antigen-independent

B-cell ontogeny can be separated into two main phases: earlier antigen-independent phase and later antigen-dependent phase (Figure 6). It should also be recalled that thymus-independent (TI) antigens have the ability to stimulate B-cells without T-cells’ help and are traditionally divided into two categories, TI-1 antigens that can activate B-cells through coengagement of Toll-like receptors (TLR), such as LPS or other bacterial polysaccharides, and TI-2 antigens that lead to extensive cross-linking of the BCR, such as polymeric protein antigens or repeated structural motifs [33]. Some antigens fall outside these and form a third category. Finally, T-cell-independent responses should be discussed, knowing that T-cells could intervene at different levels of B-cell development in response to TI antigens (For review, see [34–36]).

3.2.1 Earlier antigen-independent phase

The first phase of B-cell differentiation and maturation would be antigen-independent. It takes place in the bone marrow and results in the generation of immature B-cells expressing pair L chains with μ chains to form cell surface monomeric IgM in association with transmembrane Ig α and Ig β invariant chains, forming the BCR complex, which is able to recognize and capture antigens.

3.2.2 Later antigen-dependent phase

The second phase of B-cell activation and final differentiation is dependent on self-antigens, then on non-self-antigens within the secondary lymphoid organs (SLOs). It results in the formation of plasma cells and antigenic specific memory B-cells.

Figure 6. Phases of B-cell ontogeny. CLPs, common lymphoid progenitors; mIgM, membrane immunoglobulin M; DZ, dark zone; LZ, light zone; HLA, human leucocyte antigen; GC, germinal center; CSR, class switch recombination.

4. B-cell markers: the main molecules of the B-lymphoid lineage

The majority of human peripheral blood B-cells express on their surface IgM and IgD that have the same antigenic specificity. In addition, in humans, a large population of circulating cells expresses membrane IgG or IgA (but very little IgE). In some tissues, including intestinal mucosa, B-cells selectively express membrane IgA. In addition to the Ig α /Ig β heterodimer marker, which is part of the BCR complex, there are also other molecules on the B-cell surface that play notable roles in various cellular functions, especially in B-cell regulation, such as human complement receptor type 2 (CR2, C3d), designed as CD21, that regulates B-cell proliferative responses, and serving as a receptor for the C3d, C3dg, and iC3b proteins of complement. B-cells also express receptor for complement component C3b (CR1, CD35) as well as for Fc fragments (FcR) of certain Ig isotypes, such as IgG (Fc γ RII-B1, CD32). Of note, only Fc γ RIIB1 and Fc γ RIIB2 have an immunoreceptor tyrosine-based inhibitory motif (ITIM) sequence among the Fc γ R receptor family and are therefore inhibitory FcRs; they do not induce phagocytosis and represent an important receptor-mediated feedback circuit regulator by circulating antigen-specific IgG [24, 37]. CR2/CD21 has also been described as a receptor for the envelope glycoprotein gp350/220 of the Epstein–Barr virus (EBV) [38]. Other molecules are frequently used in routine practice as primary markers for identifying human B-cells, including CD19, CD20, and CD22 markers. CD19 is expressed at all stages of B-cell lineage, including normal plasma cells. CD20, a molecule restricted to the B-cell population, has been shown to be implicated to form calcium channels in cell membrane [39], and as an effective target for immunotherapy in treatment of B-cell lymphoma, as well as in a number of autoimmune diseases, such as type 1 diabetes [40]. It occurs at the early pre-B-cell stage of development and remains throughout all stages of B-cell maturation [41], ranging from pre-B-cells in the bone marrow to short-lived plasmablasts [42]. CD22, a B-cell-restricted surface molecule that regulates BCR signaling in mature B-cells [43], is an early marker and persists at all stages of B-cell differentiation, which has allowed it to be a useful pan marker for all mature B-cell subsets [44]. Recognized as a B-cell-specific sialic acid binding Ig-like lectin 2 (Siglec-2; B-lymphocyte cell adhesion molecule [BL-CAM]), CD22 has been exploited as a therapeutic target for humanized anti-CD22 monoclonal antibody to treat B-cell leukemia [45]. Both immature and mature mouse B-cells as well as subsets of T-cells and NK cells and subset of abnormal T-cells involved in the pathogenesis of systemic autoimmunity in MRL-*Fas*^{lpr} and MRL-*Fas*^{gld} mice express one of the five isoforms of the transmembrane tyrosine phosphatase CD45 (LCA) found on lymphocytes [46], *i.e.*, CD45R (B220, CD45RABC), which has been known to play a major role in lymphocyte signaling and activation. Other B-cell surface molecules allow them to cooperate with T-cells. They include essentially MHC II antigens (DR, DP, and DQ, in humans, and IA and IE, in mice) and CD40 (a member of the TNF receptor superfamily, also expressed on B-cell precursors in the bone marrow), which is capable of transducing extremely potent B-cell activation signals. Mutations in the gene coding for CD40 ligand (CD40L/CD154), predominantly expressed by activated CD4⁺ T-cells, lead to X-linked hyper-IgM syndrome (HIGM1), a severe primary immune deficiency (PID) in humans, characterized by recurrent infections, associated with very low or absent IgG, IgA, and IgE levels, but normal or elevated serum levels of IgM [47]. B-cells can also be identified using the inhibitory co-receptor CD72 (Lyb-2 in mice), containing an ITIM in the cytoplasmic. CD72 specifically recognizes the RNA-containing endogenous TLR7 ligand Sm/ribonucleoprotein (RNP) by C-type lectin-like domain (CTLD) located in its extracellular region and specifically inhibits B-cell responses to Sm/RNP by recruiting SH2 domain-containing phosphatase 1 (SHP-1) to the

