# ONTOGENY: DEVELOPMENT OF T AND B CELLS

ORIGINS. The immune system is part of the hematopoietic[1] system, which comprises all the cells of the blood, as well as many cells resident in other organs. This system, like the skin, is constantly renewed throughout life; unlike most parts of the brain, for example, where neurons do not turn over to any appreciable extent. Thus the development of the lymphoid or immune system, which starts in the embryo, ►is continued throughout the individual's life span, the rate decreasing with age. Hematopoietic stem cells arise early in embryogenesis from certain endothelial cells in the aorta (derived from lateral plate mesoderm). By birth, most hematopoiesis has moved into the bone marrow, which is the primary organ for it from then on.

HEMATOPOIETIC STEM CELLS. ►Stem cells are undifferentiated cells which, when they divide, give rise on average to another stem cell and a daughter committed to differentiation. That way you never run out of stem cells. They vary in their potential; the fertilized ovum is the totipotential stem cell, eventually giving rise to all other differentiated cells. The hematopoietic stem cell, HSC, is more restricted, as it gives rise to red and white cells and their derivatives, including the microglia of the brain, but not other cells—it is multipotential. ►The two differentiated daughters of the HSC are the common lymphocyte progenitor, CLP, and the common myeloid progenitor, CMP. The CLP gives rise to B and T cell progenitors. The CMP's descendants include the progenitors of erythrocytes, megakaryocytes (from which platelets bud off), eosinophils, mast cells/basophils, and the common granulocyte/monocyte progenitor from which neutrophils, macrophages, and most dendritic cells develop.
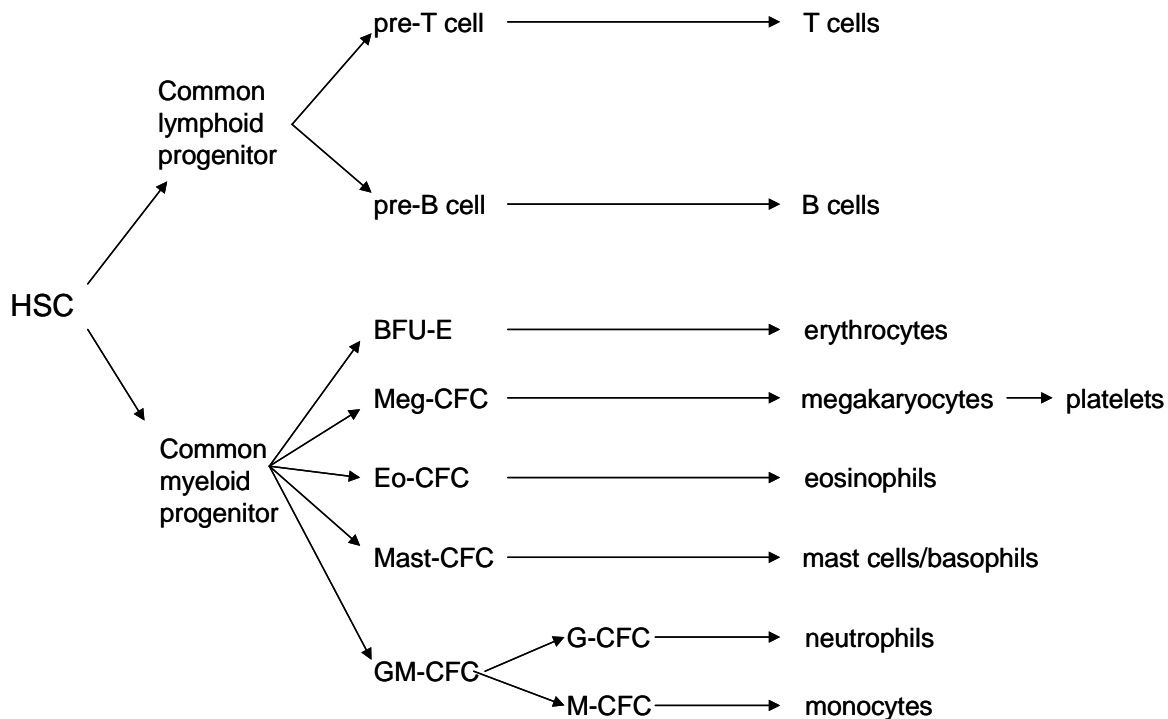

BFU-E, burst-forming unit, erythroid, a progenitor that forms shotgun-like bursts of cells in agarose gel culture of bone marrow; CFC, colony-forming cell, in similar cultures; GM-CFC, granulocyte/macrophage CFC. Note, hormones that promote growth of these cells are called colony-stimulating factors, for example GM-CSF, G-CSF, and M-CSF. E-CSF already had a name: EPO, erythropoietin.

