B-Cell Development, Activation, and Differentiation

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Lymphoid tissues

- Primary
  - Bone marrow
  - Thymus
- Secondary
  - Lymph nodes
  - Spleen
  - Tonsils
  - Lymphoid tissue within GI and respiratory tracts

Overview of B cell development

- B cells are generated in the bone marrow
- Takes 1-2 weeks to develop from hematopoietic stem cells to mature B cells
- Sequence of expression of cell surface receptor and adhesion molecules which allows for differentiation of B cells, proliferation at various stages, and movement within the bone marrow microenvironment
- Immature B cell leaves the bone marrow and undergoes further differentiation
- Immune system must create a repertoire of receptors capable of recognizing a large array of antigens while at the same time eliminating self-reactive B cells

Overview of B cell development

- Early B cell development constitutes the steps that lead to B cell commitment and expression of surface immunoglobulin, production of mature B cells
- Mature B cells leave the bone marrow and migrate to secondary lymphoid tissues
- B cells then interact with exogenous antigen and/or T helper cells = antigen-dependent phase

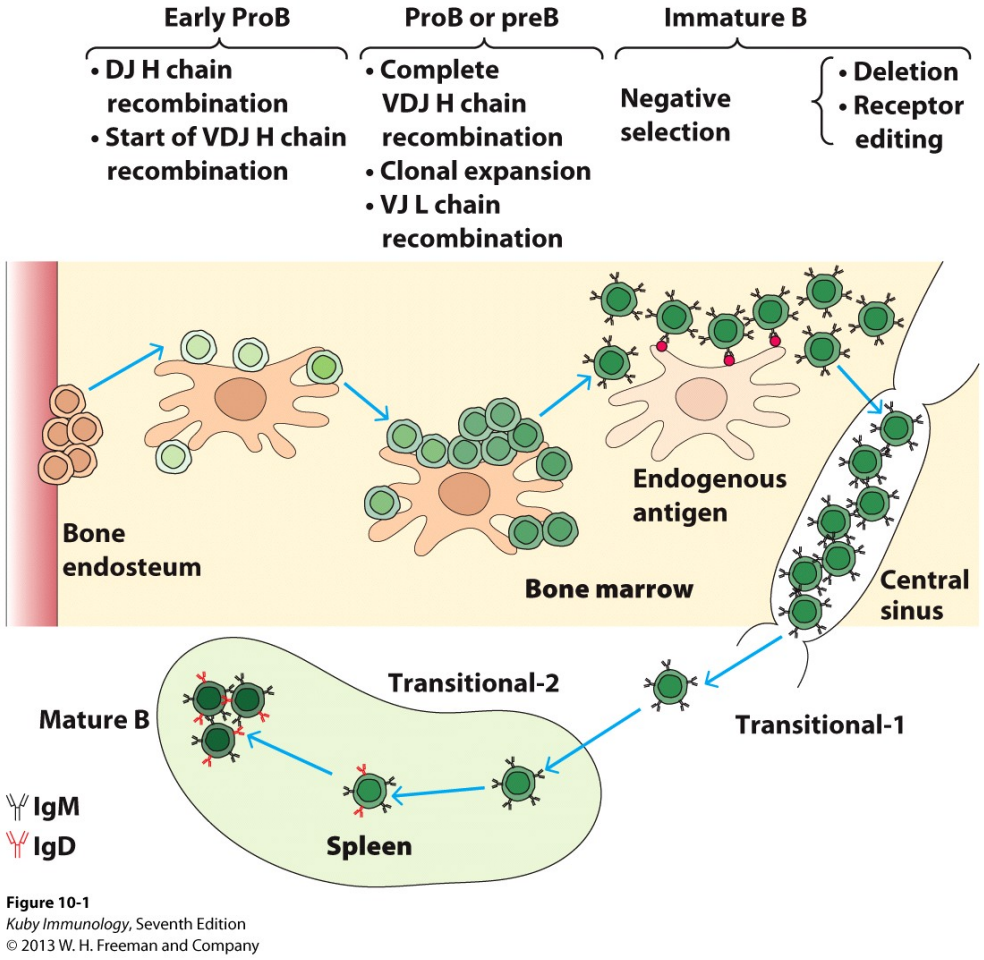

Figure 10-1
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Overview of B cells

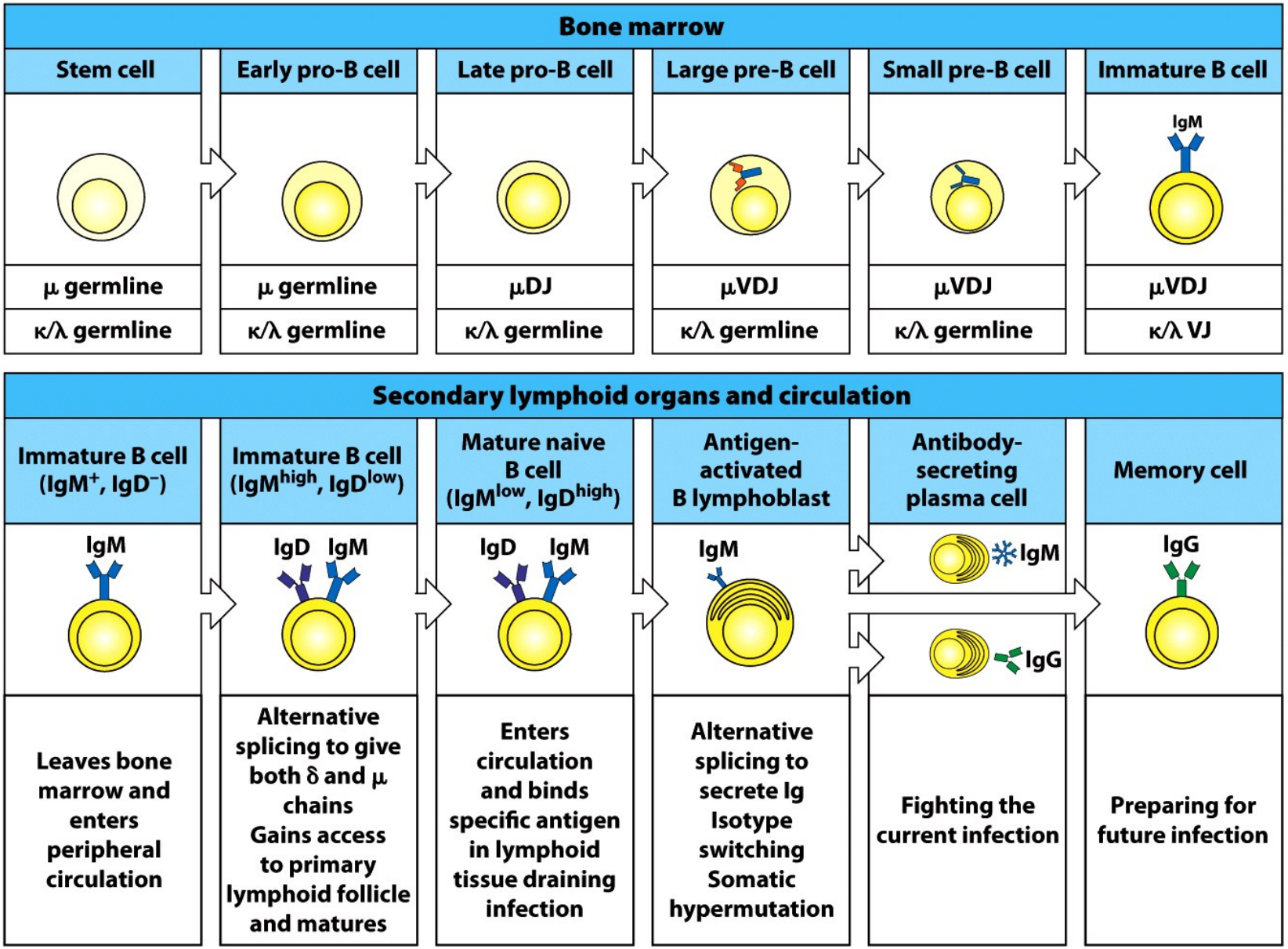
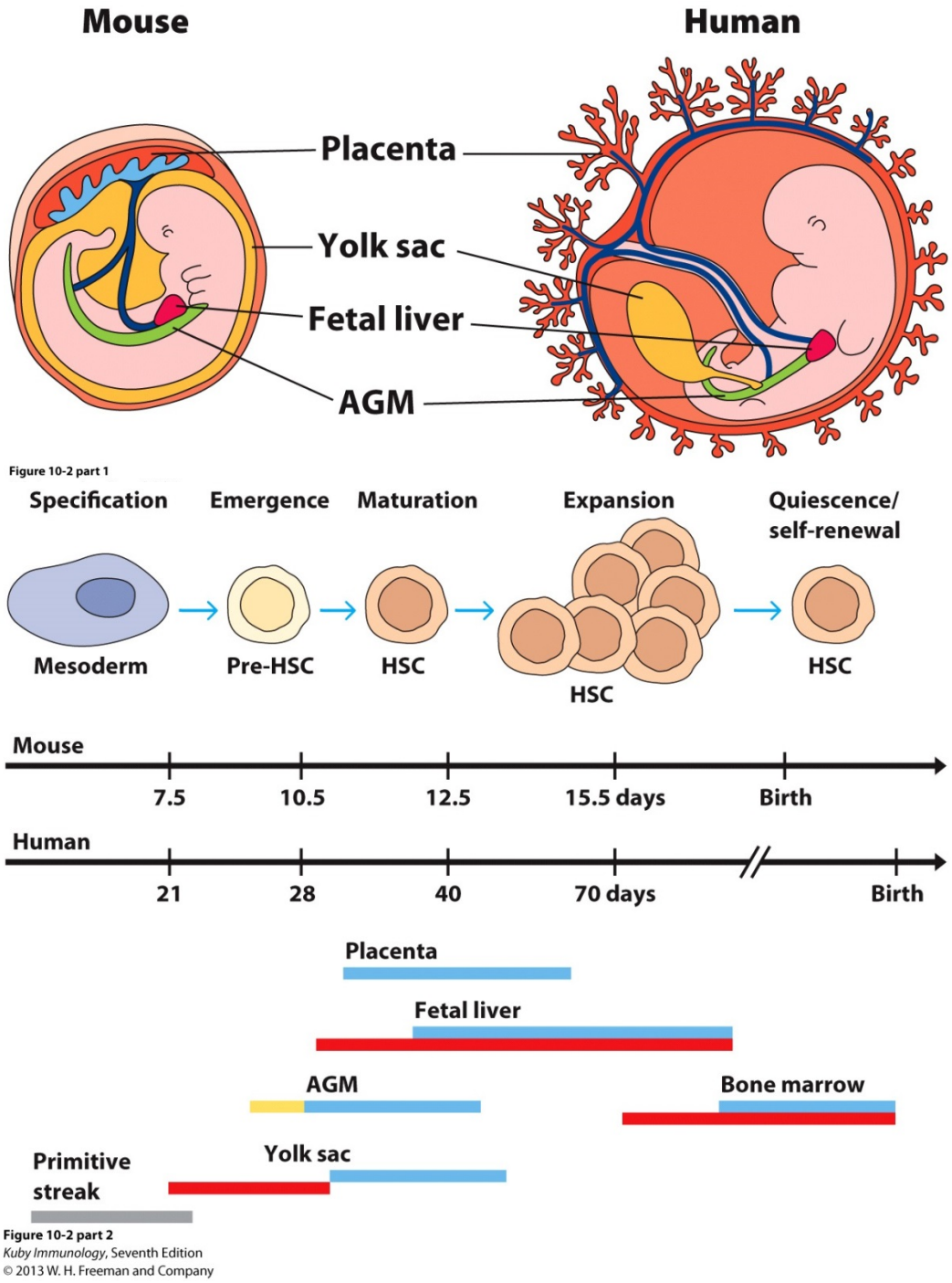


