

Clinical consequences of defects in B-cell development

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Activity Objectives

1. To describe the mechanism of B-cell development.
2. To understand the cell-surface antigens associated with B-cell development.
3. To understand the transcription factors and mediators allowing the maturation from stem cell to mature B cell.

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Abnormalities in humoral immunity typically reflect a generalized or selective failure of effective B-cell development. The developmental processes can be followed through analysis of cell-surface markers, such as IgM, IgD, CD10, CD19, CD20, CD21, and CD38. Early phases of B-cell development are devoted to the creation of immunoglobulin and testing of B-cell antigen receptor signaling. Failure leads to the absence of B cells and immunoglobulin in the blood from birth. As the developing B cells begin to express a surface B-cell receptor, they become subject to negative and positive selection pressures and increasingly depend on survival signals. Defective signaling can lead to selective or generalized hypogammaglobulinemia, even in the presence of normal numbers of B cells. In the secondary lymphoid organs some B cells enter the splenic marginal zone, where preactivated cells lie ready to rapidly respond to T-independent antigens, such as the polysaccharides that coat some microorganisms. Other

cells enter the follicle and, with the aid of cognate follicular T cells, divide to help form a germinal center (GC) after their interaction with antigen. In the GC B cells can undergo the processes of class switching and somatic hypermutation. Failure to properly receive T-cell signals can lead to hyper-IgM syndrome. B cells that leave the GC can develop into memory B cells, short-lived plasma cells, or long-lived plasma cells. The latter ultimately migrate back to the bone marrow, where they can continue to produce protective antigen-specific antibodies for decades. (*J Allergy Clin Immunol* 2010;125:778-87.)

Key words: *B-cell development, human, B-cell immune deficiencies, review*

Abnormal humoral immune function can manifest as immune deficiency, autoimmunity, or allergy. Many humoral immune disorders are caused by loss-of-function or altered-function mutations in genes involved in the regulation of B-cell differentiation, tolerance, or function. Others reflect mutations in the immunoglobulin genes themselves. In some conditions the genetic cause has been well defined. In others a genetic predisposition might have been recognized, but the underlying molecular or cellular defect remains unclear. The typical patient with a B-cell deficiency will present with a history of recurrent upper respiratory tract or pulmonary infections and will exhibit reduced serum concentrations of 1 or more classes or subclasses of IgM, IgG, or IgA. However, specific deficits in the ability to mount a protective response against certain antigens or organisms can occur even in the presence of normal serum immunoglobulin

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Abbreviations used

AID:	Activation-induced cytidine deaminase
APRIL:	A proliferation-inducing ligand
BAFF:	B-cell activating factor of the tumor necrosis family
BAFF-R:	B-cell activating factor of the tumor necrosis family receptor
BCR:	B-cell receptor
BLNK:	B-cell linker protein
BTK:	Bruton tyrosine kinase
CD:	Celiac disease
CD40L:	CD40 ligand
CSR:	Class-switch recombination
CVID:	Common variable immune deficiency
EBF:	Early B-cell factor
FDC:	Follicular dendritic cell
GC:	Germinal center
H chain:	Immunoglobulin heavy chain
NEMO:	Nuclear factor κ B essential modulator
PAX5:	Paired box protein 5
PLC γ :	Phospholipase C γ
RAG:	Recombination-activating gene
SHM:	Somatic hypermutation
TACI:	Transmembrane activator and calcium modulator and cyclophilin ligand interactor
TdT:	Terminal deoxynucleotidyl transferase
UNG:	Uracil-DNA glycosylase

levels, and some virtually agammaglobulinemic patients can be remarkably asymptomatic. Perhaps paradoxically, some patients who have difficulty defending against exogenous antigens can mount pathogenic responses to self-antigen, including red blood cells (autoimmune hemolytic anemia), platelets (idiopathic thrombocytopenic purpura), nuclear antigens (systemic lupus erythematosus), and tissues and organs of the endocrine system (eg, autoimmune thyroid disease, including Grave disease and Hashimoto thyroiditis). Others might be overrepresented in allergy clinics.

The diseases of humoral immunity often arise as a result of specific defects in the production or regulation of B cells and their antibody products. Clinical evaluation typically begins with a complete blood count and an assessment of serum immunoglobulin levels. Patients with a complete absence of immunoglobulin tend to also lack B cells, indicating an early block in B-cell development, whereas disorders of regulation typically result in abnormal levels of immunoglobulin in the presence of normal numbers of B cells (Table I).

B-CELL DEVELOPMENT BEGINS IN THE PRIMARY LYMPHOID ORGANS

B lymphocytes arise from multipotent hematopoietic stem cells that first appear in the embryo within the para-aortic splanchnopleure.¹ At 7 weeks' fetal gestation, these cells take up residence in the liver. By the middle of the second trimester, they can be found in bone marrow, which, just before birth, becomes their exclusive home. In these organs daughter cells give rise to lymphoid-primed multipotent progenitors that produce common lymphoid precursors, which can generate T cells, B cells, natural killer cells, and dendritic cells.^{2,3} Final B-cell differentiation requires the exposure of common lymphoid progenitor daughter cells to specialized microenvironments, such as those found in the fetal liver and bone marrow.³ B cells continue to be produced

in the bone marrow throughout the life of the subject, although the rate of production decreases with age.⁴

Although a sophisticated reader will recognize the oversimplification of what is a very complex process, B-cell differentiation (Fig 1) is typically conceptually viewed as a linear pathway defined by the regulated expression of specific sets of transcription factors, immunoglobulin gene products, and cell-surface molecules. Deficiencies in the function of the transcription factors that control early B-cell development often block B-cell production (Fig 1). B-lymphocyte development requires the action of cytokines and transcription factors that positively and negatively orchestrate gene expression. In mice stromal cell-derived IL-7 promotes V to DJ rearrangement and transmits survival signals.⁵ In human subjects, however, IL-7 does not appear to be essential for B-lineage differentiation, and there are active ongoing efforts to define the bone marrow stromal cell-derived cytokines that transduce the signals necessary for B-cell development in human subjects.^{6,7}

