Autoimmunity: Basic Mechanisms and Implications in Endocrine Diseases

Part II

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Abstract

Regulation of the immune response to self-antigens is a complex process that involves maintaining self-tolerance while preserving the capacity to exert an effective immune response. The primary mechanism that leads to self-tolerance is central tolerance. However, potential pathogenic autoreactive lymphocytes are normally present in the periphery of all individuals. This suggests the existence of mechanisms of peripheral tolerance that prevent the initiation of autoimmune diseases by limiting the activation of autoreactive lymphocytes. If these mechanisms of peripheral tolerance are impaired, the autoreactive lymphocytes may be activated and autoimmune diseases can develop. Several processes are involved in the maintenance of peripheral tolerance: the active suppression mediated by regulatory T cell populations, the different maturation state of antigen-presenting cells presenting the autoantigen to autoreactive lymphocytes, inducing tolerance instead of cell activation, the characteristics of B cell populations. A deeper comprehension of these mechanisms may lead to important therapeutic applications, such as the development of cellular vaccines for organ-specific autoimmune diseases. In addition, autoimmunity does not always have pathological

consequences, but may exert a protective function, as suggested by several observations on the beneficial role of autoreactive T cells in central nervous system injury.

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Introduction

Regulation of immune responses to self-antigens is a complex process that involves maintaining self-tolerance while preserving the capacity to exert an effective immune response. Mechanisms of central tolerance are insufficient to control autoimmune events. Autoreactive lymphocytes are present in most normal individuals, but only 5% of the general population is affected by autoimmune diseases. This suggests the existence of multiple mechanisms of peripheral tolerance, whose function is to keep autoimmune phenomena under control.

In this second part the processes underlying peripheral tolerance have been analyzed focusing on the cellular mechanisms.

Regulatory T Cells

Regulatory T cells (Tregs) may be defined as T cells that inhibit effector T cell proliferation in vitro and in vivo [1]. Several cells with regulatory properties exist

which may be included in two categories. Some Tregs arise after antigen exposure: Th2 cells; Th1 cells; interleukin (IL)-10 producing Tr1 cells; transforming growth factor- β (TGF- β) producing Th3 cells; CD8+ regulatory cells, and $\gamma\delta$ T cells. Other Tregs spontaneously develop: CD4+CD25+ T cells; natural killer T cells, and probably $\gamma\delta$ T cells (although they increase after antigen sensitization). A large overlap may exist among these cell subsets which exert their function as one or another subset according to their activation stage or microenvironment [2–4].

The constitutive expression of IL-2 receptor α -chain (CD25) is likely to be the most specific Treg marker. CD4+CD25+ T cells represent up to 10% of the human CD4+ T lymphocytes. However, CD25 is not an exclusive Treg marker as its expression is induced on CD4+CD25– T cells during immune responses. Nevertheless, Tregs express CD25 at a higher level and more stably, while CD4+CD25– T cells lose CD25 when the immune response switches off [5–7]. Tregs constitutively also express adhesion molecules (LFA-1, CD44, ICAM-1, CD103, CCR4 and CCR8) which guarantee rapid recruitment to the inflammation site. Tregs additionally express CD152, co-stimulatory molecule cytotoxic T-lymphocyte antigen 4 (CTLA4) and the tumor necrosis factor (TNF) super family member glucocorticoid-induced TNF receptor (GITR) family-related protein TNFRSF18. These molecules classically upregulate after T cell receptor (TCR) activation. The partially activated phenotype of Tregs may depend on the ongoing engagement of their TCRs by autoantigens, under non-inflammatory conditions. CD4+CD25– T cells also readily upregulate these markers after TCR activation, but express them only transiently [5–7]. However, the use of CD25, CTLA4 or GITR as Treg markers in the presence of inflammation may result in uninterpretable data.

FOXP3 and IPEX Syndrome

IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome) is a rare disease, with heterozygous females being asymptomatic, while affected males present severe diarrhea, lymphocyte activation, pro-inflammatory cytokine overproduction, insulin-dependent diabetes mellitus, thyroiditis and immunodeficiency [8, 9]. Scurfy mice are the counterparts of human IPEX. The causative gene in Scurfy mice was identified in FOXP3 which encodes Scurfin, a new member of the forkhead/winged-helix family of transcription factors.