Figure 6.25 The Immune System, 3ed. (© Garland Science 2009)

Hematopoiesis

- Hematopoietic stem cells (HSCs) source of all blood cells
- Blood-forming cells first found in the yolk sac (primarily primitive rbc production)
- HSCs arise in distal aorta ~3-4 weeks
- HSCs migrate to the liver (primary site of hematopoiesis after 6 wks gestation)
- Bone marrow hematopoiesis starts ~5 months of gestation



Role of bone marrow stromal cells

- At various points in development, progenitor and precursor B cells interact with specific stromal cell populations secreting specific cytokines

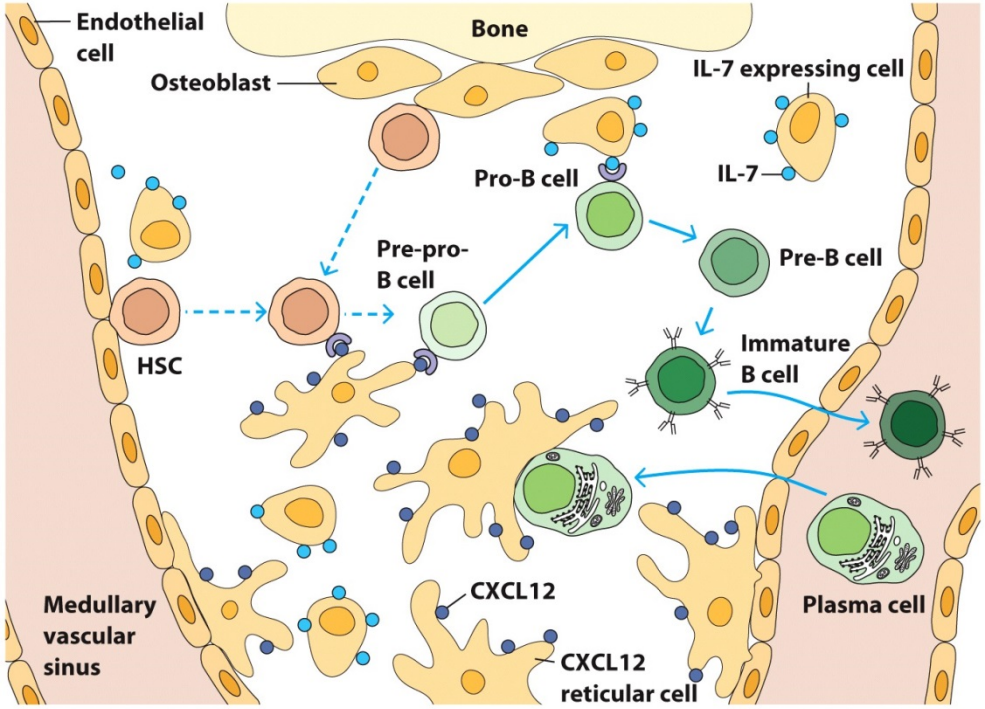


Figure 10-3
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HSCs

- Self-renewing, multipotential
- Give rise to all blood cells
- Depending on stimuli received, different transcription factors can drive HSCs down different developmental pathways
- Ikaros, PU.1 and E2A are all important transcription factors for B cell fate
- Express cKit (CD117) (receptor for stem cell factor)

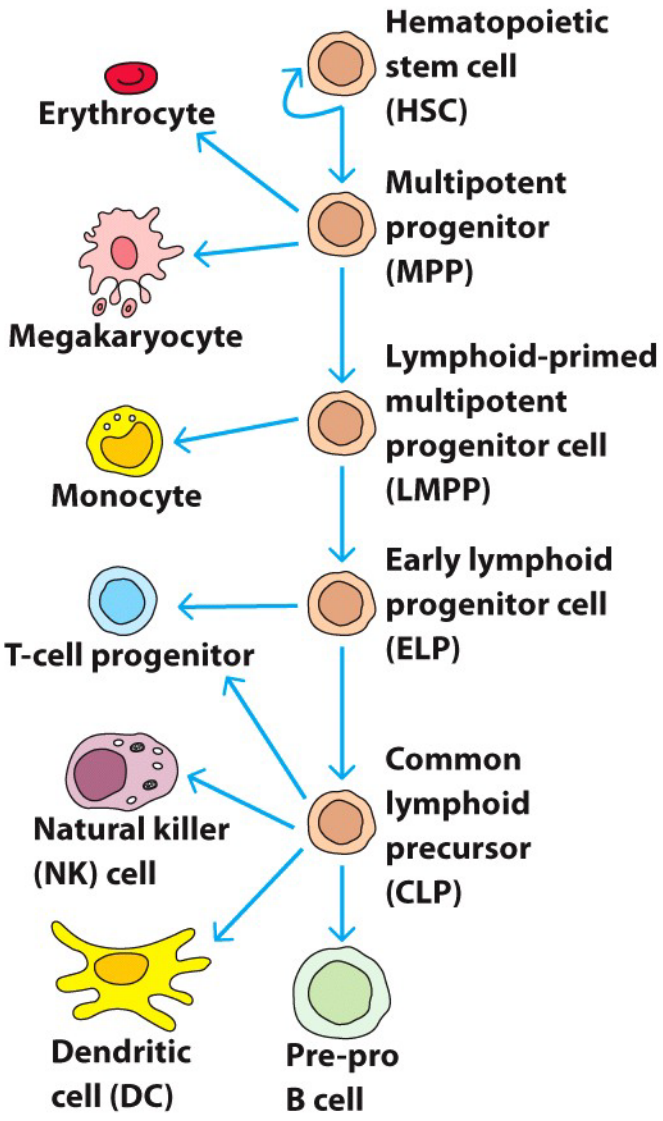

Figure 10-5
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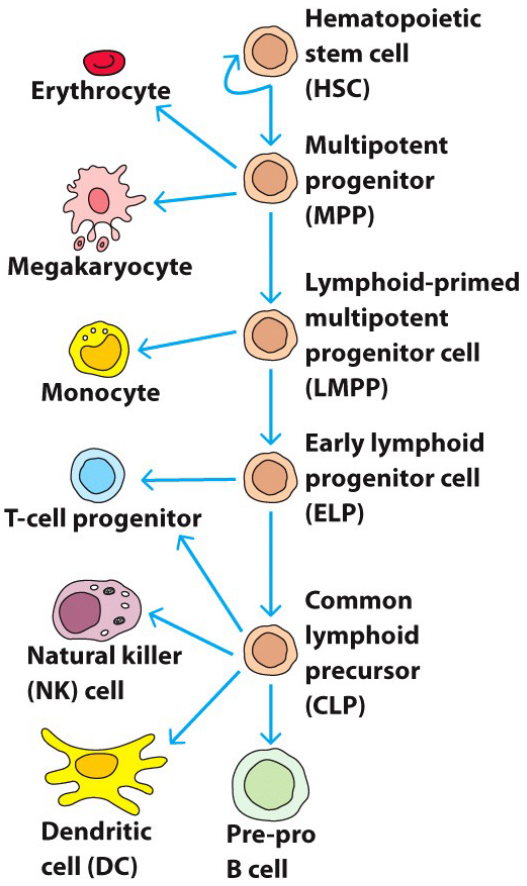
MPPs (multipotential progenitor cells)

- Generated following SCF-cKit interaction
- Lose capacity for self-renewal but can still differentiate into different lineages
- Transiently express CD34
- Express CXCR4, enables binding to stromal derived CXCL2



