Immunological Tolerance: Mechanisms

Secondary article

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Immunological tolerance refers to a reduction or complete inhibition of the ability of an individual to mount a specific immune response upon immunization.

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Introduction

An individual tolerant to a given antigen is usually unable to reject a skin graft expressing the antigen or to mount a lymphocyte-mediated cytotoxicity reaction against antigen-positive target cells or a specific antibody response to the antigen. Tolerance can be naturally acquired to the individual's own antigens, called self antigens, during development or experimentally induced following administration of the exogenous antigen according to certain regimens. Currently, tolerance is the focus of much investigation to understand the mechanisms that maintain self-tolerance and prevent development of autoimmune diseases, and to design strategies to induce tolerance in situations where it is desirable, such as organ transplantation.

Ways of Inducing Tolerance

Tolerance induction in early development

Introduction of an exogenous antigen into the fetus at the time of repertoire maturation renders the host's immune system tolerant to that antigen. This view stems from a historical observation of nonidentical cattle twins (Owen, 1945). The animals share a common placenta, allowing the two fetuses to mix and generate chimaeras, with each twin having blood cells from its sibling. As a result, the adult twins become tolerant to each other's blood cells. The technique of parabiosis in the domestic fowl also allows establishment of haematopoietic chimaerism. Birds hatched from the resulting parabiotic eggs are immunologically tolerant of each other: they do not produce antibodies against tissues injected from their partners and do not reject their partner's skin grafts. Further experiments in different species proved that immunological tolerance can be acquired (Billingham *et al*., 1953). These seminal observations led to the conclusion that confrontation of the immune system at embryonic life with an exogenous antigen creates a state of tolerance whereby the immunologically mature individual does not respond to that same antigen, regarded as a self antigen.

Tolerance induction in the adult

Although more difficult, tolerance induction in the adult is still possible. Usually, it necessitates a high dose of antigen and treatment over a prolonged period. Its induction in experimental animals is facilitated by reducing the potential of the immune system to respond by treatment with immunosuppressive drugs, antilymphocyte sera and immunotoxins, or sublethal X-irradiation. It can be induced by parabiotic fusion of two adult animals or by creating 'radiation chimaeras': bone marrow murine cells depleted of T lymphocytes are injected into a thymectomized, lethally irradiated mouse of a different strain. Induction of tolerance to live cells requires that the cells retain their ability to proliferate, to survive in the host and to induce a chimaeric state. This state of tolerance is often 'partial', leading to elimination of the antigen after a variable time period. A state of 'split tolerance' can also be observed, in which only some determinants of the inoculated antigen are tolerated. In humans, induction of immunological tolerance in the adult is an important issue in both transplantation biology and autoimmune diseases. Currently, ablation of large numbers of T cells for the purpose of tolerance induction in clinical practice is largely based on immunosuppressive (such as cyclosporin A) and antiinflammatory drugs with a number of side effects. The aim of present research is to design novel strategies to induce specific tolerance.

Systemic antigen-specific tolerance

To target antigen-specific cells, systemic administration of antigen has been proven effective in inducing effective self antigen-specific T-cell tolerance. According to the experimental protocol employed, several mechanisms have been identified. This strategy is effective in prevention of experimental allergic encephalomyelitis (EAE) and insulin-dependent diabetes mellitus (IDDM). However, several issues must be resolved before this immune intervention route becomes applicable to clinical settings and treatment of complex human autoimmune diseases.

Orally induced systemic tolerance

Early studies showed that oral administration of a hapten resulted in the suppression of systemic responses to that particular hapten (Chase, 1946). Further studies confirmed that foreign proteins that enter the body through the digestive tract suppress immune responses to those proteins instead of triggering them, creating a state of immune hyporesponsiveness to oral antigens. Oral tolerance has been used successfully to prevent, delay or treat autoimmune diseases in animal models, including collagen-induced arthritis (with type II collagen), EAE (with myelin basic protein), experimental autoimmune uveitis (with retinal S antigen or interphotoreceptor retinoid-binding protein), IDDM in NOD mice (with porcine insulin), experimental autoimmune thyroiditis (with thyroglobulin) and myasthenia gravis in Lewis rats (with acetylcholine receptor). Work from animal models has been extended into human clinical trials (multiple sclerosis, rheumatoid arthritis, diabetes, uveitis and allergy) with variable degrees of success. For example, a recent clinical trial in which patients with multiple sclerosis were treated with repeated doses of oral myelin was unsuccessful in reducing disease exacerbations. However, other results are encouraging and more work is required to identify factors that may modulate the response. Several observations indicate that, depending on the experimental system, oral tolerance operates through different mechanisms, including immune deviation, clonal deletion, clonal anergy and immune suppression (discussed below).

