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Back to Central Tolerance

Review

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The establishment and maintenance of immunological tolerance entails both central and peripheral mechanisms. The latter have been highlighted in the past several years, mostly because of great interest in the activities of regulatory T cells. However, an important role for central tolerance mechanisms has been reemphasized by recent results on human autoimmune diseases, including APECED and type 1 diabetes.

Introduction

Every day, every individual is confronted with a myriad of microbial challenges. Inevitably, certain of the microbes invade the integrity of the individual, but these are routinely disposed of by the combined forces of the innate and adaptive immune systems. To be effective against the many challenges encountered, T and B lymphocytes of the adaptive immune system exhibit extensive diversity, generated through random rearrangement of the genes encoding antigen-specific receptors during these cells' differentiation in the thymus and bone marrow, respectively. Since this is a random process, lymphocytes whose receptors can recognize one of the body's own constituents are sometimes generated, resulting in autoimmunity and, occasionally, autoimmune disease. Because of the devastating consequences such a disease can have, evolution has provided a comprehensive net of mechanisms ensuring the establishment and maintenance of lymphocyte self-tolerance.

Classically, lymphocyte tolerance mechanisms have been divided into two broad categories. Central tolerance (Ohashi, 2003; Venanzi et al., 2004b) concerns immature T or B cells as they differentiate in the primary lymphoid organs, the thymus or bone marrow. Relevant antigens, then, would be those synthesized by nurturing stromal cells, circulating hematopoietic cells, or, ubiquitously, by all cells. The major mechanisms that come into play during central tolerance appear to be clonal deletion or inactivation of self-reactive lymphocytes, in particular the former. Peripheral tolerance (Walker and Abbas, 2002), on the other hand, relates to mature T or B cells after they have exited the primary lymphoid organs and are circulating through the blood, lymph, and secondary lymphoid organs or have accessed the parenchymal tissues in response to some stimulus. Antigens of concern would primarily be those expressed in the tissues and not in the thymus or bone marrow. Clonal deletion and anergy are tolerance mechanisms also employed in the periphery, but a variety of other means are also exploited, including clonal ignorance, deviation, helplessness, and suppression.

The relative significance of central versus peripheral tolerance has been debated for decades, with sequential waves of enthusiasm supporting either one or the other. Certainly, central mechanisms are important—for example, a variety of approaches have estimated that one-half to two-thirds of thymocytes that are positively selected subsequently undergo negative selection (van Meerwijk et al., 1997; Ignatowicz et al., 1996; Tourne et al., 1997). However, central tolerance appears not to be fool-proof as all individuals harbor lymphocytes in the blood that can respond to self-antigens (e.g., Liblau et al., 1991; Sun et al., 1991). Therefore, peripheral mechanisms must be important as well. Indeed, over the past several years peripheral tolerance—in particular, the activities of regulatory T cells (Sakaguchi, 2004)—have held the limelight.

This dichotomous view of central versus peripheral self-antigens and corresponding tolerance mechanisms was the reigning paradigm for decades. Recently, however, this too-simplistic image has been brought into question. The major challenge has been a gradual accumulation of evidence that proteins usually considered to be synthesized in the periphery in a tissue-specific manner have also been detected in the thymus (reviewed in Kyewski et al., 2002). Attention was first drawn to promiscuous thymic expression of transcripts encoding tissue-specific proteins when it was reported that a transgene expressing SV40 T antigen under the dictates of the rat insulin promoter was transcribed in the thymus as well; the endogenous insulin gene was also thymically expressed (Jolicoeur et al., 1994). At first, suspicions of PCR contamination prevailed, but additional examples accrued, culminating in a listing of scores of genes encoding proteins previously thought of as tissue-specific or tissue-restricted that are also transcribed in the thymus (Derbinski et al., 2001). Proteins characteristic of a variety of tissues—for example, the pancreas, liver, eye, and nervous system—were included on the list. Some investigators still attributed this phenomenon to some uninteresting, nebulous mechanism—so-called "leaky" transcription. But even they took notice when it became clear that most of these promiscuous thymic transcripts were restricted to a very specific component of the thymic stroma, the medullary epithelial cells (MECs) (Derbinski et al., 2001).