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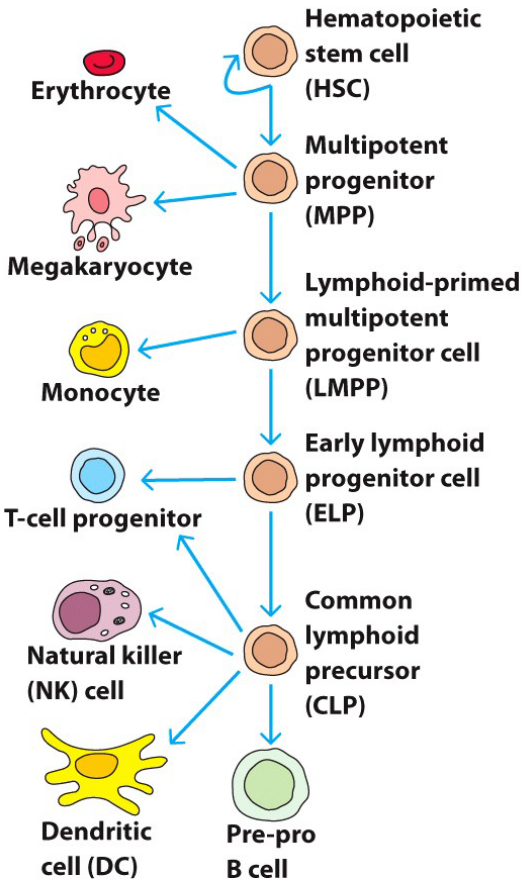
LMPPs (lymphocyte primed multilineage progenitors)

- Express flt-3 (binds to flt-3 ligand on BMSCs), leading to IL-7 receptor synthesis
- Flt-3 expression marks loss of MPP to develop into red blood cells or megakaryocytes, but still can differentiate into myeloid or lymphoid



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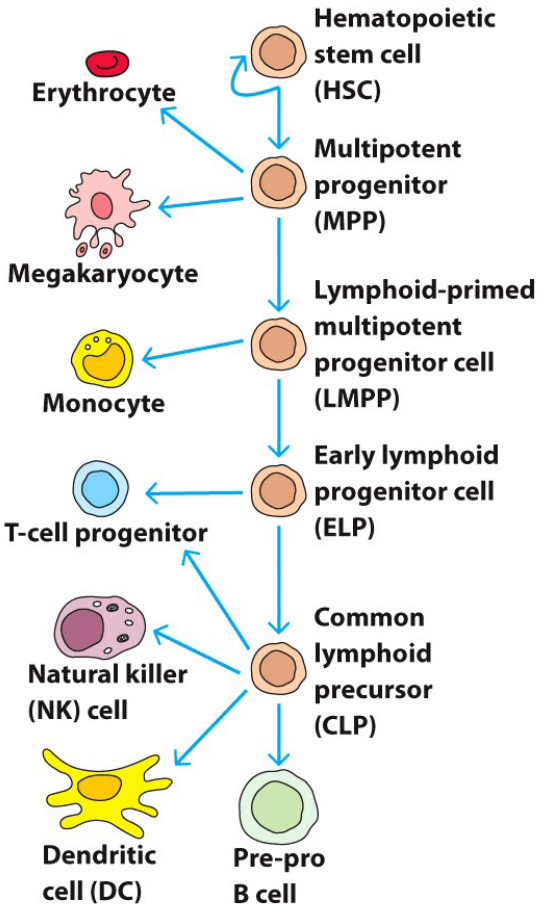
ELPs (early lymphoid progenitors)

- Express RAG1/2 (recombination activating genes)
- Some migrate to thymus, remainder stay in the marrow as B cell progenitors



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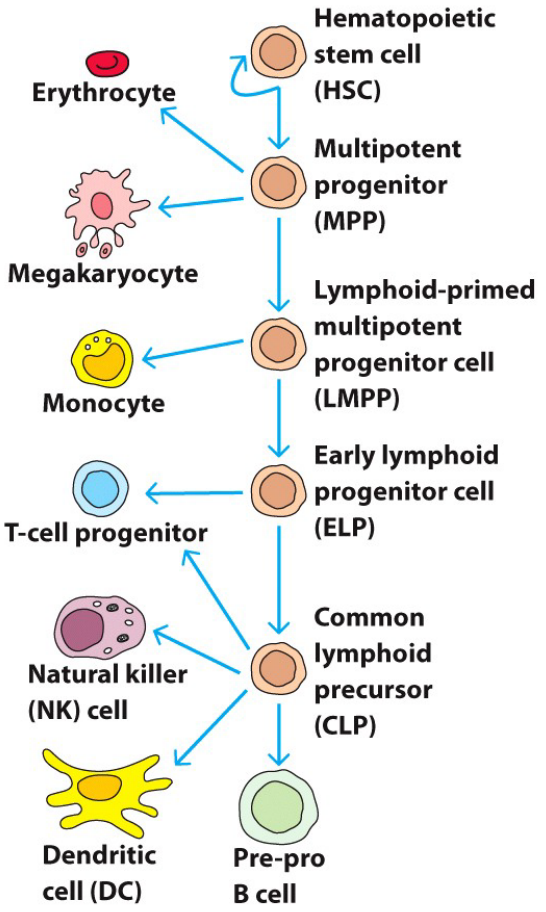
CLP (common lymphoid progenitors)

- Can mature into NKs, DCs, T cells, B cells
- Signaling through IL-7 receptor leads to increased Mcl1 (anti-apoptotic) and C-myc/N-myc (proliferative)
- Lost myeloid potential



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Immunoglobulin gene rearrangements and B cell development

- During early stages of B cell development, functional rearrangements of the heavy chain gene locus (IgH) allows for assembly of the pre-B cell receptor complex → cessation of IgH rearrangements (allelic exclusion) → light chain rearrangements (kappa, then lambda)
- Production of complete Ig (2 heavy chains, 2 light chains) allows for assembly of mature B cell receptor on the cell surface → signals cessation of light chain gene rearrangement → mature B cell stage

Pre-Pro B cells

- Express CD45R, a B-cell lineage-specific marker
- Increase expression of EBF-1
- EBF-1 and E2A bind the Ig gene, promoting accessibility of D-J_H locus, preparing for the 1^{st} step in Ig gene recombination
- EBF-1 also important for expression of other B cell proteins, including CD79α/β and genes encoding the pre-B cell receptor

Pro-B cells

- D-J_H recombination complete
- Require IL-3, IL-7, insulin-like growth factor 1, stem cell factor
- Pax5 expression (target of EBF-1)
  - Master transcription factor (essential for all subsequent stages of B cell development)
  - Promotes V_H to D recombination
- RAG1 and RAG2 expression
  - Catalyze D-JH rearrangements within the Ig heavy chain gene loci
- Surface expression of CD19
  - Component of multimolecular surface complex involved in signaling in response to antigen and T cell help
- Expression of HLA-DR and CD34
- By late pro-B cell stage, most cells have initiated V_H to DJ_H Ig gene segment recombination

Pre B cells

- Ig heavy chain genes complete V-D-J recombination
  - Allows surface expression of Ig heavy chain and surrogate light chains complex = pre-B cell receptor
- CD79a and CD79b (Ig-α and Ig-β)
  - associate non-covalently with surface Ig
  - signal transducing components of the pre-B cell receptor
  - also components of the Ig receptors on the surface of mature B cells
- Signaling through the pre-B cell receptor induces a few rounds of proliferation; at the end of this the pre-B cell receptor is lost from the surface → late pre B cell stage
- If pre B cell receptor cannot be displayed on cell surface because of nonproductive VHDJH gene rearrangement, then B cell development stops and the cell undergoes apoptosis (1^{st} checkpoint)
- Pre-B cell receptor signaling causes transient decrease in RAG1/2 and loss of Tdt
- Ensures that as soon as one heavy chain gene has been rearranged, no further recombination is possible (allelic exclusion)
- Light chain rearrangement is initiated following re-expression of RAG1/2
- Once light chain rearrangement has been successfully completed, the intact IgM receptor can be expressed
  - If light chain rearrangement does not occur successfully, then the 2^{nd} checkpoint occurs

Immature B cells

- Have functional IgM but no other Ig expression
- Express B220, CD25, IL-7R, CD19
- Once there is a functional BCR on the membrane, it has to be tested for its ability to bind self-antigens to ensure that few auto-reactive B cells are released
- Three fates if autoreactive
  - Clonal deletion via BCR-mediated apoptosis
  - Reactivation of RAG to initiate process of light chain receptor editing
  - Survive and escape the BM but become anergic
- B cell loss prior to leaving the BM = central tolerance
- Export to spleen where further development occurs
- Very susceptible to tolerance induction

Congenital agammaglobulinemia

- Genetic defects that prevent expression of the pre-B cell receptor or that prohibit the transduction of signals via the receptor leads to absence of B cells and agammaglobulinemia
- Congenital agammaglobulinemia: loss-of-function mutations in genes that encode components of the pre-B cell receptor or downstream signaling molecules
  - IgM
  - Lambda 5 surrogate light chain
  - CD79a, CD79b
  - Bruton tyrosine kinase (BTK)
  - B cell linker protein (BLNK)

Congenital agammaglobulinemia

- X-linked agammaglobulinemia (XLA)
  - Severe hypogammaglobulinemia, antibody deficiency, increased susceptibility to infections
  - Absence or near absence of tonsils and adenoids
  - Infections generally first noted between 3-18 months of age (usually recurrent bacterial respiratory tract infections)
  - Due to defects in Btk
  - Significantly reduced levels of CD19+ B cells, failure to generate plasma cells, severely decreased production of all classes of immunoglobulins and markedly defective antibody responses
  - Incidence of XLA ~1 in 190,000 male births
  - Treatment:
    - IVIG
      - Only replaces IgG, not IgM or IgA
      - Only passive immunity provided
    - Aggressive antibiotic therapy

