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Post-Module Assessment

Click here to take the online assessment after taking the course.

Then, click here to take the post-module survey (also required).

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What do antibodies do?

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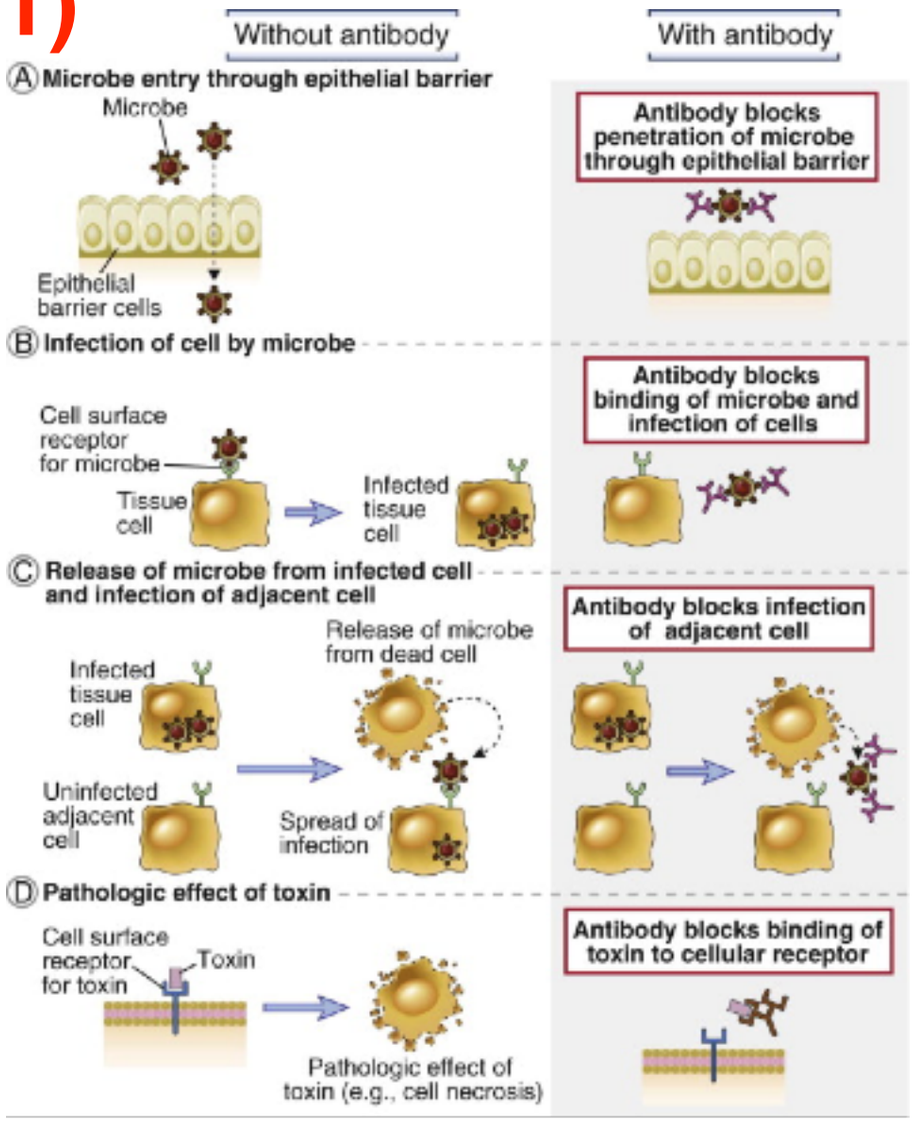

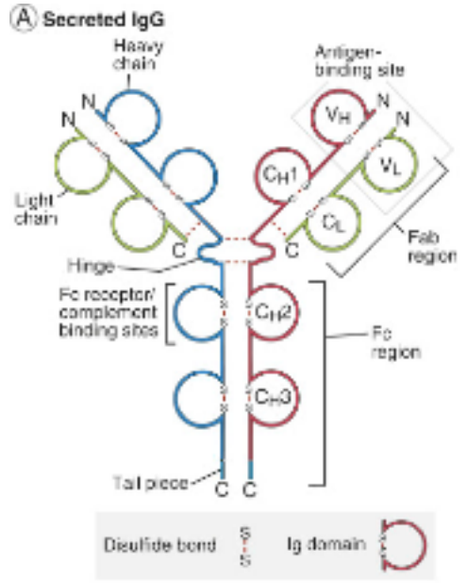

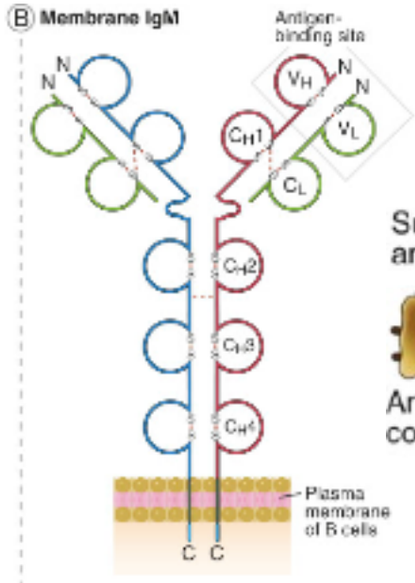

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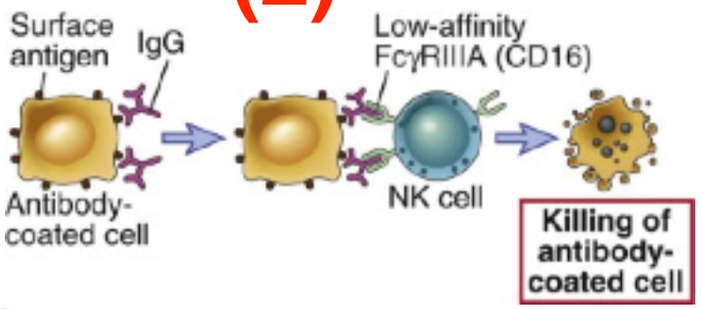

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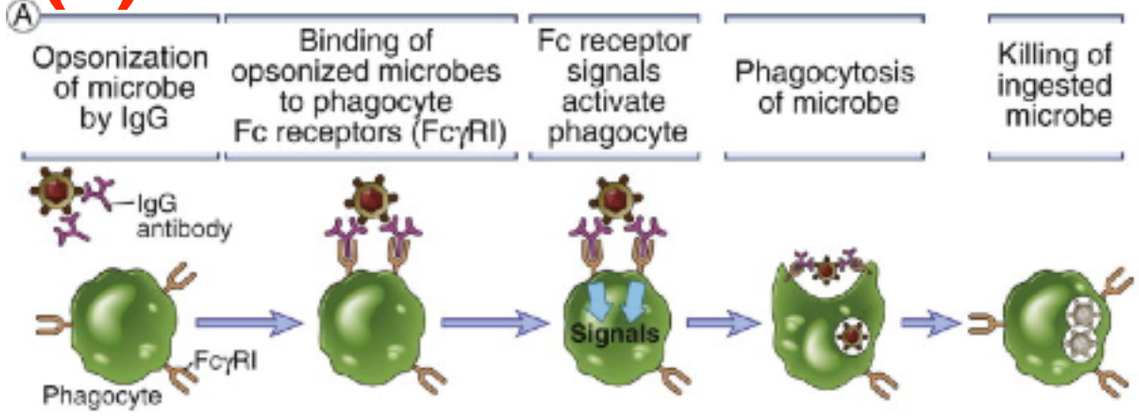


In response to specific antigen, activated B cells secrete antibodies (Abs) specific for that antigen, mediating humoral immunity. Abs can then work in several ways to enhance the effectiveness of the immune response.

