The Humoral Immune system
Structure and Diversity

Discussion:

Introduction

Our immune system protects our bodies from the harmful affects of a dizzying array of disease causing pathogens. Although our skin and mucous membranes serve as primary defense systems, many antigens are find their way into our bodies. Our immune system has developed an extensive array of cells, and sophisticated processes that help identify, and eliminate foreign invaders. Given that there are essentially billions of possible antigens it is indeed amazing that we can respond to so many in an efficient and timely manner. What is most impressive of our immune system is it's specificity, memory, and flexibility. This lesson plan will seek to explore part of our immune system: the humoral system. We will seek to understand how gene expression within the immunoglobulin variable regions is able to produce the billions of antibodies that defend us.

A. Two modes of response (See Figure 1 & 1a.) (*Teacher note 1)

The Immunity of the body is achieved by two distinct methods of immune response: the cellular immune system and the humoral immune system. The two systems work through different methods and types of lymphocytes to protect the body from invading pathogens.

1. Cellular Immunity System: (see Figure 1 &1a for overview of Cytotoxic T cells, Helper T cells and B-lymphocytes. Note the use of Helper T cells in both cases. Refer to role of the AIDS virus in the obstruction of the work of these Helper cells.)The Cellular immune system possesses three very effective sets of killing cells. They are termed T lymphocytes because they are formed in the Thymus gland. In this system, macrophages initiate destruction by engulfing pathogens, then cytotoxic T lymphocytes, NK and K (killer cells) attack and kill invading viruses and other pathogens by lysis of their cellular membranes. This is probably the immune response most students are familiar with. It is also a primary and relatively immediate form of immune response intracellular bacteria, viruses and tumor cells. For these reasons it bears mentioning. Figure 1 is a graphic of the role of T Cells and T receptor cells at work in both the humoral and cellular immune systems. An excellent description of the graphic and an explanation of the cellular immune system can be found in Voet and Voet Biochemistry pg. 1096 – 1097. A more complete explanation of figure 1 can be found in the article by Marrack, P and Knapper, J. "The T-Cell and its receptors". *Scientific American* **254** (2): 36-45 (1986).

2. The Humoral Immunity System: (See Figure 2 for the process of B cell activation by an antigen). The humoral immunity system is characterized by the activity of B cells that produce antigen specific antibodies. B cells function much like the macrophages that initiate the cellular response, in that they ingest and antigen and thence display fragments of it on their cell surface. This display binds T helper cells to the B cell membrane, which activates the B cell's production of plasma and memory cells. . Once activated these cells proliferate and produce plasma and memory cells. The plasma cells in turn secrete large quantities of the antigen specific antibody, while the memory cell clones remain ready to counteract any possible reinvasion. (Memory cells will be discussed later in this lesson plan.)

B. Description of components of the immune system * (Brief description of each)

1. Table 1 lists the surface components (what is displayed on the cell surface) and the function of each of the five types of immune cells. You may spend some time on the mode of elimination: lysis and phagocytosis as these are the end result of the work of antibodies; they are also the end result of the cellular immune system. Note that B cells and T cells both express the identity of the antigen on their surface.

C. Humoral immune system.

1. This system is the focus of the lesson plan. The main lymphocyte is the B lymphocyte that is produced from germ line DNA in the bone marrow. It is the rearrangement, recombination of these genes that help create the diversity of antibodies at our disposal. Later in the lesson, we will estimate the number of possible combinations. It is surmised the immune system can produce an antibody for any of the millions of antigen that we may encounter in our lifetime as B-lymphocytes produce one specific type of antibody for every antigen. Once the B cell ingests the antigen, it expresses fragment of the Ag on its surface. Thence with the help of T helper cells, a process of proliferation of cloned plasma cells is initiated. The plasma cells secrete the antibodies specific to the antigen bound to the B cell along with memory cells. The secreted antibodies bind to the antigens and effect one of a series of reactions that help eliminate them. (**Figure 2)** Some Igs, bind to the Ag thereby inhibiting its ability to function, some agglutinate (clump) antigens into large unwieldy particles (preparation for phagocytosis); alternately they may activate a "complement system" which initiates either phagocytosis or lysis of the bound antigen's membrane. The memory cells remain in our bodies in the event that reinvasion occurs.

