1. Immunological Tolerance and Autoimmunity

1. Tolerance

Normally we do not make immune responses against our own tissue, a concept known as "self-tolerance". Determining how the immune system distinguishes between self and foreign antigens to make the decision between tolerance and immunity has been a subject of detailed investigation during the past 50 years. In 1945, R.D.Owen made a crucial observation that suggested that tolerance to self-antigens occurred because the immune system was exposed to these antigens during early development. He came to this conclusion after observing that adult dizygotic twin cows each contained a mixture of their own and their twin's blood cells, indicating that they were equally tolerant of their own and each other's blood cell antigens. Exchange of blood between the two foetal cows occurred in utero because binovular cattle share a placenta. This crucial observation demonstrated that tolerance could be induced by exposure to antigens in utero, irrespective of whether they were self or foreign. Shortly after this, F.M.Burnet enunciated the Clonal Selection Theory, where he proposed that each lymphocyte was specific for only one antigen and if a lymphocyte met this antigen during early development it would be deleted from the repertoire. The theory was modified by J.Lederberg, who suggested correctly that it was the stage in the development or ontogeny of the lymphocyte that was critical, rather than the stage in development of the animal.

There is now a large amount of evidence to support the Clonal Selection Theory for both T and B cells. Nevertheless, deletion of lymphocytes specific for self-antigens is not the only mechanism of self-tolerance. Both T and B cells specific for self-antigens can be identified in healthy people, so there must be mechanisms that prevent such cells from becoming activated and damaging self-tissue, thereby causing autoimmune disease. Although tolerance may be induced in all lymphocyte populations, CD4+ T cell self-tolerance is the most important and occurs at a far lower antigen threshold than that for B and CD8+ cells.

2. Mechanisms of tolerance to self-antigens

(i) Central tolerance by clonal deletion: Central tolerance refers to mechanisms of tolerance acting during lymphocyte development in the thymus or bone marrow. Experimental studies show that central tolerance is mostly due to the elimination or inactivation of those T and B cells that recognise self-antigens. These cells are destroyed or inactivated after they have expressed receptors for self-antigens and before they develop into fully immunocompetent lymphocytes. Deletion of self-reactive cells at an early stage in their development has been termed 'clonal abortion' or 'clonal deletion'. In early foetal life, self-reactive lymphocytes that are part of the developing immune system are deleted when exposed to self-antigens. As mentioned above, new immunologically competent cells are generated throughout life and these must be continuously deleted or inactivated also.

(ii) Peripheral tolerance by deletion and inactivation (anergy): Peripheral tolerance refers to mechanisms acting on mature lymphocytes after they have left the primary lymphoid organs. Not all genes are expressed in the thymus so developing T cells cannot be exposed to all self-antigens. Therefore, additional mechanisms for tolerising self-reactive mature T cells are necessary. Mechanisms of peripheral B cell self-tolerance are also necessary because after stimulation with antigen B cells expand and undergo somatic mutation, generating a population of B cells with new antigen specificities. Some of these cells may be specific for self-antigens.

It is now known that at least two receptor ligand families play an important role in regulating T cell expansion in the periphery after contact with antigen and these same molecules are probably involved in deleting self-reactive lymphocytes in the periphery. Peripheral deletion of CD4+ T cells seems to be dependent on signalling through Fas. Mice and men deficient in this pathway fail to delete T cells and develop aggressive autoimmune disease.

Sometimes T cells are not deleted but become specifically unresponsive to antigen stimulation (i.e. they do not proliferate). This is called clonal anergy. If antigen is removed, these anergic cells recover their responsiveness with time. One of the molecular mechanisms responsible for inducing anergy is signalling via CTLA4, a molecule expressed by activated T cells. Mice deficient in this gene get a severe autoimmune disease, and there is some evidence that human autoimmune thyroid disease is linked to deficient expression.

(iii) Suppressor T cells: The principal mechanisms of tolerance to self-antigens are clonal deletion and anergy. It has been suggested that there may be a back-up mechanism called suppression. This is a form of dominant tolerance because suppressor cells specific for a given antigen are able to inactivate other lymphocytes specific for the same antigen. For example, under some circumstances it is possible to induce specific tolerance to allografts which appears to be maintained by suppressor T cells. Thus, when T cells are transferred from a tolerant animal (animal A) into a syngeneic animal (animal B) then they enable animal B to accept an identical allograft to the one given to animal A. T
cells specific for the allograft must still be present in animal B because if it does not receive T cells from animal A, the graft is rejected rapidly. This suggests that the transferred cells from animal A are specifically suppressing the graft-specific T cells in animal B. The mechanism of action of suppression of this sort is not clear. Possible mechanisms could be the capacity of Th2 cells which secrete IL4 and IL10 to switch off proinflammatory Th1 cells.

