

Regulation of B Cell Development and the Humoral Response II

The Germinal Center Reactions

This lecture is about what happens to B lymphocytes in the germinal centers of secondary lymphoid organs. A quick review of what's already happened to B cells by the time they reach the germinal center (also see Lecture #6 or Chapters 7 & 9): Bone marrow precursors have already become mature (but "naïve") B cells in the bone marrow. This process involved somatic recombination of antigen receptor genes, allelic exclusion, and negative selection based on reactivity to self antigen. The mature, naïve B cells – a diverse, specific, and self-tolerant repertoire – have already migrated into lymphoid follicles under the influence of chemokines that bind their CXCR5 receptors. All of the antibody produced by mature, naïve B lymphocytes exists in membrane-bound form, and in either the IgM or the IgD isotype.

In the germinal center of the follicle, four important things happen:

1. **Affinity maturation**, the development of antibodies in B cell clones that have an even higher affinity for antigen, occurs via two different events at molecular level:
 - random *somatic hypermutation* in the variable region genes, and
 - *selection* for high affinity clonal variants.
2. **Isotype switch recombination**, which diversifies the heavy-chain constant regions of antibodies.
3. **Peripheral tolerance**, the continual elimination of self-reactive clones.
4. **Final maturation of B lymphocytes into memory or plasma cells.**

It's important to remember that, even though this lecture focuses on the development of B lymphocytes, *CD4+ helper T cells are intimately involved with B cells' affinity maturation and heavy-chain isotype switch recombination in the germinal center.*

Affinity Maturation

Somatic hypermutation only happens in germinal center B cells that are receiving signals from T cells. B cells iteratively experience mutations of Ig-gene variable regions and undergo apoptosis if mutations produce antibodies with diminished antigen binding. Multiple rounds of random mutation with apoptosis-based selection after each round improve the affinity of antibody produced by activated B cells.

Somatic hypermutation of B lymphocytes' Ig genes in the germinal center occurs at 10^6 times the mutation rate of normal DNA. (According to Abbas, it's only 10^3 to 10^4 times the normal mutation rate). Remember that Ig and TCR genes are the only genes that undergo cutting and

joining. The dramatically elevated mutation rate exhibited by Ig genes while B cells are in the germinal center is another unique behavior.

Mutations are limited to 1kb around the rearranged variable regions of heavy-chain and light-chain genes. Mutations never occur in the constant regions. Somatic hypermutation is probably related to the high level of transcription of these genes. The **activation-induced cytidine deaminase (AID) enzyme** has been identified as necessary and sufficient to cause hypermutation. It is believed that short segments of ssDNA are generated during transcription of the variable regions, that AID deaminates cytidines on these segments, and that repair mechanisms subsequently cause mutations in the variable regions. The random mutations generated in this way usually result in antibody that binds antigen with diminished affinity. But some mutations improve the affinity of antibody-antigen binding.

Somatic hypermutation in the germinal center is beneficial because mutated B lymphocytes undergo **selection** after each round of mutation. In the germinal center, B cells are very pro-apoptotic. To avoid apoptosis, B cells must receive two signals:

1. Signal from antigen. **FDCs** have antigen, and if B cells bind antigen with high affinity, they get the best signal. This serves to weed out low affinity (recognition specificity) mutants, which die. The high antigen affinity mutants frequently interact with FDC, get the first signal, and internalize the antigen for presentation to CD4+ helper T cells.
2. Signal from CD4+ T-helper cells. When CD4+ helper T cells are activated by antigen, they express **CD40L (ligand), which binds CD40** on the B cells. This second signal finalizes the selection of high affinity antibody producing B cell mutants by alleviating apoptosis only in those cells that manage to activate helper T cells.

