Review article

Immunological tolerance and autoimmunity

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Immunological tolerance is a complex series of mechanisms that impair the immune system to mount responses against self antigens. Central tolerance occurs when immature lymphocytes encounter self antigens in the primary lymphoid organs, and consequently they die or become unreactive. Peripheral tolerance occurs when mature lymphocytes, escaped from negative selection during ontogeny, encounter self antigens in secondary lymphoid organs and undergo anergy, deletion or suppression. A heterogeneous family of T regulatory cells has recently been identified, which have been found to play an important role in suppressing immune responses against self. Failure or breakdown of immunological tolerance results in autoimmunity and autoimmune diseases. Such events are related to both genetic and environmental factors, the latter being mainly represented by infections. Infectious agents can indeed promote autoimmune responses either by inducing tissue inflammation and therefore an unintended bystander activation of autoreactive T cells, or by promoting T cell responses to microbial epitopes that cross react against self peptides.

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Introduction

The most remarkable characteristics of the normal immune system is its ability to react with an enormous variety of microbes. However, despite the fact that even lymphocytes with the ability to recognise self antigens are constantly being generated during the normal process of lymphocyte maturation, usually the immune system does not activate them. This unresponsiveness is due to a series of mechanisms that prevent immune responses against self antigens, and thereby enable the immune system to discriminate between self and non-self antigens. Therefore, when these mechanisms fail, the immune system may attack the individual's own cells and tissues. This phenomenon is known as autoimmunity, and the diseases it causes are called autoimmune disorders. In this review, I will first discuss the main mechanisms devoted to impair immune response against self antigens, and then the reasons why these mechanisms can fail, thereby resulting in autoimmune responses and then autoimmune diseases in some people.

Possible consequences of the encounter of lymphocyte with antigen

The encounter of lymphocytes with their specific antigen may give rise to three different possible events (Fig. 1). A first possibility is the so-called antigen ignorance, which occurs with nonimmunogenic antigens. Antigen ignorance is usually the inability of lymphocytes to interact with antigens, because these latter are present in inaccessible anatomic sites, such as central nervous system or some eye tissues. However, antigen ignorance can also occur towards accessible antigens that possess

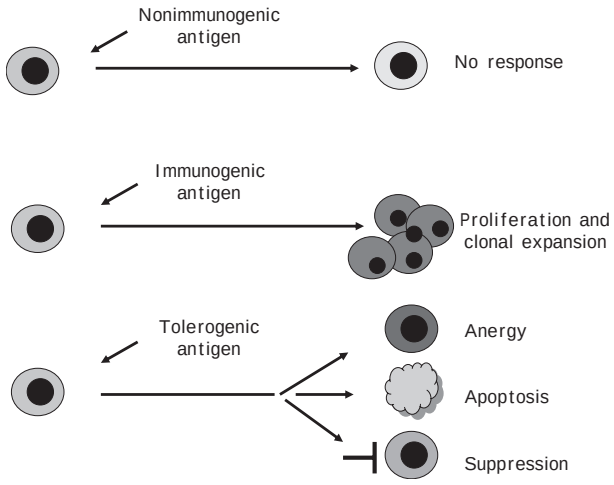

Figure 1. Possible events following the encounter between antigen and the immune system. When antigen is nonimmunogenic or inaccessible it is not recognised by the lymphocyte and no immune response occurs (antigen ignorance). When the antigen is recognised under appropriate conditions, there is proliferation of the specific lymphocyte and its clonal expansion and differentiation (immune response). When the antigen is recognised by the specific lymphocyte under inadequate conditions, the lymphocyte can die or lose the capacity to respond to the same antigen even if re-encountered under appropriate conditions.

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physic-chemical features that render them nonimmunogenic. The obvious consequence of antigen ignorance by lymphocytes is the absence of any type of immune response.

The usual response following the encounter between lymphocytes and antigens is the activation of the lymphocyte clone possessing the specific T cell receptor (TCR) for the antigen peptide, which is presented in the niche formed by class I or class II alleles of the major histocompatibility complex (MHC) on the surface of any nucleated cell type for MHC class I alleles or on the surface of professional antigen-presenting cells (APC), such as dendritic cells (DC), macrophages or B cells, for MHC class II alleles. Antigen presentation by MHC class I alleles allows the recognition of the peptide by cytotoxic CD8+ T lymphocytes and, therefore, results in a cytotoxic response, which represents the most important type of protective response against intracellular viruses. Secondly, by contrast, antigen presentation by MHC class II alleles expressed on the surface of DC, as occurs with the majority of microbes and soluble antigens, results in the recognition of the peptide by CD4+ T helper (Th) lymphocytes, and can cause a complex series of effector responses. In any case, the first effect following antigen/lymphocyte interaction is lymphocyte activation, which then results in proliferation, expansion, and functional differentiation of the antigen-specific clone (Fig. 1). This type of response represents the classic adaptive response of the immune system.

A third possibility of the encounter between lymphocytes and antigens is immunological tolerance. Immunological tolerance is the failure of a lymphocyte clone to respond to an antigen, due to its prior exposure to the same antigen that was encountered under particular environmental conditions (Fig. 1). There are two main ways by which tolerance can occur: 1) when immature lymphocytes encounter the antigen in the generative or primary lymphoid organs (central tolerance); 2) when mature lymphocytes encounter antigens in peripheral or secondary lymphoid tissues under particular conditions (peripheral tolerance)[1].