---

[1] Greek ποίησις (poiesis) = making.

B CELL DEVELOPMENT. B cells are so called because in birds (where this work was first done) their progenitors leave the bone marrow and finish their development in a separate organ called the **B**ursa of Fabricius. The bursa is located at the hind end of the gut, and many immunologists have tried to find a human bursa equivalent in Gut-Associated Lymphoid Tissues[2] (GALT); but it isn't there. ►B cells in mammals develop in the **B**one marrow.

B cell progenitors can be identified as such when they begin to synthesize immunoglobulin components. ►The first to be detectable is mu chain in the cytoplasm; then complete cytoplasmic IgM (cIgM). This indicates that B cells rearrange their heavy chain genes before their light chains. Since it may be useful, the **pro-B cell** divides a few times, so you won't have just one of a good thing. Then one of the light chain genes rearranges, making IgM. A cell with cytoplasmic IgM but no surface IgM is called a **pre-B cell**. Next to appear is surface IgM (sIgM), which is an IgM monomer with an extra membrane-embedded extension at the end of its Fc. Finally, when the cell is fully mature, both IgM and IgD (of the same specificity, of course) are found on the cell surface. All of this results from alternative splicing of the VDJ-mu-delta primary RNA transcripts, which you might like to review now. ►A functionally and diagnostically important point: an immature B cell has sIgM only, and a mature B cell has sIgM and sIgD:
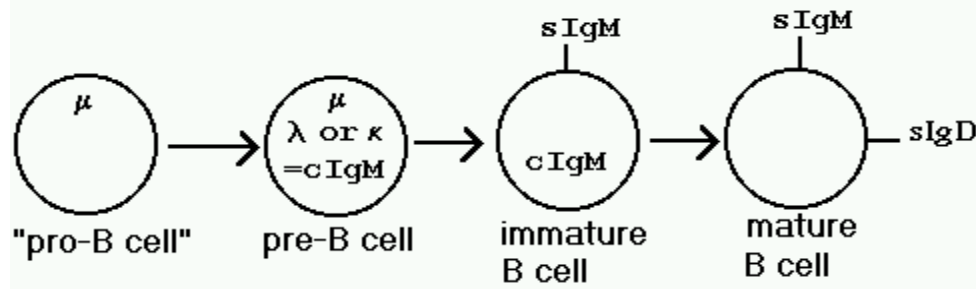

CLONAL DELETION. When a mature B cell is exposed to its correct antigen it gathers its receptors (IgD and IgM) at one spot on the surface and then takes them inside by endocytosis. The antigen is partially digested (processed), and if other conditions (that we'll learn about later) are right, the cell will go on to differentiate into an antibody secretor. ►If an *immature* B cell (sIgM but no sIgD) is similarly exposed to antigen, this signal causes the cell to try receptor editing; if that fails it activates a suicide program (apoptosis), and dies. This is called **clonal deletion**, and it partially explains why we do not make antibody to self. In the bone marrow pre-B cells are differentiating into immature B cells; you can imagine that any cell whose receptors happen to be anti-self will be likely to encounter self in the environment of the bone marrow, and it will either make a new receptor, or be deleted. If it does not encounter antigen (because its receptors are not against self) then it will mature further so that it expresses both sIgM and sIgD. Then, when it meets antigen, it will be stimulated, not deleted. Please note, though, that many anti-self B cells (usually to scarce antigens, not seen in the marrow; or B cells with low affinity to more common antigens) escape clonal deletion and other measures are necessary to keep them from becoming activated; we'll consider some of these when we discuss T cells, and autoimmunity.

> **ASK YOURSELF:** Doesn't clonal deletion seem wasteful? Can you discuss why we must make anti-self B cells in order to get a complete repertoire?