Thymic MECs are a small, heterogeneous stromal cell fraction (reviewed in Anderson and Jenkinson, 2001; Gill et al., 2003). They display MHC and costimulatory molecules at their surface, rendering them apt at antigen presentation. Interestingly, several lines of evidence argue that MECs and cortical epithelial cells (CECs) process or present antigens in distinct ways (Mizuochi et al., 1992; Kasai et al., 1996, 2000; Oukka et al., 1997). MECs have been implicated in the tolerization of mature single-positive ($CD4^+8^-$ or $CD4^-8^+$) thymocytes in a number of systems (Oukka et al., 1996a, 1996b; Kishimoto and Sprent, 1997, 1999; Kishimoto et al., 1996; Klein et al., 1998). Consequently, the notion arose that

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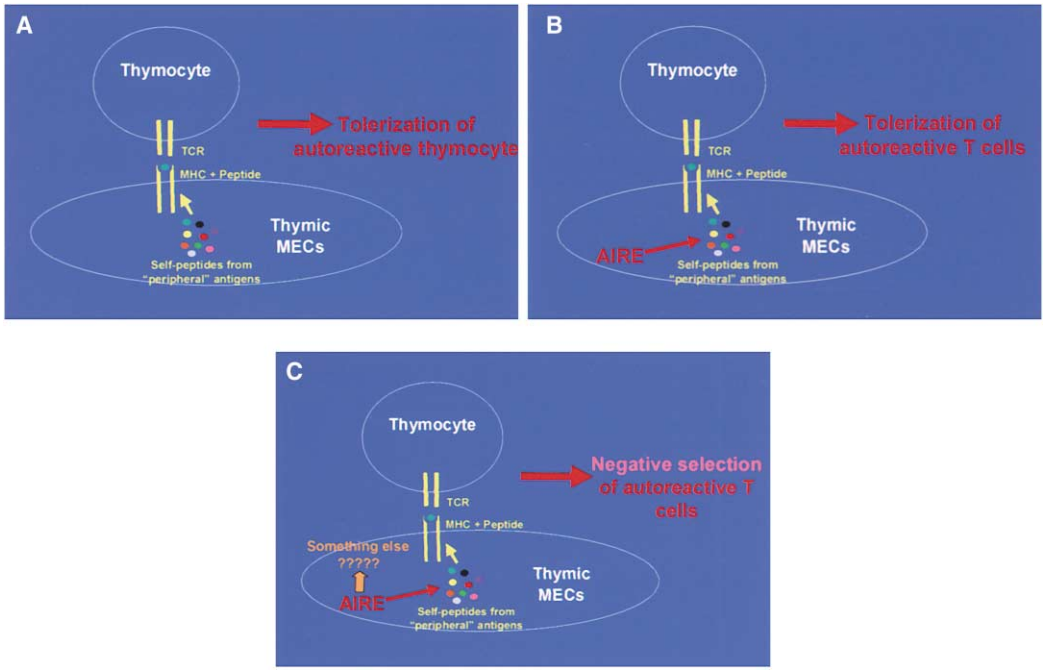

Figure 1. MEC-Mediated Central Tolerance and Its Control by AIRE/aire

the promiscuous thymic expression of proteins characteristic of peripheral organs might promote tolerance induction to those organs. As illustrated in Figure 1A, the self-proteins would be processed to peptides by the MECs, the peptides loaded onto MHC molecules, and the MHC:self-peptide complexes transported to and displayed on the MEC surface. Differentiating thymocytes would encounter the surface-displayed complexes, and, should their TCRs recognize them within a particular window of affinity/avidity, they would be dealt with in a manner resulting in tolerization of the T cell repertoire.

Thus, we are presented with a complex net of mechanisms designed to promote a self-tolerant T cell repertoire: both central and peripheral modes, entailing a diversity of cellular mechanics and involving interplay between the central and peripheral elements (display of peripheral antigens in the thymus on the one hand and thymic selection of T regulatory cells that monitor the periphery on the other hand). Which of these mechanisms dominate? Which might be artifacts of the systems used to define them? What circumstances bring particular mechanisms into play and exclude others from operation? These questions are difficult ones to answer given the sometimes complex, cryptic, and subtle nature of tolerance phenomena. A potentially powerful approach to surmounting the difficulties inherent in dissecting how tolerance can be established and maintained is to explore how it can break down as a prelude to autoimmune disease. We will discuss two illustrative examples.