Central tolerance

The first major event in the function of the immune system is the differentiation and the development of the so-called lymphocyte repertoire, which means the rising of a pool of T and B lymphocytes equipped with all possible antigen-specific receptors. These receptors in B cells are surface-bound immunoglobulins, which are capable of recognising antigen in its native form. Antigen-specific receptors in T cells are the already mentioned TCR, which interact with a peptide fragment of antigen when it is presented in the context of MHC class I or class II alleles on the surface of APC. The development of the B-cell repertoire occurs within the bone marrow during ontogeny, while the development of the T-cell repertoire occurs within the thymus. At the end of their development both mature B and T lymphocytes leave central lymphoid organs and colonise to secondary lymphoid organs (lymph nodes, spleen, mucosal lymphoid tissues), where they will encounter their specific antigen. Therefore, it is obvious that central tolerance for B lymphocytes occurs in the bone marrow, whereas for T lymphocytes it takes place in the thymus gland, where these two types of cells undergo their maturation process, respectively.

There are two main mechanisms by which central B-cell tolerance may occur (Fig. 2). Immature B lymphocytes that encounter the native antigen in the bone marrow during their process of maturation undergo apoptotic cell death[2] or, alternatively, they change their receptor specificity. The latter process is known as "receptor editing"[3].

The mechanisms responsible for central T-cell tolerance are more complex and are not yet completely understood[4-6]. This is mainly because of the need of the T-cell pool to develop TCR specific for any type of exogenous peptide, and to be able at the same time to discriminate between self and non-self peptides, which are both recognised only in the context of autologous MHC class I or class II alleles. Some steps of this complex process of maturation and selection of the useful T-cell repertoire are well known, but some other still remain unclear. It is undoubt that haemopoietic precursors emerging from bone marrow are recruited to the thymus, and enter this organ through the corticomedullary junction. The recruitment of blood-borne migrant precursors to the thymus, as well as the subsequent migratory processes of thymocytes to the different

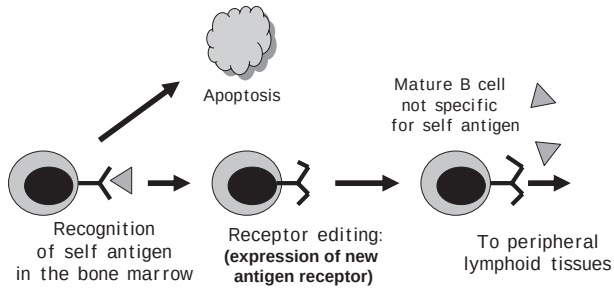

Figure 2. Mechanisms of central B-cell tolerance. When the antigen is encountered by the specific B lymphocyte during its process of maturation in the bone marrow, the cell may undergo apoptotic cell death, or express a new antigen receptor on the cell surface (receptor editing). This cell after maturation colonises to peripheral lymphoid tissues, but does not exhibit the ability to recognise the antigen already encountered in its immature state.

thymic areas and also the migration of mature selected T lymphocytes to the peripheral lymphoid organs, is an ordered process, which is regulated by chemotactic cytokines (chemokines)[7]. During this process, thymic migrants go towards a random development of any type of possible TCR (TCR rearrangement) and, therefore, they need to undergo a complex process of selection. The first step of T-cell selection occurs in the thymic cortex, where thymocytes showing no affinity for peptide-MHC, which are presented by cortical epithelial cells die of neglect[8]. These cells are double-positive (CD4+CD8+) lacking productive rearrangement of the TCRβ locus and they represent the great majority (> 95%) of cells recruited within the thymus. In other words, the neglected cells are all the cells equipped with TCR that do not possess any affinity for all possible peptide-MHC complexes encountered within the thymus (Fig. 3). This first type of choice has been named as positive selection, inasmuch as only those lymphocytes showing productive rearrangements of their TCR (meaning a TCR showing sufficient affinity for a peptide-MHC) are positively selected. The positively selected cells then migrate towards the medullary areas, where they differentiate into single positive CD4+ or CD8+ T cells. Obviously, this cell pool contains all possible T-cell clones, including not only those potentially able to recognise exogenous peptides that will be encountered in the course of life, but also those reactive with self peptides. The following process, which results in the elimination of the great majority of thymocytes able to recognise self peptides in the context of MHC, is known as negative selection (Fig. 3). Negative selection occurs because all thymocytes showing high affinity for a self peptide in the thymus undergo apoptotic cell death following self peptide interaction. Indeed, as mentioned above, central tolerance results from the encounter of the specific antigen with an immature lymphocyte[8].

