COURSE: Medical Microbiology, MBIM 650/720 - Fall 2009

TOPIC: Antibody Formation Lecture # 9 Part 1

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TEACHING OBJECTIVES:

1. To describe general characteristics of the specific immune response
2. To compare and contrast primary and secondary antibody responses
3. To describe the molecular events involved in class switching and membrane immunoglobulin expression

REQUIRED READING:

Male *et al.* Immunology, 6th Ed., pp 172-180

KEY WORDS:

Equilibrium phase, Catabolic decay phase, Immune elimination phase, Primary response, Lag/inductive phase, Log phase, Steady state phase, Decline phase, Secondary/anamnestic response, Class switching

ANTIBODY FORMATION

I. GENERAL CHARACTERISTICS OF THE ANTIBODY RESPONSE

A. **Self/non-self discrimination** - One characteristic feature of the specific immune system is that it normally distinguishes between self and non-self and only reacts against non-self.

B. **Memory** - A second feature of the specific immune response is that it demonstrates memory. The immune system "remembers" if it has seen an antigen before and it reacts to secondary exposures to an antigen in a manner different than after a primary exposure. Generally only an exposure to the same antigen will illicit this memory response.

C. **Specificity** - A third characteristic feature of the specific immune system is that there is a high degree of specificity in its reactions. A response to a particular antigen is specific for that antigen or a few closely related antigens.

N.B. These are characteristic of all specific immune responses.

II. ANTIBODY FORMATION

A. Fate of the immunogen

1. Clearance after primary injection - The kinetics of Ag clearance from the body after a primary administration is depicted in Figure 1.

   a) Equilibrium phase - The first phase is called the equilibrium or equilibration phase. During this time the Ag equilibrates between the vascular and extra vascular compartments by diffusion. This is normally a rapid process. Since particulate antigens don't diffuse, they do not show this phase.

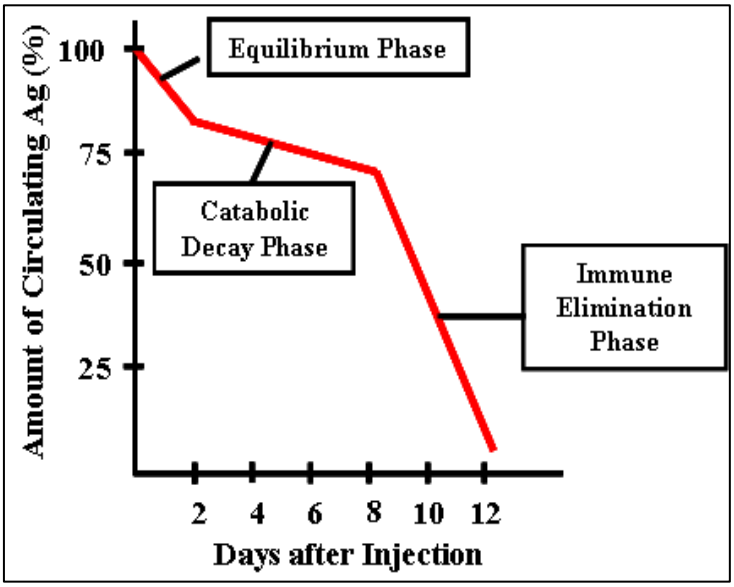

Figure 1

   b) Catabolic decay phase - In this phase the host's cells and enzymes metabolize the antigen. Most of the antigen is taken up by macrophages and other phagocytic cells. The duration will depend upon the immunogen and the host.

   c) Immune elimination phase - In this phase newly synthesized antibody combines with the antigen producing antigen/antibody complexes which are phagocytosed and degraded. Antibody appears in the serum only after the immune elimination phase is over.

2. Clearance after secondary injection - If there is circulating antibody in the serum injection of the antigen for a second time results in a rapid immune elimination. If the is no circulating antibody then injection of the antigen for a second time results in all three phases but the onset of the immune elimination phase is accelerated.