D. Antibodies:

1. Immunoglobulin are defined as globular serum proteins that typically consist of four polypeptide chains. Each has two identical heavy chains and two identical light chains. Disulfide bridges at specific positions join the chains. The ends of all the immunoglobulin end in variable regions that vary greatly within each class of antibodies. Explain that fixed regions vary very little in structure among the five, but that the variable regions (and hyper variable regions) are the areas where the greatest variation occurs. Later in the lesson you will show how this variation arises.

2. The five immunoglobulin labeled IgA – IgG are the antibodies secreted by the B cells. Each one has a specific function: see Table 2 and 2a for details of each Ab function. **Figure 3** shows the structure of IgA –IgM: **figures 4 and 4a** depict IgG.

3. Immunoglobulin G: The protein is a tetramer with 2 identical heavy chains connected by disulfide bonds to 2 identical light chains. Both chains have variable and constant regions. The L (β chain) has 214 residues divided into the V_L and C_L regions. The V_L varies amino acid sequence over the first 108 residues; residues 109 – 214 are constant. The Heavy (α chain) also has a V_H section (variable residues from 1-108) and $C_{H1\text{-}CH3}$ (constant from 109 – 446). There is a hinge section at residue 214, which gives the IgG its characteristic Y shape. Hyper variable sections (31-35,50-65,and 81-85 in the light chains and 91-102 in the heavy chains area called complementarity-determining regions.

These are the regions that help determine the immunoglobulin's antigen binding specificity.

E. Diversity in antibodies

It is assumed that each person has one antibody for any of all the conceivable antigens. The mathematics of that assumption would demand that we carry many many genes for each of these antibodies. That is not the case as a relatively small number of genes (the variable and hypervariable regions) are able to combine to form approximately 11 billion possible combinations (Voet &Voet pg. 1108). **Figure 6** may seem a bit complicated, however you can show students how the each region can contribute genes for expression. This is the heart of the lesson, thus you may spend the time needed.

For the heavy chain there are four regions of choice: V (250 variable region genes): D (10 Diversity region genes): J (6 Joining region genes). For the light chain we have 150 V region genes and 5 J region genes.

1. Recombination occurs when genes randomly combine as they code for protein sequences. Given the three regions that code for heavy chain variable segments, you can show your students that the possible combination of these genes is 250*10*6: about 15,000 possible combinations for heavy chains and 150 *5 = 750 possible combinations for the light chains.

A second method of variability arises because recombination (2) between regions is not always precise. This possibility raises the variability by a factor of 100 for heavy chain production and a factor of 10 for light chain production. (Voet & Voet: pgs 1107 and 1108). Mutations within the germ lines offer another possible area of variability. Thus the total possible combinations are in the area of 11,000,000,000 1.1 X 10^10 possibilities. This implies that when one of the millions of possible antigens enter our bodies, it is very likely that its specific antibody is available to defend against it.

F. Clonal response (see figure 7: Clonal Selection).

1. The latter step in figure 2 shows the release of the antibody producing plasma cells and the memory cells. Ask students why we need memory cells. Explain that we need to reserve a store of plasma cells at the ready in case the antigens return. To prep the last section, ask students why we get vaccines. Also elicit how they think vaccines work. Remind them that we are given a small dose of an antigen in order to build up our immunity.

G. You may close the lesson with (figure 8 Secondary response) the body's secondary response to an antigen. Explain the role of memory cells in the quick and efficient response to a second invasion by the same antigen.

Teacher Note 1

Figure 1 is a graphic of the role of T Cells and T receptor cells at work in both the humoral and cellular immune systems. An excellent description of the graphic can be found in Voet and Voet Biochemistry pg. 1096 – 1097. A more complete explanation can be found in the article by Marrack, P and Knapper, J "The T-Cell and its receptors". *Scientific American* 254 (2): 36-45 (1986).

Outline:

Leading Question: Ask students what happens when they become ill? Elicit their knowledge of immune system and its role in their lives. How does the system work? What happens when it fails? (Most children will know of some autoimmune diseases.)