APECED and AIRE

One autoimmune disease that has revealed important information about the importance of central tolerance is *a*utoimmune *p*olyendocrinopathy-candidiasis-ectodermal *d*ystrophy (APECED), otherwise known as *a*utoimmune *p*olyglandular *s*yndrome type 1 (APS-1) (reviewed in Vogel et al., 2002). This syndrome is characterized by mucocutaneous candidiasis, hypoparathyroidism, and adrenal insufficiency. In addition, a variety of secondary symptoms can be manifest, differing from patient to patient: type 1 diabetes, reproductive organ failure, alopecia, thyroiditis, etc.

APECED is a monogenic disorder, with an autosomal mode of inheritance, quite unlike that of most other autoimmune diseases, with their baroquely complex genetic influences. APECED is quite rare, although certain populations (Finns, Sardinians) show an incidence elevated to about 1 in 10,000. Finally, it has the intriguing characteristic that its secondary symptoms vary widely from individual to individual, even those harboring the same genetic lesion and seemingly exposed to the same environment. The gene underlying APECED was localized to the q22 region of chromosome 21 and was positionally cloned in 1997 (Nagamine et al., 1997).

The protein encoded by the gene responsible for APECED—*AIRE* in humans, *aire* in mice—has features evocative of a transcription factor. As concerns its structure, translation of the *AIRE* gene coding sequences and grouping of the amino acid residues into functional units reveals a domain organization similar to that of the Sp100 family of transcription factors, including SAND and PHD domains, and nuclear localization and dimerization signals. Concerning its functional activities, AIRE is localized in the nucleus, has transactivation potential, can induce transcription from the interferon-β promoter in a cotransfection assay, binds to a transcription factor (CREB binding protein [CBP]) in vitro, and has been reported to bind to DNA (although this has been chal-

lenged) (Kumar et al., 2001). Most recently, AIRE was demonstrated to have E3 ubiquitin ligase activity (Uchida et al., 2004), which might explain at least some of its influences on transcription (or might not). More than 50 mutations of the AIRE protein have been identified from the DNA of APECED patients, alterations of diverse nature (non-sense, missense, deletion) scattered throughout the protein sequence (Heino et al., 2001).

An important key to AIRE's role in promoting tolerance and preventing autoimmunity came when its pattern of expression was established. Data on humans has been controversial, but clearer results have come from the mouse, relying on quantitative assessment of *aire* gene transcripts in diverse tissues (Anderson et al., 2002; Gotter et al., 2004). The *aire* gene is transcribed primarily in lymphoid organs (thymus, lymph node, spleen), and only at low or undetectable levels in practically all parenchymal tissues, including those targeted in APECED patients. Aire is, above all, expressed in the thymus, where transcripts are confined to stromal cells and are not detected at a significant level in differentiating thymocytes. Of the diverse stromal cell populations, MECs express the most aire and dendritic cells the next-most. The pattern of *aire* gene expression was strikingly similar to the distribution of promiscuous thymic transcripts corresponding to peripheral tissue-specific proteins. This concordance has elicited the hypothesis that AIRE/aire somehow controls this promiscuous transcription (Figure 1B) and that APECED patients, owing to their mutant AIREs, cannot express such transcripts, thereby have a defect in induction of T cell tolerance to tissue-specific self-antigens, and ultimately develop autoimmune disease.

To test this hypothesis, two groups have generated mice devoid of aire (Ramsey et al., 2002; Anderson et al., 2002). The immunological phenotype of these animals was surprisingly normal, with quite standard numbers, subsets, and activities of lymphocytes in both the primary and secondary lymphoid organs. One difference noted was that there was a doubling of activated T cells in the periphery, suggestive of autoimmune activation. Indeed, the aire-deficient mice had lymphocytic infiltrates in a number of parenchymal tissues and also produced autoantibodies directed against them. Interestingly, while there was a broad autoreactivity, in the sense that many organs were targeted, it was also strikingly specific, in that within each organ only a particular structure was attacked, e.g., the rods and cones layer of the retina or the parietal cell layer of the stomach. The features of aire-deficient mice are sufficiently similar to those of AIRE-defective humans with APECED that the former can justifiably serve as a model for dissecting disease mechanisms.