Systemic tolerance induced through the airway mucosa

Profound tolerance can also be induced to soluble protein antigens delivered by aerosol through the airway mucosa. The mechanisms involved in oral tolerance and its respiratory tract equivalent seem to be similar. For allergens exposed through the airway mucosa, it seems that high levels induce tolerance dominated by anergy and deletion, and low level exposure elicits adoptively transferable immune deviation. Autoantigen administration via nasal mucosal tissue can induce systemic tolerance more effectively than oral administration in a number of experimental autoimmune diseases, including antibody-mediated experimental autoimmune myasthenia gravis.

Factors Determining the Induction, Duration and Extent of Tolerance

Competence of the immune system

Tolerance induction is easier in animals with an immature immune system or with a mature immune system that has been compromised by irradiation, drugs or thoracic drug drainage. After administration of the antigen and discontinuation of the treatment, full immunocompetence is regained but the animal remains tolerant. Not only can T and B lymphocytes undergo tolerance, but the two cell types can become tolerant independently of each other. For example, 2 months after injection of deaggregated human γ-globulin, mice carry tolerant T cells and responsive B cells. In addition, T and B cells exhibit different temporal patterns of tolerance induction. As opposed to B cells, T cells can be made tolerant rapidly and remain tolerant for longer time periods. Importantly, B cells can be tolerized in the absence of T-cell involvement when the animal is treated with tolerogenic doses of a T-independent antigen.

Molecular characteristics of the antigen

The size of the antigen is particularly important for molecules that form aggregates and for antigens that exist in monomeric and polymeric forms. Flagellin from *Salmonella adelaide*, for example, is a potent immunogen in its polymeric form (molecular weight 10^4 kDa). However, in its monomeric form (molecular weight 4 kDa), flagellin induces tolerance when administered at high dose and an immune response when administered at low dose. Furthermore, a smaller fragment (molecular weight 1.8 kDa) obtained from monomeric flagellin becomes tolerogenic at low doses. Thus, the size of the molecule consisting of the same repeating subunits underlies its propensity to induce tolerance. Similarly, serum proteins, such as albumin and γ-globulin, are strongly immunogenic in an aggregated form, but tolerogenic in a deaggregated form. Presumably, the differences reflect distinct fates of the two forms of the antigen. Aggregated molecules are taken up rapidly by professional cells, processed and presented to competent cells. Deaggregated molecules, by contrast, remain in the circulation for longer time periods and are processed slowly. When incorporated into suitable adjuvants, they become immunogeneic. Strikingly, a slight chemical modification of an immunogen may render it tolerogeneic. For example, following acetoacetylation, monomeric flagellin becomes tolerogeneic at doses that normally are immunogeneic.

Also important for tolerance induction is the epitope density. When the hapten dinitrophenyl conjugated to polymerized flagellin is incubated with spleen cells in tissue culture, the outcome of the encounter depends on the number of dinitrophenyl groups per flagellin molecule: at a low density (0.7 groups per molecule) an immune response is observed, but at a higher density (3.8 groups per molecule) a state of unresponsiveness is induced over a wide range of antigen concentrations. The chemical nature of the antigen is also critical. At the same doses, D-amino acid polymers induce tolerance and L-amino acid polymers are immunogeneic. With regard to self-tolerance and induction of autoimmune disease, there is probably no

fundamental difference between self antigens and exogenous antigens. It is rather the mechanism of exposure and the characteristics of the confrontation between the antigen and immune effectors that will determine the outcome of the response.