A. Two modes of response (Figure 1 & Figure 1a) * (Teacher note)
   1. The Cellular Immune System
   2. Humoral Immune System
B. Description of components of the immune system * (Brief description of each) (Table 1: Cells of the Immune System)
   1. B-Lymphocytes
   2. T-Lymphocytes
   3. Killer Cells
   4. Mast Cells
   5. Accessory Cells
C. Humoral immune system (Figure 2: B Lymphocyte Reaction)
   1. B cells (define function and location)
D. Antibodies
   1. Immunoglobulin (defined)
   2. Classes of immunoglobulin (Table 2: Properties of Human Immunoglobulins & Table 2a: Function of Immunoglobulins)
      a. IgA: structure and role (Figure 3: Structure of IgA-IgM)
      b. IgD: structure and role
      c. IgE: structure and role
      d. IgM: structure and role
      e. IgG: structure and role (Figure 4 & 4a: Structure of IgG)
   3. Immunoglobulin G
      a. Variable and Hyper variable regions (Figure 5:Detail of IgG variable regions)
      b. Constant Regions
      c. Antigen binding
E. Diversity in antibodies
   1. Recombination (Figure 6: Recombination of Germ Line DNA)
   2. Imprecise recombination
   3. Somatic Mutation
      a. Combination calculation (Show students how to calculate the number of possible combinations of 3 letters. Then use logic to calculate possible combinations of alleles of the heavy and light chain segments).
F. Clonal Effect (Figure 7: Clonal Selection)
   1. Memory cells
   2. Plasma cells
G. The secondary response (Figure 8: Secondary Response) (Ask students the role of vaccinations? How do they work? What happened to them when they were vaccinated? Why?

Cellular Immune Response
Figure 1

Figure showing details of Cytotoxic T cells, helper T cells, and B cells are activated for immune response. Figure contrasts cellular and humoral response, helper t cell activation is shown as the intermediary between the two responses.

Source: Biochemistry Voet & Voet. Pg. 1097. Copyright 1986.Sci. American

Overview of Immune Response
Figure 1a.

Figure showing role of cellular immune system and humoral immune system. Decision of which system is mediated by helper T cells.

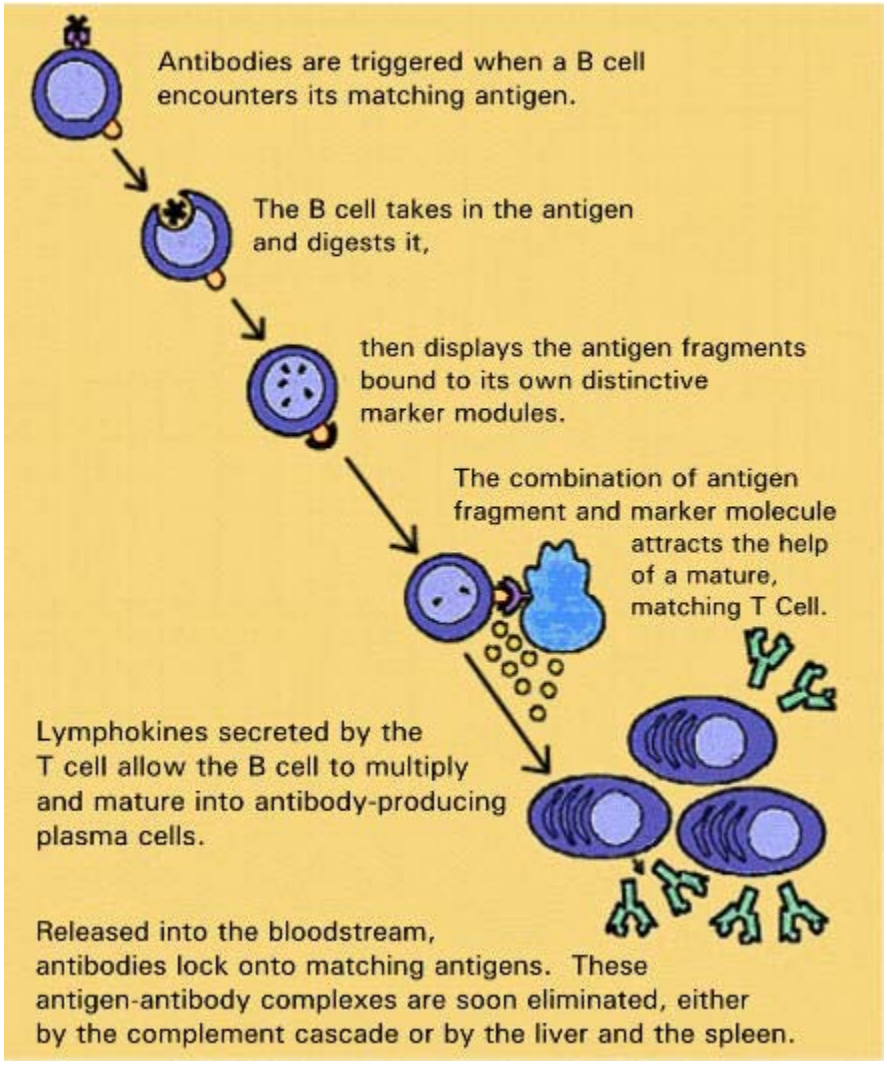
Source: Biology: Campbell 4th Edition. Page 869.