T1 and T2 transitional B cells

- T1: mIgM^{hi}, mIgD$^{-/lo}$, CD21-, CD23-, CD24+, CD93+
- T2: higher levels of mIgD, CD21+, BAFF-R
- T1 →T2 → mature B cells
- Most T1 transitional B cells differentiate to T2 within the spleen but ~25% of T2 emerge directly from the BM
- T2 cells capable of recirculating among the blood, lymph nodes, spleen
- T2 cells can enter B cell follicles

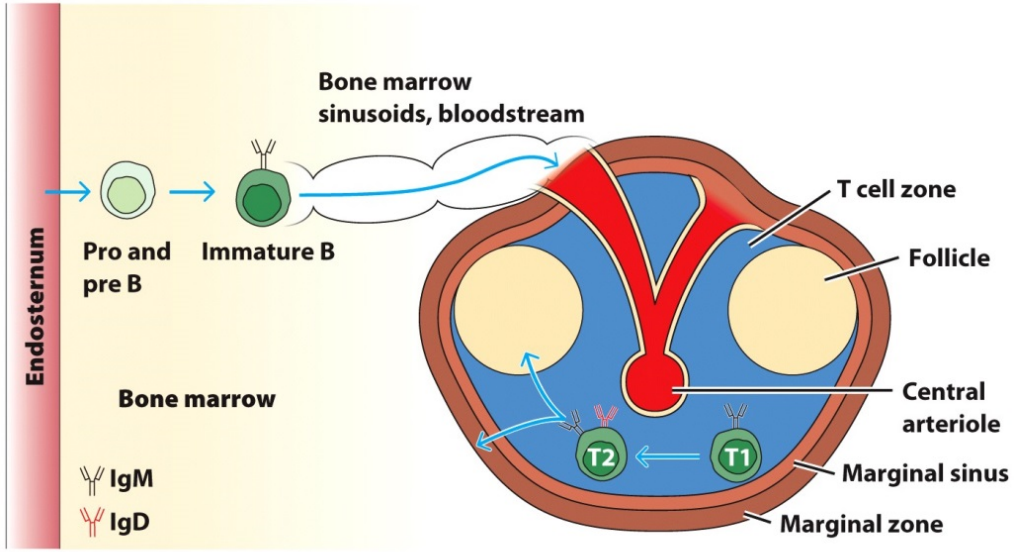

Figure 10-9
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T1 and T2 transitional B cells

- Self-reactive T1 B cells eliminated by apoptosis in response to strong antigenic signal (peripheral tolerance)
  - 55-75% of immature B cells lost this way
- T2 cells become resistant to antigen-induced apoptosis
  - Increased Bcl-X1 expression
- BAFF receptor expression first detected in T1 B cells, increases thereafter
  - Promotes survival of transitional B cells

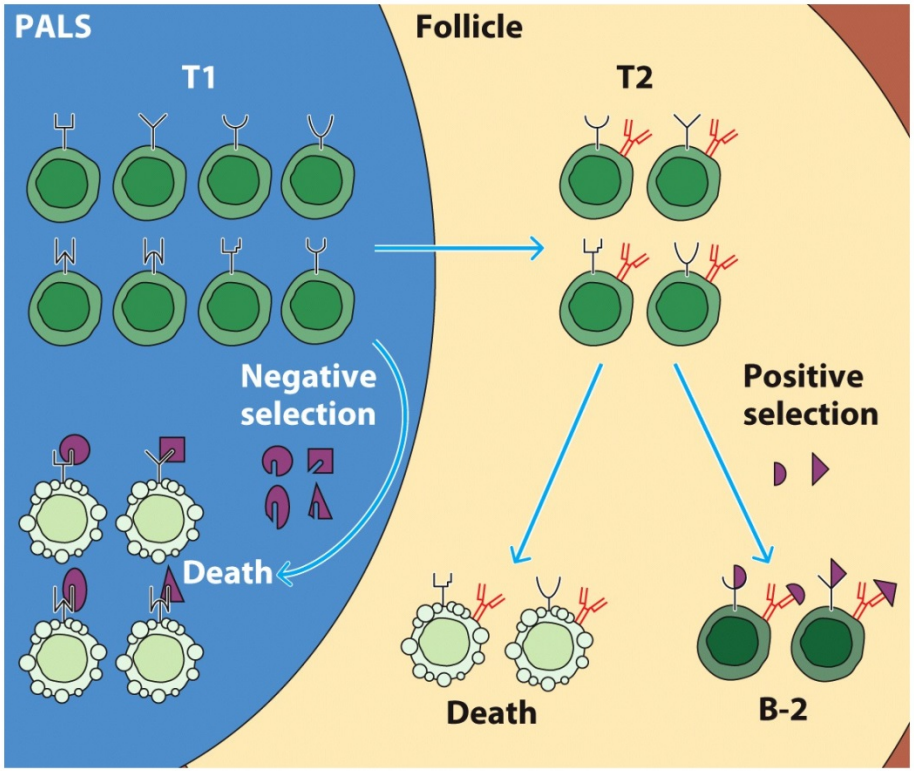

Figure 10-10
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Mature B cells

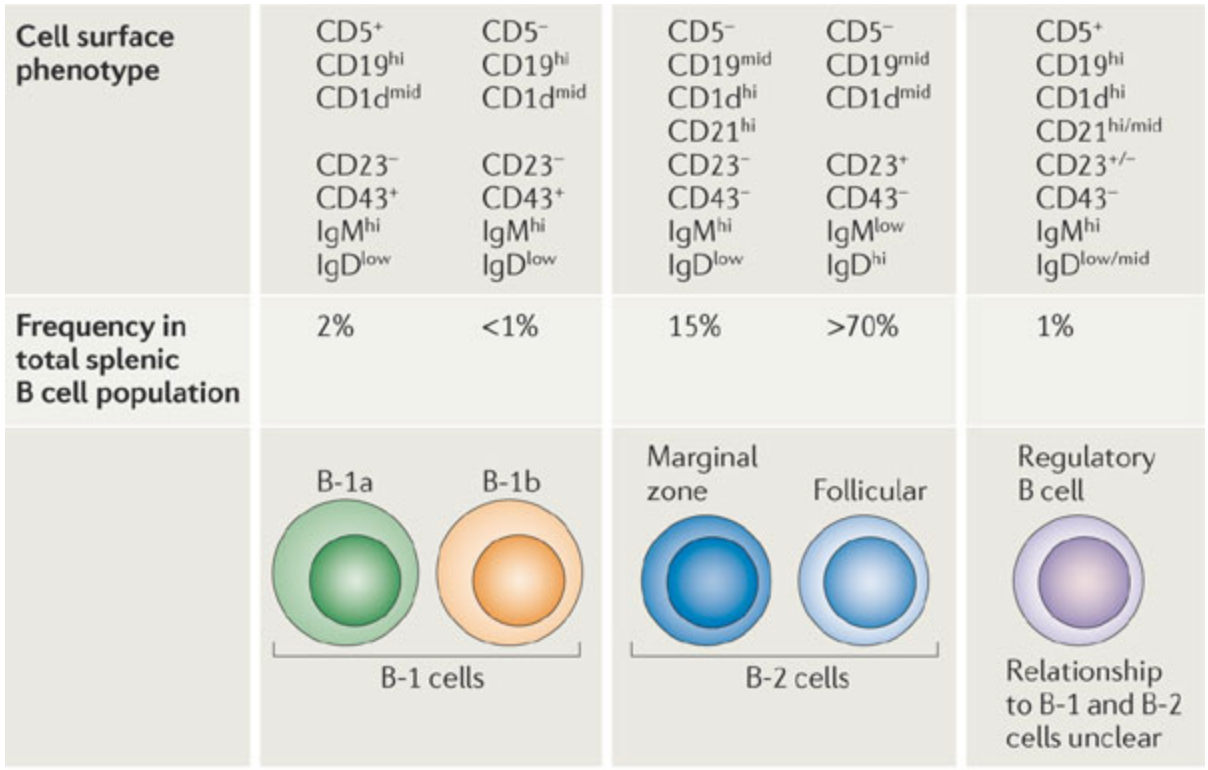
- Complete IgM molecule on cell surface
- Mature resting B cells express HLA-DR, CD19, CD20, CD40 but no longer express CD10, CD34, RAG1, RAG2, or Tdt
- Exit bone marrow, migrate to secondary lymphoid organs, then express both surface IgM and IgD as well as other molecules that mediate cell-cell and cell-ECM adhesive interactions
- Can recirculate between blood and lymphoid organs, entering B cell follicles in lymph nodes and spleen, responding to antigen encounter with T cell help, leading to antibody production

Plasma cells

- Secrete antibodies, have few surface antibodies
- Those that arise from follicular B cells are found mainly in the bone marrow and are long-lived (months)
- Those that arise from non-follicular B cells are short lived (days to weeks)
- Plasma cell differentiation involves
  - loss of Bcl6, Pax-5, CD19, CD20, and B cell activation antigens
  - Appearance of XBP-1, BLIMP-1, CD38, CD138, cytoplasmic Ig

B cell subsets

- Follicular (B-2) B cells (conventional B cells)
- B-1 B cells
- Marginal Zone B cells



