Clinical review

Science, medicine, and the future

Tolerance and autoimmunity

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Immune tolerance and autoimmunity are important clinically. Firstly, there are at least 40 known or suspected autoimmune diseases, and many are common (see box). Overall, 1 person in 31 is affected.[1] Moreover, autoimmune type 1 diabetes is the most common of all chronic diseases of children. Secondly, autoimmune disease is poorly diagnosed because the onset can be stealthy, and initial symptoms are often non-specific—tiredness, fatigue, or fever. Thirdly, patients with autoimmune disease will expect an interpretation of their illness from their doctor. Fourthly, new insights are set to revolutionise management, by replacement of blanket immunosuppression with new selective immunotherapies.

Predicted developments

Closer insights into generation of natural immune tolerance to self

Clarification of importance of apoptosis for loss of tolerance and development of autoimmunity

Enhanced understanding of how the "wrong environment" interacts with the "wrong genes" in development of autoimmunity

New selective immunotherapies that intercept the autoimmune response at critical points and induce natural immune tolerance

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Immune tolerance and the immune response

Figure 1 illustrates how tolerance is established and maintained and how it fails with ensuing autoimmunity. Specific immune and autoimmune responses involve the same elements. These include (*a*) an antigen (or autoantigen); (*b*) a response by interacting families and subsets of cells that include antigen presenting cells, T lymphocytes, and B lymphocytes; (*c*) messenger molecules, cytokines, chemokines, and their receptors; and (*d*) signalling and costimulatory molecules on cell surfaces (fig 2). Many of the functionally important cell surface molecules and their receptors are described by the cluster of differentiation (CD) nomenclature, based on their identification by characterised monoclonal antibodies.

The immune system does not normally respond to self antigens. This immunological tolerance was postulated over 50 years ago, but its multifactorial basis is still controversial.[2–4] Tolerance is generated at two levels. The "upper level" of central tolerance develops primarily in fetal life, and the "lower level" of peripheral tolerance develops postnatally as a backup process. A faulty central tolerance sows the seeds for autoimmune disease, while faulty peripheral tolerance lead to its eruption.

Central tolerance

Lymphocytes learn to react with antigens during lymphopoiesis in central lymphoid organs, thymus, and bone marrow. During the random rearrangements of genes that encode antigen receptors of nascent lymphocytes, the lymphocytes are exposed to antigenic signals from self molecules. Weak interactions with low affinity signals are stimulatory and select lymphocytes suitable for immune repertoires—positive selection. Strong interactions with high affinity signals are lethal, such that self reactive lymphocytes are eliminated by apoptosis—negative selection.[4]

In bone marrow, developing B lymphocytes receive stimulatory or deletional signals from self antigens, but selection processes continue in germinal centres of peripheral lymphoid tissues as well.[5] Exactly how these selection processes operate is uncertain, but important influences include the extent of representation and level of exposure to tolerogenic self molecules and, in the thymus, the HLA constitution of the individual.[6] In any event, not all self antigens are available for efficient negative selection, so that central tolerance is "leaky"

Prevalence of autoimmune diseases

- Thyroid diseases (includes Hashimoto's thyroiditis and Graves' disease): >3% of adult women
- Rheumatoid arthritis: 1% of general population, but female excess
- Primary Sjögren's syndrome: 0.6-3% of adult women
- Systemic lupus erythematosus: 0.12% of general population, but female excess
- Multiple sclerosis: 0.1% of general population, but female excess
- Type 1 diabetes: 0.1% of children
- Primary biliary cirrhosis: 0.05-0.1% of middle aged and elderly women
- Myasthenia gravis: 0.01% of general population, but female excess

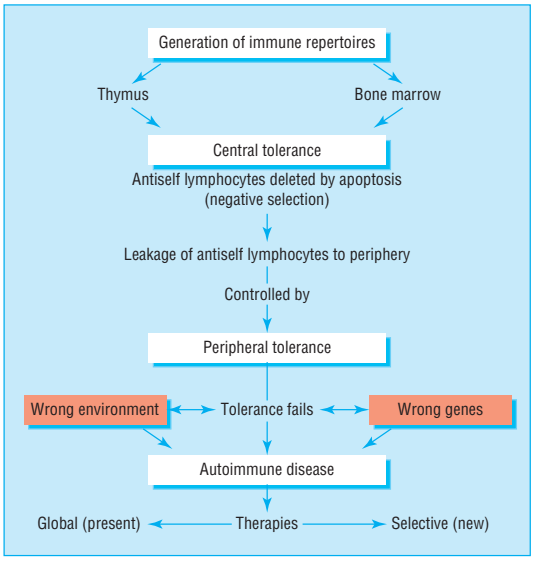

Fig 1 How tolerance is established and may fail. Generation of immune repertoires in central lymphoid organs, thymus, and bone marrow is accompanied by deletion of self reactive lymphocytes by apoptosis. The "leakiness" of this process requires back up by peripheral tolerance. Tolerance fails because of the interaction of a wrong environment with the wrong genes, resulting in autoimmune disease. Options for treatment will increasingly include new selective immunotherapies in place of present global immunosuppression

and results in export to the periphery of self reactive lymphocytes that require control throughout life.