Each fully formed germinal center contains cells derived from one or a few antigen-specific B cell clones. B cells proliferate and undergo hypermutation randomly in the “dark zone.” Then they go to the “light zone,” where they can encounter antigen from FDCs and interact with T cells. If a B cell receives the two signals essential to avoid apoptosis, it returns to the “dark zone,” where it proliferates and mutates again. This **iterative pattern** is the basis for affinity maturation in the germinal center.

Affinity maturation not only cultivates B cell clones producing antibody with higher affinity for antigen, but it also eliminates self-reactive clones (essential for **peripheral tolerance**). If a mutation yields antibody that recognizes self-antigen, then the B cell will bind antigen presented by FDCs (will receive the first signal), but it will not be able to interact with a helper T cell (will not receive the second signal) because any T cell responsive to self antigen has already been eliminated in the thymus.

Abbas writes that “**the formation of germinal centers depends on the presence of helper T cells and the interactions between CD40 and CD40L and is therefore observed only in antibody responses to helper T cell-dependent protein antigens**” (204).

Isotype Switch Recombination

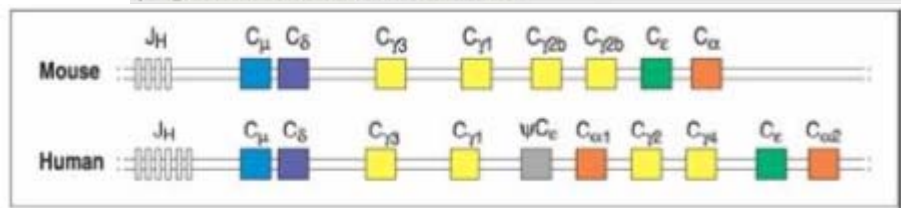
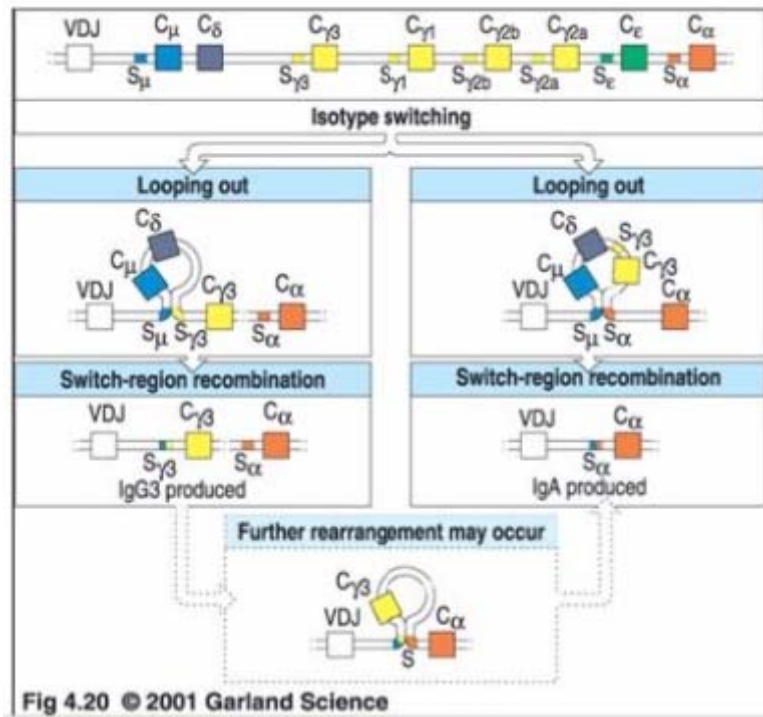
While the variable regions of B lymphocytes' Ig genes undergo somatic hypermutation, the constant regions undergo changes of their own. As mentioned above, all of the antibodies produced by the mature, naïve B lymphocytes entering the germinal center exist in membrane-bound form, and in either the IgM or the IgD isotype. But after B lymphocytes enter the germinal center and begin T cell-dependent activation, different isotypes are generated by a second DNA rearrangement (the first was the V-D-J rearrangement in the variable region that determined antigen specificity). This second rearrangement, called **isotype switch recombination** or **isotype switching**, occurs only in the heavy chain and produces diversity in the heavy chain constant regions.