(1) Ab can directly **neutralize** a toxin or pathogen by binding and blocking an important site either for toxin function or for infection of host cells. This occurs independent of the Fc region of the antibody.

(2) Ab can target a cell or pathogen for direct killing by macrophages, neutrophils or NK cells, via a process known as **antibody-dependent cytotoxicity (ADCC)**. This occurs via Fc recognition.

(3) Ab can **opsonize** a target Ag or pathogen, making phagocytosis much more efficient. In this case, the Ab-bound Ag/pathogen is efficiently engulfed by phagocytes expressing specific receptors for the constant region of Ig (these receptors are known as Fc receptors (FcR) and are specific for each Ig isotype).

(4) (not shown) Ab-bound antigen can activate the complement cascade that can then either a) promote opsonization via interaction with complement receptors expressed on phagocytes, b) promote an inflammatory response that recruits additional leukocyte effector cells, or c) directly induce the lysis of pathogens via the generation of the membrane attack complex, which creates pores in microbial cell membranes.

Why are there different isotypes?

The effector functions of Abs are regulated by the constant region, as determined by its genetic sequence, called an **isotype**. Each constant region exhibits a distinct type of effector function. Remember that binding of Ab Fc regions to FcR expressed on the surface of phagocytes and other cells occurs only when Ab has bound Ag (the exception being IgE binding to FcR of mast cells, which is why allergic IgE-mediated reactions occur so rapidly).

One important defense mechanism against the extracellular stages of most bacteria and viruses is to coat (opsonize) these microbes with antibodies and cause them to be phagocytosed by neutrophils and macrophages. This reaction is best mediated by antibody classes, such as IgG1 and IgG3 (in humans), that bind to high-affinity phagocyte Fc receptors specific for the γ heavy chain (**IgG**). Helminths, in contrast, are too large to be phagocytosed, and they are best eliminated by eosinophils. Therefore, defense against these parasites involves coating them with antibodies to which eosinophils bind. The antibody class that is able to do this is **IgE**, because eosinophils have high-affinity receptors for the Fc portion of the ε heavy chain.

Ⓑ Antibody isotype	Isotype-specific effector functions
IgG	Neutralization of microbes and toxins Opsonization of antigens for phagocytosis by macrophages and neutrophils Activation of the classical pathway of complement Antibody-dependent cellular cytotoxicity mediated by NK cells Neonatal immunity: transfer of maternal antibody across placenta and gut Feedback inhibition of B cell activation
IgM	Activation of the classical pathway of complement
IgA	Mucosal immunity: secretion of IgA into lumens of gastrointestinal and respiratory tracts, neutralization of microbes and toxins
IgE	Defense against helminths Mast cell degranulation (immediate hypersensitivity reactions)

The antibody isotype produced is also influenced by the site of immune responses. For example, **IgA** antibody is the major isotype produced in mucosal lymphoid tissues, probably because cytokines such as transforming growth factor (TGF)-β that promote switching to IgA are made in these tissues. The B cells activated in these lymphoid tissues are also induced to express chemokine receptors and adhesion molecules that favor their migration into the sites just below mucosal epithelial barriers. IgA is the principal antibody isotype that can be actively secreted through mucosal epithelia.

The diversity of pathogens is why an effective host defense requires that the immune system make different antibody isotypes in response to different types of microbes, even though all naive B lymphocytes specific for all these microbes express antigen receptors of the IgM and IgD isotypes. Thus, the nature of the **helper T cell response** to a microbe guides the subsequent antibody response, making it optimal for combating that microbe. This is how different components of the immune system are regulated coordinately and function together in defense against different types of microbes. It also explains why deficiencies in antibody production may be due to B cell or T cell defects.

How do B cells develop?

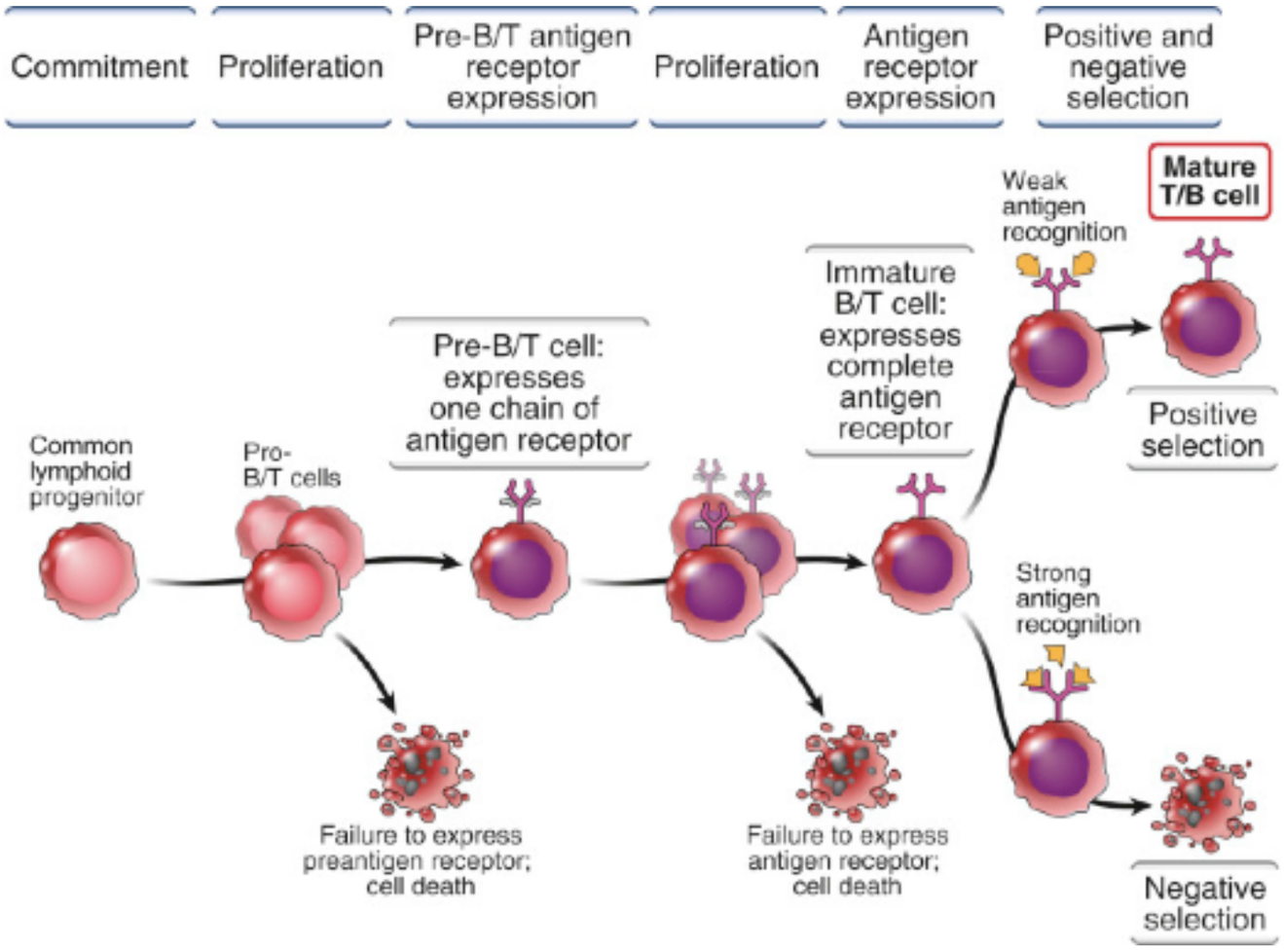
The development of lymphocytes from bone marrow stem cells involves commitment of hematopoietic progenitors to the B or T cell lineage, the proliferation of these progenitors, the rearrangement and expression of antigen receptor genes, and selection events to preserve and expand cells that express potentially useful antigen receptors. These steps are common to B and T lymphocytes, even though B lymphocytes mature in the bone marrow and T lymphocytes mature in the thymus. Each of the processes that occurs during lymphocyte maturation plays a special role in the generation of the lymphocyte repertoire.