Cells of Immune System
Table 1

Cell group	Surface components	Function
B-lymphocytes	Surface immunoglobulin (Ag recognition)	Direct antigen recognition
	Immunoglobulin Fc receptor	Differentiation into antibody-producing plasma cells
	Class II Major Histcompatability Complex (MHC) molecule (Ag presentation)	Antigen presentation within Class II MHC
T-lymphocytes	CD3 molecule	Involved in both humoral and cell-mediated responses
	T-cell receptor (TCR, Ag recognition)	
Helper T-cells (T_H)	CD4 molecule	Recognizes antigen presented within Class II MHC Promotes differentiation of B-cells and cytotoxic T-cells Activates macrophages
Suppressor T-cells (T_S)	CD8 molecule	Down regulates the activities of other cells
Cytotoxic T-cells (CTL)	CD8 molecule	Recognizes antigen presented within Class I MHC Kills cells expressing appropriate antigen
Accessory cells	Variable	Phagocytosis and cell killing
Macrophages	Immunoglobulin Fc receptor Complement component C3b receptor Class II MHC molecule	Bind Fc portion of immunoglobulin (enhances phagocytosis) Bind complement component C3b (enhances phagocytosis) Antigen presentation within Class II MHC Secrete IL-1 (macrokine) promoting T-cell differentiation and proliferation Can be "activated" by T-cell lymphokines
Dendritic cells	Class II MHC molecule	Antigen presentation within Class II MHC
Polymorphonuclear cells (PMNs)	Immunoglobulin Fc receptor Complement component C3b receptor	Bind Fc portion of immunoglobulin (enhances phagocytosis) Bind complement component C3b (enhances phagocytosis)
Killer cells	Variable	Direct cell killing
NK cells	Unknown	Kills variety of target cells (e.g. tumor cells, virus-infected cells, transplanted cells)
K cells	Immunoglobulin Fc receptor	Bind Fc portion of immunoglobulin Kills antibody-coated target cells (antibody-dependent cell-mediated cytotoxicity, ADCC)
Mast cells	High affinity IgE Fc receptors	Bind IgE and initiate allergic responses by release of histamine

Source: http://www.transplantbuddies.org/library/immunbasic.html#humoral

B Lymphocyte Reaction
Figure 2



Source: http://www.atopix.com/About%20Us/immune_globulins.htm

Table 2: Properties of Human Immunoglobulins

Property	IgM	IgG	IgA	IgE	IgD
% of Serum Ig	10	75	15	<0.01	<0.5
Structure	Pentamer	Monomer	Dimer	Monomer	Monomer
Complement Fixation	+++	+	-	-	-
Transplacental Passage	-	+	-	-	-
Allergic Response	-	-	-	+	-
Mucosal Secretion	-	-	+	-	-
Opsonization(via Complement)	+*	+++	-	-	-

Table 2a: Major Functions of Human Immunoglobulins

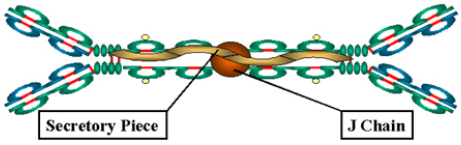
Immunoglobulin	Major Function
IgM	*Main Ig during Primary Response (Early antibody).* *Fixes Complement (most effectively).*
IgG	*Main Ig during Secondary Response (late antibody).* *Opsonization.* *Fixes Complement.* *Neutralizes Toxins, Viruses.*
IgA	*Secretory mucosal Ig* *Prevents invasion from gut mucosa.*
IgE	*Immediate Hypersensitivity.* *Mast cell and Basophil reactions.* *Activates Eosinophils in helminth infection.*
IgD	*Function Unknown.* *Mostly on the Surface of B cells.*