Dose of antigen used

Initially, it was thought that tolerance could be induced only with very high doses of antigen, referred to as high-dose tolerance, which somehow paralysed the immune system. Further studies showed that subimmunogenic doses of antigen over a prolonged time period could induce a low-dose tolerance. In general, when a given antigen is used over a wide range of concentrations, intermediate doses induce immunity, and low and high doses induce tolerance (**Figure 1**). In the newborn, induction of low-dose tolerance can be achieved by a variety of antigens. In the adult, it often requires that the host is immunologically compromised.

Route of antigen administration

The introduction route is a key variable in tolerance induction, particularly in adult animals, presumably by determining the accessibility of the antigen to professional cells. In many models, the tolerizing antigen is given by either the intraperitoneal or the intravenous route. In general, subcutaneous administration favours antigen uptake and presentation by Langerhans cells and immunity, and intravenous injection favours presentation by resting B cells and results in tolerance induction. This conclusion is supported by the observation that removal of macrophages often facilitates tolerance induction. In addition, oral administration of antigen has been reported to favour tolerance induction. Likewise, changing the route of administration of an immunogenic molecule may render it tolerogenic.

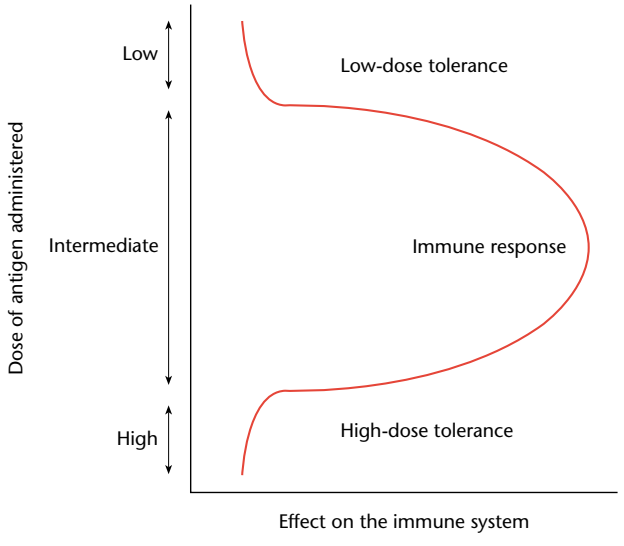

Figure 1 High- and low-dose tolerance. When a given antigen is used over a wide range of concentrations, intermediate doses induce immunity, and low and high doses induce tolerance.

Genetic susceptibility

Induction of tolerance is sometimes difficult in some inbred strains of mice. For example, unlike most strains, BALB/c mice are relatively resistant to tolerance induction by xenogeneic γ-globulin and this resistance segregates among the offspring. It is thus genetically controlled.

Completeness of tolerance

Immune recognition of a given molecule involves several clones specific for different epitopes and exhibiting different affinities. As the affinity of the interaction plays a critical role in determining tolerance induction, only some of the clones may be tolerized. The proportion of tolerant and nontolerant clones will determine the completeness of tolerance. The tolerant animal often produces small amounts of antibody, particularly if the tolerogen is a complex molecule. Therefore, the tolerant state is not absolute and tolerance is rarely complete.

Termination of tolerance

The state of tolerance does not last indefinitely. With time, it gradually wanes and eventually disappears. It can be deliberately terminated by the injection of an antigen that cross-reacts with the tolerogen used. For example, rabbits rendered tolerant to bovine serum albumin lose the state of tolerance after injection of the cross-reactive antigen human serum albumin. It is also possible to terminate tolerance by the administration of chemically altered antigens, lectins or antigen–antibody complexes.

Antigen persistence

Maintenance of tolerance depends on several factors. Notorious among these is the persistence of the tolerogen. Newborn mice may become tolerant to a single injection of serum protein for several months. However, unless the animals are challenged with the tolerogenic form of the antigen, the tolerance fades spontaneously. In the case of self-renewing antigens, such as occurs for alloantigens present in lymphoid chimaeras, tolerance may persist for life. Persistence of the tolerogen in the periphery and its accessibility to the immune system are generally required to maintain tolerance, which continuously inactivates newly emerging T and B cells that develop in primary lymphoid organs.