Nature Reviews | Immunology

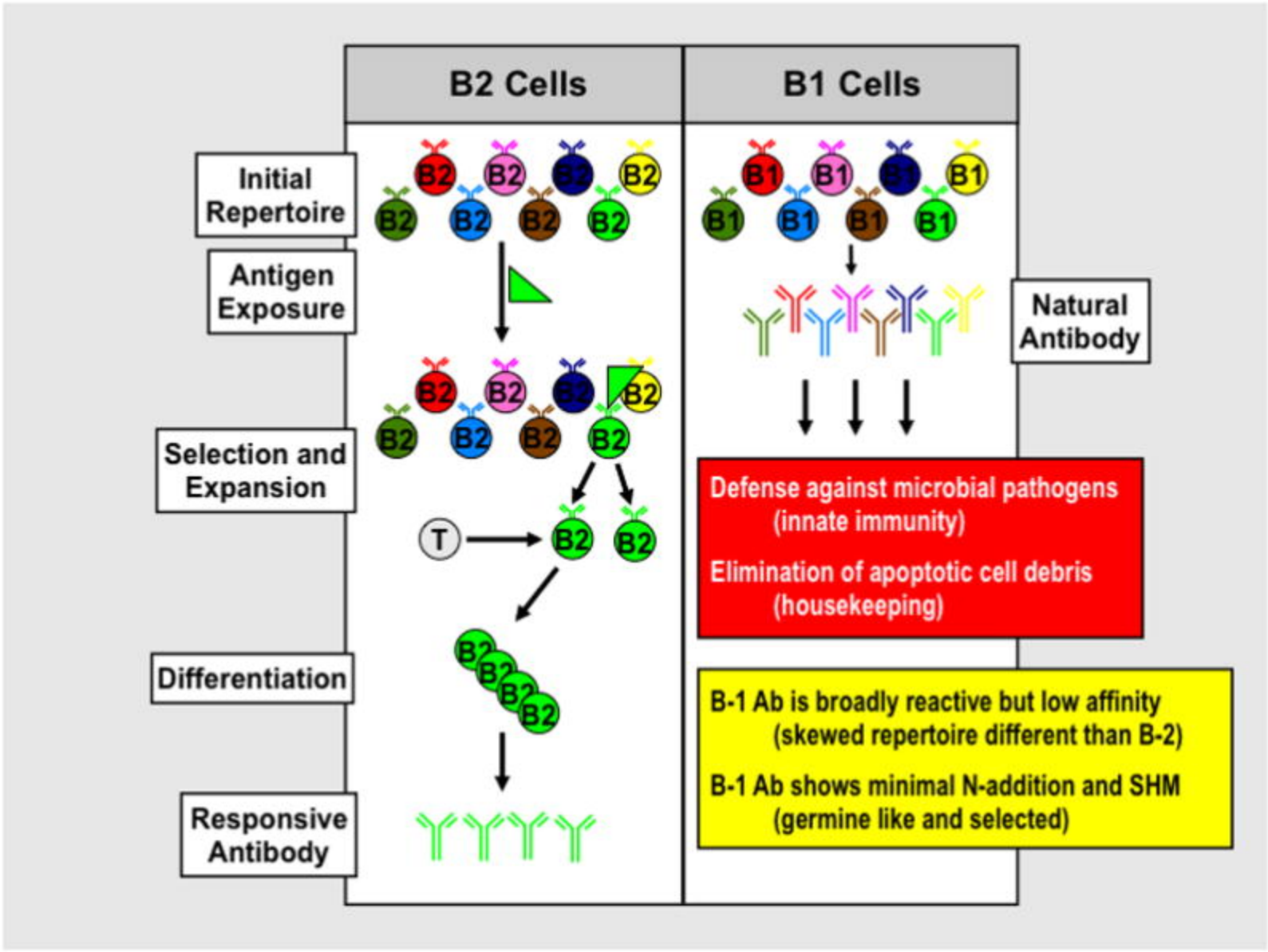
Dörner, T. *et al.* (2009) B-cell-directed therapies for autoimmune disease
Nat. Rev. Rheumatol. doi:10.1038/nrrheum.2009.141

B-1 B cells

- 30-50% of the B cells in the pleural and peritoneal cavities, only small % of splenic B cell population
- Limited receptor repertoire, primarily directed towards commonly expressed microbial carbohydrate antigens
- Transitional B-1 cells undergo apoptosis unless they interact with self-antigens
- Constantly regenerated in the periphery
  - Bone marrow ablation causes depletion of B-2 pool but not B-1 pool
- Exposure to antigen leads to plasma cell formation and to clonal expansion and persistence of antigen-specific memory B1 cells

B-1 B cells

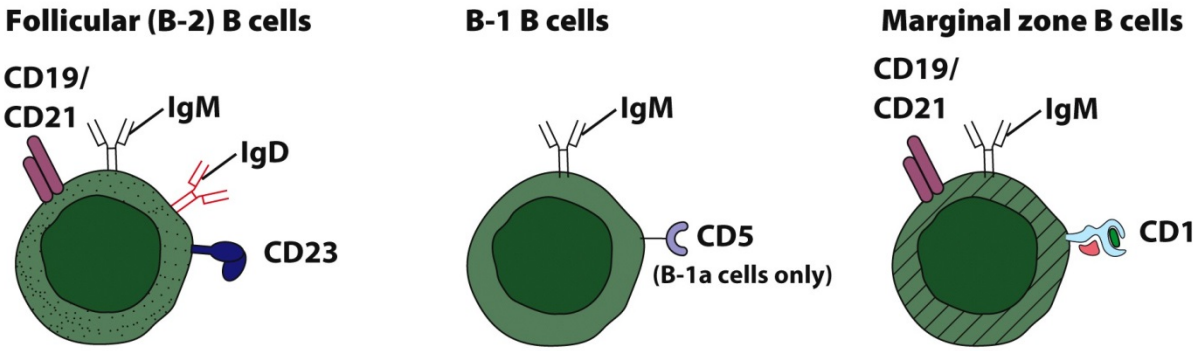
- Spontaneous, constitutive secretion of “natural antibody” that appears in the absence of infection or immunization
  - Predominantly IgM
  - 80-90% of resting serum IgM and ~50% resting serum IgA is derived from B-1 B cells
- Serum IgM is preferentially produced by B-1 B cells in the spleen
- Data suggest many B-1 cells in the peritoneum may be memory cells and that upon rechallenge of antigen, they return to the spleen to differentiate into antigen-specific high-secreting plasma cells



Ann N Y Acad Sci 1285:97-114, 2013

Marginal zone B cells

- Located in outer zones of white pulp of the spleen
- Derived from the T2 transitional population
- Specialized for recognizing blood-borne antigens
- Can respond to both protein and carbohydrate antigens
- Produce broadly cross-reactive IgM antibodies
- High levels of membrane IgM and CD21, low levels of membrane IgD and CD23
- Long lived



Attribute	Follicular (B-2) B cells	B-1 B cells	Marginal zone B cells
Major sites	Secondary lymphoid organs	Peritoneal and pleural cavities	Marginal zones of spleen
Source of new B cells	From precursors in bone marrow	Self-renewing (division of existing B-1 cells)	Long-lived May be self-renewing
V-region diversity	Highly diverse	Restricted diversity	Somewhat restricted
Somatic hypermutation	Yes	No	Unclear
Requirements for T-cell help	Yes	No	Variable
Isotypes produced	High levels of IgG	High levels of IgM	Primarily IgM; some IgG
Response to carbohydrate antigens	Possibly	Yes	Yes
Response to protein antigens	Yes	Possibly	Yes
Memory	Yes	Very little or none	Unknown
Surface IgD on mature B cells	Present on naïve B cells	Little or none	Little or none

Figure 10-12
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B cell activation

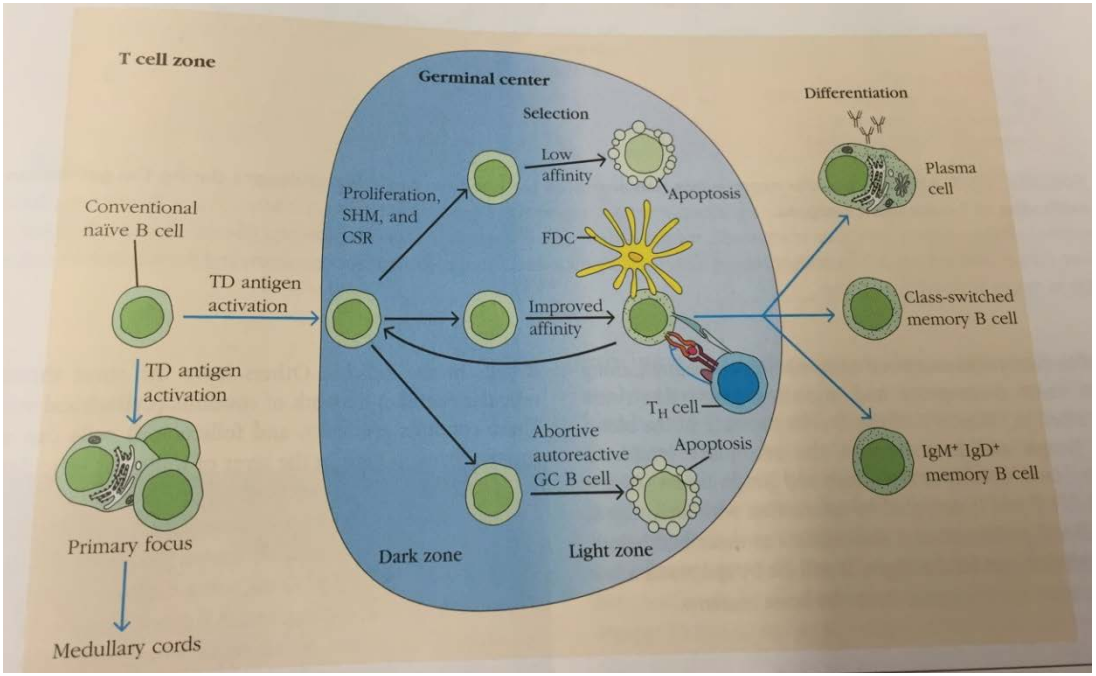
- Exposure to antigen or various polyclonal mitogens activates resting B cells and stimulates their proliferation
- Activated B cells lose expression of sIgD and CD21 and acquire expression of activation antigens
  - Growth factor receptors, structures involved in cell-cell interaction, molecules that play a role in the localization and binding of activated B cells