Source: http://sprojects.mmi.mcgill.ca/immunology/Ig_text.htm

Structure of Four Immunoglobulin Glycoproteins
Figure 3

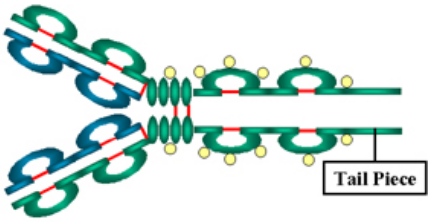
IgA

- Structure
  - Serum - monomer
  - Secretions (sIgA)
    - Dimer (11S)
    - J chain
    - Secretory component



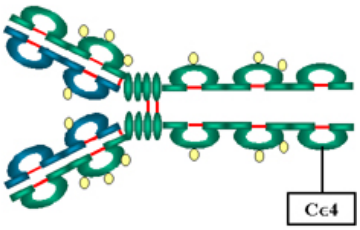
IgD

- Structure
  - Monomer
  - Tail piece



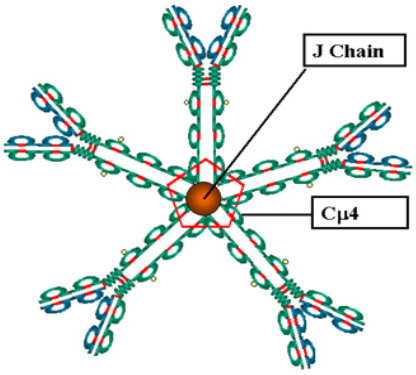
IgE

- Structure
  - Monomer
  - Extra domain (C_{H4})