Developing lymphocytes undergo proliferation at several stages during their maturation. Survival and proliferation of the earliest lymphocyte precursors are stimulated mainly by the growth factor interleukin-7 (**IL-7**), which is produced by stromal cells in the bone marrow and the thymus. Further proliferative expansion of the B and T cell lineages occurs after the developing lymphocytes have completed their first antigen receptor gene rearrangement and assembled a preantigen receptor. This step is a quality control checkpoint in lymphocyte development that ensures preservation of cells with functional receptors.

Remember, lymphocytes are selected at multiple steps during their maturation to preserve the useful specificities, with checkpoints ensuring only cells with intact, functional antigen receptors are selected to survive and proliferate. Selection is based on the expression of intact antigen receptor components and what they recognize. Pre-lymphocytes and immature lymphocytes that fail to express antigen receptors die by apoptosis.

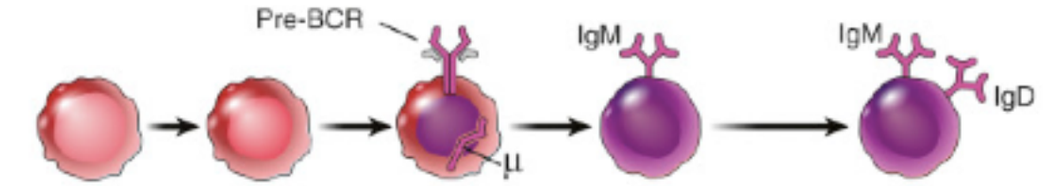
Gene rearrangements in the developing lymphocytes randomly generate antigen receptors with highly diverse specificities. T cells undergo **positive selection**, ensuring that cells that complete maturation will be capable of recognizing antigens displayed by the same major histocompatibility (MHC) molecules on antigen-presenting cells (APCs), which are the only MHC molecules these cells can normally encounter. Thus, immature T cells are selected to survive only if they recognize MHC molecules in the thymus.

B and T lymphocytes also undergo **negative selection**, which eliminates strongly self-reactive cells to prevent antigen receptors from recognizing peptides of self proteins. Negative selection is used to eliminate these potentially dangerous lymphocytes and prevent the development of autoimmune responses.

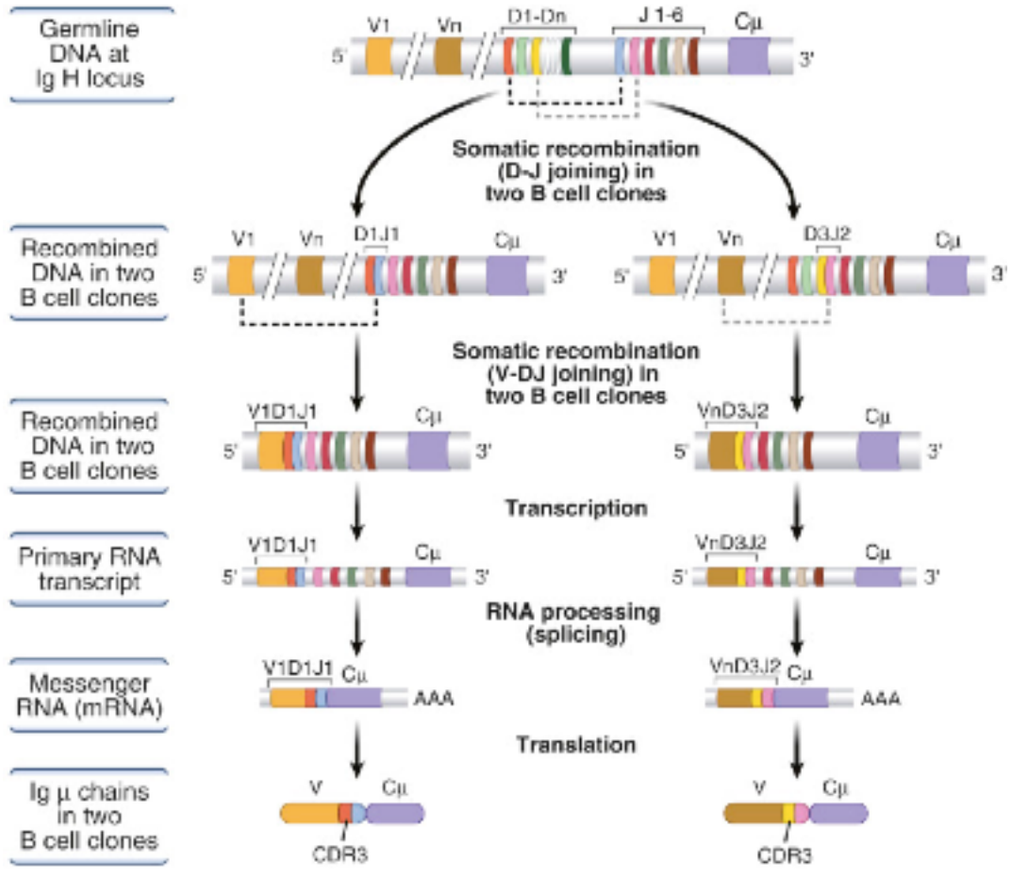


During their maturation, B and T lymphocytes go through cycles of proliferation and expression of antigen receptor proteins by gene recombination. Cells that fail to express intact, functional receptors die by apoptosis, because they do not receive the necessary survival signals. At the end of the process, the cells undergo positive and negative selection. The lymphocytes shown may be B or T cells.

How do B cells become antigen-specific?



