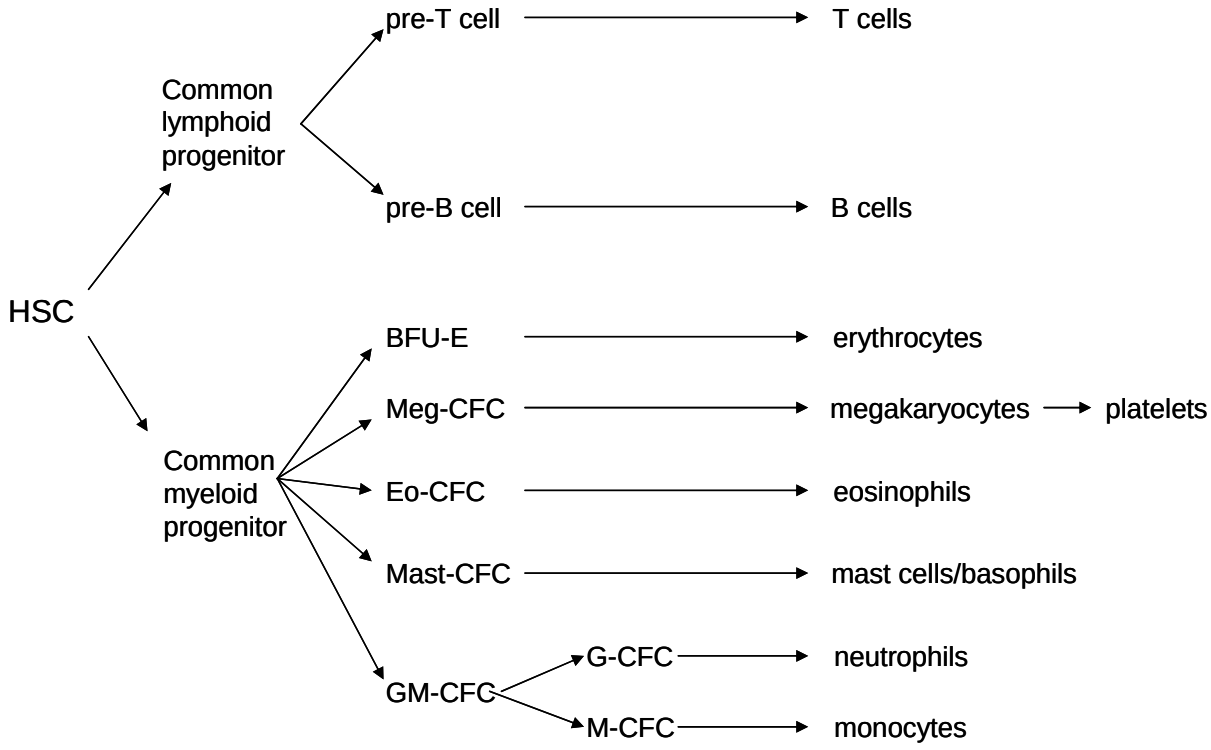
ONTOGENY: DEVELOPMENT OF T AND B CELLS

ORIGINS. The immune system is part of the hematopoietic[1] system, which comprises all the cells of the blood. This system, like the skin, is constantly renewed throughout life; unlike the brain, for example, most of whose 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 first arise early in embryogenesis from certain endothelial cells in the aorta. 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 glia 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 and macrophages develop.

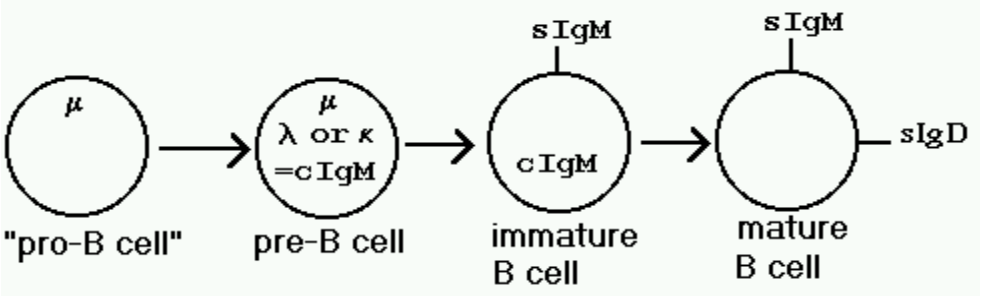


BFU-E, burst-forming unit, erythroid, a progenitor that forms shotgun-like bursts of cells in agarose gel culture of bone marrow; CFC, colony-forming cell, in similar cultures; GM-CFC, granulocyte/macrophage CFC. Note, hormones that promote growth of these cells are called colony-stimulating factors, 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. 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 important point: an immature B cell has sIgM only, and a mature B cell has sIgM and sIgD:



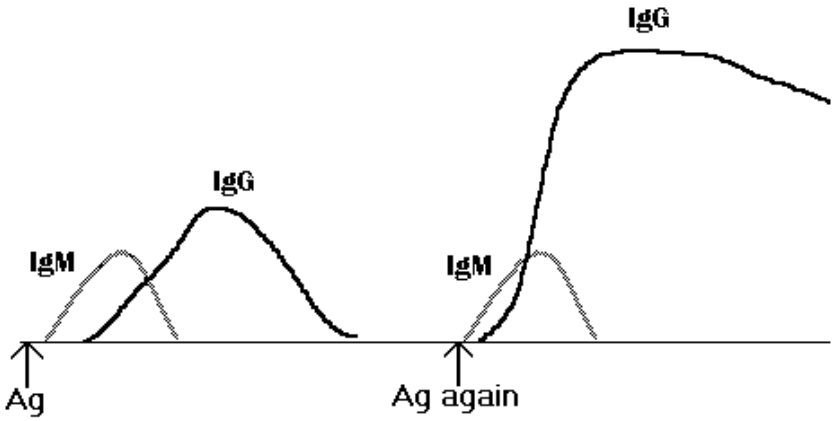
CLONAL ABORTION. When a mature B cell is exposed to its correct antigen it moves its receptors (IgD and IgM) to one spot on the surface ('caps' them) and then takes them inside by endocytosis. The antigen is partially digested (processed), and if other conditions (that we'll learn about later) are right, the cell can go on to divide and differentiate into an antibody secretor. If an *immature* B cell (sIgM but no sIgD) similarly binds antigen, this signal tells the cell it may be autoreactive, and causes it to try receptor editing; if that fails it activates its apoptosis suicide program and dies. This deletion mechanism is called **clonal abortion**. 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 pretty likely to encounter self in the environment of the bone marrow, and either make a new receptor, or be aborted. 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 aborted. Please note, though, that many anti-self B cells (usually to scarce antigens, not seen at significant concentrations in the marrow) escape clonal abortion and other measures are necessary to keep them inactivated; we'll consider some of these when we discuss T cells, and autoimmunity.

> **ASK YOURSELF:** Doesn't clonal abortion seem wasteful? Explain 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.

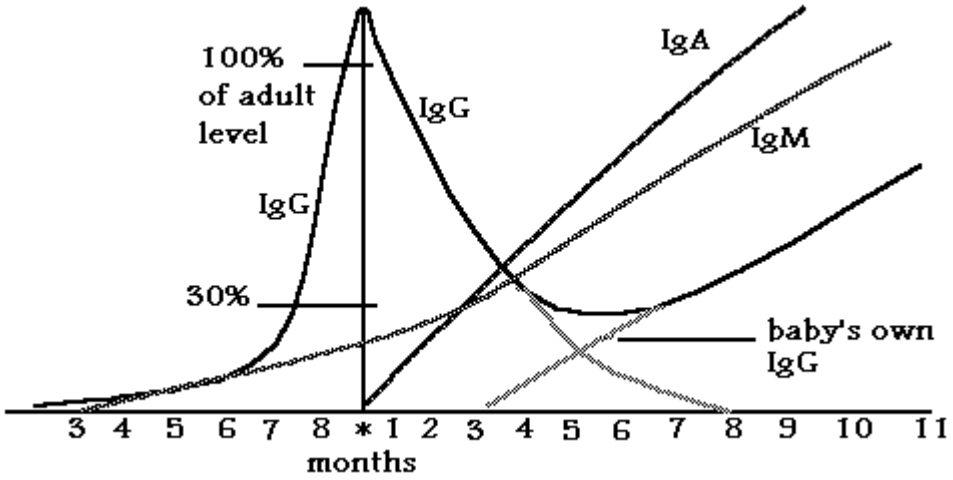
ANTIBODY RESPONSES. During primary (initial exposure) B cell responses to antigen, IgM is secreted first, then for most antigens, helper T cells get involved and there is a switch to IgG, or possibly to IgA or IgE. The helper T cells in the gut and lung preferentially drive an IgM to IgA switch. The 'switch helper' mechanism indicates that B cells in general do what T cells tell them to. As we'll see later, an inappropriate antibody response may often be the T cell's fault.

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: What would you expect to see if your patient had no functional 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:



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?

[3] This was plagiarized by the Olympics as their motto: citius-altius-fortius; quicker, higher, stronger.

COMPLEMENT DEVELOPMENT. Newborn C levels or activity are usually lower than those of adults; preemies are often quite 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. In the bone marrow one finds pre-T cells, which do not yet have the characteristic surface markers that distinguish T cells from other cells (differentiation antigens), but will express them in the right environment. These go to the thymus, where they rearrange their receptor genes 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 could thus be called an ***antigen-presenting cell***.

Once cells have been selected in the thymus, those chosen few are exported; probably not more than 1% of all cells to arise in the thymus ever leave it. The others die by apoptosis.

The thymus is a two-component organ: the lymphocytes which come in from the marrow, and the specialized supporting structure or stroma, which develops in the neck region and moves down into the chest. More on this once we've looked at the behavior of T cells.

Newborns have functional T cells, but they are biased towards a Th2 rather than a Th1 response. We'll discuss these types of helper T cells in detail soon, but for now, this means a tendency toward parasite immunity (probably highly desirable in more primitive times), or its evil twin, allergy. Eventually Th2 and Th1 balance out; but do some children get stuck at the Th2 stage? And are they the ones with allergies and asthma? There's evidence that staying at the Th2 stage is partly genetic, and partly due to inadequate exposure to environmental schmutz. I mention this now as it's part of ontogeny, but we will better understand its meaning when we do T cells, and then Type I and Type II immunopathology.

IMMUNOLOGICAL AGING. In 2006, 8% of the world's population, that is, 500,000,000 people were over 65 years of age. By 2030, the proportion will climb to 13%, or close to a billion people. Older people are more susceptible to 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. 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 their youth, but fail to respond well to 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 virus formerly known as Swine, older folks seem to have immunologic memory of a related virus, and generally did better than the young.

Learning Objectives for Ontogeny of the Immune System

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

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 abortion').

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 conception 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.