phosphorylated ITIM [48]. It has been reported to have a role in the regulation of systemic lupus erythematosus (SLE) development [49]. Its specific ligand is CD5 [50], which is usually expressed on most T-cells, but also on a subset of mature B-cells [51] and CD5⁺ B1 cells, which emerge early in development and play a major role in autoimmunity [52]. Other markers, including CD10 and CD23, are found at some stages of B-cell differentiation. CD10, a neutral endopeptidase, also referred to as common acute lymphoid leukemia antigen (CALLA), enkephalinase, and neprilysin, is expressed in early B-cell immature stages in bone marrow, but also in certain stages of immunopoiesis and is also present on antigen-activated cells in GC, in humans [53]. Defined initially as the low-affinity receptor for IgE (FcεRII) and as EBV cell surface (EBVCS) activation antigen or BLAST-2, CD23 exists in two distinct protein isoforms, CD23α (FcεRIIa), which is constitutively expressed only in normal B-cells and B-cell lines, and CD23β (FcεRIIb), which is an inducible molecule detectable on various cells [54], including most mature B-cells, follicular mantle (FM) B-cells, T-cell subsets (at low levels), NK cells, platelets, FDCs, monocytes, eosinophils, Langerhans cells, etc. CD23, a 45 kD type II transmembrane glycoprotein, can be cleaved and found in plasma as a freely soluble receptor (sCD23). In contrast to cross-linking of FcεRI that leads to degranulation of mast cells to release vasoactive mediators, engagement of membrane-bound CD23 suppresses the production of IgE by B-cells. Moreover, CD23 participates in many regulatory processes, including cytokine release, cell–cell contact, and

Figure 7.

BCR signaling. As mentioned above, the final activation of mature B-cell occurs in the SLOs, where they migrate through the blood. Within the SLOs, they receive a constant supply of antigen through the circulating lymph. The activation of B-cell is initiated after binding of an appropriate antigen to its BCR, leading to phosphorylation of the non-covalently associated Igα/Igβ transmembrane (CD79a and CD79b). The signaling mechanisms triggered during the B-cell activation can be summarized in four main steps: (i) antigen binding and starting signaling cascade, (ii) phosphorylation of Igα/Igβ ITAM, (iii) signalosome complex formation, and (iv) signalosome activation. Akt, protein kinase B/PKB; BCR, B-cell antigen receptor; BLNK, B-cell linker; Btk, Bruton's protein tyrosine kinase/non-receptor kinase; CD, cluster of differentiation; ERK, extracellular signal-regulated kinase; Fyn, proto-oncogene tyrosine-protein kinase Fyn/non-receptor Fyn proto-oncogene; Grb2, growth factor receptor-bound protein 2; ITAM, immunoreceptor tyrosine-based activation motifs; JNK, c-Jun N-terminal kinase; Lyn, Lck/yes novel tyrosine kinase; mIg, membrane-bound immunoglobulin; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B-cells; PI3K, phosphoinositide-3 kinase; PIP2, phosphatidylinositol-4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphate; PLCγ2, phospholipase C-γ2; PKCβ, protein kinase C β; SLOs, secondary lymphoid organs; SFKs, Src-family kinase/Src-protein-tyrosine kinase or Src family tyrosine-protein kinase c-Src (cellular Src kinase); Syk, spleen tyrosine kinase/non-receptor tyrosine kinases; Vav, proteins acting as guanine nucleotide exchange factors (GEFs) for small G proteins of the rho family.