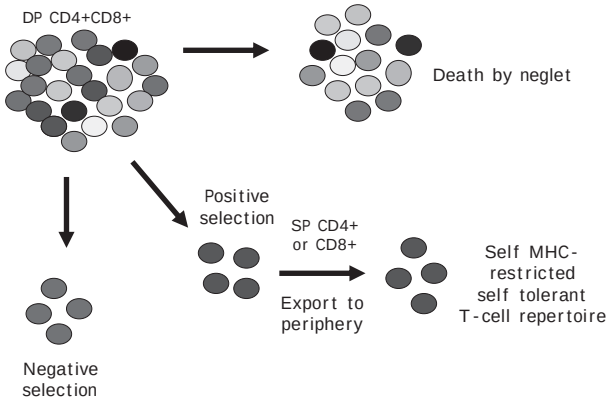

Figure 3. Mechanisms of central T-cell tolerance. Double positive (DP) CD4+CD8+ showing non-productive rearrangement of their T-cell receptor in the thymic cortex (> 95%) die by neglect. Positively selected cells migrate to the thymic medulla, where those possessing high affinity for self peptides present on the surface of medullary epithelial cells undergo apoptotic cell death (negative selection). The survived single positive (SP) CD4+ or CD8+ cells, equipped with T cell receptor showing potential specificity only for modified self, migrate to secondary lymphoid organs where they provide the entire the T-cell repertoire able to recognise on the surface of antigen-presenting cells any new complex formed by major histocompatibility complex (MHC) alleles containing within their niche the right exogenous peptide.

Two major issues that were still unsolved until recently, are the type of thymic cell able to present self peptides to thymocytes, and, mainly, how such a high number of self peptides can be encountered by T cells within the thymus. It is now clear that medullary epithelial cells (MEC), rather than DC, play the major role in the negative selection. The critical role of MEC in favouring negative selection is based on three major points: 1) MEC express on their surface many proteins that were previously thought to be tissue- or organ-specific; 2) MEC have been shown to achieve tolerance even by non-deletional mechanisms; 3) MEC are equipped with peculiar chemokine receptors. The mechanism by which MEC can present to thymocytes a number of self peptides enormously higher than that thought to be present within the thymus is the so-called promiscuous gene expression (Fig. 4)[9]. Through this mechanism, diverse tissues (lung, heart, testis, stomach, kidney, etc.) can be represented in MEC, where more than 5 000 different self-antigens have been identified. Promiscuous gene expression is part of the differentiation programme of MEC, which is regulated by a gene selectively expressed at thymus level, named as "autoimmune regulator" (AIRE). AIRE, which is located in the chromosome 21q22.3 and is encoding 545 aminoacids, probably drives the expression of certain organ-specific genes in MEC[10]. At the end of the process of negative selection, only single-positive CD4+ or CD8+ thymocytes equipped with TCR showing no high avidity for self peptides survive. T cells that escape negative selection, are expanded in the medulla by interleukin (IL)-7, migrate to the periphery and colonise to secondary lymphoid organs, where they will encounter exogenous peptides entering into the body that are captured and presented by DC.

Peripheral tolerance

It is obvious that, despite the discovery of the promiscuous gene expression at thymic level, which can explain how immature T cells can encounter in the thymus even self peptides previously thought to be restricted to organs other than thymus, not all self-reactive T cells can be deleted through the process of

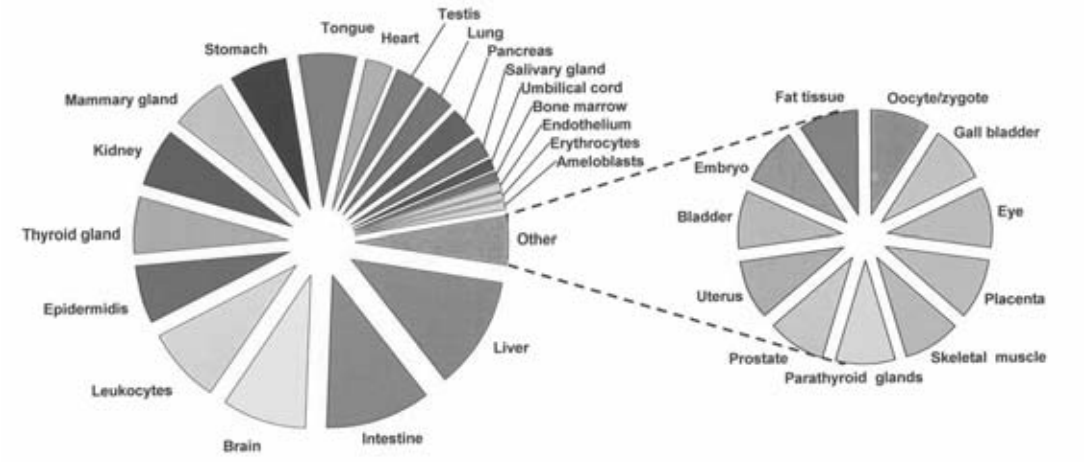

Figure 4. Representation of different tissues by promiscuous gene expression in thymic medullary epithelial cells. Murine medullary epithelial cells overexpress thousands coding and non-coding RNAs from different tissues in comparison with those expressed by cortical thymic epithelial cells, as assessed by gene chip analysis[9]. This phenomenon allows positively selected thymocytes to encounter in their immature state even peptides restricted to virtually all nonthymic tissues and, therefore, to promote the apoptotic cell death of those showing high affinity for self antigens.

negative selection. Likewise, not all self antigens can be encountered in the bone marrow by immature B lymphocytes. At birth, therefore, many clones of autoreactive mature T and B cells are present at level of secondary lymphoid organs, where they represent a high potential risk for the development of autoimmune responses during the course of life. To avoid this possibility, other mechanisms are operating on autoreactive mature lymphocytes even in the periphery.