Peripheral tolerance

Peripheral tolerance encompasses various safeguards that prevent activation of self reactive lymphocytes. These include ignorance, anergy, homoeostatic control, and regulation.

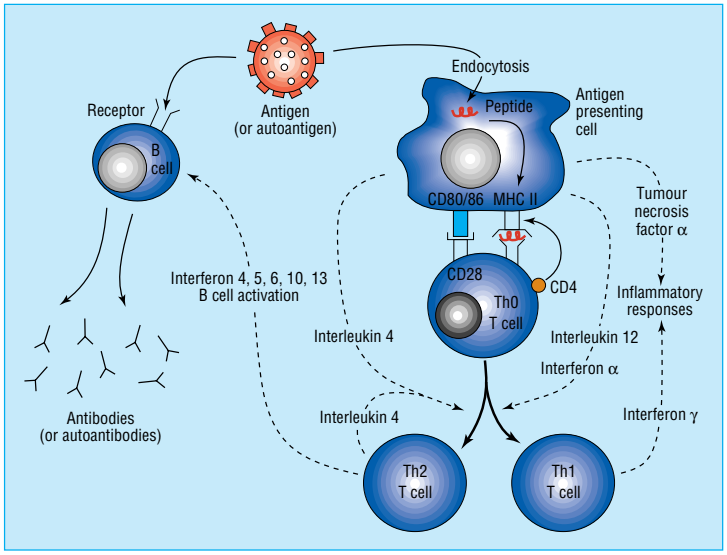

Fig 2 Components of immune (and autoimmune) responses. Antigen (or autoantigen) engages a B cell receptor directly and also is endocytosed by an antigen presenting cell (typically a dendritic cell, but B cells also serve), in which intracellular degradation generates antigenic peptides. These are presented on a class II molecule of the major histocompatibility complex (MHC II) to the receptor of a naive CD4 helper T cell (Th0). Binding is facilitated by CD4 interaction, as shown. Engagement of CD80/86 on the antigen presenting cell with CD28 on the T cell delivers a costimulatory signal necessary for activation. The naive T cell becomes a Th1 or Th2 T cell under the influence of various cytokines (dotted lines). Slightly different conditions apply to the activation of a CD8 T cell.

Ignorance—Autoimmune lymphocytes are kept in ignorance by sequestration of autoantigens behind cellular or vascular barriers; by the occurrence of cell death by apoptosis, which normally precludes spillage of autoantigenic intracellular constituents; and by the presence on the surface of potentially autoimmune (but non-activated) T lymphocytes of signalling molecules that preclude entry of the cell into tissue parenchyma.[7]

Anergy describes a state of unstable metabolic arrest affecting lymphocytes that can lead to apoptosis.[8] It occurs when a lymphocyte receives an antigenic signal without the normally necessary costimulatory second signal (see fig 2).[9] Anergy is a protective (tolerogenic) outcome after interaction between an autoimmune T cell and a self peptide on a parenchymal cell that is not competent to deliver a costimulatory signal.[10]

Homoeostatic control occurs by expression of cytotoxic T lymphocyte antigen 4 (CTLA-4, CD152) on activated T lymphocytes as an alternative to the CD28 ligand. When CD80/86 on the antigen presenting cell interacts with CTLA-4 the T cell is switched off.

Regulation by dedicated T cells inhibits the induction or effector functions of other classes of lymphocytes, either by production of down regulatory cytokines or interference with receptor signalling pathways. More information is needed on markers that identify regulatory T cells[11] and their role in the development of autoimmunity.