3. **Induction of immunity or tolerance?**

What determines immunity versus tolerance? In general, mature human T cells are extremely difficult to tolerate, which means that following transplantation lifelong immunosuppressive therapy is usually required. On the other hand, most gestating women tolerate the growing foetus and do not mount immune responses to what is effectively an allograft. The reasons for these differences are not clear but a few suggestions are laid out below.

(i) Inflammatory mediators potentiate immune responses by upregulating accessory signals and cytokines thereby promoting immunogenicity versus tolerance. For example, the presence of necrotic tissue in a cadaveric renal graft provokes an immune response whereas a healthy foetus does not.

(ii) The immune stem has been designed to respond to infectious agents, which are replicating rapidly so that the quantity of antigen present varies rapidly. As has been pointed out earlier, there are signals for switching immune responses off as well as on. During infection the balance favours immunogenicity and antigen-driven expansion. In contrast, quantities of self-antigen change slowly or remain static and there is time to allow the negative regulation of T cells to occur so that the threshold for T cell activation is never reached. This implies the rate of change of antigen concentration is as important as its absolute level in the induction of immunity. This is could be why women do not make an immune response to the foetus, but can make immune responses to self-tissues if there is a sudden release of self-antigen (e.g. autoimmune carditis following myocardial infarction, Dressler's Syndrome).

4. **Mechanisms of induction of autoimmune disease**

In any individual it is possible to find T cells which react to high concentrations of self-antigens. The important point is that T cells are tolerant to normal, physiological levels of self-antigen. However, in some people, this state of tolerance breaks down and self-destructive autoimmune disease develops. Why does self-tolerance breakdown? It appears to be multifactorial, with contributions from gene defects, which confer autoimmune susceptibility, and environmental factors. Infectious agents or tissue damage from some other cause may activate the immune system, alter expression of self-antigen, or lead to expression of self-antigens which the immune system normally does not see. When these changes in self-antigen are combined with a genetically programmed defect in immune regulation, autoimmune disease may result.

5. **Failure to delete autoreactive cells**

Expression of particular MHC molecules is associated with particular autoimmune diseases. In a landmark serendipidous experiment, D.Mathis found that a particular strain of T cell receptor transgenic mouse always developed a severe rheumatoid arthritis-like illness when one particular MHC haplotype was present. When these mice were analysed, it was found that deletion of autoreactive T cells was incomplete. The importance of this finding was that using genetic experiments it was possible to show that only the T cell receptor and the MHC were required for disease.

Some MHC molecules have strong associations with autoimmune disease. These MHC molecules often show mutations involving amino acids involved in binding peptide. One possible explanation for the disease association is that self-peptides do not bind as well to these MHC molecules, which then compromise the efficiency of negative selection of self-reactive T cells in the thymus.

6. **Cryptic Epitope Theory**

Potentially autoreactive T cells can be found in any individual. In the presence of inflammatory cytokines such as tumour necrosis factor (TNF) and interferons, costimulatory molecules are non-specifically upregulated on antigen presenting cells, which can therefore provide increased costimulation of T cells and lower the threshold for T cell activation by peptide/MHC. This is important because some self-peptides are normally expressed at levels below that required for T cell activation (and induction of T cell tolerance) so T cells specific for these self-peptide/MHC complexes remain in a state of 'clonal indifference'. Such 'invisible' peptides are called cryptic epitopes. Upregulation of costimulatory molecules means that these T cells can be activated by these cryptic self-peptide/MHC complexes, and in this way autoreactive T cells may be activated. Autoimmune phenomena such as the production of autoantibodies are a common occurrence during infections but are usually self-limiting, and subside when the patient recovers from the infection. Thus, even when self-antigens become visible to the immune system, this is not sufficient to sustain an autoimmune response and cause a chronic autoimmune disease. Despite intensive
investigation, there is still no convincing evidence that infections precipitate the major autoimmune diseases.