Peripheral tolerance for mature B cell can occur under two main conditions. The first is when the B cell encounters the specific antigen in the absence of the specific Th cell[11]. In the absence of the Th cell, the B-cell not only remains inactivated, but becomes incapable of activation even when it re-encounters the same antigen under appropriate conditions. The second possibility is that the activation of the mature B cell is partial; under this condition the B cells is excluded from lymphoid follicles (follicular exclusion)[12].

The mechanisms for peripheral T-cell tolerance are more complex. So far, at least three different possibilities have been described, which are known as anergy, deletion, and immune suppression, respectively. Anergy is a process that occurs when a T-cell encounters its proper peptide under one of these particular conditions. A first condition is that the cell expressing the peptide on its surface is a non-professional APC. This cell type may possess the MHC niche for the peptide, which is therefore recognised by the TCR, but lacks the so-called co-stimulatory molecules, such as CD80 and CD86, which are able to provide an activatory signal to the Th cell through their interaction with CD28. In the absence of this co-stimulatory signal, the Th cell not only does not undergo activation, but rather becomes unable to be activated even when it re-encounters the same peptide on the surface of professional APCs equipped with co-stimulatory molecules[13]. Another possibility is that peptide recognition by the Th cells is followed by an interaction of the co-stimulatory molecules CD80 and CD86 with the suppressive cytotoxic T lymphocyte associated antigen (CTLA)-4, instead of the activating CD28, molecule on the surface of the Th cell. Under both these conditions, the Th cell does not die and persists in the body, but becomes functionally inactive.

Deletion is another mechanism of peripheral tolerance of mature T cells, which is based on their apoptotic cell death[14]. This usually happens when T cells encounter high antigen concentrations or they are heavily activated. This process is known as "activation-induced cell death" (AICD) and is mediated through the high expression of a surface molecule known as Fas (CD95), as well as its ligand (FasL or CD95L). The interaction between Fas and of its ligand on the surface of the proliferating Th cells activates the cascade of caspase enzymes that ends with the apoptotic cell death[15].

The third mechanism of peripheral T-cell tolerance is represented by immune suppression. The existence of suppressor (Ts) cells was suggested about 30 years ago, and after a series of controversial experiments their presence has been debated for 20 years. In the last 10 years, however, the existence of T cells able to suppress the immune response has been unequivocally demonstrated. These cells have been named as T regulatory (Treg) cells, and will be extensively discussed in this review.

T regulatory cells

Treg cells represent a heterogenous family of cells (Fig. 5). First, T cells able to suppress the myelin-induced murine experimental autoimmune encephalomyelitis, which is considered as the experimental animal model equivalent of multiple sclerosis, were discovered in mice orally treated with the autoantigen before its injection together with the adjuvant. These T cells were found to be suppressive through the production of transforming growth factor (TGF)-β and were named as type 3 Th (Th3) cells[16]. Subsequently, T cells possessing suppressive activity could be induced by immunising animals with antigen in the presence of IL-10. These Treg cells were named as type 1 regulatory (Tr1) cells, and are found to exert their suppressive activity via the production of IL-10[17]. Finally and most importantly, another family of Treg cells has been discovered, based on the observation that the thymic depletion of CD4+CD25^{high} cells results in the spontaneous appearance of multiple organ-specific autoimmune disorders[18,19]. Similar cells were then described in humans, and an enormous series of data has been reported that shows a decrease of their numbers in the peripheral blood of subjects suffering of autoimmune disorders, as well as their increase in chronic infectious diseases, as well as in different tumours[20]. Based on these experiments, a critical role has been postulated for these cells in the production of several human disorders. Remember that CD25 is one of the chains of the IL-2 receptor (R), and it is consistently expressed by all T cells following activation in order to allow their interaction with the T-cell growth factor IL-2 and, therefore, their proliferation and clonal expansion. However, Treg cells express this molecule in a constitutive way (i.e. independently of their activation) and at higher levels than activated effector T cells. Despite its lack of specificity, CD25 expression has been utilised in numerous studies as a marker of Treg cells, and this may have generated oversimplistic conclusions. Other molecules expressed on the surface of CD4+CD25^{high} Treg cells are CTLA-4, and the glucocorticoid-induced tumour necrosis factor receptor (GITR). However, even these molecules are not selectively expressed by CD4+CD25^{high} Treg cells. More recently, Foxp3, another molecule associated to Treg cells even if not present on their surface, has been discovered[21]. Foxp3 is a forkhead transcription factor that plays a critical role in the development and function of CD4+CD25^{high} Treg cells in both mice and humans. The X-linked Scurfy mutation of the Foxp3 gene (Foxp3sf) results in the death of hemizygous male mice, due to a CD4+ T-cell-mediated lymphoproliferative disease[22]. After this discovery, the aggressive, fatal, autoimmune human immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) has been mapped to multiple mutations in the human homolog of Foxp3[23]. The critical role of this gene in regulating the development and function of CD4+CD25^{high} Treg cells has been definitively demonstrated by the observation that ectopic Foxp3 expression is sufficient to activate a program of suppressive function in murine peripheral CD4+CD25- T cells[21].