Immunoglobulin is the primary product of B cells, and early studies viewed B-cell development through the prism of antibody generation. Immunoglobulins are the product of a complex process of V[D]J gene rearrangement events, which typically proceed in a hierarchic pattern. Pro-B cells are marked by the initiation of recombination-activating gene (RAG) 1/RAG2-mediated rearrangement of the D_H-J_H locus. RAG-mediated rearrangement also underlies production of the T-cell receptor, and hence RAG dysfunction can prevent production of both T and B cells, yielding a severe combined immune deficiency. Incomplete blocks in V[D]J rearrangement permit the oligoclonal survival of B and T cells. This condition can manifest as Omenn syndrome, which typically presents with pruritic skin lesions, fever, lymphadenopathy, anemia, eosinophilia, and chronic diarrhea.⁸

D_H → J_H joining is followed by V_H → DJ_H rearrangement. Terminal deoxynucleotidyl transferase (TdT) catalyzes the addition of random N nucleotides between these rearranging gene segments and is thus critical for the development of a diverse antigen receptor repertoire. Human patients with a loss of function of TdT have yet to be identified.

Immunoglobulins have a very short cytoplasmic domain. They depend on their association with Ig α (CD79a) and Ig β (CD79b) to form a B-cell receptor (BCR) complex that can transduce the binding signal into the cell. Ig α and Ig β , as well as VpreB and λ 14.1 (λ 5), which together form the surrogate light chain (ψ LC), are constitutively expressed by pro-B cells. Activation of these immunoglobulin-associated genes requires transcription factors. PU.1, an ETS family loop-helix-loop (winged helix) transcription factor, appears to help regulate expression of CD79a, RAG1, and TdT, as well as the 3 immunoglobulin loci: μ , κ , and λ . Ikaro, a zinc finger transcription factor, also promotes expression of TdT, λ 14.1 (λ 5), and VpreB.^{9,10} The E2A locus encodes 2 basic helix-loop-helix transcription factors that represent 2 alternately spliced products, E12 and E47.¹¹ E47 promotes TdT and RAG1 expression better than E12, whereas E12 promotes expression of early B-cell factor (EBF) and paired box protein 5 (PAX5) better than E47. EBF, another helix-loop-helix transcription factor, promotes early B-cell development. EBF is expressed at all stages of differentiation except plasma cells, and in mice has been shown to be critical in the progression of B cells past the early pro-B cell stage. PAX5, a paired-box transcription factor, is expressed exclusively in B-lineage cells. In mice B-cell precursors require PAX5 to progress beyond the pro-B cell stage 2. The presence of PAX5 also prevents early B-lineage progenitors from transiting into other

TABLE I. Selected manifestations of abnormal B-cell development

	Humoral immunity Serum immunoglobulins				Antibody responses	Common infections	Other immunologic and clinical features
	M	G	A	E			
Predominantly B-cell deficiency							
Failure to create immunoglobulin							
Recombination-activating gene (RAG) 1/2 deficiency	↓	↓	↓	↓	—	Bacteria, viruses, fungi	Might present with Omenn syndrome
Artemis deficiency (SCIDA)	↓	↓	↓	↓	—	"	Radiation sensitivity, might present with Omenn syndrome
Cernunnos deficiency	↓	↓	↓	↓	—	"	Radiation sensitivity
DNA ligase IV (LIG4) deficiency	↓	↓	↓	↓	—	"	Radiation sensitivity, might present with Omenn syndrome
Failure to create the BCR							
Autosomal agammaglobulinemia							
Recessive: λ14.1, VpreB, Igα (CD79a), Igβ (CD79a)	↓	↓	↓	↓	—	Bacteria, <i>Giardia lamblia</i>	Normal numbers of pro-B cells
Failure to permit the BCR to transduce its signal							
Autosomal agammaglobulinemia							
Recessive: BLNK deficiency	↓	↓	↓	↓	—	Bacteria, <i>Giardia lamblia</i>	Normal numbers of pro-B cells
X-linked agammaglobulinemia (BTK deficiency)	↓	↓	↓	↓	—	"	Normal numbers of pro-B cells
Predominantly antibody deficiency							
Failure of follicular T-cell/B-cell interactions							
Hyper-IgM syndrome							
X-linked CD40L deficiency	N/↑	↓	N/↓	↓	+/—	Bacteria, viruses, fungi	ITP, AIHA, neutropenia, liver and biliary disease
X-linked IKK-γ (NEMO) deficiency	N/↑	↓	↓	↓	+/—	"	Anhidrotic epidermal dysplasia, antipolysaccharide deficiencies
CD40 deficiency	N/↑	↓	N/↓	↓	+/—	"	Neutropenia, gastrointestinal and liver/biliary tract disease
Failure to modify immunoglobulin genes							
Hyper-IgM syndrome							
AID deficiency	N/↑	↓	↓	↓	+/—	Bacteria	Lymphadenopathy
UNG deficiency	N/↑	↓	↓	↓	+/—	"	Lymphadenopathy
Alteration of BCR or B-cell survival signals							
CD20 deficiency	N/↑	N/↓	N		+/—	Bacteria	Antipolysaccharide deficiency, decreased memory cells
Selective IgA deficiency (TACI dysfunction)	N	N	↓	N	+/—	Bacteria, <i>Giardia lamblia</i>	SLE (2% to 5%), CD (3%), RA (2%), ITP (0.5%), AR, allergic rhinitis
CVID (ICOS, TACI, BAFF-R or CD19 deficiency or dysfunction)	N/↓	↓	↓	↓	—	"	AIHA, ITP, ATD, SLE
Unknown							
Selective inability to respond to bacterial polysaccharides	N	N	N	N	+/—	Bacteria	
Selective IgA deficiency (MHC associated)	N	N	↓	N	+/—	Bacteria, <i>Giardia lamblia</i>	SLE (2% to 5%), CD (3%), RA (2%), ITP (0.5%), AR
IgG subclass deficiency	N	N/↓	N/↓	N	+/—	+/— Bacteria	
CVID (MHC associated)	N/↓	↓	↓	↓	—	Bacteria, <i>Giardia lamblia</i>	AIHA, ITP, ATD, SLE