Apoptosis

Apoptosis represents physiological as opposed to pathological (necrotic) cell death.[12] It is of pivotal importance for tolerance and autoimmunity. Deficiency or dysregulation of apoptosis results in lymphocytes becoming unresponsive to death signals essential for deletional tolerance. Alternatively, when apoptosis is overwhelming or apoptotic fragments are not effectively removed, as can occur with deficiency of serum complement, there is a risk of autoimmunity.[13]

Two families of proteins mediate apoptosis. The cysteine aspartate proteases (caspases) are activated by the binding of a cell surface molecule fas (CD95) to its ligand, fas L. The Bcl2 family contains some 20 proteins, among which Bcl2 itself protects against apoptosis whereas others promote apoptosis. Since apoptosis normally eliminates self reactive lymphocytes, gene mutations that disrupt apoptosis are conducive to autoimmunity. Thus, mutations affecting fas or fas L cause autoimmune lymphoproliferative syndromes of childhood and analogous diseases in inbred mouse models.[14]

Causes of autoimmune disease

Environmental risk factors

Environmental agents can cause autoimmunity, but only the luckless few with the wrong genes will actually succumb. Infection is strongly implicated because it can readily disrupt peripheral tolerance in ways that include exposure of self to the immune system through breakdown of vascular or cellular barriers; the occurrence of cell death by necrosis rather than apoptosis; "bystander" activation of macrophages and T lymphocytes, which can then provide costimulatory signals;

and superantigen effects of bacterial products. Experimentally, mice infected with coxsackievirus with a tropism for either pancreatic islets[15] or heart[16] develop, despite viral clearance, an autoimmune response to breakdown products of islet cells or myocardium, resulting in chronic autoimmune inflammation.

In addition, there is the alternative, or perhaps complementary, process of molecular (antigenic) mimicry, whereby an antigen of a micro-organism or a constituent of food that sufficiently resembles a self molecule can induce a cross reactive autoimmune response. The mimicry idea has an attractive logic and supporting experimental evidence,[17] but clear examples are lacking for the more common human autoimmune diseases and their animal models.

Other environmental initiators of autoimmunity can act like infections by causing tissue damage, such as sunlight in lupus erythematosus, or alter a host molecule sufficiently for it to become immunogenic, as in chemical or drug induced autoimmune syndromes. Again, a permissive genetic background is also needed.

Autoimmunity might arise entirely from within, by an intracellular self molecule becoming in some way aberrantly expressed at the cell surface. The "internal" environment accounts for paraneoplastic autoimmune associations of cancers of the ovary, lung, and breast in which an antigen associated with the tumour provokes remarkable autoimmune responses that damage structures such as cerebellar, motor, or sensory neurones; nerve terminals, as in the Lambert-Eaton myasthenic syndrome; or retinal cells.[18] These syndromes reflect a misguided immune defence against the cancer since they usually precede overt expression of the cancer and even limit dissemination.

The internal environment is also indirectly relevant, since hormones influence female predisposition to autoimmunity, and autoimmune thyroid disease and type 1 diabetes may erupt in the postpartum period. Less well defined are the claimed effects of psychological stress that may act via neuroendocrine pathways.

Genetic risk factors

All autoimmune diseases probably have some genetic components. Susceptibility genes for autoimmunity may act along two tracks. One track determines tissue and disease specificity by directing the response to particular autoantigens. For example, genes that encode molecules of the major histocompatibility complex can determine which autoantigens are presented to the immune system; genes that encode the specificity of antigen receptors on T and B lymphocytes may influence which molecules are attacked; and genes may influence the susceptibility of a particular target tissue to autoimmune attack. The other track is a general susceptibility to autoimmunity via genes that influence tolerance, apoptosis, or inflammatory responses. These genes explain the tendency for autoimmunity to run in families, with variable expressions of disease in affected individuals. The genes involved are not all wrong: some alleles of the major histocompatibility complex may confer protection against autoimmunity, and the absence of these genes causes susceptibility, as for type 1 diabetes and rheumatoid arthritis.

Genetic susceptibility to autoimmune disease is now being investigated in highly informative ways.[19] Procedures include genome-wide scanning of individuals from affected families, using DNA microarrays to identify specific genetic elements. Variant alleles and their gene products are identified by linkage analysis and positional cloning. Thus studies on pairs of siblings of families with autoimmune disease can reveal susceptibility loci by sharing or otherwise of alleles at a known marker locus. Selected breeding of autoimmune strains of mice is identifying susceptibility loci for autoimmunity homologous to those identified in these human diseases.[20] Comparison of results from genome-wide scanning of autoimmune humans and mice, and use of the database of the human genome project, should provide a "blueprint" in the next few years for the estimated 20 (or perhaps more) genetic determinants for autoimmune disease. This newly acquired genetic data can then be used by clinicians to analyse complex autoimmune syndromes like rheumatoid arthritis, multiple sclerosis, and type 1 diabetes to understand their heterogeneity of expression.

Autoimmunity: what causes the damage?