IgM

- Structure
  - Pentamer (19S)
  - Extra domain (C_{H4})
  - J chain



Source http://www.phasmatodea.org/ap2/immunology2_notes.html

Structure of Immunoglobulin G
Figure 4

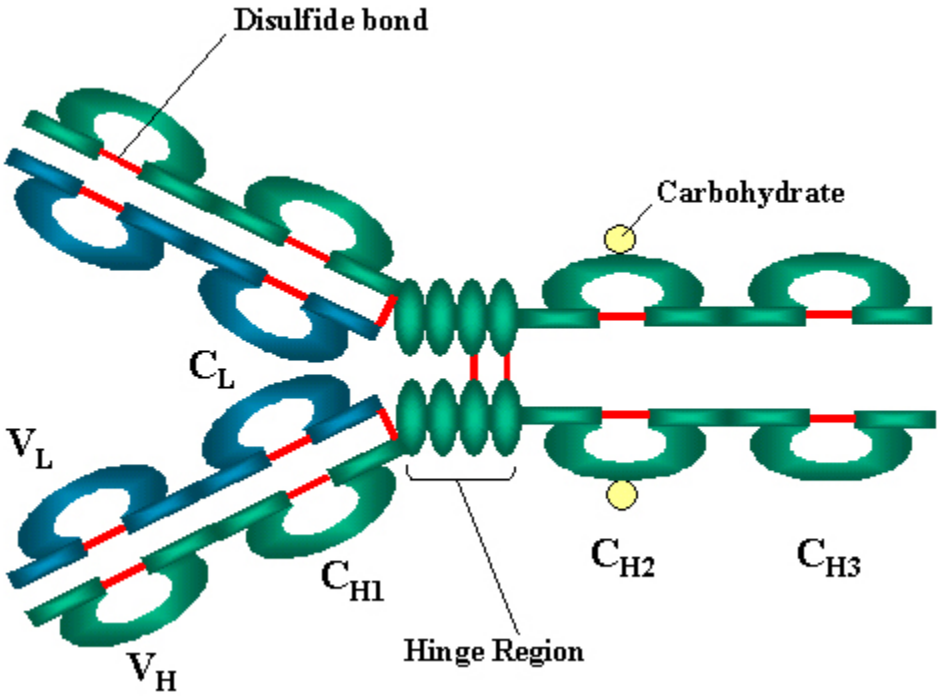
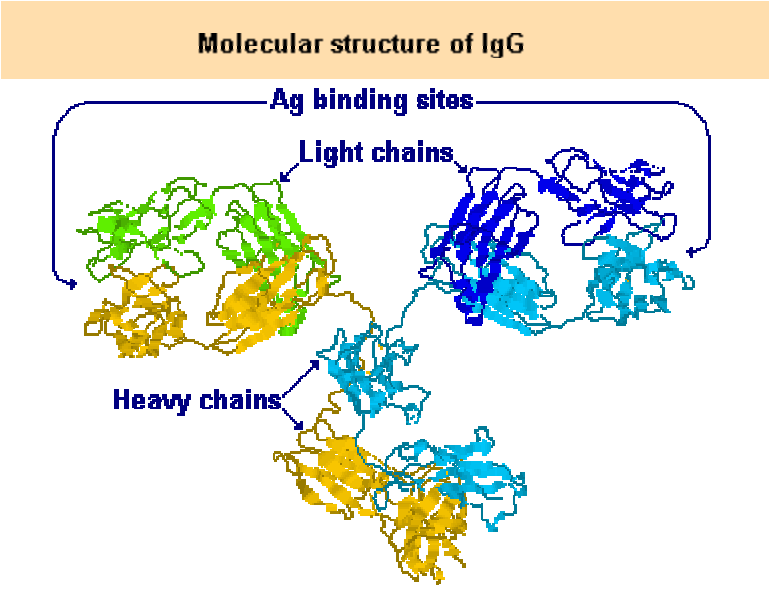
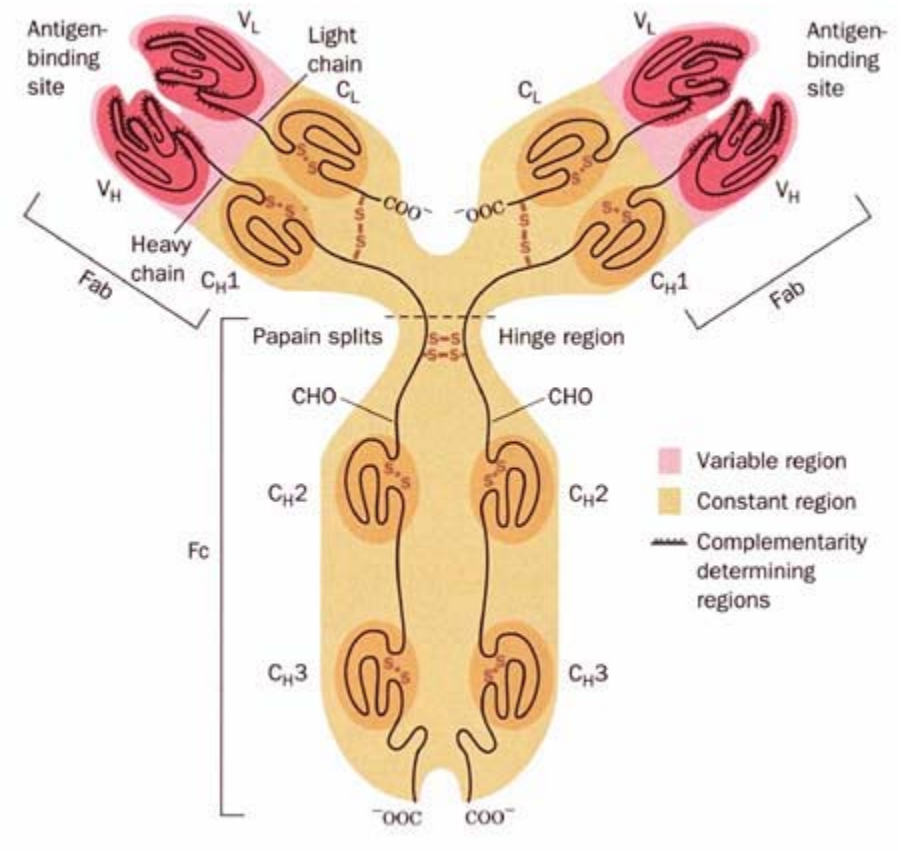