B. **Kinetics of antibody responses to T-dependent Ag**

1. Primary (1º) Ab response - The kinetics of a primary antibody response to and antigen is illustrated in Figure 2.

a)Inductive, latent or lag phase - In this phase the Ag is recognized as foreign and the cells begin to proliferate and differentiate in response to the antigen. The duration of this phase will vary depending on the antigen but it is usually 5-7 days.

b)Log or Exponential Phase - In this phase the Ab concentration increases exponentially as the B cells that were stimulated by the antigen differentiate into plasma cells which secrete antibody.

c) Plateau or steady-state phase - In this phase Ab synthesis is balanced by Ab decay so that there in no net increase in Ab concentration.

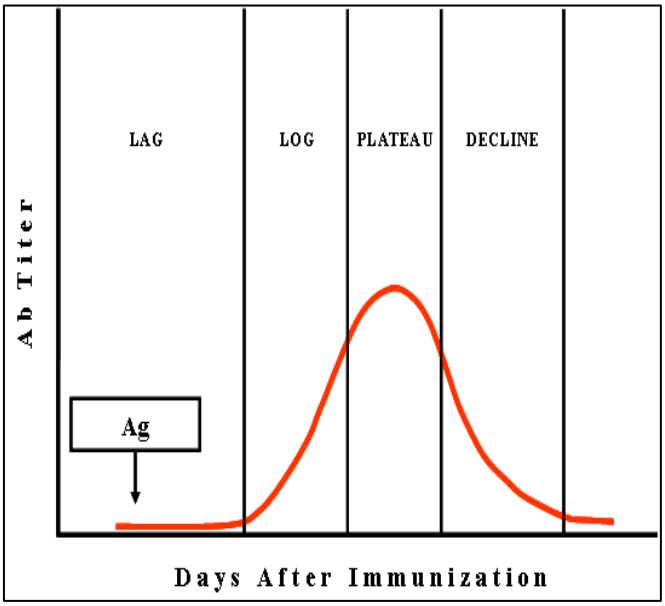

Figure 2

d)Decline or decay phase - In this phase the rate of Ab degradation exceeds that of Ab synthesis and the level of Ab falls. Eventually the level of Ab may reach base line levels..

2. Secondary (2º), memory or anamnestic response (Figure 3)

a) Lag phase - In a secondary response there is a lag phase by it is normally shorter than that observed in a primary response.

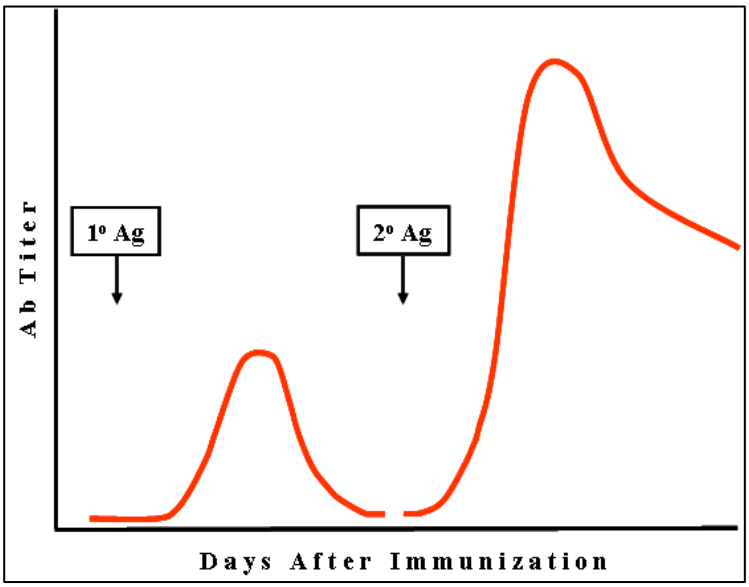

Figure 3

b) Log phase - The log phase in a secondary response is more rapid and higher Ab levels are achieved.

c) Steady state phase

d) Decline phase - The decline phase is not as rapid and Ab may persist for months, years or even a lifetime.