Also human IPEX is associated with loss of function mutations in the FOXP3 gene. FOXP3 $^{-/-}$ mice lack CD4+CD25+ T cells. A CD4+CD25+ T cell transfer completely prevents autoimmunity, suggesting a correlation between Treg absence and autoimmune diseases [9, 10]. In mice, only CD4+CD25+ T cells express FOXP3 mRNA, while activated CD4+CD25– T cells or differentiated Th1/Th2 cells do not and FOXP3 transduction correlates with CD25, CTLA4, GITR and CD103 expression [10]. However, few data about the role of human FOXP3 are available, and they show some discrepancies with murine models. In humans, there are individual differences in FOXP3 expression. Furthermore, anti-CD3/anti-CD28 stimulation induces FOXP3 expression in CD4+CD25– T cells. These cells may represent the induced Tregs [10].

Treg Development and Specificity

Tregs develop within the thymus as a functionally mature T cell subpopulation. CD4+CD25+ thymocytes express FOXP3 and present a surface phenotype similar to mature Tregs. In vitro, these cells are naturally anergic to TCR stimulation and exhibit suppressive activity [11].

Thymic development of Tregs requires an interaction between their TCRs and self-peptide/major histocompatibility complexes (MHC), expressed on thymic stromal cells. Studies with double transgenic mice demonstrated that Tregs development requires a TCR with a relatively high avidity for self-peptide/MHCs. However, TCR affinity must not be so high as to lead to deletion. In double-transgenic mice (which express moderate levels of transgenic peptides on thymic stromal cells) most of the thymocytes with a transgenic specific TCR, differentiated into CD4+CD25+ T cells [12, 13]. Treg production was abrogated in the presence either of low affinity transgenic TCRs or a high peptide concentration on thymic stromal cells, probably because of insufficient positive selection or strong negative selection, respectively. Why the relatively autoreactive Tregs are not negatively selected is unclear. Perhaps the superficial co-stimulatory molecules increase the interaction affinity between developing Tregs and stromal cells, as suggested by Treg reduction in CD28- and B7-deficient mice or in CTLA4 immunoglobulin-treated mice [13, 14].

Tregs show a polyclonal TCR repertoire, so they potentially recognize a wide array of antigens. Treg TCRs show a higher avidity for peptide/MHC complexes compared with other specific CD4+CD25– T cells. Because

of these features, Tregs may also modulate the responses to infectious agents and downregulate exuberant immune processes [14, 15].

Functional Capacities of Tregs

Tregs develop as a functionally mature T cell subpopulation, highly differentiated and able to exert their suppressive activity. When they are stimulated in vitro with a specific antigen, in the presence of CD4+CD25- T cells and antigen presenting cells (APCs), they inhibit effector T cell proliferation in a dose-dependent way [14–16]. In vitro Tregs are anergic, so they do not proliferate upon TCR stimulation. However, they require activation via TCR to express their suppressive functions, and this involves both CD4+ and CD8+ T cells [16]. Treg suppression is antigen nonspecific and does not require either TCR re-engagement or the presence of APCs [15, 16]. This may explain the mechanism of linked suppression in transplantation and evidence that histocompatibility is not necessary for Treg function [17].

Treg anergic status in vitro seems closely related to their suppressive function. Indeed, TCR stimulation with high levels of IL-2 or anti-CD28 antibodies abrogates the suppressive activity of Tregs. Removal of IL-2 or anti-CD28 antibodies restores the original anergic state and suppressive activity [18]. Nevertheless, Tregs proliferate in vivo in response to immunization, with no abrogation of their suppressive activity [19, 20]. Treg proliferation seems quite different compared with CD4+CD25- T cell responses, since it is characterized by a defective induction of IL-2, effector cytokines and CD40L. Recent studies showed a reduction in CD4+CD25- T cell number after interaction with a specific transgenic antigen, probably due to the local induction of apoptosis. Diversely, Tregs markedly increased after the encounter with the same antigen, suggesting a reduced susceptibility to apoptosis or an increased recruitment from the circulation. So interactions with autoantigens may shape Tregs repertoire at different sites [19, 20].