Figure 4a: Three dimensional image of IgG



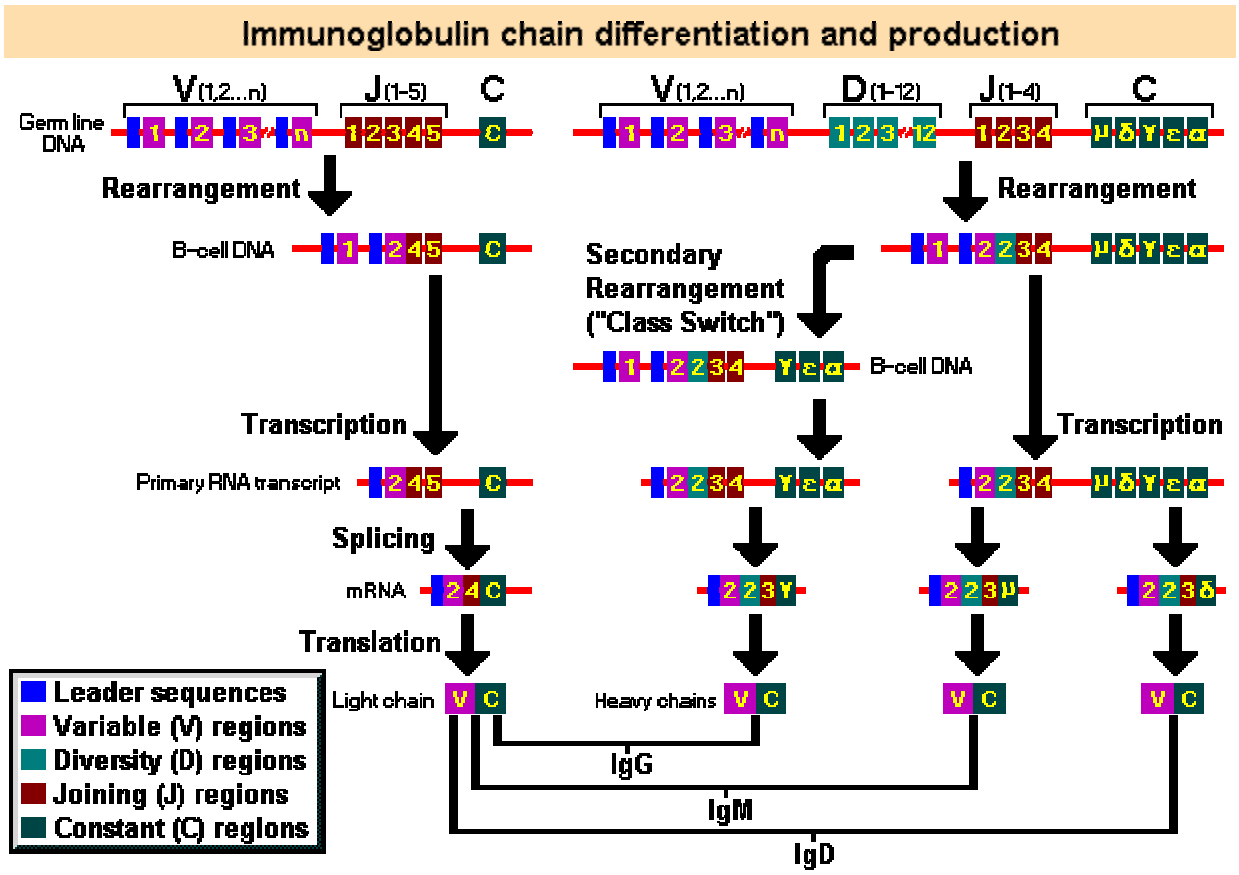
Source: http://www.cehs.siu.edu/fix/medmicro/igs.htm