	Stem cell	Pro-B	Pre-B	Immature B	Mature B
Ig DNA, RNA	Germline DNA	Germline DNA	Recombined H chain gene (VDJ); μ mRNA	Recombined H chain gene, κ or λ genes; μ and κ or λ mRNA	Alternative splicing of primary transcript to form C_μ and C_δ mRNA
Ig expression	None	None	Cytoplasmic μ and pre-B receptor-associated μ	Membrane IgM (μ+κ or λ light chain)	Membrane IgM and IgD



The maturation of B lymphocytes proceeds through sequential steps, each of which is characterized by particular changes in immunoglobulin (Ig) gene expression and in the patterns of Ig protein expression. At the transition from pro-B to pre-B or pre-B to immature B cells, failure to express Ig heavy chain or Ig light chain proteins, respectively, results in death of the cells by a default pathway of apoptosis. The pre-BCR (B cell receptor) consists of a membrane-associated Ig μ protein attached to two other proteins called surrogate light chains because they take the place of the light chain in a complete Ig molecule.

During B cell development, the expression of an Ig heavy chain involves two gene recombination events (D-J joining, followed by joining of a V region to the DJ complex, with deletion and loss of intervening gene segments). The recombined gene is transcribed, and the VDJ complex is spliced onto the C region exons of the first heavy-chain RNA (which is μ), to give rise to the μ messenger RNA (mRNA). The mRNA is translated to produce the μ heavy-chain protein. The recombination of the Ig light chain follows similarly, except it lacks D segments, so a V gene recombines directly with a J gene segment. The T cell receptor (TCR) follows essentially the same sequence using α and β chains. The TCR rearrangements result in T cell Receptor Excision Circles, or **TRECs**, which can be detected in newborn screening. *Very low or absent TRECs would suggest a T cell deficiency.*

The somatic recombination of V and J, or of V, D, and J, gene segments is mediated by a lymphoid-specific enzyme, the VDJ recombinase, and additional enzymes, most of which are not lymphocyte specific and are involved in repair of double-stranded DNA breaks introduced by the recombinase. The VDJ recombinase is composed of the recombination-activating gene 1 and 2 (**RAG-1 and RAG-2**) proteins.

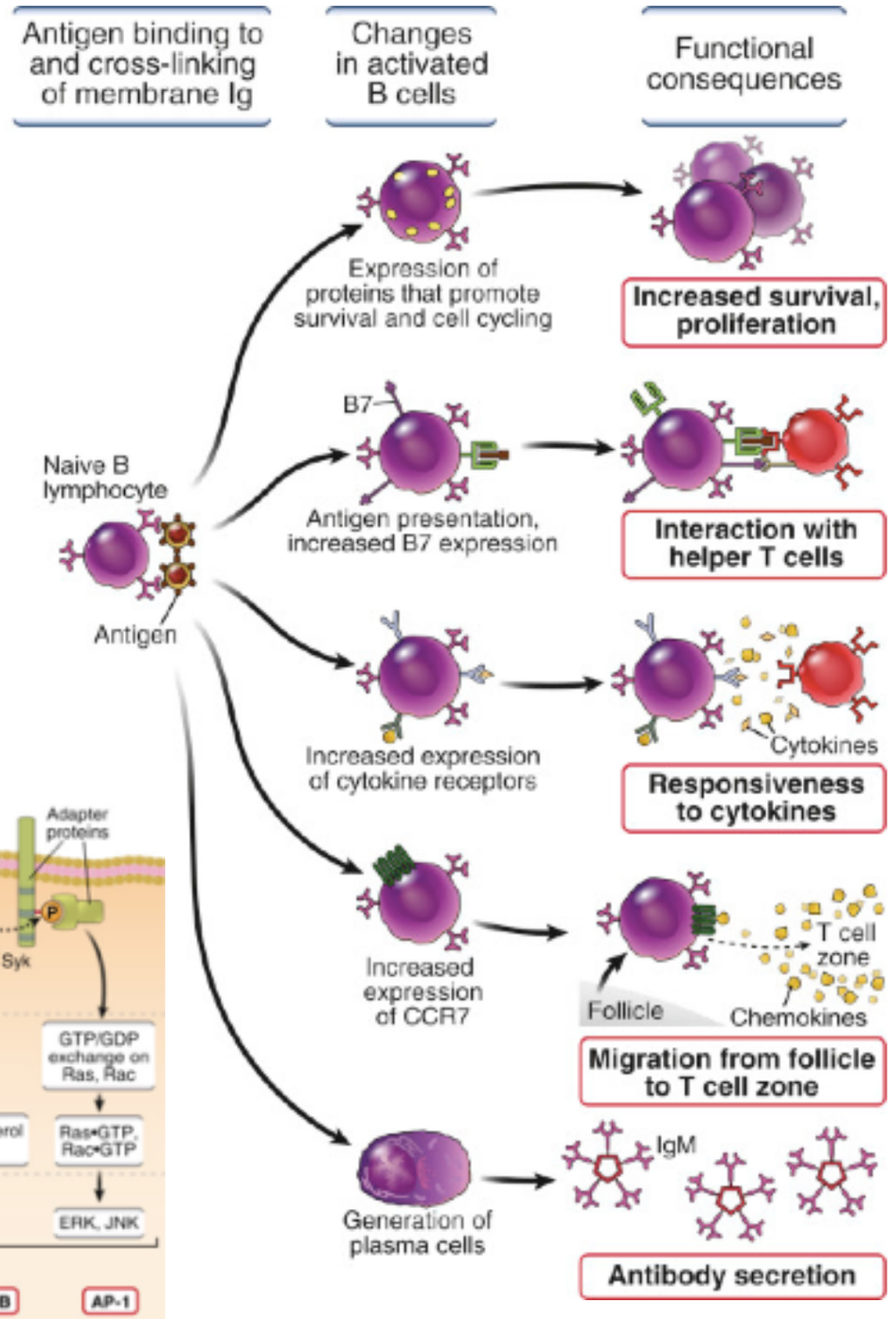
In B cells, the Ig heavy-chain locus rearranges first, and only cells that are able to make an Ig μ heavy-chain protein are selected to survive and become pre-B cells. Pre-B cells are defined by the presence of the Ig μ heavy-chain protein, mainly in the cytoplasm. Some of the μ protein is expressed on the cell surface in association with two other, invariant proteins, called surrogate light chains because they resemble light chains and associate with the μ heavy chain. The complex of μ chain and surrogate light chains associates with the Igα and Igβ signaling molecules to form the pre-B cell receptor (pre-BCR) complex. After completion, mature B cells leave the bone marrow.

How are B cells activated?

Naive B lymphocytes recognize antigens but do not secrete **antibodies**, and activation of these cells stimulates their differentiation into antibody-secreting plasma cells. The activation of naive, mature B lymphocytes upon antigen recognition results in the proliferation of antigen-specific cells, leading to **clonal expansion**, and in their differentiation into **plasma cells**, which actively secrete antibodies and are thus the effector cells of humoral immunity. This process is also under the influence of helper T cells and other stimuli. Some of the activated B cells undergo heavy-chain isotype switching and affinity maturation, and some become long-lived memory cells.