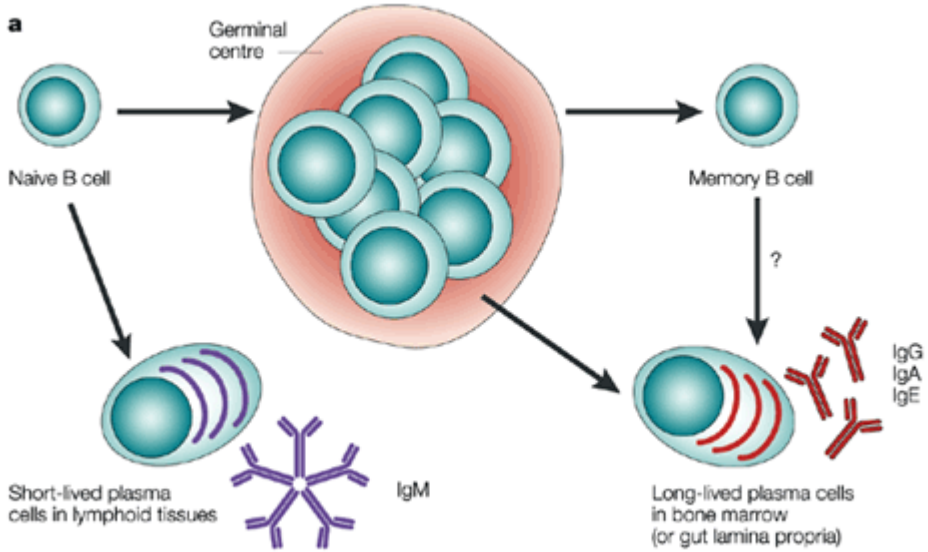
B cell activation

- Two major types: T cell dependent (TD) and T cell independent (TI)
- TD: involves protein antigens and CD4+ helper T cells
  - 1) Multivalent antigen binds and crosslinks membrane Ig receptors
  - 2) Activated T cell binds B cell thru antigen receptor and via CD40L (T)/CD40 (B) interaction
- TI: involves multivalent or highly polymerized antigens, does not require T cell help
  - TI-1: e.g., LPS. Mitogenic at high concentrations to most B cells because of binding to pattern recognition receptors (PRRs) on B cell surface. At low concentrations, only activates those B cells that bind the antigen via the Ig receptor
  - TI-2: e.g., bacterial capsular polysaccharide. Highly repetitive antigens. Not mitogenic but can crosslink Ig receptors. Many are bound by C3d.

B cell activation

- Following TD antigen activation, some activated B cells differentiate into plasma cells in **primary foci** that are outside of the follicles, then migrate to the medullary cords of the lymph node or to the bone marrow. Secrete IgM within 4 days.

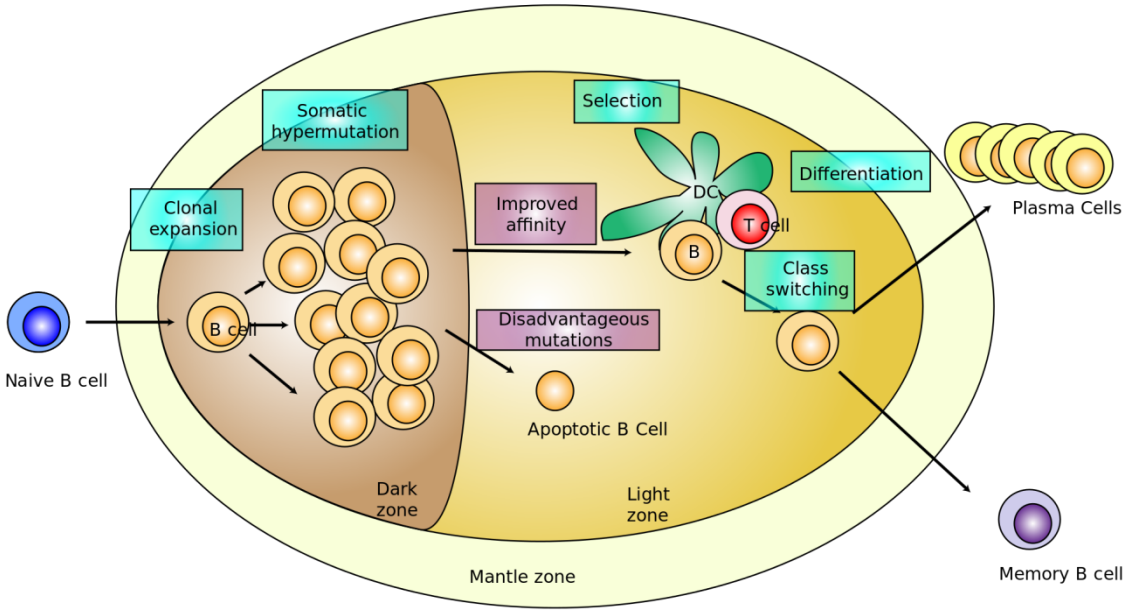




Nature Reviews Immunology 2, 60-65 (January 2002)

B cell activation

- Other activated B cells enter the follicle, divide and differentiate; germinal centers form.
- Within the germinal center, Ig genes undergo **class switching**: the μ constant regions replaced by other constant regions and the variable region is subject to **somatic hypermutation**.
- Mutated variable region subject to antigen-mediated selection
- Low affinity and autoreactive B cells die while those with improved affinity leave the germinal centers.
- Antibodies with mutations in the variable region appear in the circulation within 6-10 days



T-dependent B-Cell Response

- At conclusion of primary immune response, two sets of long-lived cells remain:
  - Memory B cells
    - On secondary exposure to the same antigen, the memory B cells will be stimulated and result in production of high-affinity, heavy-chain class-switched antibodies
  - Plasma cells
- Majority of the expanded population of antigen-specific B cells undergo apoptosis

Germinal center cell differentiation into plasma cells

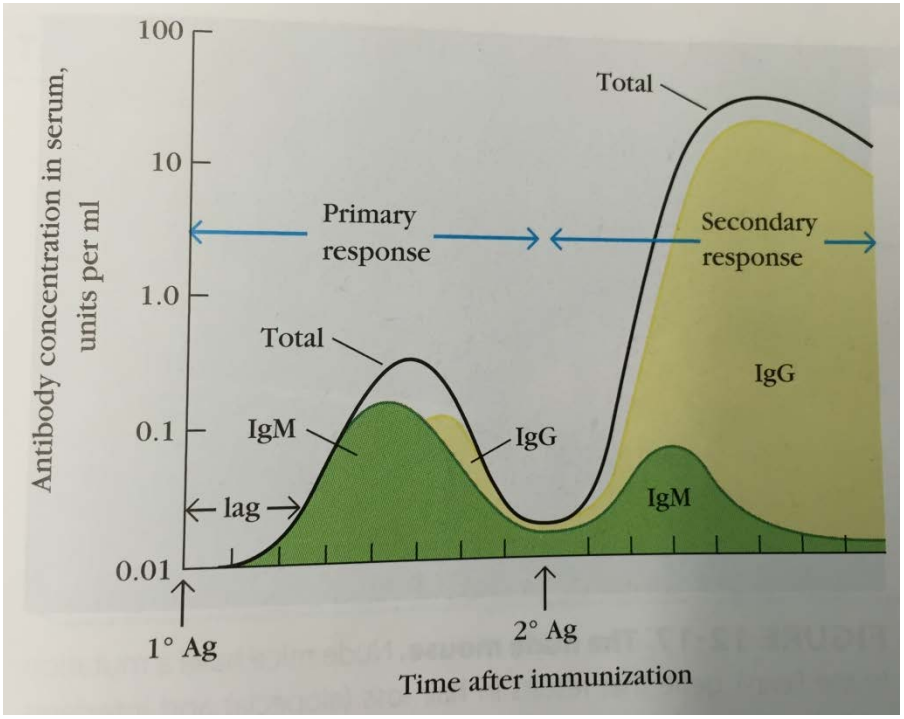
- 5-15 days after initial antigen exposure, a portion of the germinal center B cells will upregulate IRF-4 (critical for plasma cell differentiation)
  - IRF-4 knockout mice lack Ig-secreting plasma cells
  - IRF-4 overexpression promotes plasma cell differentiation
- IRF-4 → BLIMP-1 → downregulation of genes important for B cell proliferation, class switching and somatic hypermutation while upregulating synthetic rate of Ig synthesis and secretion
- Downregulation of CXCR5 (has kept the B cell in the germinal center), upregulation of CXCR4 → leave the lymph node

Class switching

- Influenced by cytokines
  - IL-4 → IgG1, IgE
  - TGF-β → IgA, IgG2b
  - IL-5 → IgA
  - IFN-γ → IgG3, IgG2a
- Loss-of-function mutations in AID, UNG, CD40, or CD40L → hyper-IgM syndrome
  - Failure of Ig class switching and marked hypoplasia of germinal center B cells
  - Normal/increased serum IgM with low IgG, IgA, and IgE as well as poor antibody function
  - Most common form is an X-linked trait due to mutations in CD40L gene
  - Recurrent sinopulmonary infections
  - Increased risk for opportunistic infections (pneumocystis, cryptosporidium, histoplasma)

Timing of memory immune response

- Primary response
  - Lag period: division/differentiation of B cells within the primary foci, movement into germinal center
  - B cells of primary foci release IgM
  - B cells that have migrated into the germinal center release IgM and IgG
  - Somatically hypermutated receptors appear, select population of B cells leaves the germinal center and enters the memory B cell compartment
- Secondary response
  - Expanded set of memory B cells with high-affinity receptors are available for immediate differentiation to high-affinity IgG secretion
  - Somatically hypermutated B cells can undergo further hypermutation with additional antigen exposure, increasing the average affinity of the antigen-specific antibodies