Treg suppression may be mediated by soluble factors or by direct cell-to-cell interaction. Treg suppression in vitro seems to correlate with a direct cell-to-cell contact. Indeed, supernatant obtained from Tregs co-cultured with CD4+CD25- T cells does not show suppressive activity, and when Tregs and effector T cells are separated by a semi-permeable membrane, the former fail to suppress the response of the latter [21, 22]. A suppression mediated by cell-to-cell contact may involve competition

for APCs (fig. 1). However, APC perturbation does not seem essential as Tregs suppress the effector T cell even without these cells [23].

The Role of Cytokines in Treg Functions

The role of cytokines in Treg suppression is largely controversial despite intense interest. In vitro neither IL-10 nor TGF- β seem involved as their removal or neutralization do not abrogate suppression. In vivo the mechanisms are more complicated and cytokines are potentially implied in suppressive activity, at least under certain conditions. Indeed, Tregs from IL-10-/- mice show a full capacity for suppressing autoimmune gastritis, but they are not able to abrogate inflammatory bowel diseases in the same mouse. The contribution of IL-10 to Treg suppression is also demonstrated in models of transplantation tolerance, graft-versus-host disease and type 1 diabetes mellitus (T1D) [24]. A recent study by Belkaid et al. [25] showed that during the chronic phase of *Leishmania* infection, IL-10 production by CD4+CD25+ Tregs is essential for protection against self-destructive inflammation, whereas during the acute phase of disease the contribution of IL-10 is not necessary. The contribution of TGF- β is quite more controversial, although the administration of anti-TGF- β receptor-blocking monoclonal antibody in mice, in the presence of both CD4+CD25- and Tregs, results in neutralization of Treg suppression and in inflammatory bowel disease development [26]. Several other factors are involved in Treg function (IL-2, CTLA4, CD28), but their role in suppression appears controversial [27–29].

Finally, Tregs may need cell-to-cell contact to exert their suppressive function both in vitro and in vivo, but in vivo this may be not sufficient. Another possibility is that additional regulatory T cell subsets exert their suppressive function by secreting cytokines, such as the induced CD4+CD25+ regulatory T cells.

CD4+CD25+ T cells can develop from mature, peripheral CD4+CD25- T cells, under certain conditions of antigen stimulation and in the presence of immunosuppressive cytokines, such as IL-10 and TGF- β , vitamin D3, dexamethasone and with CD40-CD40L blockade or immature APCs [30]. Studies in NOD mice demonstrated that iTregs can be produced in vivo, and stressed that their function is generally mediated by immunosuppressive cytokines [31]. Natural Tregs may facilitate the induction of adaptive Tregs from CD4+CD25- T cells. Probably, the two Treg subsets exert their suppressive

function in a different manner, depending on the features of the microenvironment, the nature of inflammatory response and the characteristics of antigen stimulation. It is possible that natural Tregs play a dominant role in controlling immune responses under basal conditions, i.e. in a non-inflammatory setting, where a fine balance of the homeostatic process is required and the cell-to-cell interaction seems the most efficient mechanism. On the contrary, induced Tregs probably grant a higher control of the immune response in inflammation settings, where the cell-to-cell interaction is insufficient and amplification of suppressive function may be obtained by cytokine secretion [3, 30, 31] (fig. 2).

CD4+CD25+ T Cells in NOD Mice, a Murine Model for Type 1 Diabetes

NOD mice spontaneously develop diabetes, as result of T cell-mediated destruction of pancreatic β cells. Thus, they represent a useful model for understanding autoimmune diabetes pathogenesis. CD4+CD25+ T cells have recently been described as potentially critical factors in preventing diabetes development. Thus, islet destruction might be the result of an alteration in peripheral mechanisms that normally control and suppress effector functions of autoreactive T cells [32].

Fig. 1. Potential Treg suppressive mechanisms involving APC. A suppression mediated by cell-to-cell contact may involve the competition for APCs. The high levels of chemokine and adhesion molecules enable Tregs to optimally interact with APCs physically excluding normal T cells. Another possibility is that Tregs make APCs unable to activate effector T cells, possibly by down-regulating their CD80/CD86 levels or triggering the immunosuppressive catabolism of tryptophan.

Fig. 2. CD4+CD25+ T cells with regulatory property can develop from mature, peripheral CD4+CD25- T cells, under certain conditions of antigen stimulation and in the presence of immunosuppressive cytokines, such as IL-10 and TGF- β , vitamin D₃, dexamethasone and with CD40-CD40L blockade or immature APCs. iTregs function is probably mediated by cytokines secretion, while natural Treg suppression is exerted through cell-to-cell interaction.