Remember that B cells and plasma cells secrete antibodies for **humoral immunity**, which is the principal defense mechanism against microbes with capsules rich in polysaccharides and lipids, because antibodies can be produced against polysaccharides and lipids but T cells cannot respond to nonprotein antigens.

Humoral immune responses are initiated when antigen-specific B lymphocytes in the spleen, lymph nodes, and mucosal lymphoid tissues recognize antigens. Some of the antigens in tissues or in the blood are transported to and concentrated in the B cell–rich follicles and marginal zones of these peripheral lymphoid organs. In lymph nodes, macrophages may capture and display bound antigens to B cells. B lymphocytes specific for an antigen use their membrane-bound immunoglobulin (Ig) as receptors that recognize the antigen directly, without any need for processing. B cells are capable of recognizing the native (unprocessed) antigen, so the antibodies that are subsequently secreted (which have the same specificity as the B cell antigen receptors) are able to bind to the native microbe or microbial product.



The activation of B cells by antigen in lymphoid organs initiates the process of B cell proliferation and IgM secretion and prepares the B cell for interaction with helper T cells. B cell activation proceeds through multiple different pathways and transcription factors.

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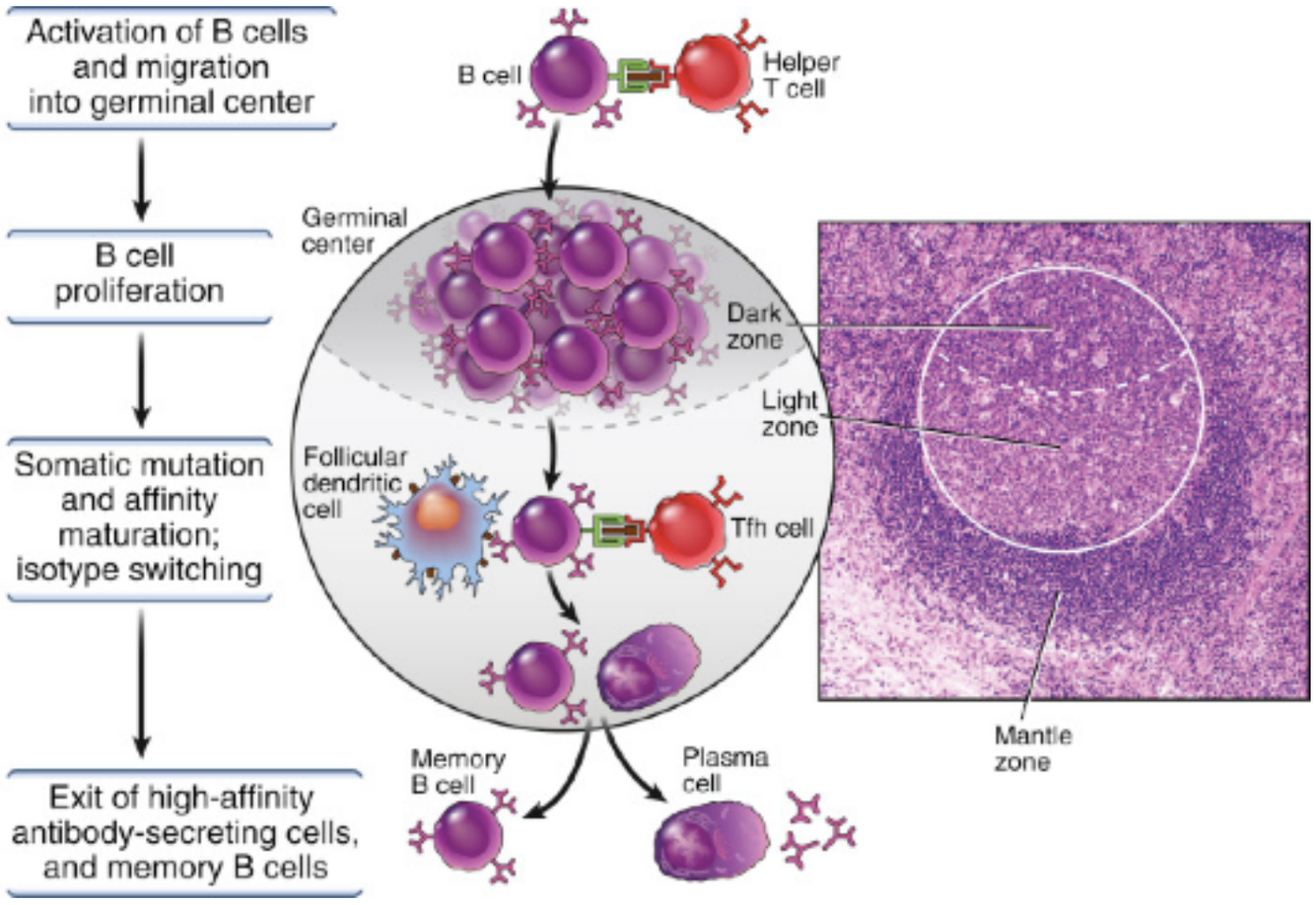
How do B cells differentiate?

B cell activation by antigen (and other signals) initiates the proliferation and differentiation of the cells and prepares them to interact with helper T lymphocytes if the antigen is a protein. The activated B lymphocytes enter the cell cycle and begin to proliferate. The cells may also begin to synthesize more IgM and to produce some of this IgM in a secreted form.

