Chapter 4. Immune responses to foreign antigens

My foreign policy: I wage war
-George Clemenceau
Prime Minister of France, 1918

IgM and IgG in Primary and Secondary Immune Responses

The primary immune response of a mouse or person to an antigen typically consists of the production of IgM antibodies specific for that antigen, followed in some cases by the production of some antigen-specific IgG antibodies. The process of giving a first dose of an antigen is called "priming" with the antigen. If a secondary immune response is evoked by giving the antigen that was injected a second time, several weeks or months after priming, the response typically consists of mainly IgG antibodies. The "foreign policy" of the immune system is to wage war on the antigen in the most effective way possible. We will learn more in chapter 10 about how such a combination of IgM and IgG antibodies permits the immune system to be stable and have memory, and thus deal effectively with antigens.

Affinity increases with time after immunization

The binding constant of antibodies to the immunizing antigen generally increases with time after immunization.[14,15] This is true both for the time following an initial injection with the antigen, and for responses to multiple immunizations. The clones that are activated in a secondary immune response have, on average, been subjected to more extensive selection to recognize the antigen, and typically have a higher affinity (equilibrium binding constant) for the antigen than those produced in the primary response. The increase in affinity with time can be simply interpreted on the basis of the clonal selection theory as follows. An initial response will include many clones of varying affinities, but as the amount of antigen decreases, clones with the highest affinity for antigen will continue to be stimulated for the longest time. The affinity may also increase due to the selection of clones with mutations in their V regions, that have higher affinity.[16]

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[14] H. Eisen and G. Siskind (1964) Variations in affinities of antibodies during the immune response. Biochemistry 3, 996-1008.

[15] J. W. Kimball (1972) Maturation of the immune response to type III pneumococcal polysaccharide. Immunochemistry 9, 1169-1184.

[16] G. M. Griffiths, C. Berek, M. Kaartinen and C. Milstein (1984) Somatic mutation and the maturation of immune response to 2-phenyl oxazolone. Nature 312, 271-275.

Tolerance

Making specific antibodies is not the only way the immune system can respond to an exposure to an antigen. The system's ability to make antibodies to the particular antigen can also be specifically switched off, which is called the *induction of tolerance to the antigen*. A third possibility is that there can be no impact on the immune system, which occurs if the applied perturbation of the system is below a certain threshold level. There are also intermediate cases; partial tolerance may be induced, or only a very weak immune response may occur. By partial tolerance, we mean that a subsequent injection of the antigen will result in an immune response, but one that is smaller than would be the case if the animal had not been previously exposed to the antigen.

Several factors determine the outcome of exposure of an individual to an antigen. These include the size and chemical nature of the antigen, its physical form (for example aggregated or deaggregated), route of injection (for example intravenous, intraperitoneal, intramuscular, subcutaneous or oral), the dose given, and whether or not it is administered together with an *adjuvant*, that is, a substance that has been found empirically to non-specifically enhance immune responses.

Aggregated and deaggregated antigens

An aggregated preparation of a foreign protein (an "immunogen") tends to induce immunity, while a deaggregated preparation of the same protein (a "tolerogen") will tend to switch the immune system off with respect to its ability to make antibodies to that protein.[17,18] Deaggregated protein antigen is obtained by removing aggregates from a protein preparation using ultracentrifugation. An experiment illustrating the induction of tolerance using a deaggregated protein antigen is shown in **Figure 4-1**. Immunization with the tolerogen followed by immunization with the immunogen results in the production of no (or very little) immune response to the antigen, while the immune response to a second, control antigen is not affected.

Dose of antigen

The dose of antigen used is also important in determining whether an immune response or tolerance is induced. Dresser showed that doses as little as 50 to 200μg of aggregate-free bovine gamma globulin (BGG, IgG from cattle) can induce tolerance to that antigen in mice.[17] Then in a famous experiment using mice and with bovine serum albumin as the antigen, Mitchison discovered that three dose ranges of antigen can be