Costimulatory signals

A number of cellular events are required for a successful adaptive immune response in which the key participants are T and B lymphocytes, macrophages and dendritic cells. To become fully activated, T cells normally need to receive two signals. The first results from interaction of the processed peptide on antigen-presenting cells (APCs) (macrophages, monocytes, activated B cells, dendritic cells and Langerhans cells) and provides specificity. The second is a costimulatory signal provided by molecules expressed on APCs and resulting from noncognate cell interactions between the T cell and the APC. Well-characterized molecules include the B7-1 (CD80) and B7-2 (CD86) ligands on APCs interacting with CD28 and cytotoxic T-lymphocyte antigen (CTLA-4 or CD152) receptors on T cells, and the CD40 molecule on macrophages, dendritic cells and B cells interacting with the CD40L on activated T cells. In the absence of the costimulatory signal, the cell may die or become unresponsive.

Activation of B cells also requires at least two signals. The first is provided by B-cell receptor (BCR) ligation and the second results from interactions between costimulatory molecules, such as class II molecules of the major histocompatibility complex (MHC) and B7, and their T-cell counterligands, T-cell receptor (TCR)/CD4 and CD28. Following TCR or BCR ligation, the decision between tolerance and immunity is affected by the amount, avidity and timing of ligation, and by the nature and the amount of the costimuli present. In the absence of a properly timed interaction with a T cell, the B cell becomes tolerant, refractory to secondary encounters with antigen.

Mechanisms of Tolerance

Because lymphocytes responsive to a given antigen are rare in the immune repertoire, *in vivo* assessment of the tolerance mechanisms and identification of the developmental stage of tolerization have been difficult. Recently, the availability of transgenic mice in which the majority of lymphocytes express an immune receptor against a given antigen has enabled considerable progress in both detailing the parameters of tolerance induction and identifying maturational stages of tolerance susceptibility. Several mechanisms were identified.

Central tolerance

Clonal deletion

Throughout development, the immune system generates potentially harmful self-reactive T and B lymphocytes that must be distinguished from useful lymphocytes (Burnet, 1959). As T lymphocytes recognize antigenic fragments presented by molecules of the MHC in a self-restricted manner, they must undergo two processes of selection in order to maintain self-tolerance. Once immature T cells have rearranged their antigen receptor genes in the thymus, they become restricted to recognition of self-MHC molecules, a process known as positive selection. Cells that fail positive selection die in the thymus. In addition, T cells specific for self peptides bound to MHC peptides are eliminated by clonal deletion, a process known as negative selection. Cell types that express antigen on their surface (dendritic cells, cortical and medullary epithelial cells, and thymocytes) can induce deletion of thymocytes from the time they express a functional TCR until they reach the single positive stage. As a result, the vast majority (over 95%) of T cells generated in the thymus die in situ by apoptosis (Kappler *et al*., 1987). Similarly, self-reactive B cells are purged from the functional repertoire during the transition from the pre-B to mature B-cell stage in the bone marrow, and even quite low-affinity interactions can lead to central deletion (Nemazee and Bürki, 1989; Chen *et al*., 1995), a process that requires BCR ligation. Antigens presented in a multivalent form are particularly efficient in B-cell deletion.

Clonal anergy

Initially, it was thought that tolerance was only the result of deleting reactive cells in primary lymphoid organs. Further studies revealed that maintenance of tolerance is a much more complicated process. The term anergy was coined to describe the functionally silent state induced in B cells (Nossal and Pike, 1980). Anergic lymphocytes persist in tolerant animals, but are functionally inactivated. They proliferate poorly, secrete little antibody upon mitogenic stimulation in the presence of the antigen, and are poorly responsive to strong immunization *in vivo*. Anergic B cells have a shortened lifespan and may be in a state of delayed deletion. They have a 90–95% reduction of surface immunoglobulin (Ig) M, but not of surface IgD. *In vitro*, B-cell stimulation in the absence of T-cell help leads to anergy, whereas stimulation in the presence of help leads to activation. This state is reversible and strong BCR ligation, together with T-cell help, can rescue cells from anergy.