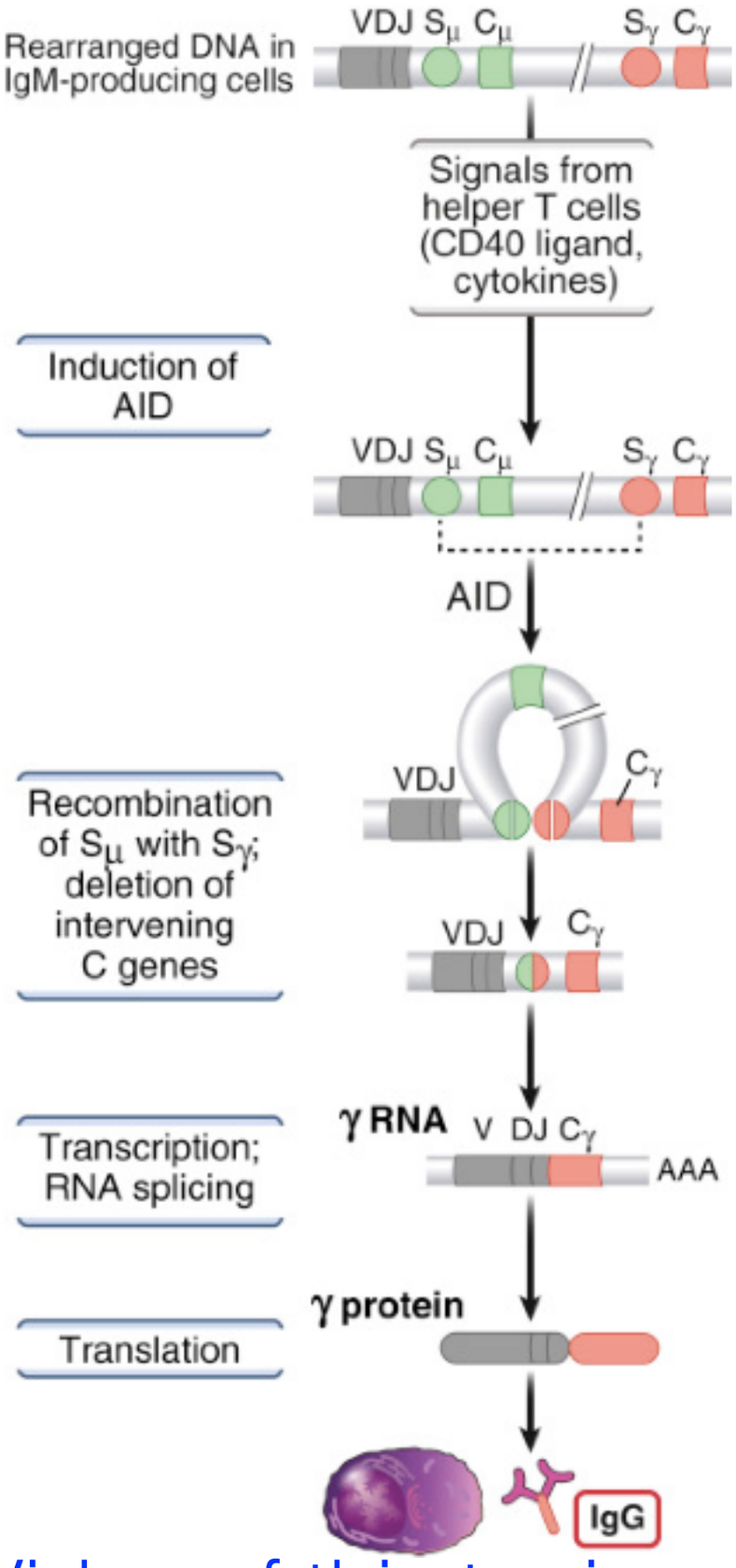
B cell activation is greatest when an antigen is multivalent, cross-links many antigen receptors, and activates complement and innate immune receptors strongly; all these features are typically seen with polysaccharides and other T-independent microbial antigens. Remember that by themselves, soluble proteins typically do not stimulate high levels of B cell proliferation and differentiation. This is because most soluble protein antigens do not contain multiple identical epitopes, so they are not capable of cross-linking many receptors on B cells. However, protein antigens can induce signals in B lymphocytes that lead to important changes in the cells that enhance their ability to interact with helper T lymphocytes.

Initial B cell activation occurs at an extra follicular focus, after which a few of the activated B cells migrate back into the lymphoid follicle and begin to divide rapidly in response to signals from T follicular helper (Tfh) cells. It is estimated that these B cells have a doubling time of approximately 6 hours, so one cell may produce several thousand progeny within a week. The region of the follicle containing these proliferating B cells is the germinal center. In the germinal center, B cells undergo extensive **isotype switching** and **somatic mutation** of Ig genes. The highest-affinity B cells are the ones that are selected during the germinal center reaction to differentiate into memory B cells and long-lived plasma cells. Proliferating B cells reside in the dark zone of the germinal center while selection occurs in the less dense light zone.



B cells that have been activated by T helper cells at the edge of a primary follicle migrate into the follicle and proliferate, forming the dark zone of the germinal center. Germinal center B cells undergo extensive **isotype switching** and **somatic mutation** of Ig genes, and migrate into the light zone, where B cells with the highest affinity Ig receptors are selected to survive, and they **differentiate into plasma cells or memory cells**, which leave the germinal center. The right panel shows the histology of a secondary follicle with a germinal center in a lymph node. The germinal center includes a basal dark zone and an adjacent light zone. The mantle zone is the part of the follicle outside the germinal center.

How do B cells change the antibody isotype?



Helper T cells stimulate the progeny of IgM– and IgD–expressing B lymphocytes to produce antibodies of different heavy-chain isotypes (classes). Different antibody isotypes perform different functions, and therefore the process of isotype switching broadens the functional capabilities of humoral immune responses. Heavy-chain isotype switching is induced by a combination of CD40 ligand (**CD40L**)-mediated signals and cytokines. These signals act on antigen-stimulated B cells and induce switching in some of the progeny of these cells. In the absence of CD40 or CD40L, B cells secrete only IgM and fail to switch to other isotypes, indicating the essential role of this ligand-receptor pair in isotype switching. Cytokines produced by follicular helper T cells determine which heavy-chain isotype is produced.

The molecular mechanism of isotype switching, called **class switch recombination (CSR)**, takes the previously formed VDJ exon encoding the V domain of an Ig μ heavy chain and moves it adjacent to a downstream C region. IgM-producing B cells, which have not undergone switching, contain in their Ig heavy-chain locus a rearranged VDJ gene adjacent to the first constant-region cluster, which is Cμ. The heavy-chain mRNA is produced by splicing a VDJ exon to Cμ exons in the initially transcribed RNA, and this mRNA is translated to produce a μ heavy chain, which combines with a light chain to give rise to an IgM antibody. Thus, the first antibody produced by B cells is IgM. Signals from CD40 and cytokine receptors stimulate transcription through one of the constant regions that is downstream of Cμ. In the intron 5′ of each constant region (except Cδ) is a conserved nucleotide sequence called the switch region. During switch recombination, the switch region 5′ of Cμ recombines with the switch region adjacent to the transcriptionally active downstream constant region, and the intervening DNA is deleted. An enzyme called **activation-induced deaminase (AID),** which is induced by CD40 signals, plays a key role in this process. AID converts cytosines in DNA to uracil (U). The sequential action of other enzymes results in the removal of the U's and the creation of nicks in the DNA. Such a process on both strands leads to double-stranded DNA breaks. When double-stranded DNA breaks in two switch regions are brought together and repaired, the intervening DNA is removed, and the rearranged VDJ exon that was originally close to Cμ may now be brought immediately upstream of the constant region of a different isotype (e.g., IgG, IgA, IgE). The result is that the B cell begins to produce a new heavy-chain isotype (determined by the C region of the antibody) with the same specificity as that of the original B cell, because specificity is determined by the sequence of the VDJ exon, which is not altered. Note that although the C region changes, the VDJ region, and thus the specificity of the antibody, is preserved. (Each C region gene consists of multiple exons, but only one is shown for simplicity.)