AIHA, Autoimmune hemolytic anemia; AR, allergic rhinitis; ATD, autoimmune thyroid disease; CD, celiac disease; ICOS, inducible T-cell co-stimulator; ITP, idiopathic thrombocytopenic purpura; N, normal; NEMO, nuclear factor κB (NF-κB) essential modulator; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

hematopoietic pathways. Aiolos, a zinc finger transcription factor, is expressed after commitment to the B-cell lineage. Deficiencies of these transcription factors have not yet been defined in human subjects, but in mice aiolos deficiency leads to autoantibody production and B-cell lymphomas.^{12,13}

Production of a properly functioning BCR is essential for development beyond the pre-B cell stage, which is classically defined by the cytoplasmic expression of a complete μ immunoglobulin heavy chain (H chain) protein. μ H chains in pre-B cells attempt to associate with VpreB and λ14.1 (λ5). Successful

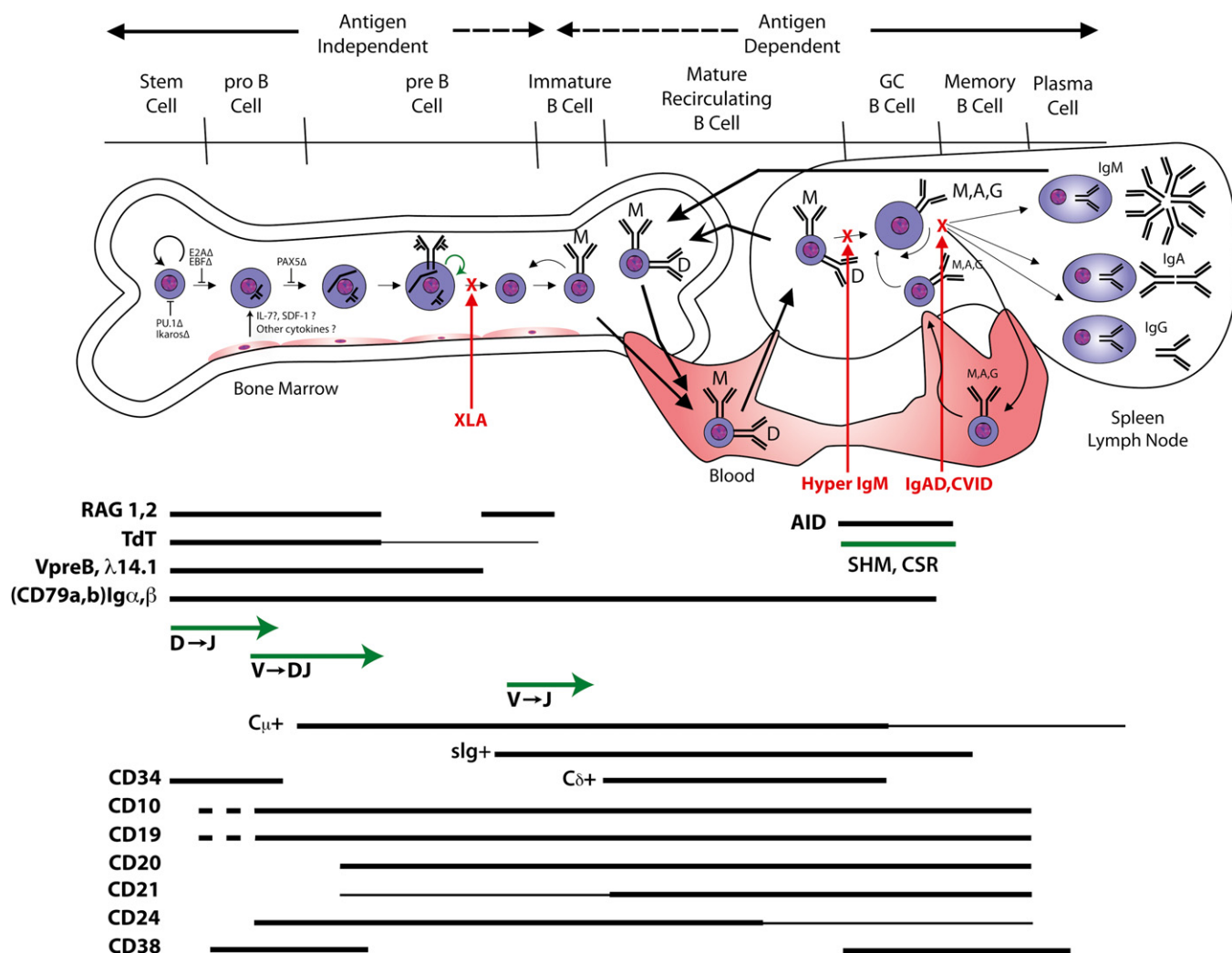


FIG 1. B-cell development illustrated as a linear progression through developmental checkpoints. Proper assembly of the B-cell antigen receptor complex is required. The expression pattern of key surface molecules during this process is marked by bars. Also shown are the developmental checkpoints at which loss of function (Δ) of selected transcription factors can influence B-cell development.

pairing creates a pre-B cell receptor, which signals the termination of further H chain rearrangement (allelic exclusion).¹⁴ This is followed by 4 to 6 cycles of cell division,² a process associated with a progressive decrease in cell size. Late pre-B daughter cells reactivate RAG1 and RAG2 and begin to undergo $V_L \rightarrow J_L$ rearrangement. Successful production of a complete κ or λ light chain permits expression of conventional IgM on the cell-surface membrane, which identifies the immature B cell. It is at this stage that the antigen specificity of the antibody begins to play the critical role in further development.