The human heavy-chain locus has the C-mu and C-delta coding regions close to each other, just after the J segment of the variable region. About 50kb after C-delta comes a series of C-gamma, C-alpha, and C-epsilon coding regions. (The mouse heavy-chain locus shown at right is almost the same as the human, but the human heavy chain locus has more C-alpha subtypes, as shown below).

When the V-D-J rearrangement occurs (in marrow), the C-mu or C-mu and C-delta regions are transcribed: all antibodies are of the IgM or IgD isotypes. But

when B lymphocytes reach the germinal center, another rearrangement leads to transcription of the C-gamma, C-alpha, and

C-epsilon regions, so IgG, IgA, and IgE isotypes can be produced. The isotype switching rearrangement involves, like the V-D-J rearrangement, cutting and looping out DNA. However, it differs from the variable region rearrangement because the cutting occurs in intervening sequences with repetitive elements called **switch regions** (one upstream of each different coding segment). The switch regions become opposed to one another, then the DNA is cut and rejoined with the C-mu and C-delta regions (or the C-mu and C-delta and C-gamma 3 regions, or any chunk up to S-alpha2) before the DNA is transcribed.



Little is known about the molecular mechanism of isotype switch recombination, but it is known that **AID** is required. Thus it's assumed that, just as in somatic hypermutation of the variable region, isotype switch recombination of the constant region involves transcription related formation of a ssDNA within the switch regions, which is deaminated to recruit cutting, joining, and repair enzymes.

What is known is how isotype switch recombination is regulated: Cytokine signals from T lymphocytes specify the transcription of a “**cryptic promoter**” (or “**I-region promoter**”) that is upstream of the heavy-chain locus. The transcript in turn specifies the switch region where the heavy-chain constant region will be cut – and thus which isotype will be produced. For example, gamma interferon released by helper T cells induces transcripts specifying recombination to gamma3 and gamma2a. Therefore a B lymphocyte that receives a gamma interferon signal from a helper T cell will produce IgG subtypes. Similarly, B lymphocytes receiving TGF-beta signals in the germinal center make I-region transcripts that promote isotype switch recombination to C-alpha, and so make IgA. **By different cytokine signals and combinations of cytokine signals, T lymphocytes instruct B lymphocytes' isotype switching in the peripheral lymphoid tissue.**

A single B lymphocyte never produces antibody with more than one specificity (variable region), but it can produce antibody in multiple isotypes (both IgM and IgD, for example).

mRNA Processing

The antibody produced by B lymphocytes is also modulated at the level of mRNA processing. Importantly, mRNA processing determines whether the antibody produced will be in **membrane-bound or secreted form**. Whether antibody will be membrane bound or secreted is determined by **differential polyadenylation and splicing**. The DNA segments encoding secreted (hydrophilic) and membrane-bound (hydrophobic) tails are side-by-side with one polyadenylation site between them, and one after them. If polyadenylation occurs between the two potential tails, the membrane-bound tail will never be transcribed or will be spliced out, and the secreted form of IgM, for example, will be produced. If polyadenylation occurs after the membrane-bound tail, then the secreted tail will be spliced out, and IgM will be membrane-bound. (See Figure 9-12 in Abbas, which is much easier to understand than my description).

Differential polyadenylation also accomplishes **the IgM to IgD transition**. As mentioned above, the B lymphocytes leaving the marrow can express antibody in both the IgM and IgD isotypes despite the fact that they will not undergo isotype switch recombination until they reach the germinal center. This happens because the C-mu and C-delta constant regions are separated by a polyadenylation site. The differentiation between IgM and IgD isotypes occurs at the level of mRNA processing, with the isotype chosen according to the location of polyadenylation.