Autoimmune responses can draw on a formidable immunological arsenal (see box). A subject of growing interest is the relation between T lymphocyte responses and chronic inflammation, which depends less on the effects of the inducing agent than on the immune responses directed to its elimination. Unfortunately, when a self molecule becomes immunogenic it cannot be eliminated; accordingly, inflammation becomes persistent and destructive. The division of CD4 helper T cells (Th) into two functional subsets, Th1 and Th2, while not as clear in humans as in mice, is a paradigm for understanding how autoimmune and allergic inflammation is orchestrated by cytokines and chemokines.[21 22] The cytokines interleukin 12 and interferon α, secreted particularly by antigen presenting cells, promote proinflammatory and cytodestructive Th1 responses via secretion of interferon γ and tumour necrosis factor α, whereas interleukin 4 promotes Th2 responses with activation of B cells (fig 2). Chemokines are chemotatic proteins secreted by various cell types and, through their interaction with specific receptors, direct the selective traffic of leucocytes throughout the lymphoid system and into inflammatory sites.[22 23] Thus polarisation

Mechanisms of autoimmune damage

Circulating autoantibodies

- Complement lysis (as in haemolytic diseases)
- Interaction with cell receptors (as in myasthenia gravis, thyrotoxicosis)
- Toxic immune complexes (as in systemic lupus erythematosus)
- Antibody dependent cellular cytotoxicity (possibly in organ specific autoimmune diseases)
- Penetration into living cells (controversial)

T lymphocytes

- CD4 cells polarised toward Th1 responses via cytokines (as in rheumatoid arthritis, multiple sclerosis, type 1 diabetes)
- CD8 cells activated to become cytotoxic T cells and cause direct cytolysis

Non-specific

- Recruitment of inflammatory leucocytes into autoimmune lesions (as in synovitis)

Selective immunotherapies for autoimmune diseases

Monoclonal antibodies or blocking antagonists

- Against T cell synapse (used for multiple sclerosis)
- Against cytokines such as tumour necrosis factor α (used for rheumatoid arthritis)
- Against receptor for cytokines such as tumour necrosis factor α (used for rheumatoid arthritis)
- Against receptors for chemokines CCR5 and CXCR3 (under development)

CTLA-4

- Downregulates activated T cells (trial use for psoriasis)

Regulatory cytokines

- Interferon β possibly inhibits interleukin 12 (used for multiple sclerosis)
- Interleukins 10 and 4 divert Th1 response to Th2 response (used in animal models)

Restoration of tolerance

- Antigen specific desensitisation (used for multiple sclerosis, type 1 diabetes)
- Stem cell replacement (used for various diseases)
- Gene therapy (under development)

towards a Th1 or Th2 response is associated with up regulation of chemokine receptors on Th1 or Th2 cells respectively.[22]

Selective immunotherapies for autoimmune diseases

The goal of replacing blanket immunosuppression by selective immunotherapy for autoimmune diseases now seems attainable, with the many possible "points of engagement" evident from figure 2. The agents in use or under development (see box) include monoclonal antibodies or blocking antagonists against the T cell synapse (represented by the binding site for the major histocompatibility complex, T cell receptor, and autoantigen), interactions between costimulatory molecules and ligands, or interactions between cytokines or chemokines with their receptors; counter-regulatory cytokines such as interleukin 10; or the T cell downregulatory molecule CTLA-4. Examples in current practice include copolymer 1 (copaxone, glatimer acetate), which interferes with the interaction between the major histocompatibility complex and a neural autoantigen to prevent relapses in multiple sclerosis[24]; monoclonal antibodies or soluble receptor for tumour necrosis factor α, which block the inflammatory effects of tumour necrosis factor α in rheumatoid arthritis[25] and inflammatory bowel disease; and CTLA-4, which limits graft versus host disease in bone marrow transplantation and alleviates psoriasis.[26]

An alternative approach is to "rewire" the immune system for tolerance. Firstly, antigen based desensitisation (oral tolerance), by repetitive mucosal administration of autoantigens, can prevent or reverse autoimmune disease in animal models. So far, results for human diseases are not so encouraging (multiple sclerosis, rheumatoid arthritis, uveitis) or not yet available (type 1 diabetes).[27] The problem may be that longstanding autoimmune disease is maintained by memory-type T lymphocytes that are difficult to render tolerant. Secondly, immune ablation by intensive immunosuppression and replacement by infusion of peripheral blood CD34 stem cells is proving successful in severe refractory autoimmune diseases, lupus erythematosus, rheumatoid arthritis, systemic sclerosis, and multiple sclerosis,[28 29] but whether this is simply due to heavy immunosuppression or indicates reprogramming for tolerance as well remains uncertain. Thirdly, for the more distant future, gene therapy for autoimmune disease has been considered for type 1 diabetes[30]; but the principles would be applicable to other autoimmune diseases.

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