receptor-mediated positive and negative feedback circuits, either as a membrane-bound glycoprotein or as sCD23 [55]. Finally, a new type of immune cell, so-called X lymphocyte, that is a dual expresser (DE) of TCR and BCR and key lineage markers of both B- and T-cells, has just recently been discovered in patients with type 1 diabetes (For review see [56]).

5. Normal mature B-cell activation and signaling mechanisms

Antigen binding to mIg induces the BCR aggregation, which leads to the rapid transmembrane disulfide-linked heterodimer phosphoprotein Igα (CD79a)/Igβ (CD79b) ITAM phosphorylation through recruitment of Syk and SFKs (Fyn, Lyn). This process begins with the formation of a “signalosome” [57]. The signalosome activation leads to three main pathways [58], including Btk, PLC-γ2, and PI3K. BCR can transactivate the B-cell co-receptor CD19, which forms, on B-cell surface, a tetrameric co-receptor complex with CD21 and CD81 (target of anti-proliferative antibody 1 (TAPA-1), a tetraspanin family member tetraspanin 1 (Tspan1, NET-1), and Leu13 (CD225)) [59]. CD19 can also be BCR-independently activated but lacks intrinsic or associated tyrosine kinase activity [60]. As CD19 has a long cytoplasmic domain, it binds and amplifies the function of the SFKs and recruits a heterodimer p85/p110 class IA PI3K concurrently, which phosphorylates a membrane phospholipid, PIP2, leading to the production of a second messenger PIP3 [61], as well as promoting Btk and Akt, and a serine/threonine, kinase phosphorylation in B-cell [62] (Figure 7).

Figure 8. Main types of diseases related to B-cell abnormalities. AID, activation-induced cytidine deaminase; Btk, Bruton’s tyrosine kinase; HIGM1, X-linked hyper-IgM syndrome type 1; CD40L, CD40 ligand (CD154); CSR, class switch recombination; GCs, germinal centers; Ig H, immunoglobulin heavy chain; BAFF, B-cell activating factor also known as B-lymphocyte stimulator (BLyS) or tumor necrosis factor (TNF) ligand superfamily member 13B (TNF-like molecule BAFF); BCL-2, B-cell lymphoma 2; EBV, Epstein-barr virus; CVID, common variable immunodeficiency; IVIg, intravenous immunoglobulin; SCID, severe combined immunodeficiency; SHM, somatic hypermutation; SLOs, secondary lymphoid organs; TACI, transmembrane activator and calcium modulator and cytophilin ligand interactor; XLA, X-linked agammaglobulinemia; HIGM2, hyper-IgM syndrome type 2 (autosomal recessive).

6. B-cell and pathologies

B-cells are threatened by various pathologies, including (i) immune deficiency, which results from a disruption in their homeostasis due to mutations or deletions, abnormalities in mechanisms of gene repair, Ig class generation, Ig affinity modulation, etc.; (ii) autoimmune disorders, in which potentially dangerous self-reactive clone B-cells escape from mechanisms of tolerance; (iii) cancerous tumors, which result from a disorder in the succession of phases of proliferation and cell death or in the processes of generation of cell diversity; and (iv) allergies that are particularly related to regulatory abnormalities involving Br1 (IL-10⁺), Br3 (TGF- β ⁺), and regulatory B-cells (Breg, Foxp3⁺) [52]. The main B-cell-associated diseases are illustrated in **Figure 8**.

Acknowledgements

Cell schematic illustrations are adapted from free Servier Medical Art (smart.servier.fr/servier-medical-art).