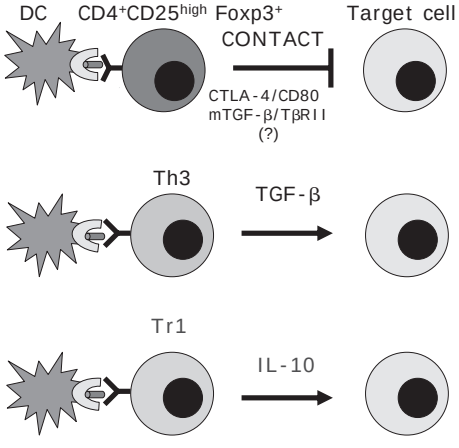

Figure 5. The heterogenous family of CD4+ Treg cells. Natural Treg cells are CD4+ T cells that originate in the thymus and probably play the most important role in the suppression of T-cell responses against self antigens. They are characterised by high constitutive expression of CD25 and of Foxp3, do not produce cytokines and act by a cell contact-dependent mechanism, where cytotoxic T lymphocyte associated antigen-4 (CTLA-4) and membrane transforming growth factor-β (TGF-β) probably play an important role. Adaptive Treg cells are induced in the periphery under particular conditions and are useful to control the extent of immune responses against exogeneous antigens. Th3 cells are induced by oral immunisation and act via the production of soluble TGF-β. Tr1 cells are induced in the presence of interleukin-10 (IL-10) and mainly act via the production of IL-10. DC, dendritic cells.

Nevertheless, several questions still remain open. First, it is clear that these cells represent a peculiar population of T cells that originate in the thymus, where they can be clearly recognized by their expression of CD25^{high}, Foxp3 and GITR, even in humans[20]. These Treg cells, whose depletion results in the development of multiple spontaneous autoimmune disorders and, therefore, seem to be mainly devoted to the suppression of the immune response against self antigens, have been named as natural Treg cells[24]. It has been suggested that natural Treg cells may be generated as an adjunctive effect of negative selection at thymic level[25]. T cells with affinity lower than deleted, but higher than surviving, self-reactive T cells may be the source of Treg cells. Nevertheless, the demonstration that CD4+CD25 T cells in the periphery can become Foxp3+ and acquire suppressive activity, raises the problem of the relation of these latter cells with the Foxp3+ cells that originate from the thymus. In other

words, whether CD25^{high} Treg cells represent a dedicated functional lineage or a plastic phenotype or both is still an open question[26]. Likewise, the relationship between CD4+CD25^{high} cells and the other types of Treg cells, such as Th3 and Tr1 cells is unsolved. At least Tr1 cells seem do not possess constitutive Foxp3. Moreover, while natural CD4+CD25^{high} Treg cells do not produce cytokines and seem to exert their suppressive activity *via* a cell contact-dependent mechanism, the other Treg cells found in the periphery, known as adaptive Treg[24], mainly act *via* the production of cytokines, such as IL-10 or TGF-β (Fig. 5). Even the contact mechanism by which natural CD4+CD25^{high} Treg cells exert their suppressive activity is controversial. The role of CTLA-4 and membrane TGF-β has been implicated, but their critical importance is not generally accepted. However, TGF-β seems to be really essential not only for the suppressive function of Treg cells, but also for maintaining their Foxp3 expression[27]. This is the reason why not only deletion of the Foxp3 gene, but also deletion of TGF-β1 and TGF-β RII genes, results in the development of lethal immunoproliferative autoimmune syndromes.

Mesenchymal stem cells

In the last few years, another probably not physiologic modality of tolerance induction has been described after discovering that allogeneic bone marrow transplantation can result in donor-specific tolerance facilitating subsequent organ transplantation. This approach has, therefore, been applied to treat autoimmune diseases, even if with a great susceptibility of recurrence[28]. Mesenchymal stem cells (MSC) present within the bone marrow seem to be critical for such an immunosuppressive effect. MSC are progenitors for several connective tissue lineages including bone, cartilage, muscle, fat and bone marrow cells. They are identified by the expression of surface markers, such as CD90, CD105, VCMA-1 and hyaluronase receptors, but they lack haematopoietic stem cell markers, such as CD34 and CD35[29]. MSC are not rejected by the immune system even after allogeneic transplantation, because they are not recognised by T cells. However, they have been shown to suppress T-cell and NK-cell responses. The mechanisms by which MSC suppress immune responses are still controversial[30].

How failure or breakdown of tolerance may result in autoimmunity

From the findings discussed above, it appears clear that autoimmunity is the result of a failure or breakdown of one or more of the mechanisms responsible for maintaining self-tolerance.

Genetic factors

In general, multiple interacting factors, both genetic and environmental, may contribute to the development of autoimmunity, but there are a few emblematic autoimmune disorders in which genetic alterations are sufficient to induce their appearance. These genetically-related diseases may be due to defects of mechanisms responsible for either central or peripheral tolerance. One classic example of autoimmune disease exclusively due a genetic defect of central T-cell tolerance, is the autoimmune poliendocrinopathy, candidiasis, ectodermal dystrophy (APECED) or autoimmune polyendocrinopathy syndrome type 1 (APS1). Indeed, mutations responsible for APECED or APS1 have been found in the hemogeneously staining region domain of the AIRE gene (see above). To date, more than 50 APS1-causing patient mutations have been established. The mutations are distributed throughout the coding region of the gene. The R257X mutation is the most prevalent in Caucasian populations[31].

