

Chapter 3. Clonal selection

I have called this principle,
by which each slight variation,
if useful, is preserved, by the term of
Natural Selection
-Charles Darwin,
On the Origin of Species, 1859

The serum concentration of antibodies is 10 mg per ml, or equivalently 10^{-4} moles per liter. If 10^6 different specificities of antibodies were present in serum at equal concentrations, each of them would have a concentration of about 10^{-10} moles per liter. A small fraction of these antibodies can be expected to bind to a particular infecting or injected antigen, and these few could hardly suffice to reliably mediate the elimination of the antigen. Hence the great diversity of antibodies leads to the requirement for a mechanism that would specifically amplify the production of just those antibodies that bind to the antigen. A simple explanation of how this can occur was formulated by David Talmadge¹⁰ and F. MacFarlane Burnet¹¹, and became known as the Clonal Selection Theory. Clonal selection is now established as a fact rather than "just a theory". It is a core component of the science of cellular immunology.

The clonal selection theory

Talmadge and Burnet postulated that each lymphocyte and its progeny are committed to the synthesis of antibodies with just one specificity (V region). A large amount of antibodies of a particular specificity can then be produced by stimulating the proliferation of lymphocytes committed to that specificity. Initially, most of the lymphocytes are small resting cells, about 7 microns in diameter, and are neither proliferating nor secreting very much antibody. A resting lymphocyte has approximately 10^5 cell-bound antibody-like molecules bearing the V region to which the lymphocyte is committed. Such cell-bound antibodies are called receptors. The antigen binds to the receptors of only those lymphocytes that have a V region specific for the antigen (that is, a shape complementary to the shape of the antigen). The interaction between the antigen and the receptors of the cell stimulates the cell to proliferate, so that there is an exponential increase in the number of

¹⁰ D. Talmadge (1957) Allergy and Immunology. Ann. Review Med., 8, 239-256.

¹¹ F. M. Burnet (1957) A modification of Jerne's theory of antibody production using the concept of clonal selection. Australian J. Science, 20, 67-69; F. M. Burnet (1959) The clonal selection theory of acquired immunity. Cambridge University Press.

antigen-specific cells. Some of these cells differentiate to become a different cell type, called a plasma cell. The plasma cell is still committed to making antibodies of the same specificity, but it is a larger, more active cell (about 10 to 12 microns in diameter), and secretes a large amount of the antibodies, namely about 2000 antibody molecules per cell per second. The selective expansion in the number of antigen-specific cells and the subsequent production of large amounts of specific antibodies leads to the elimination of the antigen. This process, called clonal selection, explains how a small number of antigen-specific lymphocytes can be amplified to a much larger number, that is then able to deal effectively with the antigen. Providing that the larger number of antigen-specific cells can be stabilized, clonal selection also provides an explanation for memory in the immune system. Clonal selection makes a lot of sense, has been confirmed to be correct in countless experiments, and is the first law of cellular immunology.

The antibody plaque assay

The clonal selection theory is now generally accepted firstly because it makes good sense, and secondly because it has a great deal of direct experimental support. Early compelling evidence came from experiments utilizing an antibody plaque assay developed by Al Nordin and Niels Jerne. This was a method for the direct visualization of lymphocytes making antibodies of just one specificity.

In a typical plaque assay experiment, a mouse is first injected with an antigen, such as sheep red blood cells. After about five days, the mouse is killed, and a suspension of its spleen cells (mainly lymphocytes) is prepared. The spleen cells are added to a monolayer of sheep red blood cells, together with a solution containing a set of serum enzymes called complement. In the vicinity of lymphocytes that secrete antibody specific for the sheep red cells, the sheep red cells have antibody bound to their surfaces. Complement enzymes attach to these antibodies, and lyse the red blood cells. Holes in the monolayer of sheep red blood cells, called plaques, are therefore formed around the small fraction of lymphocytes that secrete antibody specific for the red blood cells. The plaques are large enough to be seen with the naked eye, and by using a microscope we see that there is a lymphocyte in the centre of each plaque. By counting the plaques, we can establish that only a small fraction of the lymphocytes secrete antibodies specific for sheep red blood cells. If we do the assay at various time points following the injection of antigen, we can observe the dynamics of the increase in numbers of specific antibody-secreting cells.