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References

- [1] Cooper MD. The early history of B-cells. *Nature Reviews. Immunology*. 2015;**15**:191-197
- [2] Ribatti D, Crivellato E, Vacca A. The contribution of Bruce Glick to the definition of the role played by the bursa of Fabricius in the development of the B-cell lineage. *Clinical and Experimental Immunology*. 2006;**145**:1-4
- [3] Murphy K, Travers P, Walport M, Janeway C. *Janeway's immunobiology*. (Garland). Science. 2012
- [4] Choudhuri K et al. Polarized release of T-cell-receptor-enriched microvesicles at the immunological synapse. *Nature*. 2014;**507**:118-123
- [5] Dustin ML. What counts in the immunological synapse? *Molecular Cell*. 2014;**54**:255-262
- [6] Yuseff M-I, Lennon-Duménil AM. B-cells use conserved polarity cues to regulate their antigen processing and presentation functions. *Frontiers in Immunology*. 2015;**6**(251)
- [7] Bennett SR, Carbone FR, Toy T, Miller JF, Heath WR. B-cells directly tolerize CD8(+) T-cells. *The Journal of Experimental Medicine*. 1998;**188**: 1977-1983
- [8] Ke Y, Kapp JA. Exogenous antigens gain access to the major histocompatibility complex class I processing pathway in B-cells by receptor-mediated uptake. *The Journal of Experimental Medicine*. 1996;**184**: 1179-1184
- [9] Yefenof E, Zehavi-Feferman R, Guy R. Control of primary and secondary antibody responses by cytotoxic T lymphocytes specific for a soluble antigen. *European Journal of Immunology*. 1990;**20**:1849-1853
- [10] Barnaba V, Franco A, Alberti A, Benvenuto R, Balsano F. Selective killing of hepatitis B envelope antigen-specific B-cells by class I-restricted, exogenous antigen-specific T lymphocytes. *Nature*. 1990;**345**:258-260
- [11] Porter RR. Structural studies of immunoglobulins. *Science*. 1973;**180**: 713-716
- [12] Hovenden M et al. IgG subclass and heavy chain domains contribute to binding and protection by mAbs to the poly γ -D-glutamic acid capsular antigen of bacillus anthracis. *PLoS Pathogens*. 2013;**9**:e1003306
- [13] Scharf O et al. Immunoglobulin G3 from polyclonal human immunodeficiency virus (HIV) immune globulin is more potent than other subclasses in neutralizing HIV type 1. *Journal of Virology*. 2001;**75**:6558-6565
- [14] Chung CM et al. Thermodynamic stability of a kappa λ immunoglobulin light chain: Relevance to multiple myeloma. *Biophysical Journal*. 2005;**88**: 4232-4242
- [15] Schroeder HW, Cavacini L. Structure and function of immunoglobulins. *The Journal of Allergy and Clinical Immunology*. 2010;**125**:S41-S52
- [16] *Immunobiology: The Immune System in Health and Disease*. Garland Publ.; 2001. [animated CD-ROM inside] [u.a.]
- [17] Minegishi Y, Hendershot LM, Conley ME. Novel mechanisms control the folding and assembly of lambda5/14.1 and VpreB to produce an intact surrogate light chain. *Proceedings of the National Academy of Sciences of the United States of America*. 1999;**96**: 3041-3046