An intact and functional antigen BCR complex, which consists of the Ig α and Ig β coreceptors in association with either the pre-B receptor or mature membrane-bound immunoglobulin, must be present for the developing B cell to survive. Thus loss-of-function mutations of the μ heavy chain, the components of the surrogate light chain, or CD79 all yield a B cell-deficient agammaglobulinemia.¹⁵⁻¹⁸

The early repertoire appears to be enriched for self-reactive antibodies.¹⁹ Many of these self-reactive cells undergo repeated

rounds of light chain rearrangement that lessen but do not necessarily abolish the self-specificity of their BCR, a process termed receptor editing.²⁰ Other potentially pathogenic self-reactive B cells are inactivated by cell anergy or apoptosis of the host cell, although the details of this process in human subjects are not yet well described. Self-reactivity can also be beneficial, and some self-reactive antibodies, especially those enriched for germline sequence, contribute to what is termed the germline natural antibody repertoire.²¹ These antibodies are believed to form the first line of defense against common pathogens and are thus considered to belong to the innate portion of the adaptive immune response.²² Some of these antibodies are expressed in the marginal zone of the spleen. In patients with congenital asplenia, this population does not survive, and this line of defense never forms.²³

Immature B cells that have successfully produced an acceptable IgM BCR extend transcription of the H chain locus to include the C δ exons downstream of C μ . Alternative splicing permits coproduction of IgM and IgD. These now newly mature IgM⁺IgD⁺ B cells enter the blood and migrate to the periphery,

where they form the majority of the B-cell pool in the spleen and the other secondary lymphoid organs. The IgM and IgD on each of these cells share the same variable domains. The function of IgD remains controversial.

TYROSINE KINASES CAN PLAY KEY ROLES IN B-CELL DEVELOPMENT

It is not enough to simply express a BCR complex: that complex must be able to transduce a signal into the cell. Unsurprisingly, loss-of-function mutations in components of the signal transduction cascade also inhibit B-cell development. X-linked agammaglobulinemia, the first genetic form of B-cell immune deficiency to be recognized, serves as the classic example of the need for an intact signal cascade through the phospholipase C γ (PLC γ) pathway. Mutations in Bruton tyrosine kinase (BTK) are the genetic basis of X-linked agammaglobulinemia and account for 85% of patients.²⁴ BTK, a member of the Tec family of cytoplasmic tyrosine kinases, is part of the BCR and pre-BCR signal transduction pathway.²⁵ The B-cell linker protein (BLNK) is an Src homology 2 domain-containing signal transduction adaptor. When phosphorylated by SYK, BLNK serves as a scaffold for the assembly of cell activation targets that include GRB2, VAV, NCK, and PLC γ .^{26,27} The Src homology 2 domains of activated BTK and PLC γ 2 bind to the scaffold protein BLNK, allowing BTK to phosphorylate PLC γ 2. This leads to activation of other kinases, resulting in calcium influx and also activation of several transcription factors, which is essential for the B-lineage development and function.²⁸⁻³⁰ Both BTK and BLNK deficiency result in the arrest of B-cell development at the pre-B cell stage,^{31,32} yielding an agammaglobulinemic state.

CELL-SURFACE ANTIGENS ASSOCIATED WITH B-CELL DEVELOPMENT

Immunoglobulin is poorly or not expressed on the cell surface before the immature B-cell stage, and after that stage, immunoglobulin is expressed throughout B-cell development until the plasma cell stage. Hence early and late stages of development are typically identified through the analysis of the surface expression of other cell-surface markers. Of these, CD10, CD19, CD20, CD21, CD24, CD34, and CD38 are of particular importance (Fig 1), and assessment of their expression is often used clinically to either identify specific functionality or as a target for clinical intervention.

CD34 is expressed on a small population (1% to 4%) of bone marrow cells that includes hematopoietic stem cells. It is a highly glycosylated type I transmembrane glycoprotein that binds to CD62L (L-selectin) and CD62E (E-selectin) and likely aids in cell trafficking. CD34 expression is often used to assess, identify, or extract hematopoietic stem cells from cord blood or bone marrow.

CD10 is expressed just before initiation of DJ rearrangement. It is also known as neprilysin, neuroendopeptidase, or the common acute lymphocytic leukemia antigen. CD10 is a type II membrane glycoprotein metalloprotease that is thought to downregulate cellular responses to peptide hormones and cytokines by virtue of its protease activity. Because of its early expression pattern, CD10 is often used as a marker for pre-B acute lymphocytic leukemias and certain lymphomas.³³

CD19 is a cell-surface glycoprotein of the immunoglobulin superfamily. It is exclusively expressed throughout B-cell development from the pro-B cell stage up to, but not including, the plasma cell stage (Fig 1).³⁴ This broad expression pattern makes it the best cell-surface marker for enumerating B cells in clinical studies. Internally, CD19 associates with phosphoinositide 3-kinase and the tyrosine kinase VAV.