An initial set of studies established that aire exerts its control over autoimmunity through its expression in thymic epithelial cells (Anderson et al., 2002). Irradiation/bone marrow chimera experiments demonstrated that aire function partitioned with radio-resistant stromal cells, while thymus transplant experiments showed partitioning with the thymus rather than the peripheral lymphoid organs. In fact, all that was needed for development of an autoimmune condition like that of standard aire-deficient mice was aire's absence from thymic stromal cells. This finding argues against a required role for aire in dendritic cells of the thymus or periphery, even though this cell-type does express it at significant levels. A more directed set of studies established that aire does indeed control promiscuous thymic transcription of peripheral tissue-specific genes (Anderson et al., 2002). MECs were purified from aire-deficient mice and control littermates, RNA was isolated, and transcripts were amplified and applied to Affymetrix gene chips. RNA from MECs expressing aire included numerous transcripts encoding tissue-specific or tissue-restricted proteins, certain of which (e.g., preproinsulin and p450 1A2) are known targets of autoantibodies detected in APECED patients. RNA from aire-deficient MECs was strikingly impoverished in such transcripts. It was estimated that aire might influence the expression of around a thousand genes in MECs; this value is of the same order of magnitude as a recent estimate of the number of promiscuously expressed genes in human MECs (Gotter et al., 2004). Thus, the hypothesis that aire somehow controls the promiscuous thymic transcription of peripheral tissue-specific antigens and that the autoimmune disease of APECED patients reflects a defect in this process would appear to be correct. Proof will come when the targets of some of the autoantibodies produced in aire-deficient mice are identified and are found to be encoded by transcripts downregulated in the MECs of these animals.

An open question is the cellular mechanism by which aire exacts its control over T cell tolerance. A logical possibility would be a negative influence—aire-expressing MECs would display a diversity of MHC:self-peptide complexes on their surface, which could trigger clonal deletion of differentiating thymocytes whose TCRs recognize them at a particular affinity/avidity. However, skepticism over such a scenario has been expressed, largely due to the fact that MECs are a relatively rare thymus population and to a report contending that any particular tissue-specific protein is expressed in only a small fraction ($\sim$1%) of MECs (Derbinski et al., 2001). How can so few cells purge the entire emerging T cell repertoire? Thus, the alternative possibility of aire having a positive influence through positive selection of regulatory T cells has been evoked. Such a mechanism would be in line with reports that expression of a self-antigen on thymic epithelial cells promoted the emergence of $CD4^+25^+$ T regulatory cells in two TCR transgenic systems (Jordan et al., 2001; Apostolou et al., 2002). Nonetheless, skepticism over a negative influence might be considered ill-placed: fully mature thymocytes spend as much as 2 weeks in the medulla (Scolley and Godfrey, 1995; Rooke et al., 1997), so they have time; thymocytes display extremely active motility within the thymus, so they have the occasion (Bousso et al., 2002); and, last, there is precedence for a highly efficient clonal deletion mechanism in the ability of extremely few splenic dendritic cells or thymic hematopoietic cells to cleanse the thymocyte repertoire (Matzinger and Guerder, 1989; Merkenschlager et al., 1994).

Hence, it is important to experimentally address the cellular mechanism(s) associated with aire function. Using a double Ag/TCR transgenic system (lysozyme expression driven by the rat insulin promoter/an overabundance of lysozyme-specific T cells), Liston et al. (2003)