Anergy is also operative in T cells. *In vitro*, stimulation of T lymphocytes through the TCR in the absence of a second costimulatory signal results in functional unresponsiveness. In conjunction with a second antigen-unspecific costimulatory signal, this first signal leads to activation. *In vivo*, when self antigen is encountered intrathymically on thymic epithelium, reactive T cells may become anergic, refractory to subsequent exposure to antigen. Such functionally silenced T cells are selectively incapable of producing the autocrine growth factor interleukin (IL)-2 and proliferating upon exposure to antigen and the proper costimulatory ligands. It is an active process that is not associated with expression of low TCR levels. Remarkably, T-cell anergy is reversible and stimulation with IL-2

abolishes the unresponsive state. It has been proposed that the state of anergy results from defective antigen presentation, but recent studies suggest that this state of cell paralysis is associated with a block in signal transduction with a defect in TCR-mediated signalling along the protein kinase C (PKC)–Ras–mitogen-activated protein kinase (MAPK) activation pathway.

Receptor editing

Recent studies of mice bearing immunoglobulin transgenes indicate that B-cell tolerance occurs in newly formed bone marrow cells through receptor editing, a form of receptor processing that markedly alters the variable region genes expressed by B cells and, consequently, changes the specificity of the surface immunoglobulin. In contrast to clonal selection, where antigen encounter eliminates autoreactive clones and allows survival and maturation of unreactive B cells, receptor editing is a selection mechanism where autoantigen confrontation triggers secondary heavy- and light-chain gene rearrangements that will effectively alter the BCR specificity and extinguish the autoreactivity, allowing the primary B-cell repertoire to develop and populate secondary lymphoid organs. After they have exhausted their potential for successive immunoglobulin gene rearrangements, B cells may undergo apoptosis. Importantly, this process operates during normal B-cell development, as suggested by the induction of secondary functional light-chain rearrangements in response to antiidiotype treatment of a murine B-cell lymphoma. Hence, receptor editing is important in maintaining B-cell tolerance to self.

Peripheral tolerance

Thus, central tolerance purges the repertoire of immature lymphocytes with overt reactivity for self antigens encountered in primary organs and is crucial for preventing autoimmune diseases. However, antigens synthesized only in peripheral nonlymphoid tissues do not circulate in sufficient amounts in primary lymphoid organs. Peripheral tolerance is therefore required for lymphocytes that have not been silenced in primary organs. In the periphery, T and B cells can reach different levels of tolerance, from anergy with few phenotypic changes to physical elimination by deletion. This peripheral tolerance is highly dynamic and flexible, and often easily reversible. It also operates through additional mechanisms.

Immune deviation

Lymphocytes differentiate toward antigen-specific cells endowed with effector functions: secretion of different antibody classes for B cells and production of distinct cytokine patterns for T cells. Early studies noted a general reciprocal relationship between cell-mediated, delayed-type hypersensitivity (DTH) reactions and antibody production of particular classes as a function of antigen dose. The basis for this phenomenon is a cellular dichotomy in two reciprocally regulated, interacting T-cell populations that exhibit the same specificity for the antigen, but secrete distinct spectra of cytokines with distinct functional activities (Mosmann *et al.*, 1986). Following antigen activation, CD4 + T-cell precursors differentiate into T helper (T_H) type 1 cells (producing IL-2, interferon γ (INFγ) and tumour necrosis factor β (TNFβ), activating macrophages, and controlling DTH reactions and protection against intracellular pathogens) and T_H2 cells (producing IL-4, IL-5, IL-10 and IL-13, inhibiting macrophage functions and responsible for strong antibody responses, for controlling extracellular pathogens and for mediating allergic reactions). This polarization of the immune response seems to be related to cytokine cross-regulation, INFγ inhibiting proliferation of T_H2 cells, and IL-10 inhibiting stimulation of T_H1 cells by monocytic APCs. Recent evidence indicates that preferential activation of T-cell subsets can be a mechanism of tolerance induction. Deviating the immune response from a cell-mediated T_H1 type to an antibody-mediated T_H2 type represents a strategy to avoid responses harmful to the organism, which becomes tolerant. Thus, in orally induced tolerance, the cytokines IL-4 and IL-10 are produced, favouring T_H2 as opposed to T_H1 cells. In addition, a subset of regulatory CD4 + T cells secreting the inhibitory cytokine transforming growth factor β (TGFβ) and designated T_H3 cells, downmodulate the activity of T_H1 cells, further deviating the immune response to a T_H2-type response.