Fig. 3. In the absence of inflammation, iDCs may capture self-components derived from homeostatic cellular turnover. Thus, iDCs undergo to partial activation, perhaps correlated with TNF, and migrate into draining lymph nodes where self-antigens present in a tolerogenic manner to autoreactive T cells. This results in autoreactive T cell expansion and consequent deletion and perhaps in Treg induction.

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CD4+CD25+ T cells are present in low numbers in NOD mice, but they retain a certain capacity to suppress immune responses as demonstrated by the observation that their depletion determines a more rapid disease progression. A recent study demonstrated that Foxp3+ TGFβ+ CD4+CD25+ T cells in pancreatic lymph nodes and islets declined progressively in a age-related way in female NOD mice, whereas male NOD mice were not affected. This decline seems to be chronologically related to the development of severe insulinitis in female NOD mice [33].

NOR mice show a similar genetic background to NOD mice, but they do not spontaneously develop autoimmune diabetes. Both murine models are characterized by defects in thymic selection, allowing autoreactive T cell escape. In addition, both NOD and NOR mice spontaneously develop priming of autoreactive T cells, as demonstrated by the observation that their diabetes incidence increases in a pathogen-free environment in the absence of phenomena of cross-reactions. Transfer of CD4+CD25+ T cell-depleted spleen cells from NOR mice to NOD SCID mice induces severe insulinitis and the development of diabetes. So the state of protection from diabetes characterizing NOR mice seems to be correlated with CD4+CD25+ T cell function. Specifically, Ott et al. [34] postulated that NOR mice have a higher number of Tregs which are specific for islet cell antigens compared with NOD mice. Several data suggest that organ-specific CD4+CD25+ T cells are highly efficient in abrogating CD8-mediated autoimmune diabetes. So it is possible that a decline only in a Treg subpopulation, specific for islet cell antigens, might be the critical factor that leads to diabetes development in NOD mice [34].

This is consistent with the observation that the transfer in a lymphopenic host of Foxp3-transduced T cells with specificity for islet cell antigens suppresses diabetes progression, while the injection of polyclonal Foxp3-transduced T cells does not have the same result. Thus, Treg homing and activation in pancreatic lymph nodes seems to represent a critical factor for in vivo CD4+CD25+ T cell function [27, 28].

The comprehension of role of CD4+CD25+ T cells in diabetes pathogenesis also has important consequences in the therapeutic field. Bluestone et al. [29] observed that Tregs, derived from BDC2.5 TCR transgenic mice and expanded in vitro, were able to suppress diabetes in vivo in multiple model systems and restored normoglycemia in new-onset diabetic NOD mice. The observation that CD4+CD25+ T cells may have a protective effect on autoimmunity, also after the onset of the disease, leads to

considering the possibility of therapeutic cellular vaccines for organ-specific autoimmunity, such as T1D. Several efforts have to be made to improve knowledge about techniques to identify and selectively expand autoantigen-specific Tregs in humans. Once expanded, CD4+CD25+ T cells may be transferred into affected patients to reverse the disease and promote long-term tolerance [29].

Dendritic Cells

Dendritic cells (DCs) are the most potent and efficient professional APCs, thanks to their capacity to detect inflammation signals and induce pathogen-specific responses. Nevertheless, DCs also contribute to maintain tolerance to autoantigens [35, 36].

In order to act as APCs, DCs require a maturation process. DC maturation begins in the peripheral tissues with the activation of toll-like receptors by the antigen, which is consequently internalized. Then DCs migrate through afferent lymphatics into draining lymph nodes, where they present the processed peptides in the context of MHC molecules to naive T cells [37].

However, T cell activation needs additional factors to overcome Treg suppression. IL-6 secretion, induced by toll-like receptor stimulation, may reduce responsiveness of effector T cells to Treg inhibition, enabling their activation [3, 37]. Oldenhove et al. [38] demonstrated that Tregs may exert a certain suppressive effect on mature DCs in vivo. In fact in their study, after elimination of Tregs, these cells stimulated larger Th1 and CTL responses.