- [18] Bork P, Holm L, Sander C. The immunoglobulin fold. Structural classification, sequence patterns and common core. *Journal of Molecular Biology*. 1994;**242**:309-320
- [19] Prabakaran P et al. Structure of an isolated unglycosylated antibody C(H)₂ domain. *Acta Crystallographica. Section D, Biological Crystallography*. 2008;**64**:1062-1067
- [20] Göritz K, Maresch D, Altmann F, Obinger C, Strasser R. Exploring site-specific N-glycosylation of HEK293 and plant-produced human IgA isotypes. *Journal of Proteome Research*. 2017;**16**: 2560-2570
- [21] DeFranco AL, Robertson M, Locksley RM. *Immunité: la réponse immunitaire dans les maladies infectieuses et inflammatoires*. De Boeck; 2009
- [22] Pakkanen SH et al. Expression of homing receptors on IgA1 and IgA2 plasmablasts in blood reflects differential distribution of IgA1 and IgA2 in various body fluids. *Clinical and Vaccine Immunology*. 2010;**17**:393-401
- [23] Minegishi Y et al. Mutations in Igalpha (CD79a) result in a complete block in B-cell development. *The Journal of Clinical Investigation*. 1999; **104**:1115-1121
- [24] Chatenoud L, Bach J-F. *Immunologie. Médecine Sciences Publications-Lavoisier*; 2012
- [25] Chatenoud L, Bach JF. Antigenic modulation—A major mechanism of antibody action. *Immunology Today*. 1984;**5**:20-25
- [26] Goding JW. Cellular basis of the immune system. In: *Monoclonal Antibodies*. Elsevier; 1996. pp. 26-49. DOI: 10.1016/B978-012287023-1/50051-1
- [27] Alt FW et al. Synthesis of secreted and membrane-bound immunoglobulin mu heavy chains is directed by mRNAs that differ at their 3' ends. *Cell*. 1980;**20**: 293-301
- [28] Iype J et al. Differences in self-recognition between secreted antibody and membrane-bound B-cell antigen receptor. *Journal of Immunology (Baltimore, Md)*. 2019;**1950**(202): 1417-1427
- [29] Allende ML et al. S1P1 receptor directs the release of immature B-cells from bone marrow into blood. *The Journal of Experimental Medicine*. 2010; **207**:1113-1124
- [30] Allman D, Pillai S. Peripheral B-cell subsets. *Current Opinion in Immunology*. 2008;**20**:149-157
- [31] Enders A et al. Zinc-finger protein ZFP318 is essential for expression of IgD, the alternatively spliced Igh product made by mature B lymphocytes. *Proceedings of the National Academy of Sciences of the United States of America*. 2014;**111**: 4513-4518
- [32] Gamrekelashvili J et al. Regulation of monocyte cell fate by blood vessels mediated by notch signalling. *Nature Communications*. 2016;**7**:12597
- [33] van Nierop K, de Groot C. Human follicular dendritic cells: Function, origin and development. *Seminars in Immunology*. 2002;**14**:251-257
- [34] Vinuesa CG, Chang P-P. Innate B-cell helpers reveal novel types of antibody responses. *Nature Immunology*. 2013;**14**:119-126
- [35] Lentz VM, Manser T. Cutting edge: Germinal centers can be induced in the absence of T-cells. *Journal of Immunology (Baltimore, Md)*. 2001; **150**(167):15-20