7. **T cell help for an autoantigen is provided by an exogenous protein**

Tolerance in B and CD8\(^+\) lymphocytes is less profound than in CD4\(^+\) cells as outlined above. This usually does not matter, as most CD8\(^+\) and B cell expansion is controlled by CD4\(^+\) cells. However, if linked CD4\(^+\) help is provided to these self-reactive lymphocytes, autoimmunity can result. Linked help describes an immune response in which CD4\(^+\) T cells respond to foreign peptides and then provide T cell help to self-reactive CD8\(^+\) T cells or B cells. Such a mechanism was shown quite elegantly by Zinkernagel and coworkers who expressed a viral antigen in pancreatic \(\beta\)-islet cells (i.e. antigen transgenic mice). When these mice were crossed with CD8\(^+\) T cell receptor transgenic mice, which could react with the transgenic viral peptide expressed on islet cells, the T cells were not deleted but the mice did not destroy their islets and did not get diabetes. When the mice were infected with live virus, however, diabetes ensued.

The diagram below illustrates in outline how linked help might be generated. Imagine a situation where a virus has infected a cell, and viral proteins are using the cell's replication machinery to produce more virus. When the cell dies, fragments of DNA with both viral and self proteins attached get taken up by dendritic cells and presented to CD4\(^+\) T cells. Peptides from viral protein, should be able to activate T cells. These T cells can then provide linked help for B cells and CD8\(^+\) cells that recognise epitopes on the DNA and on the self-protein. This is one potential explanation for how autoantibodies are generated. An interesting example of a human autoimmune disease where such a mechanism may be operating is Wegener's granulomatosis. This is a form of systemic vasculitis and is normally treated with cytotoxic agents. After patients have entered remission, there is evidence that antibiotics decrease the frequency of relapses suggesting that pathogens (exogenous antigens) might be driving the autoimmune response. Another example which fits this model is coeliac disease where patients are sensitive to the exogenous protein gluten. Gluten can form complexes with the self-protein enzyme transglutaminase present in all cells, potentially leading to cytopathic T cells recognising self antigen. When gluten is withdrawn from the diet, the disease abates because the disease is driven by help from the CD4\(^+\) T cells specific for gluten.

8. **Role of autoantibodies in autoimmune disease**

B cell tolerance is most profound to soluble antigens expressed in the serum or on the surface of cells. Normally, B cells specific for these self-antigens are deleted in the bone marrow or shortly afterwards. By contrast, autoantigens which elicit high titre, high affinity autoantibodies are usually sequestered inside cells. This indicates that B cells specific for intracellular self-proteins escape clonal deletion. However, some autoantibodies do recognise extracellular cell-surface self-antigens. For example, some autoantibodies responsible for autoimmune haemolytic anaemia are sticky complement fixing IgM antibodies, which are evoked by infection (e.g. infectious mononucleosis or mycoplasma pneumonia). B cells secreting the haemolytic antibody are also present in normal individuals, but the level of expression is so low that there is no red cell destruction. When the level of IgM antibody is increased by infection, autoimmune haemolysis occurs. However, IgG antibodies can cause haemolytic anaemia. It is not known how these B cells escaped deletion.

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Model for linked help to self antigens

[Diagram of linked help to self antigens]

Self DNA binding protein

Viral Protein

DNA
Legend Complex of viral protein, DNA and self DNA binding protein is taken up by Dendritic cells. T cell help for the viral protein provides linked help for B cells and CD8⁺ cells which recognise self antigens.

If T cell help is present and autoreactive B cells are not deleted, the immune system cannot help making autoantibodies. The measurement of these autoantibodies is very useful in the diagnosis and subsequent monitoring of many autoimmune diseases. While some autoantibodies are clearly involved in disease pathogenesis, in other situations they are epiphenomena, which are nevertheless useful for diagnostic purposes.

To confirm that autoantibodies are involved in the pathogenesis of a disorder there are several criteria which should be met:

1. Reproduction of the disease following introduction of the antibodies into man or animals.
2. Induction of a lesion similar to the disease by immunization with the autoantigen.
3. In vitro simulation of the lesion.
4. Isolation of the autoantibody from a typical lesion.
5. Correlation of the antibody levels with disease activity.