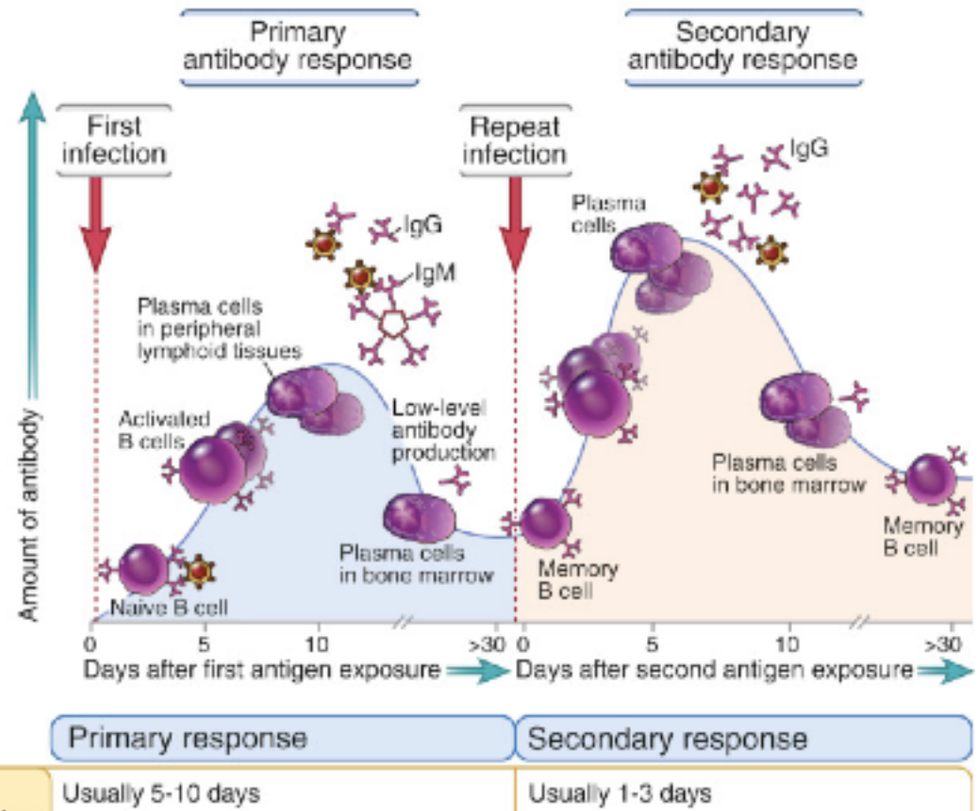
How do B cells acquire memory?

In the germinal centers, the activated B cells not only under class switch recombination (CSR) but they also undergo rapid proliferation and accumulate mutations in their immunoglobulin (Ig) V genes. These B cells produce antibodies with different affinities for the antigen. Follicular dendritic cells (FDCs) display the antigen, and B cells that recognize the antigen are selected to survive. FDCs display antigens by utilizing Fc receptors to bind immune complexes or by using C3 receptors to bind immune complexes with attached C3b and C3d complement proteins. B cells also bind the antigen, process it, and present it to follicular helper T (Tfh) cells in the germinal centers, and signals from the Tfh cells promote survival of the B cells. As more antibody is produced, the amount of available antigen decreases, so only the B cells that express receptors with higher affinities can bind the antigen and are selected to survive.

Activated B cells in germinal centers may differentiate into long-lived **plasma cells** or **memory cells**. The antibody-secreting cells enter the circulation and are called plasmablasts. From the blood they tend to migrate to the bone marrow or mucosal tissues, where they may survive for years as plasma cells and continue to produce high-affinity antibodies, even after the antigen is eliminated. It is estimated that more than half of the antibodies in the blood of a normal adult are produced by these long-lived plasma cells; thus, circulating antibodies reflect each individual's history of antigen exposure. These antibodies provide a level of immediate protection if the antigen (microbe or toxin) reenters the body. Think about the antibody titers you've had checked post-vaccination before enrolling in medical school.

A fraction of the activated B cells, which often are the progeny of isotype-switched high-affinity B cells, do not differentiate into active antibody secretors but instead become memory cells. Memory B cells do not secrete antibodies, but they circulate in the blood and reside in mucosal and other tissues. They survive for months or years in the absence of additional antigen exposure, undergo slow cycling, and are ready to respond rapidly if the antigen is reintroduced. Therefore, memory from a T-dependent antibody response can last for a lifetime.

Ⓐ



Ⓑ

Lag after immunization	Usually 5-10 days	Usually 1-3 days
Peak response	Smaller	Larger
Antibody isotype	Usually IgM>IgG	Relative increase in IgG and, under certain situations, in IgA or IgE (heavy-chain isotype switching)
Antibody affinity	Lower average affinity, more variable	Higher average affinity (affinity maturation)

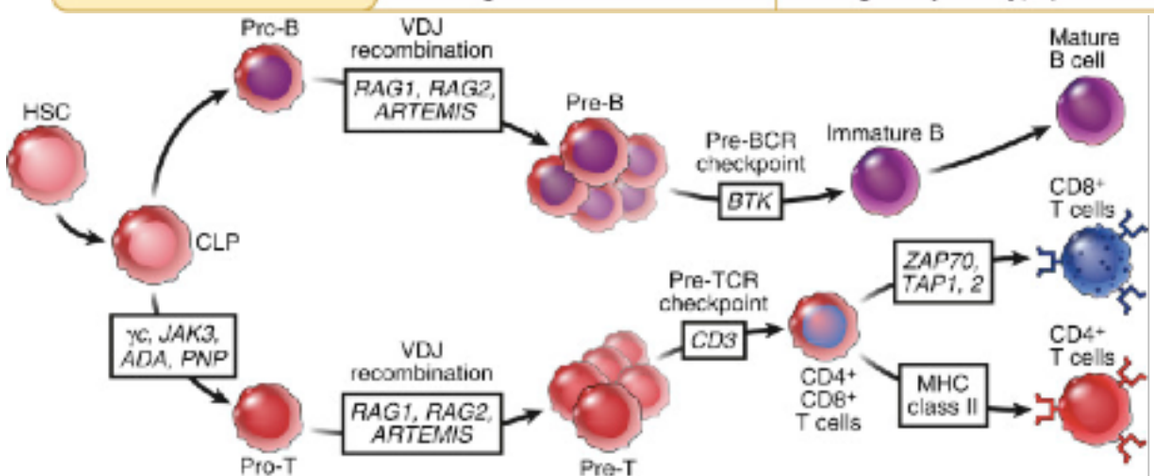
Primary and secondary antibody responses differ in several respects. In a primary response, naive B cells in peripheral lymphoid tissues are activated to proliferate and differentiate into antibody-secreting plasma cells and memory cells. Some plasma cells may migrate to and survive in the bone marrow for long periods. In a secondary response, memory B cells are activated to produce larger amounts of antibodies, often with more heavy-chain class switching and affinity maturation. These features of secondary responses are seen mainly in responses to protein antigens, because these changes in B cells are stimulated by helper T cells, and only proteins activate T cells (not shown). The kinetics of the responses may vary with different antigens and types of immunization.