---

[2] The appendix seemed like a good bet, but it turned out it isn't a lymphoid source; on the contrary, it may be a storehouse for bacteria to replenish the gut after an illness.

ANTIBODY RESPONSES. During primary (initial exposure) B cell responses to antigen, ►IgM is secreted first, then for most antigens, helper T cells get involved and there is a switch to IgG, or possibly to IgA or IgE. The helper T cells in the gut and lung preferentially drive an IgM to IgA switch. The 'switch helper' mechanism indicates that B cells in general do what T cells tell them to. We discuss all this more in a little while.

In secondary (booster) responses the IgM response is about the same as in a primary, but the IgG response, efficiently helped by T cells, is sooner, faster, higher[3] and more prolonged:
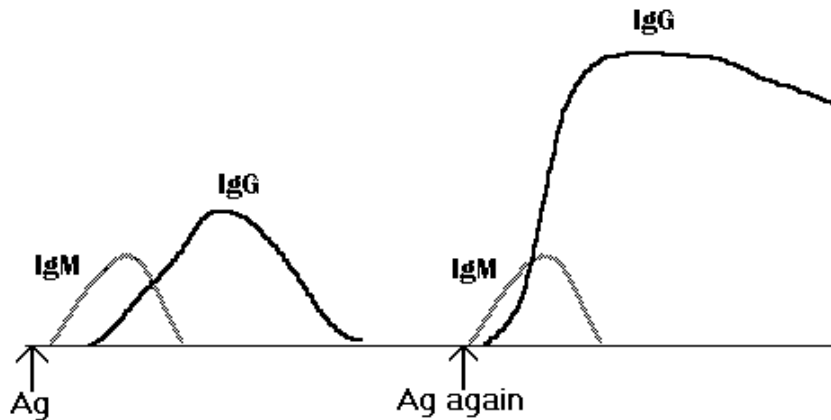
> **ASK YOURSELF:** Given what we've just been discussing, what would you expect to see in response to these immunizations if your patient had no functional helper T cells?

ONTOGENY OF ANTIBODY RESPONSES. ►The fetus makes IgM before birth, but only begins to acquire the capacity to make IgG 3 or 4 months postnatally. However, at birth it has as much IgG in its blood as does an adult; this IgG is maternal, because IgG crosses the placenta, by active transport, from mother to fetus (no other class of immunoglobulin does). ►**The half-life of IgG is about 3 weeks**, so in 7 half-lives = 21 weeks after birth there is less than 1% of the starting amount of maternal IgG left; fortunately, the infant begins to make reasonable amounts of its own IgG at about 12 weeks. IgA starts about when IgG starts.
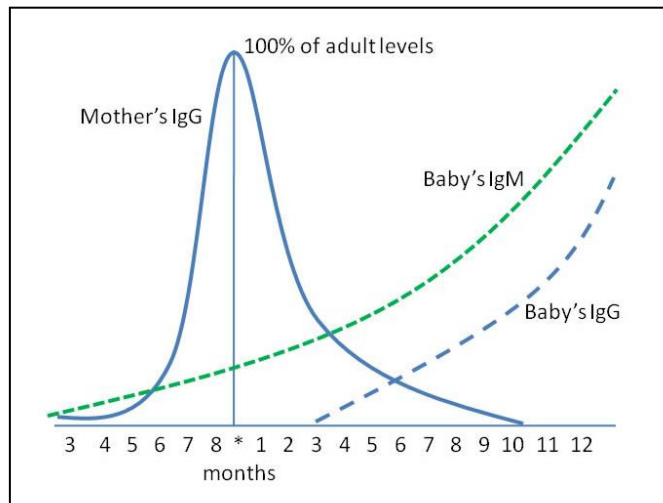
> **ASK YOURSELF**: A baby is born and fails to thrive. At three weeks its serum level of antibody to cytomegalovirus is high. Did it have an intrauterine infection with CMV? Do you have enough information to decide?

---

[3] Was this borrowed by the Olympics for their motto: citius-altius-fortius; quicker, higher, stronger?

COMPLEMENT DEVELOPMENT. Newborn C levels or activity are usually around those of adults; preemies are often low. Complement components are mostly made in the liver, though white blood cells also contribute.