Harrington injected himself with the serum from a patient with immune thrombocytopenic purpura (ITP) and induced a dramatic fall in his own platelets. Less enthusiastic researchers have relied on neonatal autoimmune disease due to transplacental passage of autoantibodies from affected mothers as evidence that the IgG autoantibodies are pathogenic. By this criterion, autoantibodies in myasthenia gravis, in Graves’ disease, and Goodpasture’s syndrome are pathogenic, but smooth muscle and mitochondrial antibodies are not. Transfer of autoantibodies to experimental animals may also confirm their pathogenic role, although this approach is limited by interspecies differences in target antigens. The lesion of pemphigus can be induced in infant mice by the injection of intercellular cement substance (ICCS) antibodies. Glomerular deposition of IgG identical with that found in Goodpasture’s disease occurs in monkeys after the injection of human glomerular basement membrane (GBM) antibodies. Pathogenic autoantibodies are more commonly associated with non organ-specific than systemic autoimmune diseases. One interesting exception is neonatal lupus where transplacental passage of SS-A and SS-B antibodies from mothers with Sjogren’s syndrome or SLE may directly damage the foetal cardiac conduction system to cause neonatal heart block. In this case, the disease manifestations in the neonate are distinct from those in the mother suggesting that the pathogenic effects of the autoantibodies are confined to the developing heart.

There are five principle mechanisms by which autoantibodies can produce autoimmune disease:

1. Complement dependent lysis of the target cell. Paroxysmal cold haematuria (a form of autoimmune haemolytic anaemia originally described in association with congenital syphilis but now more commonly observed in patients with mumps or measles) is an example of autoimmune disease mediated by complement-fixing IgM antibodies.
2. Opsonisation. This is the mechanism of most forms of haemolytic anaemia as the density of Ig is insufficient to allow cross-linking and activation of C1q.
3. Formation of immune complexes. e.g. Glomerulonephritis with serum sickness. Due to the negative charge of the glomerular basement membrane, immune complexes with a net positive charge tend to deposit there.
4. Blockade of receptors for physiological ligands. e.g. Myasthenia gravis (anti-acetylcholine receptor antibodies), pernicious anaemia (anti-intrinsic factor antibodies).
5. Stimulation of cell surface receptors. e.g. Graves’ disease (antibodies mimic actions of TSH).

These mechanisms are perhaps an oversimplification. For example, myasthenia gravis cannot be induced in an animal model in mice which lack IFNγ, although the mice generate autoantibodies to acetylcholine receptors. This implies that tissue damage by Th1 T cells plays an important role in addition to antibodies in the pathogenesis of autoimmune diseases.

The question which always arises with regard to autoantibodies and systemic autoimmune disease pathogenesis is how the antibodies react with intracellular antigen. There is evidence that some of these intracellular antigens are released to the circulation and immune complexes are generated (e.g. DNA ab). Also, there is evidence that nuclear antigens are pathologically expressed on the cell surface of apoptotic cells in patients with systemic autoimmune
In summary, most auto-reactive lymphocytes are eliminated during lymphocyte development in a process called clonal deletion. However, autoimmune diseases reveal that lymphocytes reactive to certain self-antigens do exist in the body. In some circumstances, the self-antigen may have been previously hidden from the immune system so that lymphocytes have not had an opportunity to become tolerant (clonal ignorance). For example, trauma to one eye can lead to autoantibodies that damage the healthy eye, a condition called sympathetic ophthalmia. In many cases (and in normal circumstances), B cells and CD8+ T cell immune responses do not occur since helper T cells specific for the self-antigen have been deleted, suppressed, or rendered anergic. Therefore, immunocompetent T and B cells which recognise the self-antigen and which require T cell help in order to be activated are unable to respond. In other words, a state of tolerance to certain self-antigens is maintained at the level of the T helper cell. The T helper cells may be activated by foreign antigen to provide help for autoimmune B cells or CD8+ T cells (linked help). In this situation, the appearance of a new antigenic determinant in association with a self-antigen may allow a new population of helper T cells to provide help to pre-existing self-reactive T and B cells. Finally, autoreactive cells may be activated by a potent inflammatory stimulus. In experimental animals this is achieved by injecting antigen with adjuvant, and this is probably a way of mimicking what happens when antigens are introduced into the body in the form of pathogenic organisms. In this case, presentation of antigen with an adjuvant may bypass regulatory mechanisms. Thus, there are multiple factors involved in both the establishment of either an immune response or a state of unresponsiveness to an antigen. Therefore, there are a number of different ways in which a breakdown of tolerance can occur leading to autoimmunity.