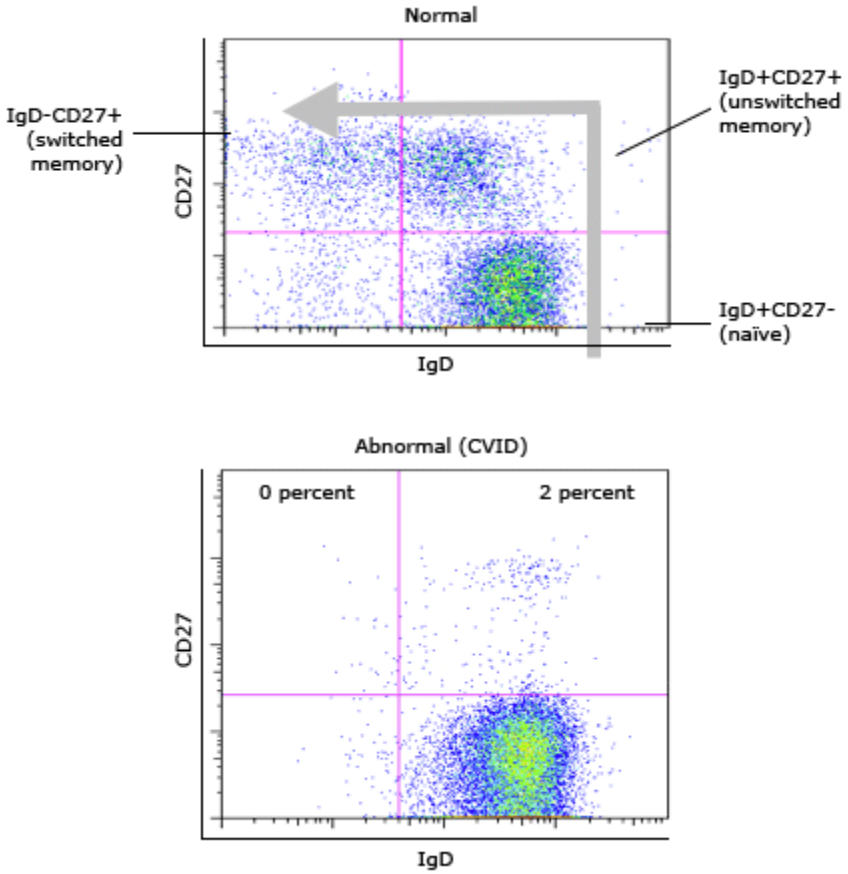


Primary (naïve) vs Secondary (memory) B cells

	Naïve	Memory
Lag period after antigen exposure	4-7 days	1-3 days
Time of peak response	7-10 days	3-5 days
Magnitude of peak antibody response	Variable	10-1000 times higher than primary response
Antibody isotype	IgM	IgG (IgA in mucosal tissues)
IgM/IgD expression	Positive/positive	Mostly negative/negative
Antibody affinity	Low	High
Life span	Days to weeks	Long-lived
Recirculation	Yes	Yes

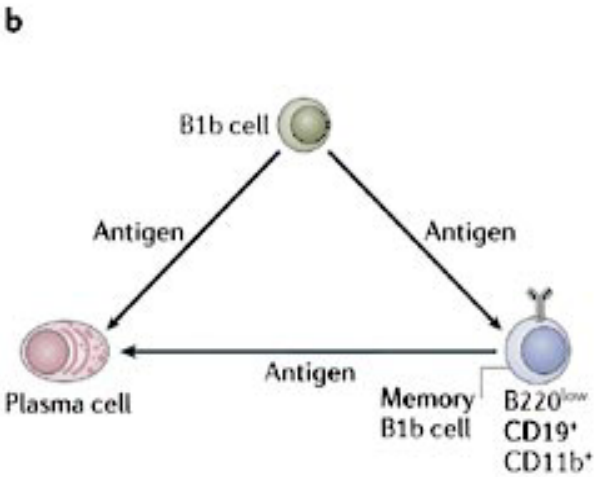
Common variable immunodeficiency (CVID)

- Characterized by markedly low serum IgG levels and low IgA or IgM, poor/absent response to immunization
- Recurrent sinopulmonary infections, autoimmune disorders, enhanced risk of malignancy
- Failed B cell differentiation with impaired secretion of immunoglobulin
- B cell number normal, but reduced percentages of isotype switched memory B cells
- Variety of gene defects, most of which are sporadic.



Maturation of activated B cells in absence of T cells

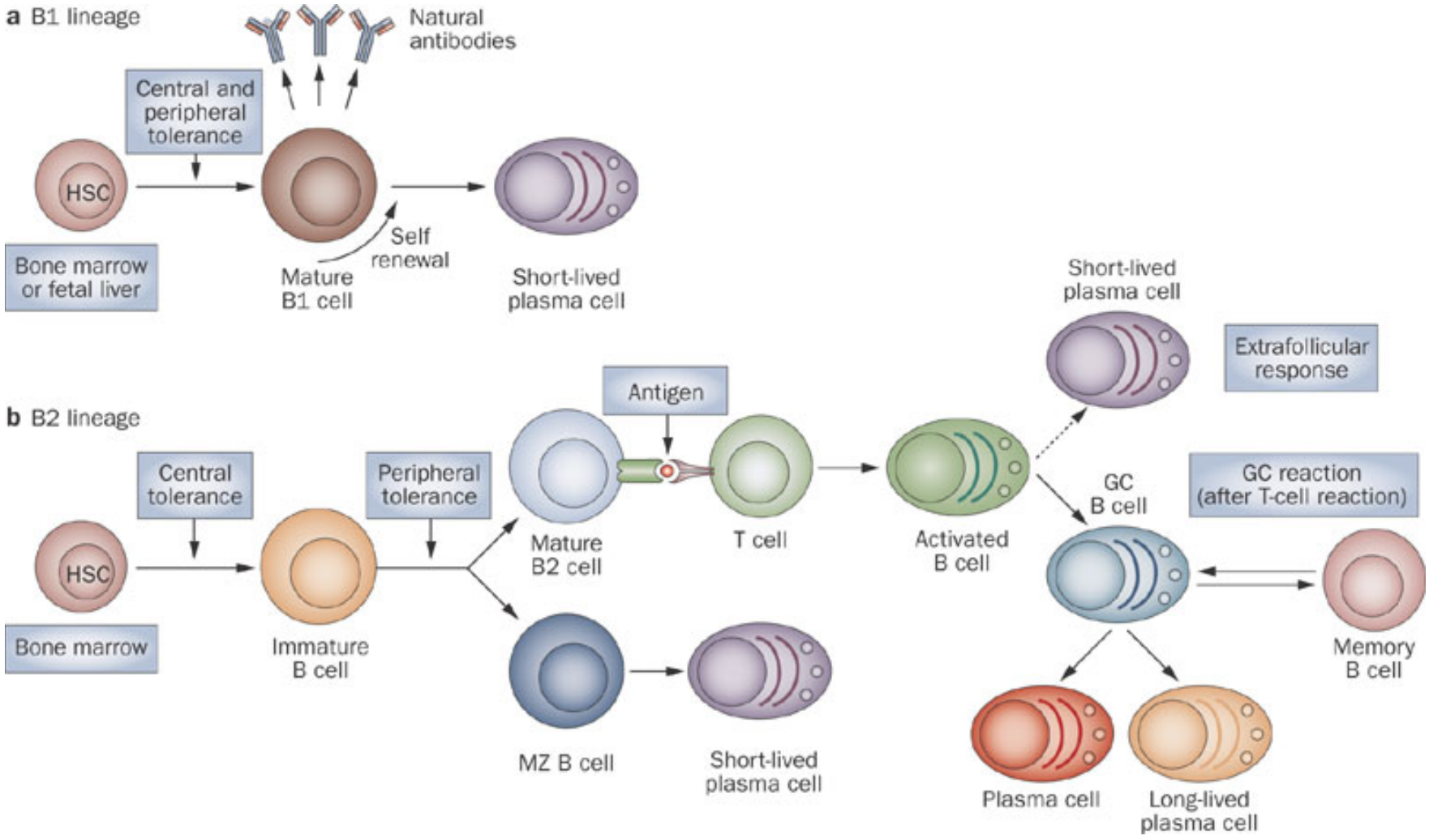
- Rapidly mature into short-lived plasma cells without undergoing somatic hypermutation or class switching
- Secrete IgM antibodies of low affinity
- Do not contribute to memory B cell pools
- B-1 cells may preferentially follow this non-follicular differentiation pathway as they appear to be much less dependent on T cell help for antibody production



Tarlinton *Nature Reviews Immunology* **6**, 785–790 (October 2006) | doi:10.1038/nri1938

Summary of thymus-dependent vs thymus-independent antigens

	TD antigens	TI type I antigens	TI type 2 antigens
Antigen type	Soluble protein	Bacterial cell wall components (e.g., LPS)	Polymeric protein antigens, capsular polysaccharides
Humoral response			
Isotype switching	Yes	No	Limited
Affinity maturation	Yes	No	No
Immunologic memory	Yes	No	No
Polyclonal activation	No	Yes (high doses)	No