Suppression

In at least some situations, tolerance is a process that can be transferred by lymphocytes from tolerant animals to naive recipients by T lymphocytes. This phenomenon is thought to be mediated by suppressor T cells and cytotoxic T cells (Bloom *et al.*, 1992). Presumably suppressor cells, which have been noted in several experimental systems of autoimmunity and graft rejection, inhibit lymphocyte activity of T cells that are capable of causing tissue damage. In orally induced tolerance, high doses of antigen trigger deletion and anergy, and low doses favour active suppression whereby locally produced CD4 + and CD8 + regulatory T cells migrate to the systemic immune system. Cytotoxic T cells can also suppress immune responses by eliminating APCs or by lysing antigen-specific B cells. Even though suppression has been a dominant theme in cellular immunology, it has not been possible to clone the T-cell clones or to isolate the genes controlling this activity.

Immune privilege

It has been known for some time that certain anatomical areas are more favourable for grafting than others and that immune privileged sites are locations where allogeneic and

xenogeneic tissues are frequently tolerated. For example, tumour cells placed in the anterior chamber of a rabbit's eye grow progressively and corneal allografts are well tolerated in the absence of tissue matching or immunosuppressive therapy. Sites that seem to be exempt from immune responses include the brain, the eye and the testis. Initially, privileged sites were thought to lack lymphatic drainage and to be separated from the immune system by natural blood–tissue barriers, making them inaccessible to immune effectors. The current view is that immune privilege results from active dynamic phenomena and that it represents the consequence of interactions between the immune system and specialized tissues (Van Parijs and Abbas, 1998; O'Connell *et al*., 1999). For example, CD95L, the ligand for the death factor CD95, is expressed constitutively in privileged sites, such as the eye and the testis, and activated CD95 + T cells that enter these sites undergo apoptosis. Thus, in addition to other factors, such as the lack of lymphatic drainage and the presence of anatomical barriers, apoptosis through interactions between CD95L + cells and CD95 + inflammatory cells in immune-privileged sites may represent a powerful tolerance mechanism.

Network-mediated regulation

It has been proposed that normal recognition of self antigens involves a network of B and T lymphocytes interacting with one another and maintaining the homeostasis of the immunoregulatory system (Jerne, 1984). These circuits prevent the immune system from attacking self components and ensure the potential for efficient responses to exogenous antigens (Zouali *et al*., 1996). There are examples where the B-cell repertoire can influence the nature of the T-cell repertoire and *vice versa*. Thus, treatment of mice with anti-IgM antibody alters their repertoire of suppressor T cells. Administration of highly connected antibodies (derived from newborn animals and capable of interacting with the variable region of other antibodies) to neonatal mice can perturb the immune repertoire and affect the antibody response. It is, however, unclear whether such interactions have a major impact on immune response and tolerance.

Coreceptor modulation

In addition to these various mechanisms, TCR or coreceptor modulation may lead to peripheral tolerance to self antigens. It has been proposed that the immune system does not distinguish between self and nonself, but between dangerous and harmless entities, and that APCs are the primary distinctive elements (Ridge *et al*., 1996). When exposed to 'alarm signals' (such as apoptotic cells and pathogen-derived products), APCs are capable of being activated to upregulate costimulatory molecules. As a result, lymphocytes are tolerant when they recognize antigen in the absence of costimulatory signals. This line of argument is used to propose that the nature of APCs determines the outcome of neonatal exposure to an exogenous antigen (i.e. tolerance or immunization). In this view, T cells may be activated to either type of response by appropriate APCs, costimulatory signals and antigen.

In summary, several mechanisms are involved in induction and maintenance of tolerance, including deletion, anergy, editing, receptor downmodulation and lymphocyte sequestration. The number of APCs, the nature and amount of antigenic peptides generated, and the presence of costimulatory signals in a particular tissue are also important. Depending on the sites and the levels of antigen expression, different states of peripheral B- and T-cell tolerance will be reached. In certain situations, they could act in an additive manner.

Situations Where Tolerance Induction is Desirable

Understanding the mechanisms that maintain tolerance is important to understand the physiology of the immune system. It also is useful for designing novel means of inducing or restoring tolerance in conditions where it is not functioning properly. Situations where tolerance induction is desirable represent a major cause of morbidity and mortality in humans. They include autoimmune diseases, rejection of transplants and hypersensitivity reactions.