- [36] Mongini PK, Stein KE, Paul WE. T-cell regulation of IgG subclass antibody production in response to T-independent antigens. *The Journal of Experimental Medicine*. 1981;**153**:1-12
- [37] Smith KGC, Clatworthy MR. FcγRIIB in autoimmunity and infection: Evolutionary and therapeutic implications. *Nature Reviews. Immunology*. 2010;**10**:328-343
- [38] Jabs WJ, Paulsen M, Wagner HJ, Kirchner H, Klüter H. Analysis of Epstein-Barr virus (EBV) receptor CD21 on peripheral B lymphocytes of long-term EBV- adults. *Clinical and Experimental Immunology*. 1999;**116**: 468-473
- [39] Silverman GJ, Khanna S. B-cell modulation in rheumatology. *Current Opinion in Pharmacology*. 2007;**7**: 426-433
- [40] Hamouda L, Miliani M, Hadjidj Z, Messali R, Aribi M. Rituximab treatment modulates the release of hydrogen peroxide and the production of proinflammatory cytokines by monocyte at the onset of type 1 diabetes. *Endocrine, Metabolic & Immune Disorders Drug Targets*. 2019;**19**: 643-655
- [41] Rehnberg M, Amu S, Tarkowski A, Bokarewa MI, Brisslert M. Short- and long-term effects of anti-CD20 treatment on B-cell ontogeny in bone marrow of patients with rheumatoid arthritis. *Arthritis Research & Therapy*. 2009;**11**:R123
- [42] Pröbstel A-K, Hauser SL. Multiple sclerosis: B-cells take center stage. *Journal of Neuro-Ophthalmology*. 2018;**38**:251-258
- [43] Tedder TF, Tuscano J, Sato S, Kehrl JH. CD22, a B lymphocyte-specific adhesion molecule that regulates antigen receptor signaling. *Annual Review of Immunology*. 1997;**15**: 481-504
- [44] Erickson LD, Tygrett LT, Bhatia SK, Grabstein KH, Waldschmidt TJ. Differential expression of CD22 (Lyb8) on murine B-cells. *International Immunology*. 1996;**8**:1121-1129
- [45] Wen T et al. The pan-B cell marker CD22 is expressed on gastrointestinal eosinophils and negatively regulates tissue eosinophilia. *Journal of Immunology (Baltimore, Md)*. 2012;**190**(188):1075-1082
- [46] Benbijja M, Mellouk A, Bobé P. Sensitivity of leukemic T-cell lines to arsenic trioxide cytotoxicity is dependent on the induction of phosphatase B220/CD45R expression at the cell surface. *Molecular Cancer*. 2014;**13**:251
- [47] López-Granados E, Cambronero R, Ferreira A, Fontán G, García-Rodríguez MC. Three novel mutations reflect the variety of defects causing phenotypically diverse X-linked hyper-IgM syndrome. *Clinical and Experimental Immunology*. 2003;**133**: 123-131
- [48] Akatsu C et al. CD72 negatively regulates B lymphocyte responses to the lupus-related endogenous toll-like receptor 7 ligand Sm/RNP. *The Journal of Experimental Medicine*. 2016;**213**: 2691-2706
- [49] Tsubata T. CD72 is a negative regulator of B-cell responses to nuclear lupus self-antigens and development of systemic lupus erythematosus. *Immune Network*. 2019;**19**:e1
- [50] Van de Velde H, von Hoegen I, Luo W, Parnes JR, Thielemans K. The B-cell surface protein CD72/Lyb-2 is the ligand for CD5. *Nature*. 1991;**351**: 662-665

- [51] Biancone L et al. Identification of a novel inducible cell-surface ligand of CD5 on activated lymphocytes. *The Journal of Experimental Medicine*. 1996; **184**:811-819
- [52] Noh G, Lee JH. Regulatory B-cells and allergic diseases. *Allergy, Asthma & Immunology Research*. 2011; **3**:168-177
- [53] Jackson SM, Wilson PC, James JA, Capra JD. Human B-cell subsets. *Advances in Immunology*. 2008; **98**: 151-224
- [54] Yokota A et al. Two species of human Fc epsilon receptor II (Fc epsilon RII/CD23): Tissue-specific and IL-4-specific regulation of gene expression. *Cell*. 1988; **55**:611-618
- [55] Acharya M et al. CD23/FcεRII: Molecular multi-tasking. *Clinical and Experimental Immunology*. 2010; **162**: 12-23
- [56] Ahmed R et al. A public BCR present in a unique dual-receptor-expressing lymphocyte from type 1 diabetes patients encodes a potent T cell autoantigen. *Cell*. 2019; **177**: 1583-1599.e16
- [57] Corcos D et al. Pre-B-cell development in the absence of lambda 5 in transgenic mice expressing a heavy-chain disease protein. *Current Biology*. 1995; **5**:1140-1148
- [58] Woyach JA, Johnson AJ, Byrd JC. The B-cell receptor signaling pathway as a therapeutic target in CLL. *Blood*. 2012; **120**:1175-1184
- [59] Inabe K, Kurosaki T. Tyrosine phosphorylation of B-cell adaptor for phosphoinositide 3-kinase is required for Akt activation in response to CD19 engagement. *Blood*. 2002; **99**:584-589
- [60] Okkenhaug K, Burger JA. PI3K signaling in normal B-cells and chronic lymphocytic leukemia (CLL). *Current Topics in Microbiology and Immunology*. 2016; **393**:123-142
- [61] Jimenez C, Hernandez C, Pimentel B, Carrera AC. The p85 regulatory subunit controls sequential activation of phosphoinositide 3-kinase by Tyr kinases and Ras. *The Journal of Biological Chemistry*. 2002; **277**: 41556-41562
- [62] Morbach H et al. CD19 controls Toll-like receptor 9 responses in human B-cells. *The Journal of Allergy and Clinical Immunology*. 2016; **137**: 889-898.e6