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## CHAPTER 7

### CELLULAR BASIS OF ANTIBODY DIVERSITY: CLONAL SELECTION

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The *specificity* of humoral immune responses relies on the huge DIVERSITY of antigen combining sites present in antibodies, *diversity which is generated in an antigen-independent fashion*. The process by which antigen (during an immune response) stimulates the clonal expansion and differentiation of antibody-forming cells of predetermined specificity is known as CLONAL SELECTION.

The basic principles of clonal selection are illustrated in this chapter by two classic experiments, and are applied to our understanding of some of the fundamental features of the adaptive immune response, namely TOLERANCE, MEMORY and AFFINITY MATURATION, as well as the presence of NATURAL ANTIBODIES.

A humoral immune response typically generates antibodies with a wide diversity of combining sites; even a simple hapten will elicit a highly heterogeneous population of antibodies. In order to provide a framework for understanding how the diversity of the antibody response is generated, we can set out three facts regarding antibody specificity which are based on experimental findings:

- 1) **An animal can produce antibodies to many different epitopes.** This has been known since the early days of immunology, based simply on the variety of distinct molecules that antibodies can distinguish. How many antibody combining sites exist in nature? For a start let's say there are at least one million different combining sites (although this is certainly a gross underestimate).
- 2) **A single antibody-secreting cell produces only one kind of antibody.** All molecules of antibody produced by a single plasma cell are identical with respect to isotype, allotype and idiotype specificities. The heterogeneity of antibody responses, therefore, reflects the heterogeneity of antibody-forming cells, and *not* that of the products of each individual cell. This explains the *homogeneity* of myeloma proteins, since they are the products of clonally derived tumor cells. (NOTE: One important consequence of this fact is **allelic exclusion**, which will be discussed later [see Chapter 9].)
- 3) **The specificity of antibodies is determined by the primary structure (*i.e.*, the amino acid sequence) of the light and heavy chain variable regions.** Antibody diversity *cannot* be explained by different folding patterns of molecules with identical amino acid sequences (as had erroneously been proposed in the 1940's).

Let's examine the problem of the generation of immune responses at the cellular level. How does a particular antibody-forming cell "know" which of the million possible combining sites it is "supposed" to produce? Two general kinds of schemes can be invoked to explain this, namely those of **instruction** and **selection**. These two schemes differ in the assumptions they make about the hypothetical *precursors* of the antibody-forming cells ("*AFCP*"s).

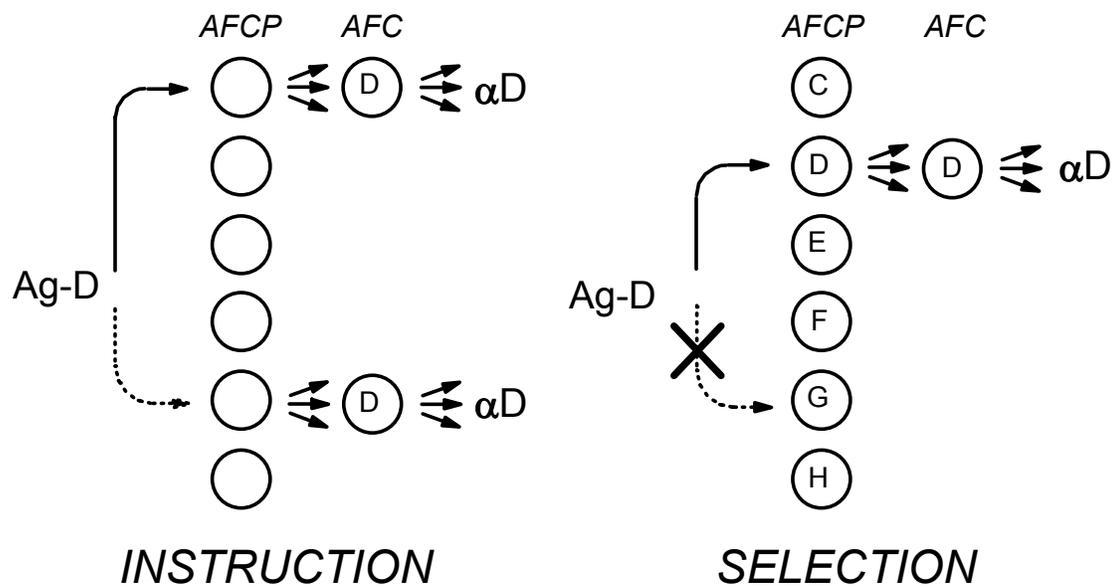


Figure 7-1

Under INSTRUCTIONAL schemes, the precursor of an antibody-forming cell is *not* precommitted, but has the potential of making any one of a million different antibodies. This multipotential precursor cell, following exposure to antigen, somehow recognizes which of the million possible antibodies is needed and proceeds to differentiate into an antibody-forming cell producing that particular antibody; proliferation also takes place, expanding the number of antibody-forming cells over the number of precursors. Under such a scheme *every* precursor cell can potentially respond to *any* antigen.