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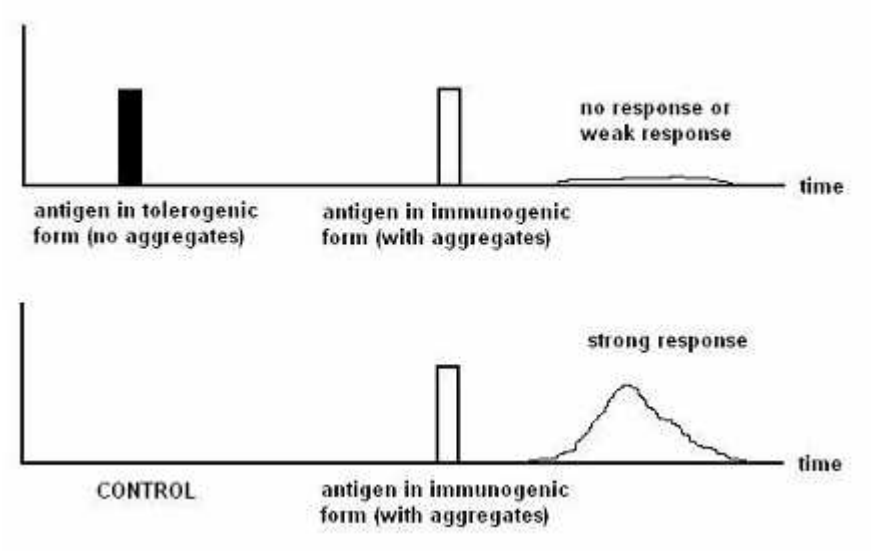
[17] D. W. Dresser (1962) Specific inhibition of antibody production. II. Paralysis induced in adult mice by small quantities of protein antigen. Immunology 5, 378-388;.

[18] J. M. Chiller and W. O. Weigle (1971) Cellular events during induction of immunologic unresponsiveness in adult mice. J. Immunol. 106, 1647-1653; W. O. Weigle (1973) Immunological unresponsiveness. Adv. Immunol. 16, 61-122.

Figure 4-1. Induction of tolerance (no response or a relatively weak immune response) using a protein antigen that has been deaggregated by ultra-centrifugation. Aggregated antigen settles in the bottom of the centrifuge tube while aggregate-free antigen is in the supernatant.



important. Low doses of about 10 micrograms given three times per week for one to six weeks induced tolerance, intermediate doses of about 1 mg three times per week for 4 to 8 weeks induced immunity, while triweekly doses of 10 to 100 mg for 2 to 16 weeks again induced induced tolerance (Figure 4-2).[19] In subsequent work using more potent antigens, that may be more efficient at cross-linking receptors, it was found that low dose tolerance can be induced with multiple doses of antigen that are several orders of magnitude lower than this, as shown in Figures 4-3a and 4-3b. This became known as *ultra-low dose tolerance*. Doses of antigen as low as 10^{-3} picograms (10^{-15} g) per gram of body weight, given daily for two weeks, were found to be effective in inducing tolerance in newborn rats.[20,21] The phenomena of low dose tolerance and ultra-low

[19] N. A. Mitchison (1964) Induction of immunological paralysis in two zones of dosage. Proc. Royal Soc. London B161, 275-292; D. W. Dresser and N. A. Mitchison (1968) The mechanism of immunological paralysis. Advan. Immunol. 8, 129-181.

[20] G. R. Shellam and G. J. V. Nossal (1968) The mechanism of induction of immunological paralysis. IV. The effects of ultra-low doses of flagellin. Immunology 14, 273-284.

[21] G. L. Ada and C. R. Parish (1968) Low zone tolerance to bacterial flagellin in adult rats: a possible role for antigen localized in lymphoid follicles. Proc. Nat. Acad. Sci. (USA), 61, 566-561.

Figure 4-2. The experiment by Mitchison on dose dependence of the immune response that led to the concept of low dose and high dose tolerance, separated by a dose range that induces tolerance. The curves are a polynomial computer fit to the actual data, showing the size of the response as a function of dose and duration of treatment. The antigen, bovine serum albumin (BSA), was given three times per week for the number of weeks and doses shown, then ten days later they were immunized with BSA together with an adjuvant, a substance that non-specifically enhances the magnitude of immune responses. The size of the immune response of mice that had not been pre-treated with antigen is 100%. Reproduced from N. A. Mitchison (1964) Proc. Royal Soc. London B161, 275-292.