Tolerance to self antigens

Throughout development, two major opposing pressures act on the immune system. The first drives the generation of sufficient immune receptor diversity to recognize the wide variety of exogenous antigens. The second must avoid aggressive immune responses against self components. In normal conditions, self antigens are available to the immune system early in ontogeny and the corresponding clones become tolerant to the body's own components, preventing the organism from mounting an immune response to self antigens and from developing autoimmune disease. Although lymphocyte tolerance is induced continuously in central organs, T cells reactive with self antigens not present in sufficient amounts can develop, escape censorship, and migrate to the periphery. As a result, lymphocytes from normal individuals can be activated *in vitro* against a variety of self antigens. However, they remain silent *in vivo*, causing no damage. Factors that maintain this tolerance include low-level expression of MHC molecules and absence of costimulatory molecules on most nonlymphoid tissue cells, insufficient expression of target autoantigens, low precursor frequency of autoreactive cells and low affinity of their immunoreceptors, and restricted pathways of lymphocyte homing. If one of these factors is overcome, an

autoimmune disease may develop. The mechanisms responsible for activation of sufficient numbers of self-reactive lymphocytes and for induction of clinical symptoms remain the focus of investigation.

Tolerance to environmental antigens

In addition to self-tolerance, the maintenance of immunological homeostasis requires tolerance to exogenous, nonpathogenic antigens present ubiquitously in the environment, including airborne antigens and food antigens. Orally administered antigens encounter a well-developed immune network, called the gut-associated lymphoid tissue, which is able to discriminate effectively between harmful pathogens and essential innocuous nutrients. While avoiding a response to food antigens, the intestinal mucosal immune system is able to guard against invasion by pathogens. This selective immunoregulation is dependent on APCs (dendritic cells) that process and present gut antigens.

Although failure to tolerate proteins that are part of the normal diet is rare, failure to tolerate antigens present in biological dusts (including molecules of both plant and animal origin) is relatively common, resulting in immune reactions at the level of the mucosal respiratory tract with clinical consequences, such as allergic rhinitis and allergic bronchial asthma. It is thought that very early exposure to allergens predisposes to long-term allergic sensitization and that high-level exposure to airborne allergens during the first 3 months of life, as occurs by birth during the pollen season, can markedly increase the probability of developing an allergic disease during adulthood. Multiple mechanisms are engaged in tolerance to ubiquitous nonpathogenic environmental antigens and their breakdown probably explains the appearance of allergic reactions in the adult. Among the treatment and prophylactic strategies for controlling allergy, induction of peripheral tolerance is a promising approach. Therapeutic applications of oral tolerance to autoimmunity are in progress both experimentally and clinically, while those to allergies have been poorly investigated.

Transplantation tolerance

Transplantation of tissues and organs from one individual to another has become a potent treatment for chronic failure of the kidney, heart, liver and lungs. Because of the shortage of donor organ tissues, current studies attempt to use animal organs and tissues for transplantation. This success in transplantation of major organs is due to the availability of drugs able to control the immune response of the recipient against the graft and to prevent its rejection. Immune responses leading to graft rejection consist of both innate immunity (natural antibodies, complement and natural killer cells) that pre-exists the transplantation and adaptive immune responses elicited by the grafted tissue. Natural antibodies generally do not trigger an immediate rejection response in allotransplantation when the blood groups of the two individuals are compatible. However, natural killer cells can be responsible for early failure of bone marrow transplantation. Adaptive T cell-mediated immune responses play an important role in the rejection of allografts and xenografts. While graft acceptance is T_H2 mediated, T_H1-dependent effector mechanisms, such as DTH and cytotoxic T-lymphocyte activity, play a central role in acute allograft rejection. In the absence of immunosuppressive agents, they cause acute cell-mediated rejection and can destroy the graft in days or weeks. Cytotoxic drugs that attack proliferating lymphocytes and suppress immunity, and immunosuppressive agents (cyclosporin and mycophenolic acid) that inhibit cell activation, allow tolerance induction to transplanted organs. However, the transplant recipients become more susceptible to infections and tumours.