SELECTIONAL schemes, however, hypothesize that the precursors of antibody-forming cells are themselves *precommitted* to producing antibody of a particular specificity. Only a small fraction of the precursor cells is capable of responding to any particular antigen. The consequences of antigen exposure are the same as described for the instructional scheme, *i.e.* differentiation into mature antibody-forming cells, and multiple rounds of proliferation which greatly expand the number of antibody-forming cells.

### CLONAL SELECTION

One formulation of the selectional class of schemes is known as CLONAL SELECTION, and was developed in the 1950's by Burnet based on work of Jerne and Talmadge. *This hypothesis has become the foundation of modern immunology*, and states the following:

- 1) Each Antibody-Forming Cell Precursors (AFCP) is *precommitted* to making antibody of a particular specificity, *even before it ever encounters a target antigen*.

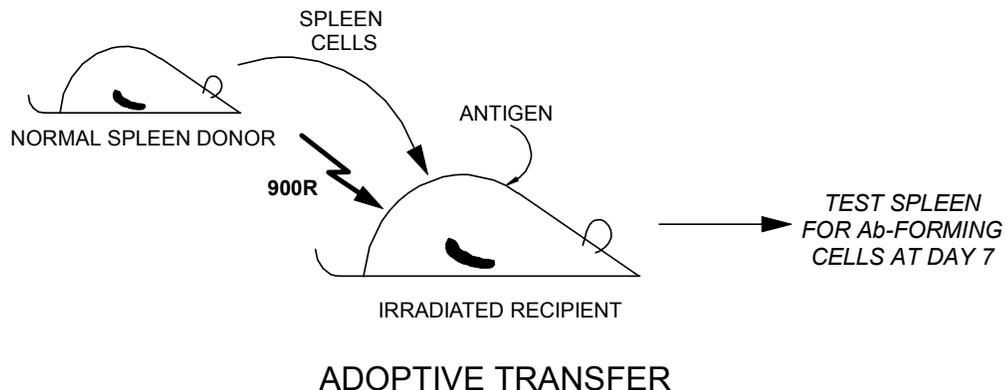
- 2) Each AFCP bears **membrane-bound immunoglobulin** displaying the combining site it is capable of making, in effect, a "sample of its wares".
- 3) This membrane Ig acts as a **specific receptor molecule** for the particular epitope to which it is directed. Upon binding to an AFCP's surface Ig, the antigen stimulates that cell to differentiate into an antibody-forming cell, and to proliferate (**clonal expansion**).

Two central features of Burnet's Clonal Selection Hypothesis should be emphasized. First, it makes the simplifying assumption that **the only molecule that can specifically recognize an epitope is antibody** itself. By hypothesizing the presence of antibody pre-existing on the surface of the AFCP, Burnet avoided the problem of how a non-committed precursor cell "looks" at antigen to determine which of the many possible antibodies is needed. Second, it states that **the development of the immunological repertoire is antigen-independent**. The *differentiation* which results in the appearance of a particular AFCP must be a developmentally controlled process, and must be able to take place *in the absence of any antigen stimulation*.

## EXPERIMENTAL CONFIRMATION OF CLONAL SELECTION

Let's examine two key experiments, carried out in the late 1960's, which provided compelling evidence for the idea that AFCPs are precommitted to a particular antigen. In one case specific AFCPs are killed as a result of binding a specific radioactive antigen, in the other the AFCPs are killed as a result of their proliferative response to their particular antigen.

**Hot Antigen Suicide.** Ada and Byrt (1969) reasoned that if AFCPs bear surface receptors which can specifically bind antigen, then these cells should be susceptible to being killed if presented with a toxic form of antigen. They used an ADOPTIVE TRANSFER system, consisting of injecting normal spleen cells together with antigen into a lethally irradiated recipient. Testing the recipient's spleen for the presence of antibody-forming cells showed a high response at the end of seven days.