C. Specificity of 1° and 2° responses

Ab elicited in response to an antigen is specific for that antigen although it may also cross react with other antigens which are structurally similar to the eliciting antigen. In general secondary responses are only elicited by the same antigen used in the primary response. However, in some instances a closely related antigen may produce a secondary response, but this is a rare exception.

D. Qualitative changes in Ab during 1° and 2° responses

1. Ig class variation - In the primary response the major class of Ab produced is IgM whereas in the secondary response it is IgG (or IgA or IgE) (Figure 4). The antibodies that persist in the secondary response are the IgG antibodies.

2. Affinity - The affinity of the IgG Ab produced increases progressively during the response, particularly after low doses of antigen (Figure 5). This is referred to as affinity maturation. Affinity maturation is most pronounced after secondary challenge with antigen.

   One explanation for affinity maturation is clonal selection as illustrated in Figure 6. A second explanation for affinity maturation is that, after a class switch has occurred in the immune response, somatic mutations occur which fine tune the antibodies to be of higher affinity. There is experimental evidence for this mechanism, although it is not known how the somatic mutation mechanism is activated after exposure to antigen.

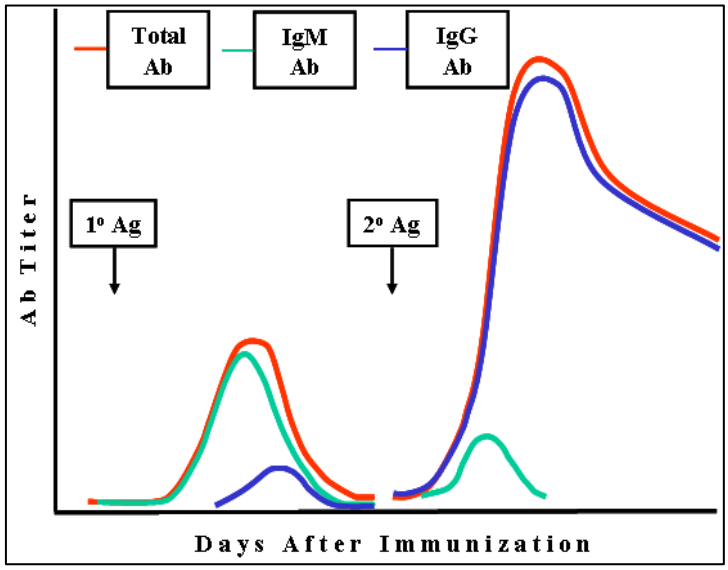

Figure 4

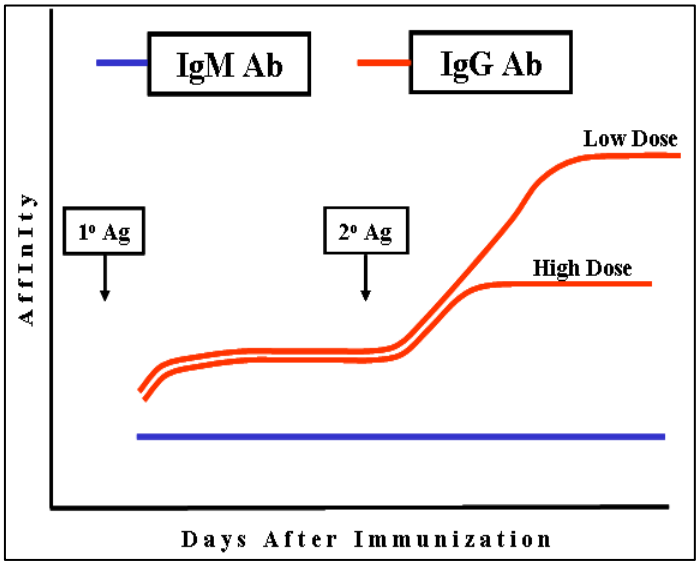

Figure 5

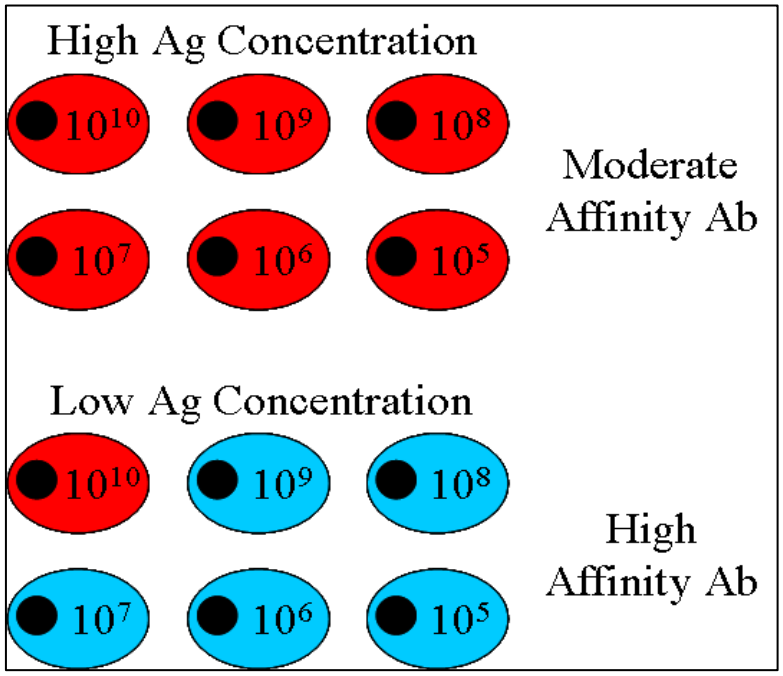

Figure 6

3. Avidity - As a consequence of increased affinity, the avidity of the antibodies increases during the response.