CD19 exists in a complex with CD21 (complement receptor 2), CD81 (target of antiproliferative antibody 1 - TAPA-1), and Leu 13. CD21 (complement receptor 2) is a cell-surface protein that contains a small cytoplasmic domain and an extracellular domain consisting of a series of short consensus repeats termed complement control protein domains. These extracellular domains can bind 3 different products of complement C3 cleavage: iC3b, C3dg, and C3d. With the help of CD21, CD19 can bind the complement C3 cleavage product C3d. The simultaneous binding of sIgM and CD19 to a C3d-antigen complex enables CD19 and the BCR to interact and thereby provide a link between innate and adaptive immune responses. EBV can use CD21 as a receptor, allowing entry into mature B cells. Once integrated into the cell, EBV gene products transform the cells, permitting creation of long-lived B-cell lines. These lines are an excellent source of replenishable DNA or monoclonal human immunoglobulins.

CD19 is not essential for transduction to occur, and thus it is not required for B-cell production. However, it is required for appropriate modulation of that signal. In the absence of CD19, full activation and maturation of the B cell is inhibited, yielding panhypogammaglobulinemia in the presence of normal numbers of B cells in the blood.³⁵ This phenotype is a characteristic of common variable immune deficiency (CVID), the most common phenotype of antibody deficiency under the care of the clinical immunologist, although CD19 deficiency contributes to only a small subset of patients with CVID.

CD20 appears to function as a B-cell Ca²⁺ channel subunit and regulates cell-cycle progression. It is a member of the CD20/Fc ϵ RI β superfamily of leukocyte surface antigens. CD20 can interact directly with MHC class I and II molecules, as well as members of another family of 4 transmembrane domain proteins known as the TM4SF, such as CD43, CD81, and CD82. It also appears to interact indirectly with LYN, FYN, and LCK and can thus also modulate signal transduction in B cells.³⁶ Deficiency of CD20 can lead to hypogammaglobulinemia in the presence of normal B-cell numbers and an inability to mount antipolysaccharide responses.³⁷

The pattern of expression of CD20, which is present on mature B cells through plasmablasts but not on early stem cells or plasma cells, makes it an outstanding target for B cell-ablative therapy. Antibodies directed against CD20, such as rituximab, effectively reduce B-cell numbers without affecting the ability to produce new B cells or inhibiting the production of protective antibodies by long-lived plasma cells. Although originally developed to treat lymphoma, it is being used successfully for the treatment of rheumatoid arthritis and other autoimmune diseases.³⁸

CD24 is a glycosylphosphatidylinositol-linked (GPI-linked) sialoprotein that serves as a ligand for P-selectin (CD62P). It is expressed on progenitor, immature, and mature B cells. Its expression decreases in activated B cells and is lost entirely in plasma cells. This cell-surface marker can be used to characterize the state of B-cell development for diagnostic purposes.

CD38 is first expressed on pre-B cells but is downregulated in immature and mature B cells. It is a bifunctional enzyme that can

synthesize cyclic ADP-ribose from nicotinamide adenine dinucleotide and also hydrolyze cyclic ADP-ribose to ADP-ribose. It is presumed that the enzyme exists to ADP-ribosylate target molecules. CD38 is re-expressed in activated B cells and early plasma cells, but its expression is again lost in mature plasma cells. This pattern of expression makes CD38 an excellent marker to identify B cells from the blood or lymphoid organs that are activated or proceeding to the plasmablast stage. CD38 expression has also been used to distinguish between aggressive and more benign forms of chronic lymphocytic leukemia.³⁹

B-CELL ACTIVATING FACTOR OF THE TUMOR NECROSIS FAMILY AND A PROLIFERATION-INDUCING LIGAND CAN PLAY KEY ROLES IN THE DEVELOPMENT OF MATURE B CELLS

B cells leave the bone marrow while still undergoing initial maturation, demonstrating progressively higher levels of IgD expression with a commensurate decrease in IgM levels. They need the splenic environment to complete this maturation process. Immigrant splenic maturing B cells pass through a series of transitional stages, which balance responses between B-cell activating factor of the tumor necrosis family (BAFF) and its receptor, BAFF-R, with activation of the BCR by self-antigen. Only a minority of newly arisen cells will successfully make this transition. Death signals triggered through interaction of the BCR with self-antigen might be counterbalanced by stimulation of BAFF-R, which is expressed primarily on B cells.⁴⁰ BAFF-R activation enhances expression of survival factors, such as BCL-2, and at the same time downregulates proapoptotic factors.

BAFF and a second TNF family member, a proliferation-inducing ligand (APRIL), can also induce isotype switching in naive human B cells.⁴¹ APRIL, as well as BAFF, can bind to B-cell maturation antigen and transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), both of which are members of the TNF receptor family. B-cell maturation antigen is exclusively expressed on B cells, whereas TACI is expressed on B cells and activated T cells.

The importance of BAFF-R and TACI for the long-term survival of B cells is underscored by the effect of loss-of-function or altered function mutations, which promote the development of the phenotypes of selective IgA deficiency or panhypogammaglobulinemia with normal numbers of newly arisen B cells (CVID).

B-CELL DEVELOPMENT IN THE PERIPHERY

The lifespan of mature B cells expressing surface IgM and IgD appears entirely dependent on exposure to antigen. Mature unstimulated cells can live for only days or weeks. Antigens from peripheral sites are transported into the secondary lymphoid organs by dendritic cells, macrophages, and other highly specialized cells. Circulating lymphocytes within these organs then survey the antigens. As originally postulated by Burnet's clonal selection theory, B cells are rescued from apoptosis by their response to a cognate antigen. The reaction to antigen leads to activation, which might then be followed by diversification.

Spleen

In the secondary lymphoid organs T and B cells are segregated into clearly defined areas. Fig 2 shows the pattern observed in the

white pulp of the spleen. It is in these areas where the antigen-dependent B-cell activation occurs and where the cells subsequently undergo further differentiation. The marginal sinuses, the site of entry for lymphocytes, macrophages, and dendritic cells, separate the white pulp from the red pulp. These sinuses are lined with a mucosal addressin cell adhesion molecule 1-expressing endothelium. In the marginal sinuses one finds a specialized layer of metallophilic macrophages that are thought to control the entry of antigen into the white pulp.