provided evidence in favor of aire control of clonal deletion of self-reactive thymocytes. Some questions have been raised about this conclusion concerning, in particular, the perhaps too-early timing of deletion or the level and site of expression of the neo-self-antigen in the thymus. However, experiments of similar design employing an ovalbumin-based double-transgenic system have yielded the same conclusion (Anderson et al., 2004). It remains unknown whether the observed effects on clonal deletion are direct ones that can be attributed to MECs themselves or are more indirect, operating via crosspresentation through an intermediary antigen-presenting cell of hematopoietic lineage, such as a dendritic cell or macrophage. A role for aire in inciting negative selection of thymocytes does not rule out an additional activity to promote positive selection of regulatory T cells. However, aire-deficient mice were reported to have normal numbers of $CD4^+25^+$ T cells in all lymphoid organs (Anderson et al., 2002; Liston et al., 2003). In addition, this population appears to function normally in the standard in vitro and a variety of in vivo assays of regulatory T cell function, the latter including protection from lymphopenia-induced wasting and colitis in lymphocyte transfer experiments as well as multiorgan autoimmunity in double-thymic ($aire^+$ plus $aire^-$) transfers (Venanzi et al., 2004a).

Aire's molecular mechanism is also an intriguing issue. As discussed above, there are many structural and functional suggestions that aire might act as a transcription factor (according to the loose definition of a molecule that somehow promotes gene expression). Given that it probably targets several hundreds of genes in thymic MECs and that these show radically different patterns of expression in peripheral parenchymal tissues, it seems counterintuitive to suppose that aire functions by binding directly to individual promoter/enhancer elements. Some epigenetic mechanism—involving chromosomal imprinting, chromatin acetylation, DNA methylation, or chromatin remodeling—would appear to be more likely—in short, some process that might turn on or off sets of genes, whether adjacent or nonadjacent. In support of such a notion, it was recently reported that the list of promiscuous transcripts expressed in human MECs contains several sets of chromosomally clustered loci, including a group of genes encoding S100 family members and neighbors ultimately expressed in the liver and a group of genes encoding molecules involved in epidermal cell differentiation (Gotter et al., 2004). Aire-controlled genes in murine MECs are also preferentially ones that are clustered along chromosomes (Venanzi et al., 2004a). This is clearly an important area for future study, one that seems in its neonatal period.

It is also worth considering whether aire might not function in ways other than to control the promiscuous transcription of tissue-specific proteins. This possibility is raised by the fact that aire regulates, either positively or negatively, a variety of MEC transcripts other than those encoding peripheral antigens (Anderson et al., 2002). Included among these are transcripts of chromosomally clustered genes encoding a diversity of MHC molecules and various chemokines. The newly described E3 ubiquitin ligase activity of aire (Uchida et al., 2004) also opens the possibility of activities in addition to transcriptional control. These might affect MEC differentiation, antigen processing or presentation, or chemokine-mediated attractions of thymocytes (e.g., Kwan and Killeen, 2004).

Insights into the molecular and cellular pathways that aire participates in may come from the identification of molecules that impinge on its expression or operation. Two recent studies have drawn attention to similarities in the autoimmune phenotypes of aire-deficient mice and mice with a defect in the lymphotoxin (LT) pathway (either LT-α or LT β receptor) (Chin et al., 2003; Boehm et al., 2003). Both studies found a reduction in thymic expression of aire, but one attributed this to a direct effect on *aire* gene transcription (Chin et al., 2003), while the other invoked an influence on the differentiation of MECs, i.e., on the number of cells capable of transcribing the *aire* gene (Boehm et al., 2003). The more careful analysis of purified MECs (rather than whole thymus) in the latter study argues for the latter point of view. However, the former study did report an acute effect (within 6 hr) on thymic levels of *aire* (and *insulin-1*, but not *keratin-14*) gene transcripts after systemic engagement of the LT β receptor with an agonistic mAb, a finding difficult to integrate into the MEC differentiation scenario. One member of a second cytokine family also appears to control AIRE expression, at least in vitro: treatment of human dendritic cells with thymic stromal lymphopoetin (TSLP) activated their antigen presentation capabilities and turned on AIRE (Watanabe et al., 2004). The in vivo relevance of this finding remains to be determined, but it was noted that TSLP is also expressed in Hassal's corpuscles, a specialized epithelial cell type found in the thymic medulla.