INTRODUCTION TO T CELL DEVELOPMENT. T cells are very interesting. They carry out their development in three different locations: the bone marrow, then the **T**hymus, and finally the peripheral lymphoid organs. Lymphoid precursors in the bone marrow have not yet decided what to become; the default, if they stay there, is to become a B cell. Others go to the thymus, where a very high concentration of Notch ligands induce T cell differentiation. They rearrange receptor genes (V(D)J, but a completely different set of genes from those of the H and L families) and then are selected for their responsiveness to 'self plus antigen.' This concept will be made clear in the next few units; for now, please remember that ►while B cells see free antigen in solution, T cells **only** see antigen on the surface of another cell, which is therefore called an *antigen-presenting cell*.

Developing lymphocytes in the thymus display a variety of differentiation antigens which allow us to understand their maturation. Newly-arrived pre-T cells divide energetically at the thymus periphery, while they are trying to make T cell receptors (TCR); at this stage they express neither of the markers, CD4 and CD8, but soon those that successfully made TCR become CD4+/CD8+ "double positives." As these filter through the thymus from cortex to medulla, they undergo selection, the topic of upcoming discussions. The result is a few mature T cells that get exported as CD4+ (only) helper T cells, or CD8+ (only) cytotoxic T cells. About 99% of thymocytes don't survive selection, and die within the thymus.

►The thymus is a two-component organ: the lymphocytes ("thymocytes") whose precursors came in from the marrow, and the supporting structure or stroma, which develops in the neck region and moves down into the fetal chest. More on this once we've looked at the behavior of T cells.

IMMUNOLOGICAL AGING. In 2010, 7.7% of the world's population, that is, 530,000,000 people were over 65 years of age. By 2050, the proportion will climb to 16%, or 1.55 billion people. Older people are more susceptible to most infectious diseases and usually get sicker and take longer to recover. The explanations are probably spread around just about all body systems; for example, the cilia in lungs beat less efficiently so bacteria aren't as easily cleared. But T cells and B cells age, too, though the numbers in the blood do not decline. We know that the thymus gradually becomes replaced with fat, though there are islands of healthy-looking lymphoid tissue in it up to a great age. ►People can completely reconstitute their T cell numbers and diversity up to about 40 years of age, then diversity becomes increasingly limited, and more and more cells show a 'memory' phenotype while fewer are naïve; old people have fewer but larger clones than do the young. A similar change takes place in B cells, too, possibly a decade or two later. ►So older folks generally make good responses to antigens they saw in the past, but fail to respond well to completely new antigens. This may help explain why the recent SARS epidemic— featuring a brand new pathogen—was disproportionately fatal in the elderly, as is West Nile Fever, brand new in America since 1999. It may also suggest that flu shots in the elderly (unless they are cross-reactive with an earlier strain of virus) are not as useful as we would like to think. And if H5N1 avian flu becomes widespread in humans? *Fuhgeddaboudit!* However, for the H1N1 virus that caused the great flu of 1917-8, older folks had cross-reactive immunologic memory of a related virus, and generally did better than young adults.

Learning Objectives for Ontogeny of the Immune System

1. Define:
   stem cell
   B cell
   T cell
   pre-B cell
   pre-T cell
   self-tolerance
   titer

2. Draw an outline diagram which shows bone marrow, thymus and spleen or lymph node. Indicate the development and movement of cells of the B and T lines, starting with the hematopoietic stem cell and ending with mature T and B cells.

3. Define the Bursa of Fabricius, and discuss where its functions take place in mammals.

4. Describe the sequence of appearance of cytoplasmic and surface immunoglobulins in developing B cells. Using these data, derive a model that could explain self-tolerance at the B cell level ('clonal deletion').

5. Draw a graph showing the antibody response to a typical antigen in a primary and in a secondary response. Show both IgM and IgG antibody levels.

6. Draw a graph which shows relative IgG and IgM levels in a normal infant from fetal life to one year of age. Distinguish maternal from infant's antibodies.

7. Given a newborn's antibody titer, interpret its significance if the antibody is IgG, or IgM. If IgG, calculate what the titer will be at 4 months of age, and state the assumptions you made when you did the calculation.

8. Discuss in cellular and clonal terms the decrease in diversity seen in the immune repertoire of older people.

9. Discuss the relative values of immunizing the young and the old in an epidemic of a novel respiratory virus.