Immature DCs (iDCs) are highly specialized in endocytosis while expressing low levels of co-stimulatory and MHC molecules. In the steady state, iDCs may capture autoantigens and present them to T cells in lymphoid organs, with the induction of peripheral tolerance, through abortive expansion, deletion and anergy of the specific T cells [39]. Diversely, if antigen is targeted to iDCs together with anti-CD40 antibodies, promoting APC maturation, T cell activation occurs. In murine intestinal epithelium, the presence of iDCs was demonstrated in the steady state loaded with apoptotic epithelial cells, suggesting that iDCs, located in peripheral tissues, may capture inhaled, ingested proteins or dying cells generated during normal cell turnover. Upon antigen endocytosis, in the absence of inflammation, DCs remain immature and migrate to regional lymph nodes, where they induce peripheral tolerance [40] (fig. 3).

Actually, DC migration and antigen presentation imply at least a partial maturation, with upregulation of MHC and other co-stimulatory molecules. Pulmonary DCs triggered with antigen induce peripheral tolerance and present features of mature DCs [41]. Semi-mDCs may express relatively high levels of MHC molecules but secrete low levels of pro-inflammatory cytokines and present low levels of CD86 and CD40 [37]. TNF- α plays an important role in inducing partial activation of DCs [42]. In vitro TNF- α exposed self-peptide loaded DCs were transferred into mice and prevented experimental autoimmune encephalomyelitis [42].

The antigen presentation by iDCs in draining lymph nodes, in the absence of activation signals, may induce Treg differentiation. Dhodapkar et al. [43] demonstrated that injection of mature DCs, pulsed with MHC class I-restricted influenza matrix peptides, determined the expansion of peptide-specific CD8⁺ T cells, secreting IFN- γ . By contrast, the injection of peptide-charged iDCs increased influenza-specific IL-10-secreting T cells which suppress IFN- γ -secreting T cells. The suppression was contact mediated and IL-10 independent, like Tregs [44]. However, additional factors are probably needed in order to induce Treg generation.

1,25(OH) $_2$ D $_3$ represents an important growth factor with a relevant role in several immune processes, thanks to its interaction with the vitamin D receptor (VDR). VDR (belonging to the superfamily of nuclear hormone receptors) is a transcription factor, present in many immune cells where it influences the expression of specific DNA sequences (vitamin D responsive elements). The interaction between VDR and its ligands inhibits DC maturation by reducing IL-12 production and the expression of co-stimulatory molecules, such as CD80, CD86, CD40. Furthermore, VDR activation upregulates the inhibitory receptor ILT3, correlated with DC tolerogenic properties [45].

DCs might play a critical role in T1D development because of their ability to present antigens to effector T cells but also to mediate peripheral tolerance. This is consistent with the observation that the first phase of T1D progression is characterized by the infiltration of the islets by DC, macrophages and B cells [46].

Several studies have documented a reduced DC generation in NOD mice, with bone marrow precursors that preferentially differentiate into macrophages or macrophage-like DC. In addition, the few DCs present in NOD mice seem to be defective in interacting with allogenic T cells, and this may contribute to alter central and peripheral tolerance mechanisms [46].

Angelini et al. [47] demonstrated that DC in children affected by T1D display a lower expression of co-stimulatory molecules, such as B7-1 and B7-2, with a consequent potential alteration in T stimulatory function. This impaired DC phenotype might affect also CD4⁺CD25⁺ T cell generation and homeostasis, and enhance autoimmune phenomena [47].

In addition, alterations in DC development have been shown both in children with T1D and in healthy children with a genetic predisposition to develop T1D, suggesting a correlation to the genetic background, independent of disease development [48].

In this scenario DCs may also represent useful therapeutic tools. Tolerogenic DCs, induced by short treatment with 1,25(OH) $_2$ D $_3$ or its analogs, probably mediate the property of this hormone to enhance Tregs involved in transplantation tolerance. Furthermore, before insulinitis onset, treatment of NOD mice with 1,25(OH) $_2$ D $_3$ or its analogs seems to inhibit T1D progression. The slowing of T1D is associated with an increase in Tregs in pancreatic lymph nodes, which may be DC mediated. However, a combination of 1,25(OH) $_2$ D $_3$ and steroids in vitro is able to induce differentiation of naive CD4⁺ T cells in Tregs, even in the absence of DCs [45].

B Lymphocytes and Type 1 Diabetes

The pathogenesis of type 1 diabetes is T cell-dependent, however, B cells probably play a significant role, even if the exact mechanism involved is still unknown.