Follicular dendritic cells (FDCs) are highly specialized cells that present antigen to B cells. In contrast to other types of dendritic cells, FDCs do not process antigen. Instead, FDCs have abundant complement receptors and immunoglobulin Fc receptors that allow accumulation of antigen in the form of immune complexes within the B follicle. FDCs are crucial for B-cell maintenance, as well as for their activation and differentiation.

Mature B cells are not homogenous. Functionally and developmentally distinct subsets exist. In the spleen follicular B cells have a key role in the adaptive immune response, whereas marginal zone B cells are now seen as major players at the interface between the initial innate immune response and the delayed adaptive response.⁴² The ability of marginal zone B cells to rapidly respond to encapsulated bacteria by differentiating into antigen-specific plasma cells helps keep such infections under control. Marginal zone B cells take time to develop, and the marginal zone is not fully populated until after the age of 2 years. Asplenic patients have difficulty producing normal numbers of marginal zone B cells,²³ and a poor response to blood-borne infections can be observed.⁴³

T-independent responses

The nature of the activation process is critical. Unlike T cells, which require presentation of antigen by other cells, B cells can respond directly to an antigen as long as that antigen is able to cross-link the antibodies on the B-cell surface. Such antigens, especially those that by nature cannot be recognized by T cells (eg, DNA or polysaccharides) can induce a B-cell response independent of T-cell help. T-independent responses can lead to class switching, depending on the cytokine milieu. For example, antipolysaccharide responses are typically linked to production of IgG2. B cells that are activated by antigen alone do not take part in a germinal center (GC) reaction.

Clinical evaluation of the ability of the patient to produce IgM T-independent responses can be made by assessing serum isohemagglutinin titers because the sugars that define blood types A and B are also found on bacteria. Vaccination with 23 valent Pneumovax with a subsequent comparison of prevaccination and postvaccination titers to pneumococcal polysaccharides permits assessment of the ability to produce T-independent IgG antibodies.

T-dependent responses

Binding to a cognate antigen activates antigen-specific B and T cells. Activated B cells express both MHC class I and II molecules on their cell surface. They can thus present both intracellular and extracellular antigens to CD4 T_H and CD8 T-cytotoxic lymphocytes. Their role as antigen-presenting cells is enhanced when they present peptides from the same antigen they have taken up with their antibodies. Cognate recognition of the same antigen by both a B cell and a T cell permits each of these cells to

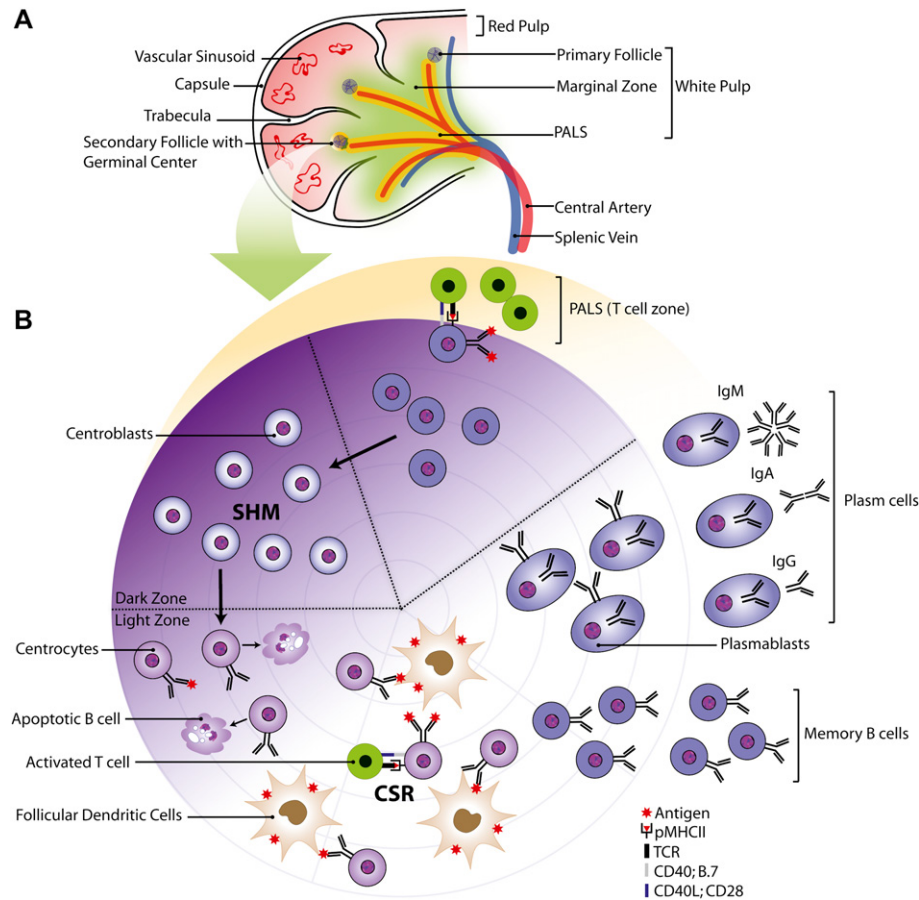


FIG 2. Schematic morphology of the spleen (A) and the GC reaction (B). The white pulp consists of a central artery surrounded by the periarterial lymphatic sheath (PALS) or T cell zone, the marginal zone, and the follicles. Antigen-specific B and T cells interact at the border of the follicle. The B cells differentiate into centroblasts and undergo clonal expansion in the GC dark zone.