Immunoglobulin G (Detail of Regions)
Figure 5



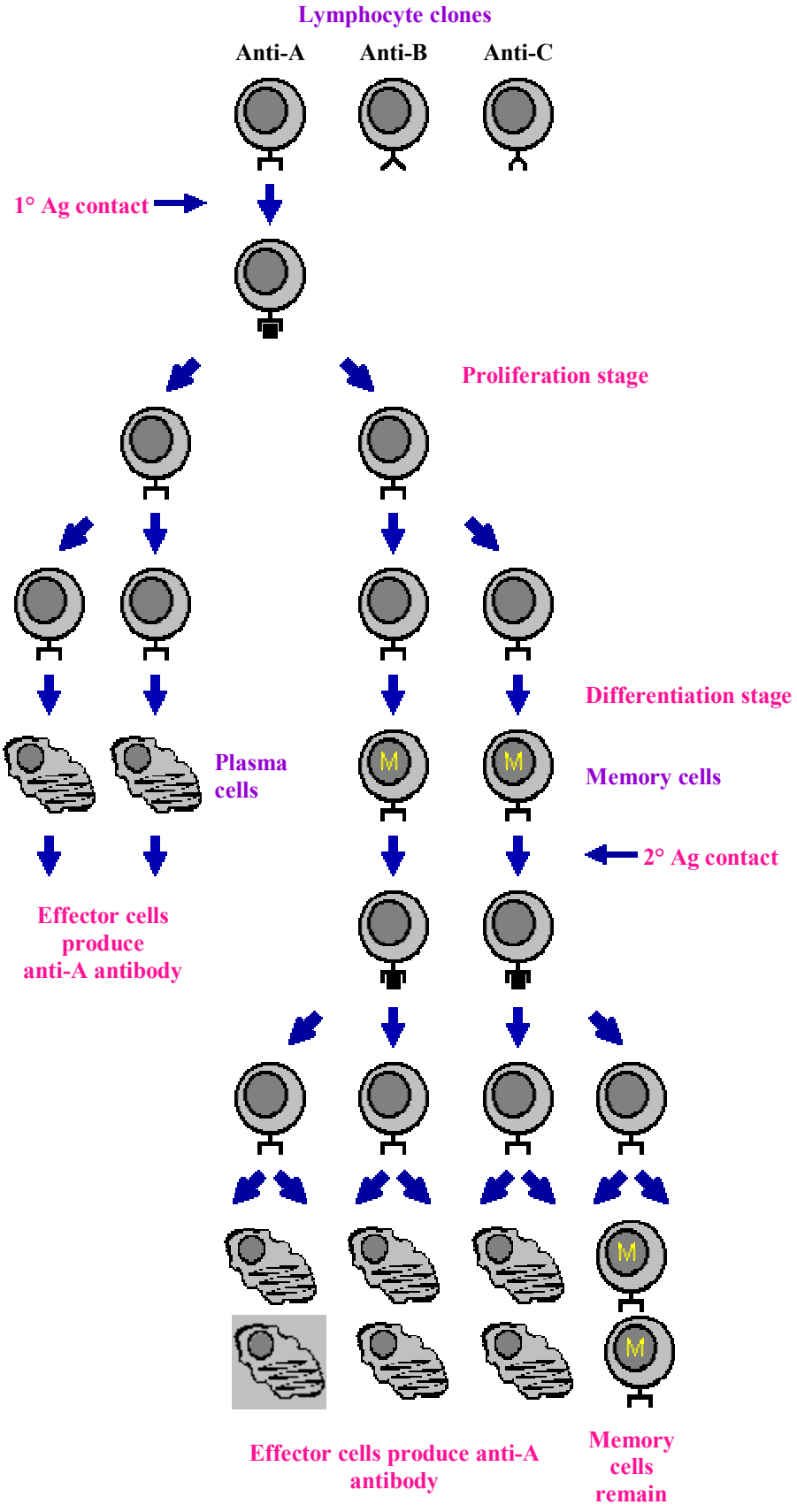
Source: http://info.bio.cmu.edu/Courses/03231/LecF03/Lec11/V&V34-18.jpg

Recombination of Germ Line DNA (Heavy Chain)
Figure 6:



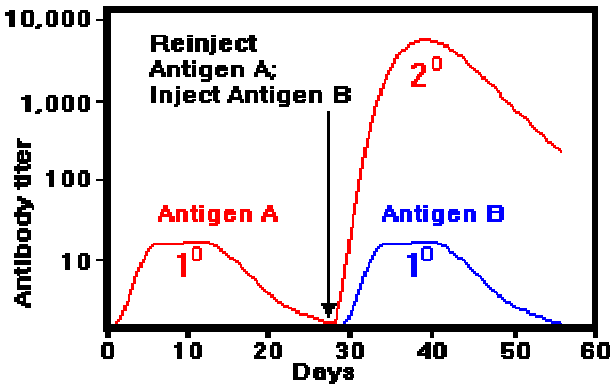
Source: http://www.transplantbuddies.org/library/immdiff.html

Clonal Selection
Figure 7



http://www.transplantbuddies.org/library/clonal.html

Secondary Response
Figure 8



Source: http://www.transplantbuddies.org/library/immunbasic.html#humoral