The fascinating nature of the cellular and molecular machinations of aire have reawakened interest in central tolerance induction. This single protein has a strong impact on immunological tolerance, which reads out as a multiorgan autoimmune disease in both humans and mice. Current thought and effort are focused on better defining just how aire operates. Yet, intriguing lateral thoughts are also emerging. Why don't aire-deficient mice show more widespread organ attack? What controls the expression of promiscuous MEC transcripts not regulated by aire—is there an aire-2 or aire-3? Might mutations in such molecules underlie the related polyendocrine autoimmune disease APS-2? Might mutations in other elements of the pathway through which aire operates, e.g., LT or TSLP, be responsible?

Type 1 Diabetes

Insulin-dependent diabetes mellitus, or type 1 diabetes, is one of the classic examples of an organ-specific autoimmune disease (for reviews see Bach, 1994; Tisch and McDevitt, 1996). It consists of two stages: an occult phase, termed insulitis, when a mixed population of leukocytes invades the islets of Langerhans of the pancreas, eventually provoking specific destruction of the insulin-producing β cells; and an overt phase, diabetes, when the bulk of β cells has been destroyed and insulin production is no longer sufficient to regulate blood glucose levels, resulting in hyperglycemia. Although autoimmune diabetes is an ancient and increasingly frequent disease, we remain surprisingly ignorant of its etiology and pathogenesis.

Faced with the difficulties of studying disease in humans, many investigators have turned to small animal models of type 1 diabetes—in particular, the nonobese diabetic (NOD) mouse strain. Developed in the late 1970s, the NOD strain is now the most commonly used animal model of autoimmune diabetes (for review see Bach and Mathis, 1997). Disease develops spontaneously in these mice, sharing several critical features with the human disorder. As in man, the course of pathology in NOD animals is protracted: insulitis begins at about 4 weeks of age, but diabetes is not evident until 15 to 25 weeks. Again as in man, diabetes in these animals is primarily T lymphocyte mediated, although other cell types, like B cells or macrophages, may also play an important role. Finally, the human and murine diseases are both under complex polygenic control, by far the most important contributor being the major histocompatibility complex (MHC). Strikingly, comparison of the three-dimensional structure of the NOD MHC class II molecule, A^{g7}, with those of the human HLA-DQ8 and -DQ2 class II molecules, both major genetic risk elements for diabetes development, revealed pronounced similarities in the antigen (Ag) binding pockets, suggesting that similar autoAg presentation events may prelude diabetes in mice and humans (Lee et al., 2001). The NOD mouse strain has permitted many of the outstanding issues in the diabetes field to be addressed by direct experimentation, and results on these mice have heavily colored our view of diabetes pathogenesis.

A battery of studies on the NOD mouse has established that T lymphocytes are central to disease pathogenesis in this model of type 1 diabetes (reviewed in Adorini et al., 2002; Atkinson and Eisenbarth, 2001). Immunohistological analyses reveal that most of the leukocytes in the islet infiltrate are T cells, and T lymphocyte autoreactivity to β cells is readily demonstrable. Disease does not develop in NOD mice genetically athymic or T lymphopenic or in mice thymectomized at birth; likewise, it is dampened or even abrogated by reagents that interfere with T cell function. Finally, diabetes can be transferred by injecting T cells from diseased donors into healthy NOD recipients, the least equivocal demonstration being inoculation of a single T cell clone into a lymphocyte-deficient NOD mouse. There is ample indication that the human disease is also mediated by T lymphocytes, exhibiting a similar islet histology, T cell autoreactivity to β cell antigens, and positive response to treatment with T cell inhibitors.

The preeminent role of β cell-reactive T lymphocytes in type 1 diabetes implies that individuals with this disease harbor some deficit in the induction and/or maintenance of T cell tolerance to self-antigens. An antigen-dependent defect has been postulated in the case of humans (reviewed in Pugliese and Miceli, 2002). The only well-established diabetes susceptibility locus outside the MHC is *IDD2*, which encompasses the single human *insulin* gene. A correlation has been made between the number of copies of a particular repeat sequence found at the 5′ end of the *insulin* gene, levels of insulin expression in the thymus, and incidence of type 1 diabetes. This observation is highly suggestive and interesting, but it remains essentially correlative at present. Modeling of the human phenomenon by engineering mice with different levels of insulin expression in the thymus has lent support to the pathological relevance of this correlation (Chentoufi and Polychronakos, 2002) as have the recent findings that mice carrying a *proinsulin-2* gene deletion, and thereby lacking thymic insulin expression, develop more aggressive type 1 diabetes (promoted by islet-restricted expression of insulin-1) than wild-type mice (Dubois-Lafforgue et al., 2002; Moriyama et al., 2003). This set of observations might be considered an antigen-specific manifestation of AIRE's influence on central tolerance.