To improve the outcome of organ transplantation, other strategies are being sought, including modulation of donor grafts to reduce immunogenicity, encapsulation of tissue, and induction of a state of immunological tolerance without chronic use of immunosuppressive drugs. Strategies for tolerance induction include inhibition of alloreactive T lymphocytes in an antigen-specific manner by interfering with costimulatory receptors or with the TCR. It would be possible to trigger activation and proliferation of cells with a potential specifically to inhibit or divert the effector functions of undesirable lymphocytes. Another possibility for inducing donor-specific T-cell unresponsiveness is to transplant bone marrow cells to the donor, causing elimination of lymphocytes that would attack the graft.

Tolerance of the fetus

Under physiological conditions, the successful implantation of the fetus expressing histocompatibility antigens of the father in the uterus of the histoincompatible mother is an intriguing example of natural tolerance. Despite the presence of maternal T cells specific for paternally inherited antigens, the semiallogeneic fetus survives, and the mother's T-cell phenotype and responsiveness are restored after delivery, indicating that the pregnant woman transiently acquires tolerant lymphocytes specific for paternal alloantigens. The reasons for this tolerance are not fully understood and it is thought that elucidation of the mechanisms might be applicable to organ transplantation.

Normal pregnancy is characterized by a lack of strong maternal antifetal cell-mediated immunity and a dominant humoral immune response. To account for the success of the fetal allograft, it was proposed that, under the

particular hormonal environment of the pregnant female, confrontation of fetal antigens with maternal lymphocytes triggers a state of temporary silencing. Parenthetically, tolerance induction may account for the temporary amelioration of certain autoimmune diseases during pregnancy, as seen in multiple sclerosis and rheumatoid arthritis. After delivery, fetal cells carrying the self antigens disappear and the disease is reactivated.

Recent studies indicate that fetus-derived forces operate to immunosuppress the mother and to deviate her immune response towards a T_H2-like pattern, and that T_H1-type cytokines may damage the placenta directly or indirectly. The maternal response seems to be modulated by several cells and soluble factors, such as progesterone-induced blocking factor, placental suppressor factor, trophoblast cell-derived factor and cytokines (IL-10, TGFβ). It is likely that progesterone-mediated production of T_H2 cytokines contributes to the maintenance of a successful pregnancy. Also, the nonclassical class I antigen, human leucocyte antigen (HLA)-G, has been shown to play a role in fetomaternal tolerance by interacting with inhibitory receptors to downregulate natural killer and T-cell cytotoxic functions. Thus, it seems that it is the fetus who successfully eludes the mother's immune attack by diminishing initiation of the response, shifting it towards a nonaggressive response, and avoiding its destructive effects.

Undesirable tolerance of tumours

The majority of tumours express antigens potentially recognizable by T cells, including peptides derived from mutated oncogenes, tumour-suppressor genes and viral antigens (in virus-associated malignancies), nonmutated self proteins not normally expressed in adult tissues but transcriptionally activated within the tumour, and lineage-specific differentiation antigens shared by the tumour and the cell lineage from which the tumour arose. Despite the existence of these target antigens, the immune system is unable to reject cancers that develop *de novo*. Also intriguing are observations that there is no increased incidence of common tumours in animals and humans that lack a competent immune system.

Obviously, the mechanisms used to induce self-tolerance are relevant to understanding the strategies adopted by tumours to escape immune surveillance. One possibility is that *in vivo* unresponsiveness to tumours is the result of abnormal T-cell help. This has led to studies of the role of APCs in determining whether development of a tumour will result in tolerance or activation of effector lymphocytes, and several studies have highlighted the importance of antigen presentation. The CD95–CD95L system also seems to be part of an escape strategy used by tumour cells in various neoplastic malignancies. Several other mechanisms are being investigated. For example, malignant human melanoma cells can exhibit high levels of HLA-G and inhibit natural killer cytolysis, thus potentially impeding elimination of malignant cells by antitumour immune effectors.

In summary, immune tolerance is an active field of research where the experimental approaches and the concepts are evolving rapidly. It holds promise for designing novel strategies to modulate autoimmune, allergic and tumour diseases, and to prevent rejection of transplants.

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