reciprocally activate the other. In particular, T cell-activated B cells express the costimulatory molecules CD80 and CD86, which are in turn required for activation of T cells through CD28, as well as their inactivation by CD152 (cytotoxic T lymphocyte-associated antigen 4). Because B cells do not express IL-12, they do not induce expression of IFN- γ in the activated T cells but rather favor the differentiation of activated T cells into IL-4-, IL-5-, IL-10-, and IL-13-expressing T_H2 cells. These cytokines can support the CD40-induced expansion of memory B cells (IL-4), OX40- or CD27-induced differentiation into short-lived and long-lived plasma cells (IL-10), and CD40-induced class-switch recombination (CSR) to IgG4 or IgE (IL-4). Costimulation also permits T_H cells to induce somatic hypermutation (SHM), permitting affinity maturation.⁴⁴

GCs

The GC is the microenvironment in which affinity maturation of the humoral immune response and class switching takes place.^{45,46} GCs develop only after T cell-dependent activation of B cells. Their full function is dependent on the interaction between CD40 expressed on B cells and CD40 ligand (CD40L; CD154) expressed on activated T cells. Patients with loss-of-function mutations in CD40L have high serum levels of IgM and recurrent infections (hyper-IgM syndrome).⁴⁷ After the

common pattern, disruption of normal regulation through CD40/CD40L, including through its signal transduction cascade (eg, NEMO deficiency), permits the production of pathogenic IgM autoantibodies that are frequently directed against hematopoietic cells, including red blood cells, white blood cells, and platelets.

It takes about a week for the complex GC structure to develop in a primary immune response. After activation of antigen-specific B and T cells, small clusters of proliferating B cells are observed at the border of the T-cell zone and the primary B-cell follicle.⁴⁸ The rapidly expanding antigen-activated B-cell clone seems to push the naive B cells toward the edge of the primary follicle. Naive B cells form a mantle zone around the newly developing GC, and the primary follicle changes into a secondary follicle containing within it a network of FDCs and follicular T cells. About the second week after immunization, the GC matures into a classical structure that contains a dark zone and a light zone. In the fully developed GC, dividing cells are termed centroblasts, whereas differentiating cells within the FDC network are termed centrocycles (Fig 2, B).

In the dark zone proliferating B cells induce SHM of their immunoglobulin variable domains, consisting of single nucleotide changes or microdeletions.⁴⁹ By chance, 1 or more of these mutations might give rise to a receptor with altered affinity for the stimulating antigen, creating a clone of variants expressing

antigen receptors of various affinities. B cells expressing higher-affinity receptors are presumed to compete more effectively for the antigen, which becomes rate limiting. B cells can move from the dark zone to the light zone and back again.⁵⁰ In the light zone B cells can undergo class switching.

Only the few B cells with a high-affinity receptor for the antigen ultimately survive. The rest of the B-cell variants die of apoptosis, presumably because of the lack of sufficient stimulation through the B-cell antigen receptor complex. In the light zone, which is surrounded by a network of FDCs, the B cells can differentiate into plasma cells or memory cells (Fig 2, B).

SHM and CSR

Immunoglobulin SHM and CSR are essential mechanisms for the generation of a high-affinity, adaptive humoral immune response. They allow the generation of effector plasma cells secreting high-affinity IgG, IgA, and IgE antibodies.

Hypermutation occurs only during a narrow window in B-cell development. The mechanism is induced during B-cell proliferation within the GC dark zone.⁵¹ With a high rate of about 10^{-3} to 10^{-4} /base pair/generation, single nucleotide exchanges are introduced in a stepwise manner into the rearranged V region and its 3' and 5' flanking sequences. Mutations are randomly introduced, although there is a preference for transitions (cytidine → thymidine or adenosine → guanine) over transversions. Analysis of the pattern of somatic mutations has revealed that the sequence of the complementarity-determining regions, the loops that form the antigen-binding site, have been selected to form mutation hot spots.

B cells can switch from expression of their V_HDJ_H exon with C_μ to expression of the same V_HDJ_H exon with any of the downstream C_H genes (eg, $C_{\alpha 1,2}$, $C_{\gamma 1,2,3,4}$, or C_ϵ) in a process known as class switching. Class switching, like SHM, is a hallmark of B-cell activation. It can be induced by T cell-independent signals (eg, LPS) or by signals derived from T cells (eg, CD40L). CD40L-deficient human subjects (X-linked hyper-IgM syndrome) are severely impaired in the expression of immunoglobulin classes other than IgM.⁴⁷

The choice of C_H gene targeted for switch recombination in a particular B cell appears to be dependent on external cytokine signals, such as those from T cells. IFN- γ targets CSR to IgG2, IL-4 to IgG4 and IgE, and TGF- β to IgA.

Both molecular mechanisms, CSR and SHM, are dependent on an activation-induced cytidine deaminase (AID)⁵²⁻⁵⁴ and uracil-DNA glycosylase (UNG). AID converts cytidine nucleotides into uracil. UNG removes the uracil, permitting its replacement with any of the 4 nucleotides facilitating SHM. AID activity at sites of runs of cytidine, such as the switch regions upstream of individual heavy chain genes, followed by UNG elimination of the resultant runs of uracil, allow the DNA double helix to fray, permitting the double-stranded breaks necessary to allow class switching. Patients with homozygous AID or UNG loss-of-function mutations present with a hyper-IgM syndrome⁵⁵ but one that is not associated with the production of autoantibodies.

Both mechanisms, SHM and CSR, have to be tightly controlled because the introduction of mutations and double-strand breaks into the DNA can not only pose a risk to the longevity of the B cell but also lead to activating translocations and mutations of oncogenes.⁵⁶ For example, for Burkitt lymphoma cells and plasma cell-derived myeloma cells, the translocation and ectopic expression of the c-myc gene is an apparent consequence of abnormal SHM and CSR.