An important function of B cells is their ability to act as APCs. Thanks to their receptor specificity, B cells are able to efficiently capture, process and present autoantigens to diabetogenic T lymphocytes. Moreover, once activated, B cells may enhance antigen processing and express a broad array of different peptides on their surface. This may favor the diversification and amplification of T cell responses, by the recruitment of different clones of autoreactive T cells [49].

Studies with the progeny derived from inter-crosses between NOD mice and μ MT-/- mice, which lack B cells, show a reduction and a delay in the onset of insulinitis in these mice. This suggests that the absence of B cells may confer a sort of protection from the development of spontaneous diabetes. A similar result is obtained in treatment with anti-IgM antibodies. This tolerance status seems to be correlated with an impaired activation of islet-reactive CD4 T cells in pancreatic lymph nodes, sug-

gesting a key role for B cells as APCs for diabetogenic T cells [50].

Moreover, B cell-deficient NOD mice lack T cell responses against 65-kDa glutamate decarboxylase (GAD65) and heat shock protein 60 (HSP60), two of the most important autoantigens in autoimmune diabetes pathogenesis. Falcone et al. [49] demonstrated that these responses are lacking since the activation of GAD65- and HSP60-specific T cells requires the presence of B cells as unique APC.

However, even if with a lower frequency, diabetes can also develop in B cell-deficient NOD mice, suggesting that B lymphocytes are not the unique mechanism operating. In addition, chimeric models with B cells entirely derived from NOD mice are characterized by a tolerance status, demonstrating that B cells may play a pathological role but their presence is not sufficient to determine the occurrence of diabetes [51]. Other alterations have to be present to break tolerance, such as defects in non-B cell APC subsets. This is a critical point because it implies that therapeutic options depleting the B cell compartment (such as rituximab, which has been proposed for the prevention of diabetes) may confer protection from the disease, but probably further interventions, such as manipulations to restore professional APC subsets, are needed to ensure a stable tolerance [51].

Another important feature of B cell function is related to autoantibody production. A body of evidence documents the presence of autoantibodies in patients with diabetes, also in the prediabetic phases, but it is still unclear if they play a pathogenetic role or are simply epiphenomena. There are several proteins targeted by the autoimmune response, such as insulin, the GAD65 and the tyrosine phosphatase insulinoma-associated protein-2/islet cell antibody 512. The association between some autoantibody patterns and peculiar HLA haplotypes seems to be highly predictive of the progression to diabetes in relatives of patients affected by the disease [50]. Moreover, Greeley et al. [52] demonstrated that the abrogation of autoantibody transmission from a NOD mother to her progeny seems to protect from the development of spontaneous diabetes.

However, autoantibodies fail to transfer disease in mouse models. In addition, reports document the development of autoimmune diabetes in a patient affected by X-linked agammaglobulinemia, suggesting that neither autoantibodies nor B cell function are critically engaged in the pathogenesis of autoimmune diabetes [53].

Fig. 4. Schematic representation of rearrangements and expression of the genes of the heavy chains of the B cell receptor.

Receptor Editing and Its Role in Autoimmunity

T1D is characterized by alterations enabling the generation of autoreactive T cells; however, defects in B cell development may also represent a critical event to initiate autoimmunity.

Receptor editing represents a fundamental event in B cell development [54]. At the stage of pro-B cell, the rearrangement of variable (V), diversity (D), joining (J) genes of heavy chains (HCs) occurs, and leads to pre-B cells (fig. 4). Pre-B cells express a μ HC, whose synthesis induces light chain (LC) gene rearrangement. LC rearrangement involves first the κ locus and leads to a κ LC. If this process is effective, a λ LC will be synthesized. If no LC is appropriate, the developing B cell dies (fig. 5). If the pre-B cell receptor (BCR) is appropriate, unknown signals suppress the V(D)J recombination, with the allelic exclusion at the HC locus. Appropriate signals also grant pre-B cell proliferation and development progression. Inappropriate or underexpressed pre-BCRs stimulate a secondary V(D)J recombination at the HC locus, involving DJ rearrangement or V segment replacement [55]. So, receptor genes are replaced with the generation of non autoreactive pre-BCR and without cell loss. This mechanism is very useful, since autoreactivity is a very common event in B cells. Wardemann et al. [56] showed that the most part (55–75%) of developing BCRs presents self-reactivity.