Dörner, T. *et al.* (2009) B-cell-directed therapies for autoimmune disease
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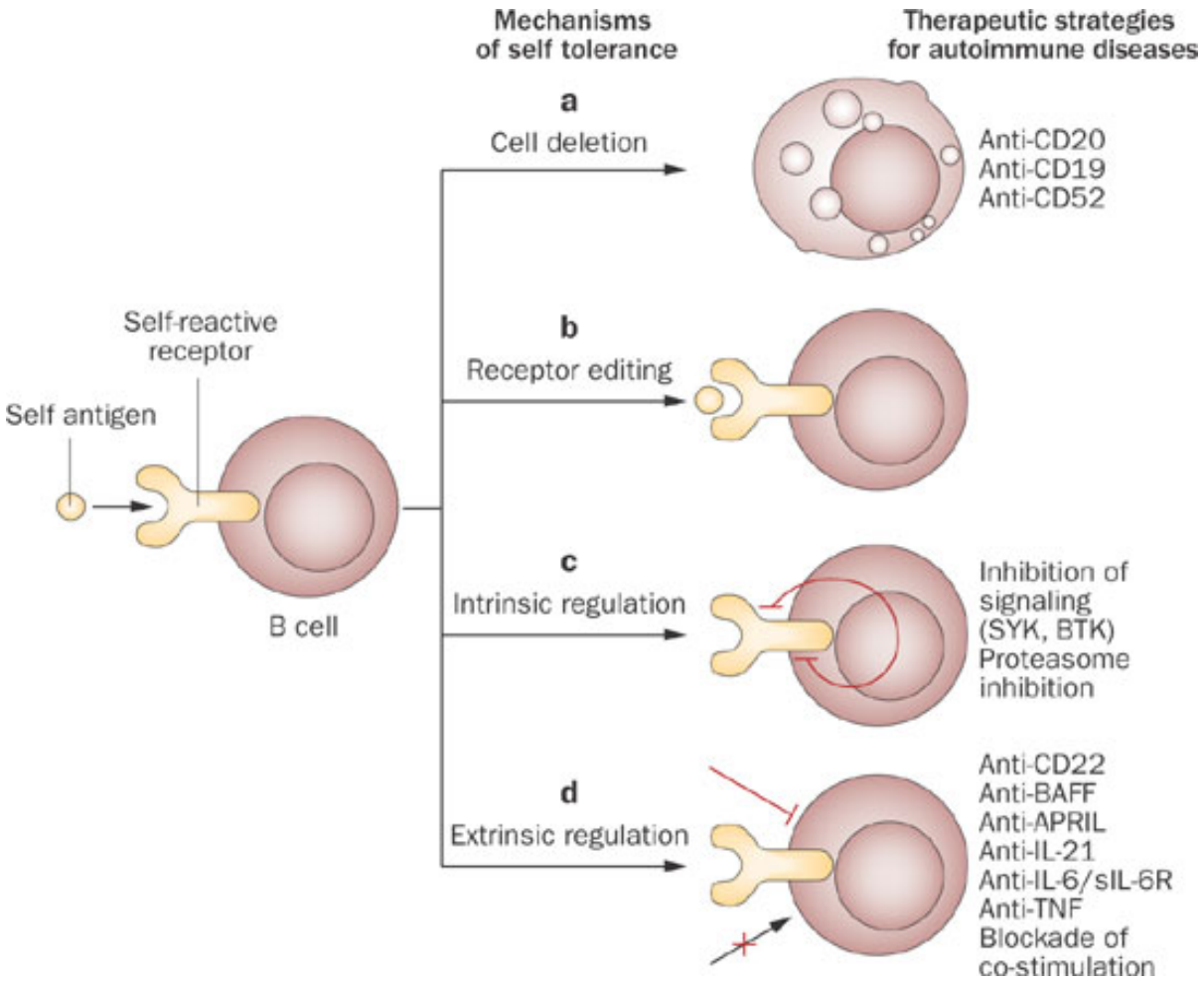
Negative regulation of B cell activation

- CD22
  - Transmembrane protein associated with the BCR
  - Has ITIM (immunoreceptor tyrosine-based inhibitory motif)
  - Activation of B cells → phosphorylation of ITIM → association of SHP-1 tyrosine phosphatase → dephosphorylation of neighboring signaling complexes
  - In the presence of antigen, constant phosphorylation/dephosphorylation of adapter molecules
  - As antigen levels decrease, balance shifts towards dephosphorylation
- FCγRIIb receptor (CD32)
  - Recognizes immune complexes containing IgG
  - Has a cytoplasmic ITIM domain
  - Co-ligation of B cells FCγRIIb receptor with BCR by specific Ag-Ab complex → activation of FCγRIIb signaling cascade and phosphorylation of the ITIM domain
  - SHIP binds to ITIM, hydrolyzes PIP_3 to PIP_2, interfering with membrane localization of Btk and PLCγ2 → decreased B cell signaling
- B-10 B cells
  - Population of B cells which secrete IL-10 upon stimulation



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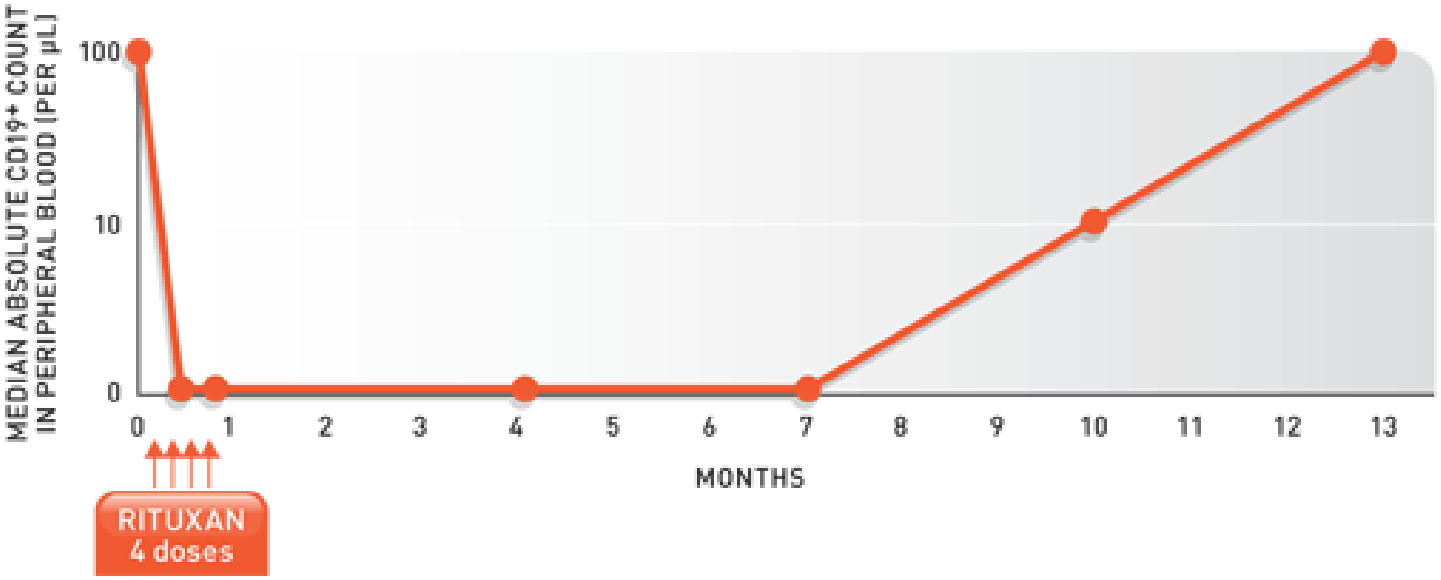
Therapeutic B cell depletion

- Indications: Autoimmune disorders or B-cell malignancies
- Rituximab
  - Anti-CD20 monoclonal antibody
  - Depletes B cells
    - Fc receptor gamma-mediated antibody-dependent cytotoxicity
    - Antibody-dependent complement-mediated cell lysis
    - Growth arrest
    - B cell apoptosis
  - Does not significantly alter immunoglobulin levels because long-lived plasma cells are CD20-



McLaughlin et al. JCO 1198; 16:2825-2833

Splenectomy

- Asplenic patients at risk for fatal septicemia
- Immune compromise
  - Spleen efficiently clears IgG-coated bacteria and is critical for clearance of encapsulated bacteria that are not opsonized by antibodies or complement
  - Reduction in serum IgM antibodies to polysaccharides
  - Reduction in memory B cells producing IgM antibodies
  - Delayed and lower magnitude of response to vaccination
- Most common cause of sepsis: *S. pneumoniae*

Surface markers during B cell development-I

	Antigen-independent phase					Antigen-dependent phase			
	Pre-pro B cell	Pro-B cell	Pre B cell	Immature B cell	Mature B cell	Activated B cell	Blast B cell	Memory B cell	Plasma B cell
MHC Class II	+	+	+	+	+	+	+	+	
CD10	+	+					+		
CD19	+	+	+	+	+	+	+	+	
CD20		+	+	+	+	+	+	+	
CD21			+	+	+	+			
CD23				+	+	+	+		
CD25, IL-2R alpha						+	+		
CD32, Fc gamma RII				+	+	+	+	+	
CD34	+	+	+						
CD35, CR1				+	+	+	+	+	
CD40		+	+	+	+	+	+	+	
CD80/86, B7-1/2						+	+		

IL: interleukin; R: receptor; alpha: alpha chain.

Data from Bona, CA, Bonilla, FA, Textbook of Immunology, 2nd edition, Harwood Academic Publishers, Amsterdam, 1996. page 102 (no table number).

Surface markers during B cell development-II

	Antigen-independent phase					Antigen-dependent phase			
	Pre-pro B cell	Pro-B cell	Pre B cell	Immature B cell	Mature B cell	Activated B cell	Blast B cell	Memory B cell	Plasma B cell
CD121, IL-1R						+	+		
CD122, IL-2R beta				+	+	+	+	+	
CD123, IL-3R alpha	+	+	+						
CD124, IL-4R alpha				+	+	+			
CD125, IL-5R alpha						+	+		
CD126, IL-6R alpha						+	+		
CD127, IL-7R alpha	+								

IL: interleukin; R: receptor; alpha: alpha chain.

Data from Bona, CA, Bonilla, FA, Textbook of Immunology, 2nd edition, Harwood Academic Publishers, Amsterdam, 1996. page 102