**Figure 7-2**

They then took a sample of the antigen (Salmonella flagellin, or "Fla") and made it highly radioactive by coupling it with Iodine-125. This radioactive antigen was incubated overnight with the normal spleen cells *before* transfer into the irradiated recipient. The rationale is that if precursor cells are *precommitted* to a particular antigen, and if they have *cell surface receptors* specific for the antigen, then those cells precommitted to the antigen Fla, and *only* those cells, should *bind the radioactive antigen* and thus commit "suicide" due to the radioactivity.

The results they obtained are summarized below:

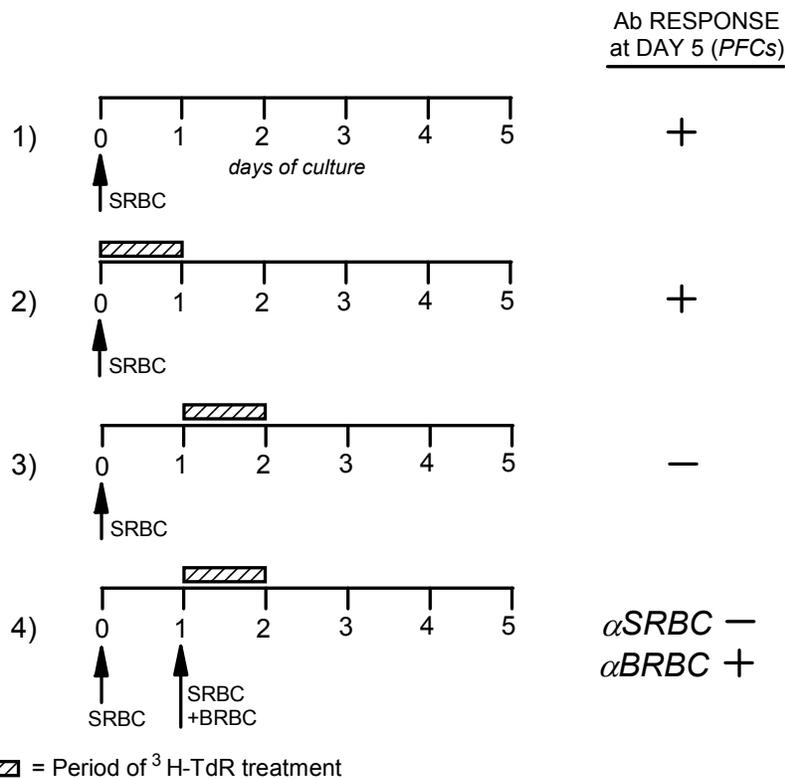
	Ag used for preincubation	Ag transferred with cells	Resulting 7-day Ab response
1)	none	none	none
2)	none	Fla	high
3)	<sup>125</sup> I-Fla	Fla	low
4)	<sup>125</sup> I-Fla	Fla + BGG	low anti-Fla high anti-BGG

Spleen cells incubated with "hot" Fla can no longer transfer responsiveness to Fla, although their response to an unrelated antigen is (BGG) unaffected.

These results show that the precursors of antibody-forming cells for the anti-Fla response can be eliminated by incubating the spleen cells with highly radioactive Fla, but that this treatment does *not* affect the precursors for the anti-BGG response. *The precursors of anti-Fla antibody-forming cells are a different population from the anti-BGG precursors, and this precommitment is reflected in their ability to specifically bind the antigen to their surface.* These are the two key elements of Burnet's Clonal Selection Theory.

**<sup>3</sup>H-TdR Suicide.** Mishell and Dutton (1966) took a slightly different approach when they developed a system in which they could generate an *in vitro* primary antibody response. Under a carefully specified set of culture conditions, they incubated normal mouse spleen cells together with antigen, and after five days they were able to demonstrate the presence of large numbers of antibody-forming cells (PFC's, for Plaque-Forming Cells) in the culture dish (line 1 in Figure 7-3).

They then used highly radioactive tritium-labeled thymidine (<sup>3</sup>H-TdR) to selectively kill those cells undergoing proliferation. (Thymidine is a DNA precursor which is taken up and incorporated only by those cells synthesizing DNA, *i.e.*, proliferating cells; "resting" cells are not affected by this treatment.) By treating with "hot" thymidine during different 24-hour periods, they were able to show that the precursors of antibody forming cells were proliferating (and therefore could be killed by <sup>3</sup>H-TdR) between 24 and 48 hours after initiation of the culture with the antigen SRBC (sheep red blood cells; see line 3); treating the cells during the *first* 24 hours after initiation had no effect on the resulting response (seen in line 2).