4. Cross-reactivity - As a result of the higher affinity later in the response there is also an increase in detectible cross reactivity. An explanation for why increasing affinity results in an increase in detectible cross reactivity is illustrated by the following example.

	Affinity of Ab for Ag	
	Early	Late
Immunizing Ag	10^{-6} +	10^{-9} ++
Cross reacting Ag	10^{-3} -	10^{-6} +

If a minimum affinity of 10^{-6} is needed to detect a reaction, early in an immune response the reaction of a cross reacting antigen with an affinity of 10^{-3} will not be detected. However, late in a response when the affinities increase 1000 fold, the reaction with both the immunizing and cross reacting antigens will be detected.

E. Cellular events during 1° and 2° responses to T-dependent Ag

1. Primary response (Figure 7)

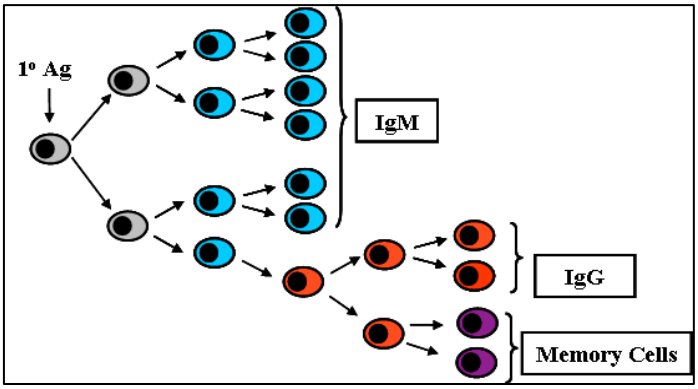

Figure 7

a) Lag phase - Clones of T and B cells with the appropriate antigen receptors bind antigen, become activated and begin to proliferate. The expanded clones of B cells differentiate into plasma cells which begin to secrete antibody.

b) Log phase - The plasma cells initially secrete IgM antibody since the C_μ heavy chain gene is closest to the rearranged VDJ gene. Eventually some B cells switch from making IgM to IgG, IgA or IgE. As more B cells proliferate and differentiate into antibody secreting cells the antibody concentration increases exponentially.

c) Stationary phase - As antigen is depleted, T and B cells are no longer activated. In addition, mechanisms which down regulate the immune response come into play. Furthermore, plasma cells begin to die. When the rate of antibody synthesis equals the rate of antibody decay the stationary phase is reached.

d) Decline phase - When no new antibody is produced because the antigen is no longer present to activate T and B cells and the residual antibody slowly is degraded, the decay phase is reached.