ELISA

The antibody plaque assay has now been largely superseded by the enzyme-linked immunosorbant assay (ELISA). There are many different ELISA assays. As one example, an ELISA assay to detect the presence of antibodies specific for the protein antigen bovine serum albumin (BSA) in (say) a mouse serum is as follows,

and is shown in Figure 3-1. Wells in a plastic plate are coated with BSA, then the plate is washed with a buffer to remove excess BSA. The serum is then added so that anti-BSA antibodies bind to the immobilized layer of BSA. The plate is washed again to remove unbound antibodies. Then, for example, rabbit anti-(mouse Ig) antibodies are added to quantitate the amount of bound mouse anti-BSA antibodies. Excess rabbit anti-(mouse Ig) is washed away. The rabbit antibodies had been coupled to an enzyme, for example a phosphatase. Finally, a substrate of the enzyme is added, and each bound molecule of enzyme can convert many molecules of the substrate to a form with a different optical density. The measurement of optical density then gives a measure of the amount of anti-BSA antibodies in the serum. The IgM component of the response can be determined by using a rabbit anti-(mouse IgM), and the IgG component can be determined using a rabbit anti-(mouse IgG).

There are numerous varieties of ELISA assays. Another simple example is to have the serum on the plate and use biotinylated antigen at the second step. ELISA assays can be used to quantitate antigen using antibodies as well as the other way around. A sensitive variation is the capture ELISA for detecting an antigen, in which antibodies to two separate determinants of an antigen are used. The first antibody binds to the plate, then comes a solution, for example a serum, containing the antigen, and then the second antibody in (for example) biotinylated form, and then so on as before.

The immune system has memory: primary and secondary responses

If a particular foreign substance (antigen) is injected into a mouse, it makes antibodies to that substance within a few days as illustrated in Figure 3-2. The synthesis of antibodies resulting from a first exposure to an antigen is called a *primary immune response*. If the same antigen is injected again several weeks, months or even years later, the mouse typically makes specific antibodies much more rapidly and vigorously; this is called a *secondary immune response*. The change in state of a person's immune system caused by a primary immune response accounts for the fact that he or she does not get sick twice from pathogens such as measles. The immune system "remembers" measles from the first encounter, and is then equipped to deal with a second infection so effectively, that the individual does not even notice the second infection. The immune system is thus said to have "memory". The difference between the primary and secondary immune responses can be ascribed to changes in population levels of lymphocytes that are caused by the primary exposure to the antigen. A straightforward understanding of this follows directly from the clonal selection theory above; the antigen expands the number of antigen-sensitive cells.

Figure 3-1 An example of an ELISA assay (enzyme linked immunosorbent assay). In this example the antigen is bovine serum albumin, BSA. The BSA, like many antigens, has non-specific affinity for the surface of a plastic plate, and is used to coat the plate. Excess BSA is washed off. Then a serum sample containing anti-BSA antibodies is added and anti-BSA antibodies bind to the BSA. Unbound antibodies and other unbound serum components are washed off. The next layer is rabbit anti-(mouse IgG) antibodies to which a small molecule, biotin, has been covalently coupled. The protein avidin has a high affinity for biotin, and the next step is to use a conjugate of avidin and an enzyme, in this case alkaline phosphatase. Then a substrate for the enzyme is added. The enzyme changes the colour of the substrate, and this change in colour can be precisely measured. The change in colour is proportional to the number of antibodies bound to antigen molecules. One molecule of the enzyme can process many substrate molecules, giving an amplification of the effect of one bound antibody bound to one bound antigen molecule.