One classic example of autoimmune disease exclusively due to a genetic defect that results in a failure of peripheral T-cell tolerance, is the autoimmune lymphoproliferative syndrome (ALPS), initially known as the Canale-Smith syndrome[32]. The majority of these patients have a lymphoproliferative disease, autoimmune cytopenias and susceptibility to malignancy. Similar syndromes also exist in lpr/lpr, as well as in gld/gld, mice and are equally due to genetic defects in the Fas/FasL-mediated apoptosis. Comparable pathological features have been described in patients showing gene mutations at level of Fas (type Ia), FasL (type Ib), caspase 8 or 10 (type II), or other still unknown (type III) mutations of genes involved in the cascade of Fas/FasL-mediated apoptosis.

Finally, there is a third example of autoimmunity selectively due to genetic defects involving the process of immunological tolerance. This is represented by the already mentioned IPEX syndrome, which is characterised by Foxp3 mutations that impair the suppressive function of Treg cells[23]. In this case, both central and peripheral tolerance may be affected, based on the still undefined relationship between natural and adaptive Treg cells (see above).

Apart from these three prototypic examples of autoimmune disorders, which are exclusively due to genetic defects of the most important mechanisms that preside over the tolerance towards self antigens and provoke the failure of these mechanisms, the development of the great majority of human autoimmune disorders results from the interaction between genetic and environmental factors resulting in the breakdown of self tolerance. Multiple genes may predispose to autoimmune diseases, even if the genetic alteration is not sufficient for their triggering in the

absence of contributing environmental stimuli[33]. Certainly, the most important of these genes are MHC genes, inasmuch as MHC alleles play such an important role in the presentation of both self and non-self peptides. Associations between particular MHC class II alleles and autoimmune diseases have been made repeatedly, and they are usually different in different diseases. In addition, other genes are also involved that act directly on the cells of the immune system, thus altering the immunoreactivity of the host. These genes are frequently not disease-specific. Genes that predispose towards autoimmunity by affecting immunoreactivity might not necessarily act by making the immune system more reactive. For example, several strains of animals that are predisposed towards autoimmunity actually have lower lymphocyte counts and lowered ability to mount immune responses. It is also well known that human beings suffering from common variable immunodeficiency have a higher risk to develop autoimmune diseases than normal individuals. However, the genetic alterations that predispose to the development of autoimmune disorders by interacting with the environmental stimuli are so complex that they would require a special review and therefore will not be discussed in detail here.

Environmental factors

There are different environmental factors that possibly are responsible for the breakdown of self tolerance and the consequent development of autoimmunity. Classically, ischemic injury or trauma, may trigger autoimmune disorders, as exemplified by post-traumatic uveitis or by orchitis following vasectomy. Under these conditions, probably sequestered self antigens become accessible to the immune system, thus overcoming the process of antigenic ignorance mentioned above. Hormones have also been thought to be involved in the pathogenesis of autoimmunity, inasmuch as the prevalence of some autoimmune diseases, such as systemic lupus erythematosus and systemic sclerosis, is at least 10 times higher in women than in men. However, whether the increased prevalence of these diseases in women is due to sex hormones or other gender-related factors is still unclear. Apart from tissue injuries and hormonal influences, it is undoubtedly true that microbial infections are the most important environmental factors contributing with the genetic predisposition to the breakdown of immunological tolerance and the development of autoimmunity.

It is of note that the Th cell-mediated effector responses that play a major protective role against most infectious agents exhibit a type 1 Th (Th1) profile, inasmuch as these cells produce IL-2, interferon-γ and tumour necrosis factor, which are not only responsible for the so-called cell-mediated immunity that involves the activation of macrophages, but also collaborate with B cells for the production of opsonising antibodies. Thus, Th1 cells should be considered as essential for the highly protective phagocyte-dependent effector immune responses[34]. However, if despite this type of response the microbe is not rapidly removed, Th1 cells may become potentially dangerous for the host just because of their ability to activate macrophages. In this context, it is of note that a role for Th1 responses and for cytotoxic CD8+ T cells (that usually associate with Th1 cells) in the pathogenesis of different organ-specific autoimmune disorders, such as Hashimoto's thyroiditis, type 1 diabetes mellitus, and multiple sclerosis, and autoimmune gastritis, has been proposed[35]. Recently, however, a major role of a novel subset of Th cells, named as Th17 cells in the pathogenesis of some inflammatory and autoimmune disorders, including rheumatoid arthritis, lupus erythematosus, and experimental autoimmune encephalomyelitis (which is the experimental animal model equivalent to multiple sclerosis), has been suggested (Fig. 6). Th17 cells are probably a distinct subset of effector T cells, induced as a consequence of IL-23 production by DC. Th17 cells produce IL-17 and IL-17F, and these cytokines evoke inflammation largely by stimulating fibroblasts, endothelial cells, epithelial cells and macrophages to produce chemokines, as well as granulocyte colony-stimulating factor (CSF) and granulocyte macrophage CSF, with the subsequent recruitment of poly-morphonuclear leukocytes. In some settings, however, IL-17 and IL-17F-induced inflammation is dominated by macrophages, with subsequent production of IL-1, IL-6, metalloproteinase-3, and inducible nitric oxide synthase[36,37] (Fig. 6). More recently, a functional antagonism between Th17 and Foxp3+ Treg cells has been reported[38]. Th17 seem to originate not only because of the production of IL-23 but also from the combined activity of IL-6 and TGF-β.