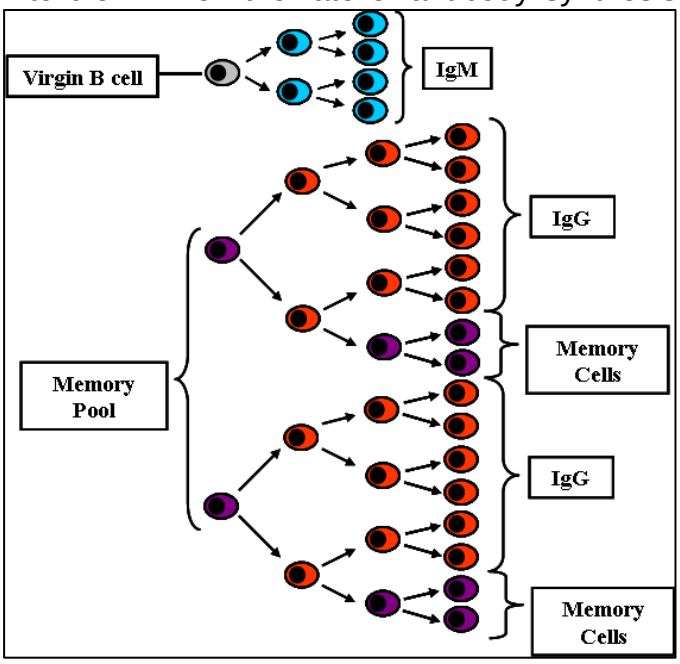

Figure 8

2. Secondary response (Figure 8)

Not all of the T and B cells that are stimulated by antigen during primary challenge with antigen die. Some of them are long lived cells and constitute what is refer to as the memory cell pool. Both memory T cells and memory B cells are produced and

memory T cells survive longer than memory B cells. Upon secondary challenge with antigen not only are virgin T and B cells activated, the memory cells are also activated and thus there is a shorter lag time in the secondary response. Since there is an expanded clone of cells being stimulated the rate of antibody production is also increased during the log phase of antibody production and higher levels are achieved. Also, since many if not all of the memory B cells will have switched to IgG (IgA or IgE) production, IgG is produced earlier in a secondary response. Furthermore since there is an expanded clone of memory T cells which can help B cells to switch to IgG (IgA or IgE) production, the predominant class of Ig produced after secondary challenge is IgG (IgA or IgE).

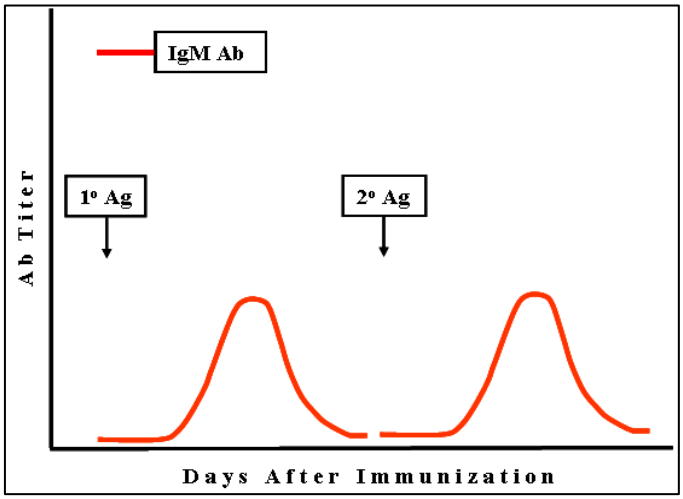

Figure 9

F. **Ab response to T-independent Ag**

- Responses to T-independent Ag are characterized by the production of almost exclusively IgM Ab and no secondary response. Secondary exposure to the Ag results in another primary response to the Ag as illustrated in Figure 9.