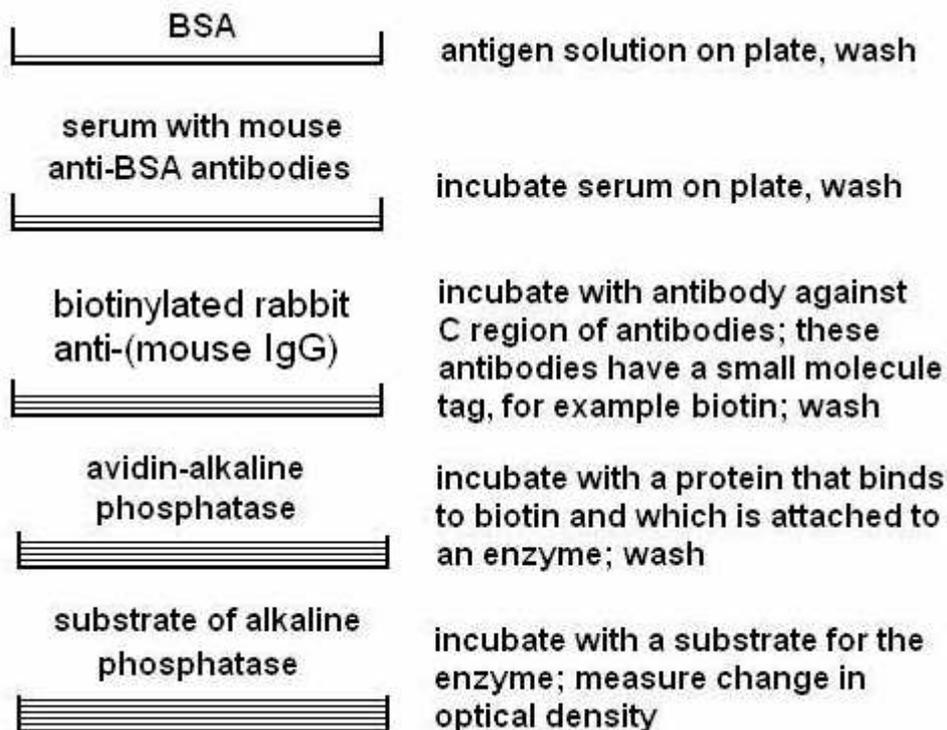
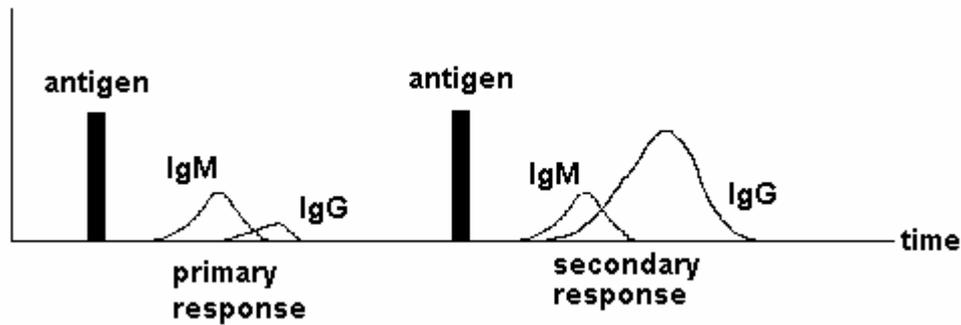


Figure 3-2 The primary and secondary responses to an antigen. A first immunization with an antigen typically causes an IgM immune response followed by a small IgG response. There is memory associated with the IgG response, but not with the IgM response. A second immunization with the same antigen causes a larger IgG response.



Switching on a B cell

A key, early step in an immune response to an antigen is the binding of antigen to the specific receptors of a B cell. This binding then has to be "noticed" by the cell. The information that an antigen is bound to cell's receptors has to be transmitted across the cell's membrane. Extensive studies have shown that antigen binding does not always result in signal transmission. It has been shown experimentally that the necessary and sufficient condition for an activating signal to be registered by the cell is that specific receptors be aggregated on the cell surface.¹² The antigen brings two or more receptors into close proximity by binding to them simultaneously. This aggregation process at the cell surface is also called the cross-linking of receptors. The cross-linking signal is the process that mediates specific stimulation of a particular lymphocyte, as opposed to signals that act on all lymphocytes regardless of their specificity. Additional, non-specific signals are needed by a B cell for it to secrete antibodies. Cross-linking is also the mechanism used for the specific stimulation of T cells.

The specific receptors are trans-membrane proteins, so aggregation at the cell surface inevitably causes aggregation also of the other end of the receptor, inside the cell. This process results in the formation of a structure inside the cell, that is the basis for further signal transmission.

When B cells are stimulated by antigen and receive the appropriate additional non-specific signals, they can differentiate from being IgM secreting cells to being large IgG secreting cells called "plasma cells". Alternatively, they can become

¹² The extensive evidence proving that this is the case is presented in Chapter 9.

smaller “memory cells”, that can subsequently respond in a secondary response to antigen by making antigen-specific IgG. Plasma cells may with time also become memory cells. During these differentiation steps the B cells mostly retain the same V region, or in some cases mutational variants are selected, especially variants that have higher affinity for the antigen.

Allelic exclusion

Consider an individual that has two versions (“alleles”) of a gene, namely one inherited from its mother and one from its father. When the gene is switched on to produce the corresponding protein, in most cases both alleles are active, and two versions of the protein are produced. This makes sense, because if one of the two is defective, the other one can still do the required job. In the case of B cells making antibodies, however, this is not the case. Each B cell makes only one complete V region, comprised of one heavy chain and one light chain, a phenomenon known as “allelic exclusion”. This ensures that the B cell has receptors with only one V region, thus enabling clonal selection to work as described above.