Since TGF-β. is involved in the generation of Treg cells[27], the fact that IL-6 production inhibits their development suggests the existence of a dichotomy in the generation of pathogenic (Th17) cells that can induce autoimmunity and Foxp3+ Treg cells that inhibit autoimmune tissue injury[38].

Based on these findings, it is reasonable to suggest that infectious agents can induce autoimmunity in two main different ways. The first way is the so-called "by-stander activation" (Fig. 7). Infectious agents indeed cause tissue inflammation, thus inducing the accessibility of sequestered autoantigens, or the activation of APC via the production of pro-inflammatory cytokines, or the aberrant expression of co-stimulatory molecules by APC; moreover, infectious agents themselves can also act as mitogens or superantigens[39,40]. Under these conditions, tolerance against self peptides may be broken, and Th1-, Th17- or CD8+-mediated autoimmune responses take

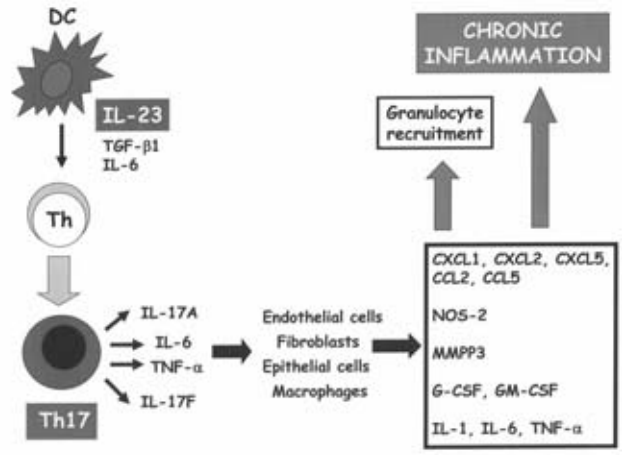

Figure 6. The possible role of Th17 cells in the inflammation of some autoimmune disorders. While dendritic cells (DC) that produce interleukin (IL)-12 promote the development of Th1 cells, DC that produce IL-23 promote the development of Th17 cells. The presence of IL-6 and transforming growth factor-β1 (TGF-β1) also contribute to Th17 cell development. Th17 cells produce IL-17A, IL-17F, IL-6 and tumour necrosis factor-α (TNF-α), which act on different cell types, such as fibroblasts, endothelial and epithelial cells, and macrophages. This results in the production of several chemokines, granulocyte colony stimulating factor (G-CSF) and granulocyte macrophage colony stimulating factor (GM-CSF), which induce the recruitment of granulocytes. The production of inflammatory compounds, such as IL-1, IL-6, TNF-α, metalloproteinase-3 (MMPP3) and nitric oxide synthase-2 (NOS-2), also occurs and contributes to maintain chronic tissue inflammation.

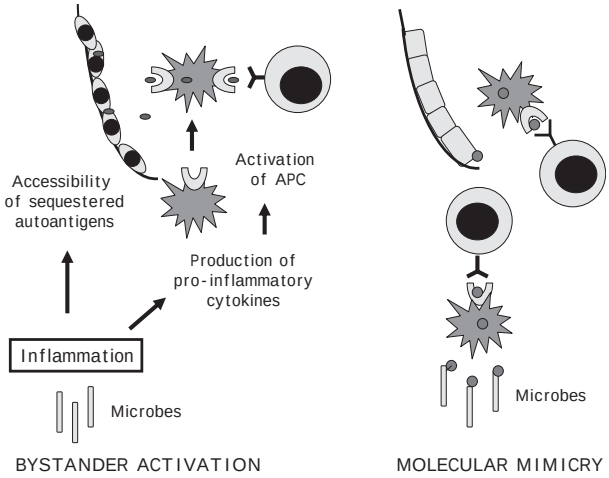

Figure 7. Main mechanisms by which infectious agents can induce autoimmune responses in genetically predisposed individuals. Microbes induce inflammation and this process may favour the accessibility of sequestered self antigens to antigen-presenting cells (APC) and therefore to autoreactive T cells, or the production of pro-inflammatory cytokines that may induce the overactivation of non-professional APC and the expression of co-stimulatory molecules, thus promoting their ability to present self peptides to Th cells *(left)*. Some microbial components may also act as mitogens or superantigens, thus inducing the direct activation of several Th-cell clones, including one or more autoreactive clones (not shown). This series of mechanisms are known as "by-stander activation". Some microbes can express peptides identical to self peptides. During the immune response against the microbe, Th cells can also react against tissues expressing self peptides identical to microbial peptides ("epitope or molecular mimicry") *(right)*.

place. The effects of infection and inflammation are not limited to the activity of professional APC, but may also involve cells that are not normally included in this category, such as endothelial or epithelial cells. Indeed, inflammation can induce expression of MHC class II alleles and also co-stimulatory proteins, such as CD40 and CD40 ligand by these cells. Increased expression of MHC by non-professional APC will lead to increased exposure to T cells of peptides from proteins expressed within these cells. Because T cells are less likely to become tolerant in the thymus to peptides from such specialised cells, this might be a particular threat to the individual, especially in combination with increased levels of co-stimulatory proteins, which will ensure that the T-cell responses are productive.