Fig. 5. Schematic representation of rearrangements and expression of the genes of the light chains, on the κ or λ locus.

An effective first receptor editing induces B cell progression and the expression of surface IgM. At this stage, immature B cells are able to interact with antigens and underlie the processes of positive and negative selection. While negative selection deletes autoreactive B cells, positive selection tests the presence of an appropriate signaling, generated by BCR. This signaling, called 'tonic', seems to be the result of a balance between enhancing and inhibiting regulatory molecules, and is ligand independent [57]. B cells expressing signals that are too high or too low escape positive selection and undergo a secondary LC gene recombination. Positive selection is critical, since interaction between autoantigens and B cells, expressing incompetent autoreactive BCR, may result in B cell selection rather than in apoptosis, promoting autoimmune events [58].

Silveira et al. [59] demonstrated that in NOD mice autoreactive B cells specific for soluble self-antigens are not efficiently deleted both in bone marrow and spleen. This defect of tolerance mechanisms may be due to an imbalance between apoptotic and anti-apoptotic molecules or to a weak signal through the autoreactive BCR upon the encounter with self-antigen (as has been proposed for defects in autoreactive T cell selection in T1D). In NOD mice, self-antigens recognized by specific T cells are likely to be soluble, thus autoreactive B cells might represent

the most efficient APC subset in expanding diabetogenic T cells [59].

Negative selection correlates with affinity and the signal threshold of BCR. The interaction of autoreactive BCR with self-antigens leads to a new developmental arrest, with the most autoreactive B cells undergoing LC receptor editing [58, 60].

Recent studies in systemic lupus erythematosus (SLE)-prone models, demonstrated an apparent correlation between autoantibody production and consistent LC receptor editing. These apparent contradictions may be explained with the 're-editing' of mature B cells, which leads to autoreactive B cell generation from previously non autoreactive cells. This acquired autoreactivity may derive from alterations in receptor revision, probably induced by B cells carrying a low affinity BCR. In this context, the re-editing process may play an important role in affinity maturation, but may also induce the appearance of autoreactivity [61, 62].

Autoreactive B cells that escaped central selection and entered the peripheral repertoire may become tolerant. The interaction of their BCR with self-antigens determine a cascade of events that leads to a blockade of the differentiation process (functional silencing). B cells also become unable to activate several pathways correlated to BCR activation (anergy). Anergic autoreactive B cells are

more susceptible to apoptosis as they do not efficiently home to lymphoid follicular areas compared to normal B cells, and do not produce actively fundamental survival factors [63].

Several studies demonstrated that in murine models of systemic autoimmune diseases, such as SLE, this mechanism is not fully operating, since autoreactive B cells are not blocked in their development after the encounter with the antigen. This process has been evidenced in SLE-prone strains, where systemic antigens may stimulate at high levels and continuously autoreactive B cells. Organ-specific autoimmune diseases, such as T1D, are characterized by limited phenomena of autoimmunity, directed against specific antigens on target organs. Thus, autoantigens are presented only at low levels and they may condition autoreactive B cell function in a different way. A recent study demonstrated that, in a transgenic model of NOD mice, the maturation process and the expression of co-stimulator molecules on the surface of autoreactive B cells are not affected upon the encounter with self-antigen. Only the B cell capacity to proliferate becomes defective. This new tolerance state for autoreactive B cells, characterized by the dissociation between anergy and developmental arrest, may allow them to interact with dia-

betogenic T cells and promote diabetes onset. Recent studies have opened new scenarios about the role of B cells in the pathogenesis of T1D, with potentially important effects also on therapeutic strategies [64].

Protective Effects of Autoimmunity: The Beneficial Role of Autoreactive T Cells in Central Nervous System Injury

Autoreactive T cells may also exert a protective function, at least in the central nervous system (CNS). Indeed, they seem to protect the injured CNS from damage correlated with self-destructive factors produced during inflammation.

In murine models, Yoles et al. [65] demonstrated that autoreactive T cells, specific for CNS myelin basic protein, enhance recovery after optic nerve crush injury or spinal cord contusion. Moreover, active or passive immunization with CNS myelin-associated antigens or passive transfer of T cells reactive to myelin proteins limit the extent of CNS damage. Thus, T cell-mediated autoimmune responses may represent the physiological response to CNS damage [65].

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