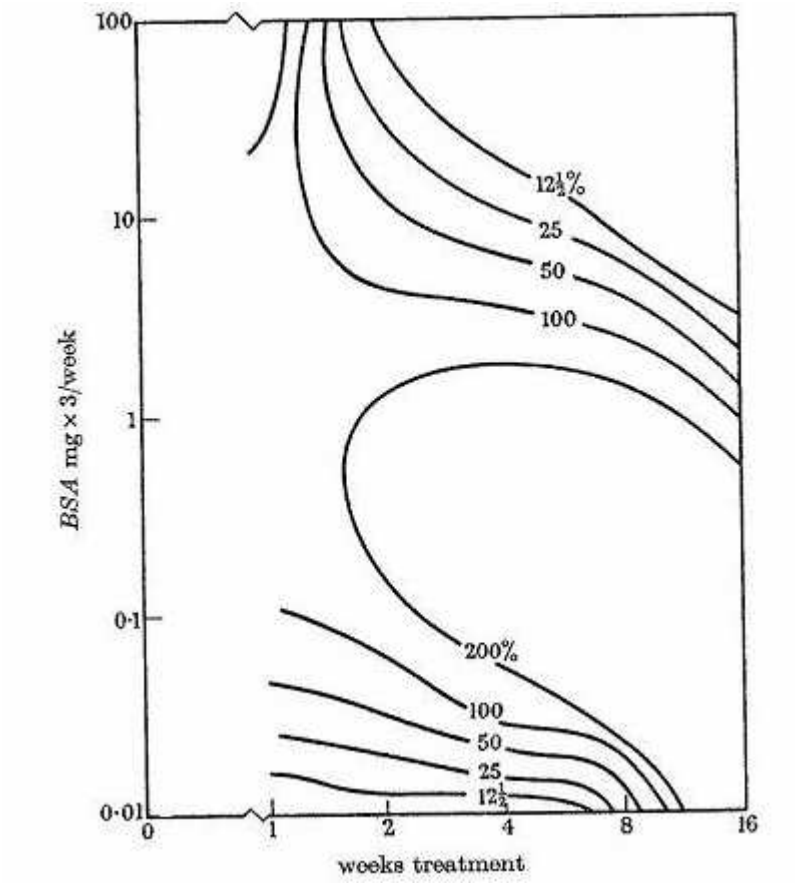
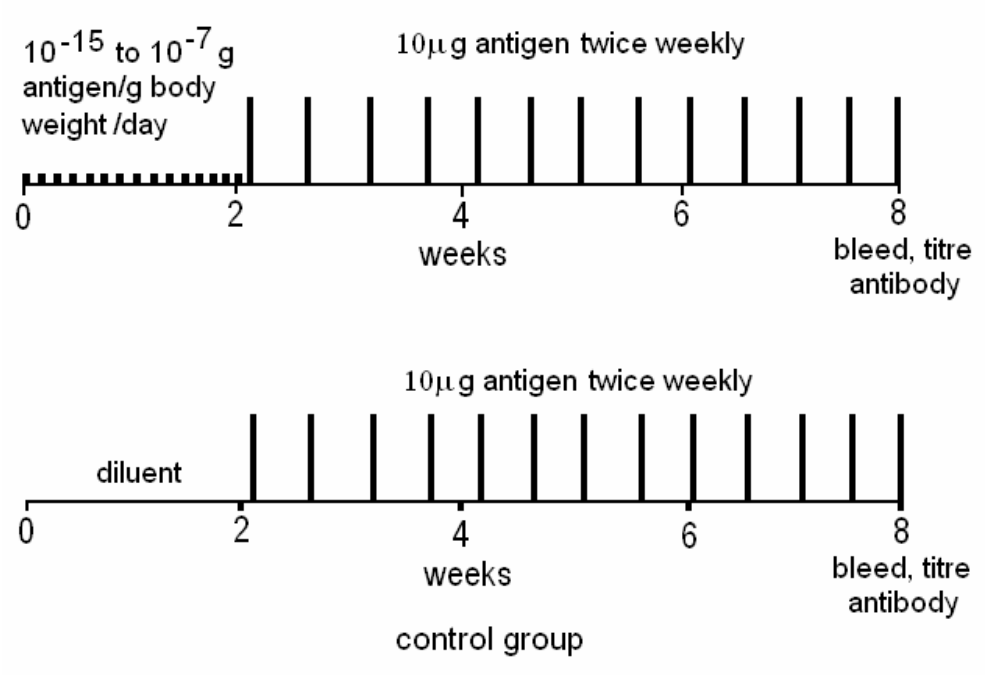


Figure 4-3a. An experiment by Shellam and Nossal demonstrating two zones of tolerance, including ultra-low dose tolerance, in rats. New-born rats were injected with the amount of the antigen flagellin shown per gram body weight daily for two weeks. The rats were then given twice weekly injections of 10 micrograms of flagellin, until they were bled at 8 weeks of age and the anti-flagellin antibody levels in the sera were measured.