An antigen-independent defect in central tolerance induction has been hypothesized in the case of NOD mice, i.e., incomplete deletion or anergization of autoreactive cells differentiating in the thymus (Lee et al., 2001; Carrasco-Marin et al., 1996), and there now exist multiple lines of experimental data in support of this notion. Initial evidence included the demonstration of unusually high syngeneic T lymphocyte reactivity (Ridgway et al., 1996, 1998; Kanagawa et al., 1998) and unexpected T cell responses to injected self-antigens (Ridgway et al., 1996, 1998; Kanagawa et al., 1998). However, in both cases, aberrant peripheral tolerization remained an explanation. More recently, abnormal clonal deletion of NOD thymocytes was reported using two systems. First, engagement of TCRs by systemic injection of an anti-CD3 mAb or a superantigen was less effective at inducing deletion of NOD than of C57Bl/6 thymocytes (Kishimoto and Sprent, 2001), although this result was later challenged (Villunger et al., 2003). Second, coupling of a TCR transgenic mouse line with a second transgenic line expressing cognate antigen in the thymus resulted in more extensive clonal deletion on the NOD than on the C57Bl/6 genetic background (Lesage et al., 2002). Certain of these aberrant properties segregated with NOD MHC genes (Kanagawa et al., 1998; Ridgway et al., 1998), although the abnormalities in thymocyte deletion were specified by non-MHC genes (Kishimoto and Sprent, 2001; Lesage et al., 2002) and were T cell intrinsic (Lesage et al., 2002).

Extensive evidence of ineffective peripheral tolerance induction/maintenance in NOD mice has also been provided (Kreuwel et al., 2001; Markees et al., 1999; Quinn et al., 2001; Pearson et al., 2003a, 2003b; Molano et al., 2001; Grohmann et al., 2003; Makhlouf et al., 2002). These abnormalities have usually been associated with non-MHC loci (Kreuwel et al., 2001; Markees et al., 1999; Quinn et al., 2001). It may be possible that, at least to some degree, they reflect reported aberrancies in numbers and/or activity of regulatory T cell populations (Gombert et al., 1996; Baxter et al., 1997; Salomon et al., 2000; reviewed in Bach and Chatenoud, 2001).

The relative importance of defects in central versus peripheral tolerization in promoting diabetes in NOD mice has been heavily debated. However, it is possible that, at least in part, the two classes of defect are manifestations of the same genetic lesion(s). For example, apoptosis plays a role in tolerance induction/maintenance in both the thymus and peripheral lymphoid organs, and there have been many reports that apoptosis or its executors are subnormal in NOD mice (Kishimoto and Sprent, 2001; Quinn et al., 2001; Penha-Goncalves et al., 1995; Leijon et al., 1994; Garchon et al., 1994; Colucci et al., 1997; Lamhamedi-Cherradi et al., 1998; Bergman et al., 2001, 2003; Decallonne et al., 2003;

Arreaza et al., 2003). It is also possible that genetic variation that promotes T cell hyporeactivity results in less efficient central tolerance, but is balanced by less aggressive autoimmune attack in the periphery.

Conclusion

Thus, of late, we are back to appreciating the important influence of central mechanisms of tolerance induction. This by no means denies the significance of peripheral mechanisms. The fact that aire-deficient mice and AIRE-defective humans actually exhibit a rather restrained autoimmune disease surely reflects MHC restriction of autoantigen presentation but just as certainly must be an indication of the operation of regulatory T cells and other peripheral processes. The multiorgan autoimmune diseases of mice lacking Foxp3 (Fontenot et al., 2003) and Foxj1 (Lin et al., 2004) also argue that central and peripheral tolerance mechanisms should be considered as successive barriers, imperfect on their own but powerful in synergy, to autoimmune disease.

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