What happens if something goes wrong?
Immunodeficiencies Part 1 of 2

Severe combined immunodeficiency (SCID)		
Disease	Functional deficiencies	Mechanism of defect
X-linked SCID	Markedly decreased T cells; normal or increased B cells; reduced serum Ig	Cytokine receptor common γ chain gene mutations, defective T cell maturation due to lack of IL-7 signals
Autosomal recessive SCID due to ADA, PNP deficiency	Progressive decrease in T and B cells (mostly T)	ADA or PNP deficiency leads to accumulation of toxic metabolites in lymphocytes
Autosomal recessive SCID due to other causes	Decreased T and B cells; reduced serum Ig	Defective maturation of T and B cells; may be mutations in *RAG* genes and other genes involved in VDJ recombination or IL-7R signaling

B cell immunodeficiencies		
Disease	Functional deficiencies	Mechanism of defect
X-linked agammaglobulinemia	Decrease in all serum Ig isotypes; reduced B cell numbers	Block in maturation beyond pre–B cells, because of mutation in Bruton tyrosine kinase (BTK)
Ig heavy chain deficiencies	Deficiency of IgG subclasses; sometimes associated with absent IgA or IgE	Chromosomal deletion involving Ig heavy-chain locus at 14q32

Disorders of T cell maturation		
Disease	Functional deficiencies	Mechanism of defect
DiGeorge syndrome	Decreased T cells; normal B cells; normal or decreased serum Ig	Anomalous development of 3rd and 4th branchial pouches, leading to thymic hypoplasia



CLP (common lymphoid progenitor) HSC (hematopoietic stem cell)

There are two big categories of immunodeficiency relevant to this case. Determining which parts of a patient's immune system are normal or abnormal can help narrow the underlying genetic cause, but also guide treatment, including prophylactic antibiotics, vaccination strategies, or determining if the patient is a candidate for a stem cell transplant. Newer treatment strategies of genome alteration may also become available.

(1) Defects in Lymphocyte Maturation

Many congenital immunodeficiencies are the result of genetic abnormalities that cause blocks in the maturation of B lymphocytes, T lymphocytes, or both. Some example proteins shown include JAK3 (Janus kinase 3), a kinase involved in signaling by many cytokine receptors; ARTEMIS, a protein involved in antigen receptor gene recombination; BTK (Bruton tyrosine kinase), a kinase that delivers signals from the pre–B cell receptor (BCR) and BCR; ZAP70, a kinase involved in TCR signaling; TAP proteins, which transport peptides for presentation by class I MHC molecules; ADA (Adenosine deaminase) and PNP (purine nucleoside phosphorylase), enzymes involved in purine metabolism important for lymphocytes; and RAG1, RAG2 (recombination-activating gene), enzymes which mediate V(D)J recombination.

What happens if something goes wrong?
Immunodeficiencies Part 2 of 2

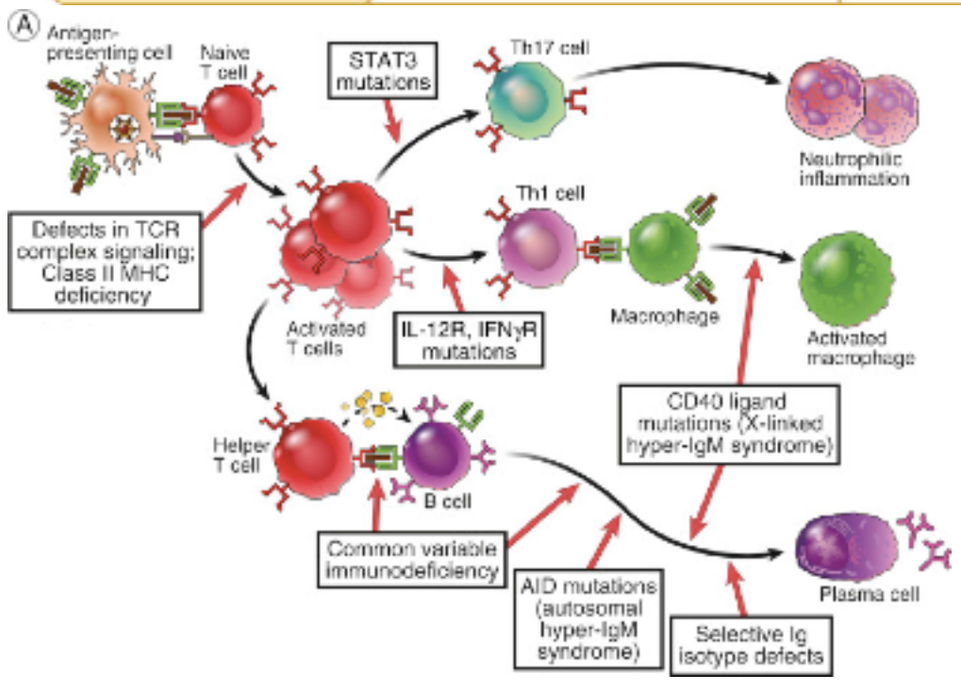
There are two big categories of immunodeficiency relevant to this case. Determining which parts of a patient's immune system are normal or abnormal can help narrow the underlying genetic cause, but also guide treatment, including prophylactic antibiotics, vaccination strategies, or determining if the patient is a candidate for a stem cell transplant. Newer treatment strategies of genome alteration may become available.

(2) Defects in Lymphocyte Activation and Function
Congenital immunodeficiencies may be caused by genetic defects in the expression of molecules required for antigen presentation to T cells, T or B lymphocyte antigen receptor signaling, helper T cell activation of B cells and macrophages, and differentiation of antibody-producing B cells. Examples include AID (Activation-induced deaminase) an enzyme which mediates class switch recombination (CSR); SAP (SLAM-associated protein) and ZAP-70 (ζ chain–associated protein of 70 kD) which are signaling molecules in T cell activation. Defects in memory B and T cells can also occur (not shown). Note that abnormalities in class II MHC expression and TCR complex signaling can cause defective T cell maturation as well as defective activation of the cells that do mature.

B

Disease	Functional Deficiencies	Mechanisms of Defect
X-linked hyper-IgM syndrome	Defects in helper T cell–dependent B cell and macrophage activation	Mutations in CD40 ligand
Common variable immunodeficiency	Reduced or no production of selective isotypes or subtypes of immunoglobulins; susceptibility to bacterial infections or no clinical problems	Mutations in receptors for B cell growth factors, costimulators
Defective class II MHC expression: the bare lymphocyte syndrome	Lack of class II MHC expression and impaired $CD4^+$ T cell activation; defective cell-mediated immunity and T cell–dependent humoral immunity	Mutations in genes encoding transcription factors required for class II MHC gene expression
Defects in T cell receptor complex expression or signaling	Decreased T cells or abnormal ratios of $CD4^+$ and $CD8^+$ subsets; decreased cell-mediated immunity	Rare cases due to mutations or deletions in genes encoding CD3 proteins, ZAP-70
Defects in Th1 differentiation	Decreased T cell–mediated macrophage activation; susceptibility to infection	Rare cases due to mutations encoding the receptors for IL-12 or interferon-γ
Defects in Th17 differentiation	Decreased T cell–mediated inflammatory responses; mucocutaneous candidiasis, bacterial skin abscesses	Rare cases due to mutations in genes encoding STAT3, IL-17, IL-17R
X-linked lymphoproliferative syndrome	Uncontrolled EBV-induced B cell proliferation and CTL activation; defective NK cell and CTL function and antibody responses	Mutations in gene encoding SAP (an adaptor protein involved in signaling in lymphocytes)

A



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