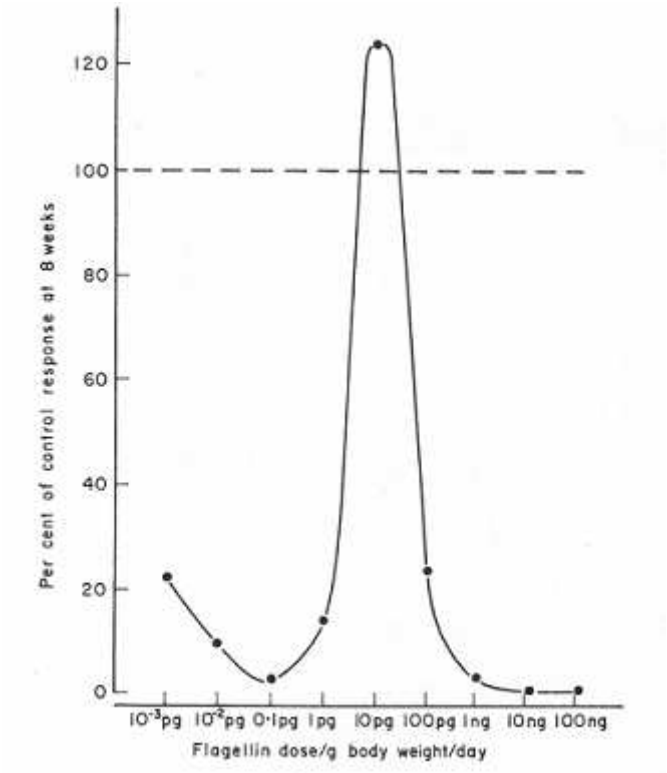


dose tolerance were intriguing for immune network theorists, since they represented switching of the system between states of the system using a minimal stimulus, and were not readily explained by basic clonal selection theory in the absence of any network interactions.

T cells and B cells

The events leading to specific antibody formation are more complicated than implied by our description of clonal selection in chapter 3. In the 1960s, soon after the formulation of the clonal selection theory, it was found that there are two major classes of lymphocytes, called B cells and T cells. Only the B cells make antibodies, while T cells regulate immune responses by B cells, in addition to making immune responses of

Figure 4-3b. The results of the experiment of Figure 4-3a, namely the mean flagellin-specific antibody levels as a percentage of those for control rats that received the 10 microgram immunizations but not the low tolerizing doses. From G. R. Shellam and G. J. V. Nossal (1968) Immunology, 14, 273-284.



their own called cellular immunity. The symmetrical network theory introduced in chapter 10 includes the key regulatory role played by T cells in antibody production by B cells.

The names “T cells” and “B cells” are derived from the organs in which the cells develop. T cells mature in the thymus, while B cells mature in the bone marrow (for mammals) or in the bursa (for birds). Roughly half of the lymphocytes belong to each of these classes. The regulatory function of T cells has two aspects. In some cases T cells help B cells to respond (“helper T cells”), and in other cases T cells suppress the immune response of B cells (“suppressor T cells”). What is meant by "helping" and

"suppressing" immune responses is defined by the experiments that led to the discovery of these functions. In chapter 6 we will describe some of those experiments.

Types of immune response

The immune system can respond to exposure to an antigen either positively with antigen-specific immunity, or negatively with antigen-specific tolerance. Antigen-specific immunity can be either an antibody response, or B cell immunity, or T cell immunity. The focus of much of this treatise is on IgM and IgG immune responses to foreign antigens. IgM and IgG antibody immune responses are not mutually exclusive. Two types of T cell immunity are delayed type hypersensitivity (DTH) and cytotoxic T lymphocytes (CTL). A DTH response involves primarily helper T cells. Another, unpleasant type of immune response is allergic reactions, that involve IgE antibodies.

A DTH response occurs when an animal or person is immunized with an antigen, then subsequently a small amount of the antigen is injected into the skin. There is local inflammation at the site of the second injection, that appears in about 10 hours, and reaches a maximum in 48 to 72 hours. It is called delayed type hypersensitivity because this is a much slower reaction to the antigen than that of an allergic reaction, which is also called immediate type hypersensitivity. An inverse relationship has been observed between antibody responses and DTH responses.[22] An immunization that induces a strong antibody response induces a weak DTH response, and vice versa.

The antigen can also induce antigen-specific tolerance or unresponsiveness. In some cases suppressor T cells can be shown to play a role in tolerance, while in other cases this cannot be shown to be the case.