B-CELL MEMORY

Plasma cells

One of the key features of the immune system is immunologic memory for antigens encountered in the past. Immunologic memory of B lymphocytes is dependent on T_H lymphocytes. Although primary B-cell responses start with secreted low-affinity IgM antibodies after a lag phase of 1 to 2 days and only gradually develop high-affinity antibodies of other classes, secondary responses start faster and with high-affinity antibodies of IgM and other classes. This protective humoral memory is provided by long-lived plasma cells,⁵⁷ which are generated in the secondary lymphoid organs and then migrate to the bone marrow or to a site affected by inflammation. In the bone marrow they can survive for long periods, providing long-term protection against previous activating antigens.

Memory B cells

Memory B cells are derived from naive B cells activated by antigen and T-cell help in extrafollicular or GC reactions (Fig 2, B). The differentiation to memory B cells is critically dependent on CD40 of the B cell and CD40L expressed by T cells and FDCs. Inactivation of the CD40 or CD40L genes by targeted mutation in the murine germline or by accidental mutation in human subjects leads to a hyper-IgM syndrome and a general lack of B-cell memory.⁵⁸

In human subjects CD27 expression is often used as a marker of the memory B-cell phenotype. Three types of CD27⁺ memory B cells have been identified in the circulation: IgM⁺IgD⁻CD27⁺ IgM-producing memory B cells, IgM⁻IgD⁻CD27⁺ class-switched memory B cells, and IgM⁺IgD⁺CD27⁺ marginal zone-like memory B cells.⁵⁹ This latter population has been shown to produce antipolysaccharide antibodies. Recently, it has been shown that some memory B cells might not express CD27⁶⁰ but instead express Fc receptor-like 4,^{61,62} complicating the analysis of this critical B-cell subset.

LOCAL PRODUCTION OF IMMUNOGLOBULIN IN THE MUCOSAL TISSUES

The gastrointestinal and respiratory mucosal epithelia, which are constantly exposed to the external environment, are sites of complex interactions between the body microorganisms, ranging from commensal bacteria to parasites. Proper defense appears to require the local production of immunoglobulin, especially IgA and IgE. Although serum IgA concentration is a fraction of the level of IgG, local mucosal production of IgA makes it the most abundant immunoglobulin isotype in the body, comprising at least 70% of the total immunoglobulin produced.⁶³ Indeed, approximately 80% of IgA-secreting cells reside in the gut mucosa. The mechanisms that regulate the preferential migration of IgA-producing cells to the gut and other mucosal surfaces, such as mammary glands of lactating woman, salivary glands, and the alveolar tract, are an active area of investigation. Special adhesion molecules and the local environment appear to be contributing elements.⁶⁴

In the absence of parasites, local IgE production in the epithelia and mucosa often permits allergic reactions. Local restriction to the mucosal tissues at sites of exposure to antigen reduces the likelihood of systemic anaphylaxis.⁶⁵

DEVELOPMENT OF ECTOPIC LYMPHOID TISSUE IN PATIENTS WITH AUTOIMMUNE DISEASE

In patients with immune diseases, such as myasthenia gravis, Sjögren syndrome, Hashimoto thyroiditis, or rheumatoid arthritis, ectopic lymphoid tissue can develop in the affected tissue or organ.^{66,67} The presence of proinflammatory cytokines might support the development of additional lymphoid tissue. In some patients well-organized, large lymphocyte structures can develop that are similar in appearance to the lymphoid follicles seen in the secondary lymphoid organs. At the center of these cell clusters one finds a network of FDCs. Antigen presented by the FDCs appears to activate B cells, which induces proliferation. A layer of T cells, which might support B-cell differentiation into plasma cells, surrounds the central B cells. There are indications that normal processes of antibody repertoire selection might not work properly within such ectopic lymphoid tissue, contributing to the production of potentially hazardous antibodies. This and other questions remain active topics of investigation.

CONCLUDING REMARKS

The study of B-cell development has led to a greater understanding of the mechanisms that underlie the pathogenesis of B-cell immune deficiencies, as well the development of reagents, such as antibodies directed against critical cell-surface molecules, that can be used for the diagnosis or treatment of immune disorders. Conversely, the study of immune deficiencies has yielded greater understanding of the mechanisms that underlie normal development of the humoral immune response and the recognition that B-cell immune deficiencies are largely the consequence of abnormal B-cell development. Although the molecular nature of the majority of known B-cell immune deficiencies has now been defined, the molecular nature of the most common B-cell immune deficiencies, such as selective IgA deficiency, symptomatic IgG subclass deficiencies, selective deficits in the ability to respond to bacterial polysaccharides, and CVID, remain unknown. Based on past history, it is likely that the dynamic of twin investigations into development in normal and diseases states will ultimately yield the answers to these remaining clinical conundrums and lead to new diagnostic tools and therapeutic agents.

What do we know?

- The anatomic sites of early B-cell development and the basic mechanisms that permit rearrangement of immunoglobulin genes and production of soluble antibody
- The molecular basis of most Mendelian immune deficiency disorders
- The molecular basis of basic BCR signal transduction pathways

What is still unknown?

- The signals that control early B-cell development in human subjects
- The signals that control the migration and activation of IgA- and IgE-producing cells in the mucosa and the development of marginal zone B cells
- The mechanisms that underlie the development of most cases of CVID

- The mechanisms that underlie most selective antipolysaccharide deficiencies
- The mechanisms that regulate the composition of the natural antibody repertoire
- The mechanisms that determine which B cells will be permitted to form long-lived plasma cells and which will form memory B cells
- The nongenetic (environmental?) mechanisms that predispose to the development of CVID and hypogammaglobulinemia
- The mechanisms that trigger autoimmunity in immune-deficient hosts

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