### $^3\text{H-TdR}$ SUICIDE OF ANTIGEN-SPECIFIC AFC PRECURSORS

**Figure 7-3**

The key part of the experiment is in line 4. A  $^3\text{H-TdR}$  pulse between 24 and 48 hours should eliminate the response to the original SRBC challenge (as in line 3). However, if AFCs are not precommitted, then it should *not* affect the response to a second dose of SRBC given at 24 hr (see line 2), nor should it effect the response to a different antigen (BRBC, burro red blood cell) given at the same time. But what they found was that while the response to BRBC was high, as expected, the response to the second dose of SRBC was very low.

These results show that the population of precursor cells which proliferate in a *specific response* to antigenic challenge are *different* for the closely related antigens SRBC and BRBC (burro red blood cells). Following the "hot" pulse at 24-48 hr, which kills all those precursors which had responded to SRBC, there is *no other* remaining population of precursors which can respond to SRBC (as there would be under an "instructional" scheme), although the precursors for a different antigen have not been effected by this treatment.

These two sets of experiments, and many more in later years, confirmed the basic features of CLONAL SELECTION--*separate precommitted populations of precursor cells respond (by proliferation and differentiation) to different antigens, and each have antigen-specific receptors on their surface.*

## CLONAL SELECTION AND TOLERANCE

One of the most striking features of Burnet's Clonal Selection theory was its simple explanation of the phenomenon of SELF-TOLERANCE, an organism's normal lack of ability to make immune responses against its own "self" components, while simultaneously being able to respond to any "foreign" antigen. Burnet hypothesized that during the development of an antibody-forming cell precursor, it goes through a *short-lived stage in which exposure to its specific antigen will result in its own death*. This would ensure that no precursor can develop which recognizes antigens ordinarily present in the organism, but all other precursors would develop normally. This represents tolerance by the mechanism of CLONAL ABORTION, and was the most attractive explanation of the phenomenon of tolerance for many years.

The phenomenon of tolerance will be discussed at greater length in Chapter 18, and we will find that while clonal abortion is known to take place and to play an important role, it clearly fails to account for some of the features of natural or experimental tolerance. It can be shown in many cases, for instance, that some clonally specific precursor cells specific for "self" antigens *do* exist in normal animals; therefore, the fact that they do *not* ordinarily respond and become antibody-forming cells must be explained on some basis *other* than clonal abortion.

## CLONAL SELECTION AND MEMORY

Antigen-dependent proliferation of clonally precommitted cells has at least two important consequences.

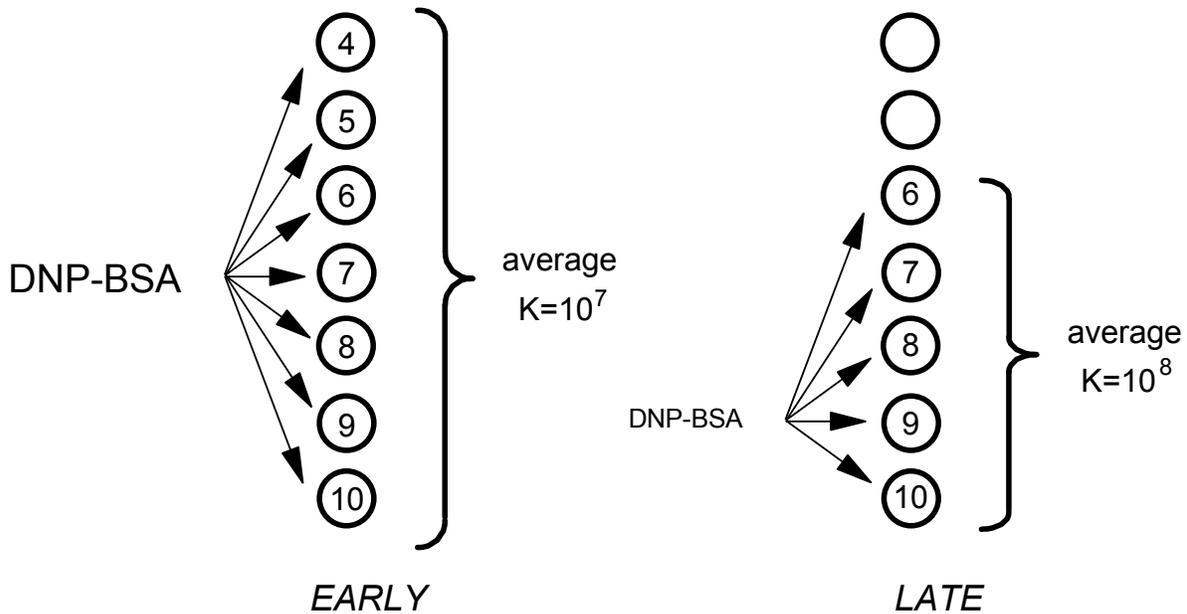
- 1) Production of a large number of effector cells of that particular specificity (**Clonal Expansion**). A single antigen-reactive precursor may give rise to hundreds of thousands of antibody-forming cells.
- 2) Production of an expanded number of **memory cells**. These memory cells are themselves antigen-reactive cells, which can respond to antigen stimulation by proliferation and differentiation into antibody-forming cells. However, they differ from the original "virgin" precursors in several ways, including that they are considerably more numerous., and that they respond much more rapidly to antigenic stimulation,