Beyond the Germinal Center

Both somatic hypermutation/selection and isotype switch recombination happen **at the same time**, in the germinal center. Whereas B lymphocytes in the marginal zones of peripheral lymphoid organs encounter a lot of T cell independent antigens, which give rise to low affinity IgM antibodies, B lymphocytes in the germinal center undergo iterative rounds of proliferation, somatic hypermutation, and selection, as well as isotype switch recombination, in close

cooperation with CD4+ helper T cells. The germinal center reactions thus cultivate high affinity antibodies with diverse effector functions.

After 10-12 days, the germinal center is resolved, and B lymphocytes are differentiated. Two cell types result from B lymphocytes that have undergone the germinal center reactions:

Plasma cells making high affinity antibody that's usually a switched isotype (via isotype switch recombination) and in secreted form (via differential polyadenylation). Plasma cells have a great deal of ER, which enables them to secrete a great deal of antibody (they no longer express surface Ig or surface proteins that would permit them to interact with T cells). Plasma cells are terminally differentiated; their lifespan thus **limits the antibody response in time**. (Sometimes plasma cells home back to the bone marrow, where they stimulate stromal cells to secrete the cytokine IL-6, which sustains them; thus their lifespan is lengthened).

Memory cells have high affinity surface Ig, also usually a switched isotype. Usually memory cells enjoy a long lifespan, even without antigen. They circulate and reside in the blood, lymph nodes, and the marginal zone of the spleen. Memory cells **respond rapidly** to a secondary stimulation – they have already undergone the germinal center reactions, so they produce high affinity antibody ready without having to wait 10-12 days for affinity maturation. That's why the secondary immune response is enhanced. Upon binding antigen, some memory cells become plasma cells rapidly; others remain memory cells.

Antibodies eliminate antigen in five ways:

1. Secreted antibodies neutralize pathogens (e.g., keep viruses from binding cell receptors and infecting cells).
2. Antibody-dependent cell death: antibodies coat infected cells (antigen on surface) or pathogens; then NK cells, which have FC receptors on their surface to bind the **FC regions** of antibodies, kill the target cell.
3. IgM and IgG antibodies can activate the complement system (proteolytic reactions that end up killing cells). (Remember that IgM is pentameric when secreted; it binds epitopes on a cell and is recognized by components of the complement system as such).
4. **Opsonization**, or coating the pathogen, which makes endocytosis more efficient because macrophages, etc. can bind the FC regions of antibodies.
5. Mast cells have FC receptors for IgE and bind IgE all the time. When a pathogen binds a resident IgE' (or multiple resident IgEs') variable region(s), it causes the mast cell to degranulate. The released products produce an allergic reaction.

In general, IgG is the primary antibody in serum. IgA is prominent on mucosal surfaces and in mucosal secretions. IgE, as mentioned above, is bound to mast cells and involved in allergic

reactions. And IgM is present on developing B lymphocytes and can activate the complement system. The FC regions of different isotypes are different and contribute to this diversity of function.

Pathology

Hyper IgM syndrome (HIM) describes the patients with a lot of IgM and not a lot of other isotypes. Several mutations can cause HIM. A mutation in either CD40 or CD40L, which are necessary for the survival of B lymphocytes that are undergoing somatic hypermutation and isotype switching in the germinal center, can cause HIM. A mutation in AID, which is also required for the germinal center reactions, can also cause HIM. Without the molecules critical to T cell-dependent B cell development, somatic hypermutation and isotype switching in the germinal center are impossible. B lymphocytes can only produce low affinity IgM.

Multiple myeloma is a malignancy of B lymphocytes, specifically of plasma cells. It begins in the bone marrow, when a single clone becomes malignantly transformed. Malignant plasma cells are usually IL-6 dependent and cause destruction in bone. They produce a tremendous amount of antibody, which appears in the blood and urine, and can cause kidney disease. Kidney disease and bone destruction typically cause death in patients with multiple myeloma.