The other mechanism that can promote the breakdown of self-tolerance inducible by infectious agents is the so-called "epitope or molecular mimicry" (Fig. 7). This means that under certain conditions infectious agents can express antigens that are cross-reactive with self determinants. Infectious agents provide their own adjuvants, therefore T-cell responses against microbial antigens are particularly vigorous. If the microbial antigen codes for a peptide that is closely related to a peptide of the host, such vigorous responses might powerfully induce T cells that can then react, less strongly perhaps, but still effectively, against cells bearing the cross-reacting self antigen. Thus, a protective immune response against a microbial antigen may become dangerous for the host. The most classic examples of epitope mimicry are the cross-reaction between a *Borrelia burgdorferi* antigen and a peptide from human LFA-1, that underlies the chronic arthritis in some patients with Lyme's disease, between streptococci and heart antigens, that accounts for heart damage in rheumatic fever, and between H+/K+ ATPase and *Helicobacter pylori*, possibly responsible for autoimmune gastritis[41-43].

Role of innate immunity

The role of infectious agents in triggering autoimmunity appears still more complex after the discovery that cells of the innate immunity (DC and NK cells), but also B cells, possess on their surfaces a series of receptors able to interact directly with highly conserved structures of many bacteria and viruses. These receptors have been

named Toll-like receptors (TLR)[44]. For example, animals that lack TLR4 and therefore cannot detect lipopolysaccharide are not susceptible to any of the potential effects of this inflammatory agent, including presumably its ability to induce autoimmunity[45]. The family of TLRs is instrumental in activating APCs and initiating inflammation, and they are triggered by a variety of microbial components. For example, double-stranded RNA binds to TLR3 and lipopolysaccharide activates TLR4. Some recent investigations shed more light on the potential role of TLR cross-linking in offsetting autoimmune processes. In these studies, TLR agonists were administered with model autoantigens to trigger autoimmunity in non-diabetes-prone animals that expressed the same antigen in their ß-cells as a transgene. Intriguingly, divergent outcomes were observed. In one study, autoimmune disease occurred readily, whereas in the other one, autoimmune responses were transiently invoked[46,47]. The crucial difference was the need for autoantigen-specific CD4 Th responses. If added to the latter model, autoimmune disease developed. Thus, for autoantigenic immunisations in conjunction with TLR ligation to precipitate disease in naïve animals, a variety of factors likely need to coincide. Therefore, an effect of TLR ligation on autoimmunity is more likely to occur if an autoimmune process is already established, as would be the case in prediabetic individuals. Indeed, investigations from the BB rat model support this notion. If TLR agonists were administered during the prediabetic phase in this genetically determined model of autoimmune type 1 diabetes, development was strongly accelerated[48]. Thus, breaking of tolerance to autoantigens requires very strong inflammatory stimuli, unless autoreactive T cells are already activated. Ultimately, the development is strictly dependent on numbers of autoaggressive T cells available, and thymic tolerance can prevent development of autoimmunity even in the presence of TLR agonists[49].

Concluding remarks

In the last years, the mechanisms by which the immune system discriminates between self and non-self antigens, thus avoiding dangerous responses against its own cells or tissues, have been extensively investigated and most of them have been clarified. The complex series of processes involved in the protection against responses towards self antigens is known as immunological tolerance. Mechanisms operating at level of primary lymphoid organs, such as bone marrow and thymus (central tolerance) and at level of secondary lymphoid organs, such as spleen, lymph nodes and mucosal lymphoid tissue (peripheral tolerance) have been described. Among these mechanisms, negative selection of self-reactive T lymphocytes through AIRE gene-regulated promiscuous gene expression by thymic MEC and the generation of Foxp3 gene-controlled autoantigen-specific Treg cells seem to play the most important protective role.

Failure or breakdown of mechanisms responsible for the development or maintenance of immunological tolerance, result in immune responses against autoantigens and, therefore, in the development of autoimmune diseases. In some cases, this can occur simply because of genetic alterations that involve critical pathways of central or peripheral tolerance, as it occurs in patients with APECED, due to genetic mutations of the AIRE gene, in those with IPEX, due to genetic mutations of the Foxp3 gene or in those with ALPS, due to mutations of genes that control AICD (Fas, FasL, or caspase). In the majority of autoimmune disorders, however, both genetic and environmental factors contribute together to induce the breakdown of tolerance against self-antigens. Infections are certainly critical in triggering the autoimmune responses through different mechanisms. They can increase or alter the function of APC by rendering easier their collaboration with T cells (by-stander activation), or can induce Th1 and/or Th17 cell responses against self peptides cross-reactive with microbial peptides (epitope mimicry). A linking also seems to exist between TLR present in APC and the development of, or the protection against, autoimmune responses.

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