*The increased speed of response of memory cells, as well as their higher numbers, is the basis of immunological memory.* This will be discussed in more detail in Chapter 9 (see Figure 9-3).

## CLONAL SELECTION AND AFFINITY MATURATION

We have already noted that secondary (memory) responses produce antibody of higher affinity than primary responses. This is one manifestation of the phenomenon of **affinity maturation** which can also be readily understood in the context of clonal selection. The heterogeneity of antibody responses is a reflection of the heterogeneity of AFCs. Many *different* precursors will exist, for example, which can recognize the hapten DNP, each with its own characteristic affinity. Let's assume, for the argument, the existence of seven different precursor cells with affinities for DNP ranging from  $10^4$  to  $10^{10}$ . In a typical

immune response there will be enough antigen to trigger all seven (they will all differentiate and proliferate), resulting in an average antibody affinity of about  $10^7$  (see the left hand side of Figure 7-4).



## AFFINITY MATURATION BY ANTIGEN-DRIVEN SELECTION

**Figure 7-4**

As the response progresses, one of its consequences is the elimination of antigen (by "processing", opsonization and phagocytosis, *etc.*). Before the antigen is completely eliminated, however, the small amount that still remains may continue to trigger available precursor cells. However, *if the amount of remaining antigen is limiting* and there is not enough to trigger the entire population of available precursors, *those precursors for higher affinity antibody will be selectively triggered*. If, in the example above, only those precursors in the range of  $10^6$  to  $10^{10}$  are re-stimulated later in the response, they will be selectively expanded and the average affinity of serum antibody will increase toward  $10^8$  (shown on the right-hand side of Figure 7-4).

*Thus, affinity maturation is a logical consequence of the heterogeneity of precommitted AFCs, and of the fact that antigen can continue to trigger precursor cells as long as it is present. (The process of "somatic hypermutation", discussed in Chapter 9, increases the diversity of antigen-combining sites during immune responses, thus contributing to even more effective affinity maturation.)*

### "NATURAL" ANTIBODIES?

As we have seen, antibodies are produced following stimulation by specific antigens. The total immunoglobulin in our serum, which typically amounts to 10-15 mg/ml (1-1.5 gm/dl), is the result of extensive exposure to many immunogenic substances in our food and on the

microorganisms which inhabit and infect us, as well as from deliberate vaccinations. Experimental animals which are raised under germ-free and antigen-free conditions have little or no detectable serum Ig; if they are subsequently exposed to a conventional environment, however, their Ig levels rapidly rise to reach the normal range.

Are there any antibodies which can truly be called "natural" and which are not the result of specific antigen stimulation? Perhaps, but only in a limited sense. It is important to recognize that immune responses often include *non-specific* components -- in addition to resulting in a specific antibody response, antigenic stimulation may result in non-specific stimulation of nearby "bystander" B-cells, a phenomenon known as POLYCLONAL ACTIVATION (we will discuss some of the mechanisms involved later in Chapters 12 and 15.) This is thought to reflect the non-specific stimulation of pre-existing memory cells (among others) and, therefore, will tend to be biased toward the products of previous immune responses. In general, the term "natural antibody" simply means antibody which is not the result of a specific and *known* antigenic stimulation.

#### CHAPTER 7, STUDY QUESTIONS:

1. How does CLONAL SELECTION differ from *instructional* theories in explaining antibody diversity?
2. How do the experiments of Ada and Byrt (*hot antigen* suicide) and Mishell and Dutton (*hot thymidine* suicide) support Clonal Selection? How do they differ from one another?
3. Does either of the above experiments *exclude* the possibility that a single AFCP may be capable of producing *two* different antibodies with different specificities? Does this situation occur in nature?
4. Why is affinity maturation a logical consequence of Clonal Selection?