Cytotoxic T cells

A third class of T cells in addition to helpers and suppressors is called "cytotoxic T cells". They are not known to be involved in regulating antibody responses by B cells. They kill target cells specifically by cell-cell contact. The regulation of both B cells and cytotoxic T cells involves helper T cells and suppressor T cells.

T-independent antigens

A strong immune response to most antigens, including for example protein antigens, requires the presence of both B cells and T cells. Some antigens however, called "T-independent antigens" cause immune responses in the presence of no T cells or very few T cells. The immune response then consists of the production of only IgM antibodies, with only temporary immunity (no memory). T-independent antigens are

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[22] C. R. Parish (1972) The relationship between humoral and cell-mediated immunity. Immunol. Rev. 13, 35-66.

typically polymeric, highly flexible molecules, for example polysaccharides, and hence are very efficient cross-linkers of the receptors for antigen on B cells.

Route of injection

The injection of an antigen intravenously (directly into the blood-stream) is more likely to cause tolerance than injecting the same antigen at a local site, for example intramuscularly or subcutaneously.

Genetic factors

Immune responses to foreign antigens can be influenced by the genes that encode antibody V regions and also genes that encode major histocompatability complex (MHC) proteins. In many systems antibody V region genes have been shown to play a role in the idiotypes of the antibodies that are produced. "Histocompatability" refers to the ability of a graft from one individual to be accepted (not immunologically rejected) by another individual. The MHC proteins play an important role in the selection of the repertoire of T cell V regions, and have regulatory influences on immune responses. We will describe the MHC and its significance in the context of immune network theory in chapter 12.

An antigen given in a specified form at a specified dose via a specified route may cause an immune response in one strain of mouse but not in another. For example, Table 4-1 shows the proliferative response to a collagen antigen *in vitro* (Latin: "in class") following *in vivo* priming with the antigen of mice that have two different MHC genotypes.[23,24] A stimulation index (ratio of amount of proliferation of cells obtained with the antigen to the amount obtained without the antigen) of over 10 is observed already with a priming dose of 0.05 μg in an H-2^s mouse, while the comparable level of response in an H-2^b mouse (a different strain) requires priming with 1000-fold more antigen, namely 50μg. (The H-2 genes are the MHC genes in mice.)

A simple (albeit incomplete) way to view these phenomena is that the responses obtained to different antigens and different strains may be related to thresholds that are important for the dynamics of the response. With an antigen A or a strain X, a crucial threshold may be reached, while with an antigen B or strain Y the threshold is not reached. In a highly non-linear system, small differences in thresholds can conceivably result in large differences in the size of responses, thus accounting for much

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[23] C. Pfeiffer, J. Murray, J. Madri and K. Bottomly (1991) Selective activation of Th1- and Th2-like cells in vivo - response to human collagen IV, Immunological Reviews 123, 65-84.

[24] MHC means Major Histocompatability Complex. The MHC of an animal has a big impact on its repertoire of V regions on T cells, and hence its response to many antigens; see chapter 12.

Table 4-1. Two strains of mice with different MHC genes (different H-2) need priming with markedly different amounts of a peptide fragment of collagen IV in order for their T cells to subsequently make a strong proliferative response to the peptide in vitro.

H-2 genotype	Dose of antigen used for priming in μg	Stimulation index
$H\text{-}2^s$	50	19.7
	5	44.1
	0.5	13.3
	0.05	13.4
$H\text{-}2^b$	500	21.8
	50	17.6
	5	5.6
	0.5	0.9
	0.05	0.7

unpredictability in the system. At the same time the detailed analysis of such sensitivity has the potential for providing important insights concerning the non-linearities inherent in the system.

The immune system may respond with an immune response (or tolerance) to an antigen A given in a specified form at a specified dose via a specified route, and yet fail to respond in the same way to a physically similar antigen B given in the same form at the same dose via the same route. In many cases such differences are related to the T cell repertoire of V regions and the influence of the MHC. Such findings underline the key role played by T cells in regulating the immune system.

Responses to self antigens

One of the most remarkable aspects of the immune system is its ability to respond to things that are foreign, but not to antigens that are part of the body itself. In the development of network theory we will focus firstly on responses to foreign antigens, and subsequently on the relationship of the system to self antigens, with which the system has a long-term stable relationship.

Is the diversity of phenomena due to a diversity of mechanisms?

There are many phenomena in immunology. Is there a new mechanism that underlies each new phenomenon, or can we find a theory that shows there is a unity underlying the diversity? Belief in such a unity is what motivates theoretically inclined immunologists.