G. **Class switching**

During an antibody response to a T-dependent antigen a switch occurs in the class of Ig produced from IgM to some other class (except IgD). Our understanding of the structure of the immunoglobulin genes, helps explain how class switching occurs (Figure 10).

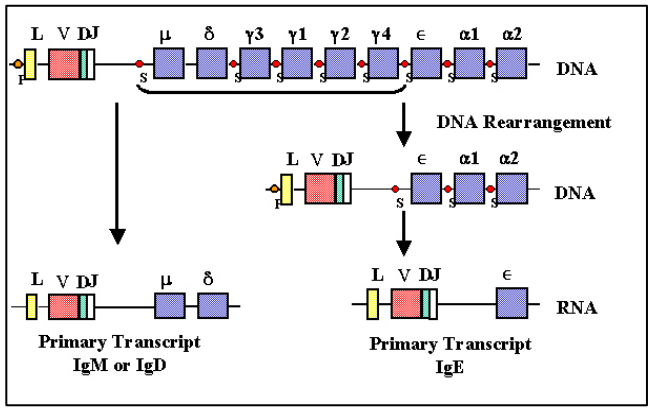

Figure 10

During class switching another DNA rearrangement occurs between a switch site (S_μ) in the intron between the rearranged VDJ regions and the C_μ gene and another switch site before one of the other heavy chain constant region genes. The result of this recombination event is to bring the VDJ region close to one of the other constant region genes, thereby allowing expression of a new class of heavy chain. Since the same VDJ gene is brought near to a different C gene and since the antibody specificity is determined by the hypervariable regions within the V region, the antibody produced after the switch occurs will have the same specificity as before.

Cytokines secreted by T helper cells can cause the switch to certain isotypes.

H. Membrane and secreted immunoglobulin

The specificity of membrane immunoglobulin on a B cell and the Ig secreted by the plasma cell progeny of a B cell is the same. An understanding of how the specificity of membrane and secreted Ig from an individual B cell can be the same comes from an understanding of immunoglobulin genes (Figure 11).

There are two potential polyA sites in the immunoglobulin gene. One after the exon for the last heavy chain domain and the other after the exons that code for the trans- membrane domains. If the first polyA site is used, the pre-mRNA is processed to produce a secreted protein. If the second polyA site is used, the pre-mRNA is processed to produce a membrane form of the immunoglobulin. However, in all cases the same VDJ region is used and thus the specificity of the antibody remains the same. All C regions genes have these additional membrane pieces associated with them and thus after class switching other classes of immunoglobulins can be secreted or expressed on the surface of B cells.

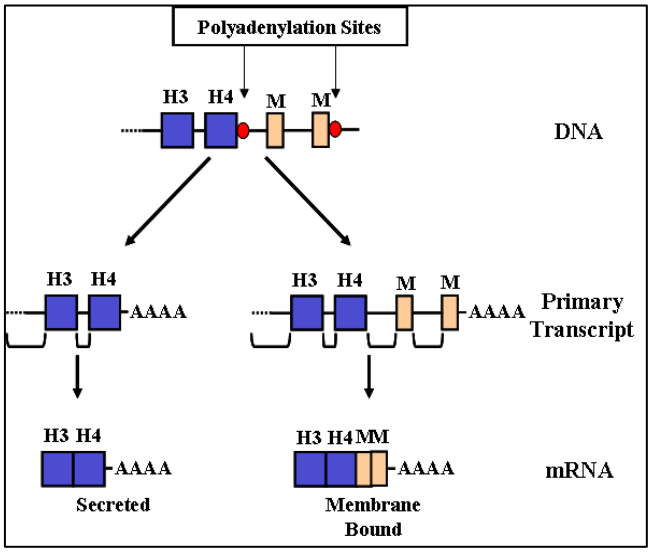

Figure 11

Adapted from Dr. E.P.Mayer