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Levees of immunological tolerance

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Immunological tolerance guards against spurious immune responses to body constituents. Tolerance encompasses a network of mechanisms: central and peripheral, cell-autonomous and cell-interactive. Our understanding of these mechanisms has improved greatly over recent years, often reflecting new insights into the processes underlying particular autoimmune diseases. Yet it is possible that important tolerance mechanisms remain to be discovered, perhaps an explanation for the so-far disappointing clinical translation to the prevention and cure of autoimmune diseases.

This Focus issue contains four Reviews and two Perspectives that discuss the breakdown of immunological tolerance from a variety of viewpoints. Reading this body of work, we were struck by the many analogies with Holland's famous dike system. Just as an elaborate network of levees was built to repel the waters from the North Sea and the inland canals, a series of tolerance mechanisms has evolved to keep autoimmunity in check. In both cases, leaks can spring up, borders be skirted or saboteurs emerge.

Cracks and side steps

The Dutch levee system is not a monolithic dam. Analogously, several successive T and B lymphocyte tolerance mechanisms together compose a largely watertight system to prevent autoimmune damage. First, taking T cells, and as reviewed by von Boehmer and Melchers[1], the repertoire of nascent thymocytes is purged of cells displaying T cell antigen receptors (TCRs) with reactivity to self-peptides presented in the thymus, reflecting the multicellular thymic proteome, including ectopically expressed peripheral-tissue antigens. Removal of self-reactive thymocytes occurs most radically by maturation blockade or clonal deletion, or more subtly by clonal deviation into alternative lineages where the TCR's potentially damaging reactivity is reused after being defused by alternative co-receptor usage (as in CD8αα T cells) or alternative cell phenotypes (for example, natural killer (NK) T or Foxp3$^+$ T regulatory (T_{reg}) cells). Second, the destructive potential of cells that escape this first filter can be dealt with by cell-autonomous peripheral tolerance mechanisms, reviewed here by Mueller[2]. However, the riddle of self–nonself discrimination is harder to solve in the periphery than in the shielded thymus; only in the latter case can any encountered element be taken as self. Persistent recognition of complexes of peptide–major histocompatibility complex (MHC) molecules detected in the absence of infection may be the common denominator leading to pathways described operationally as receptor tuning, anergy or exhaustion. Finally, immunocyte populations with immunoregulatory properties, the best characterized of which are the Foxp3$^+$CD4$^+$ T_{reg} cells reviewed by Wing and Sakaguchi[3], further limit peripheral reactivity to self, much as they control many immune responses. It is probably fruitless to argue the relative importance of central versus peripheral tolerance, as natural and experimental loss of function have demonstrated that ultimately each is required. Perhaps the clearest demonstration of this complementarity was provided by *Aire*$^{-/-}$*Foxp3*$^{-/-}$ mice, which show faster and more extensive disease than either *Aire*$^{-/-}$*Foxp3*$^{+/+}$ or *Aire*$^{+/+}$*Foxp3*$^{-/-}$ mice[4].

Although autoimmune disease has usually been considered to be the inverse of tolerance, disorders that appear in humans or in animal models probably only incompletely reflect tolerance mechanisms, much as a few cracks do not inform concerning the entire dike. Each disease may reflect only one breach or bypass of the edifice of immune tolerance, and may ultimately represent a rare exception, considering the number of potentially self-reactive lymphocytes generated on a daily basis by mammalian organisms. For the huge majority of autoreactive cells, the successive levels and multiple redundancies of tolerance mechanisms perform very well indeed. In addition, although the above-mentioned tolerance modes are established textbook material, we may still be missing large facets. Remember that, although the importance of ectopic thymic antigen expression had been proposed for some time, only less than 10 years ago was its role firmly established with the discovery of how AIRE deficiency promotes autoimmunity in humans with autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED) and in *Aire*$^{-/-}$ mice[5,6].

It is also important to keep in mind that autoimmune diseases represent a range of failures, rather than unique entities. Some diseases result from genetic deficiencies in elements essential for facilitating major facets of the global tolerance process. For example, APECED and IPEX (immune deficiency–polyendocrinopathy–X-linked) are diseases provoked by mutations in *AIRE* and *FOXP3*, respectively, and result in impaired clonal deletion and immunoregulation, respectively; as might be expected, multiorgan pathology occurs in these instances. Other disorders, including myasthenia gravis, Hashimoto's thyroiditis, and perhaps type 1 diabetes, result from the failure of tolerance to a very specific antigen. Here the emergence of autoimmune cells may require a particular combination of events.

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Just as an elaborate network of levees was built to repel the waters from the North Sea and the inland canals of Holland, a series of tolerance mechanisms has evolved to keep autoimmunity in check.

One of the most noteworthy examples is found in the experimental allergic encephalomyelitis mouse model of human multiple sclerosis induced by immunization with proteolipid protein (PLP)[7,8]. Although both H-2^b and H-2^s-bearing mouse strains can potentially present PLP peptides, the immunodominant peptide presented by antigen-presenting cells from H-2^s mice happens to map within an alternatively spliced exon that is missing in thymic PLP1 mRNA transcripts; in contrast, the immunodominant epitope for H-2^b mice is in a constant exon, so T cells reactive to it are properly tolerized. Such a combination (defective expression in the thymus coinciding with the MHC-preferential epitope) may be required for certain autoreactive TCRs to sneak through. Indeed, as pointed out by von Boehmer and Melchers[1], autoimmune TCRs and their targets are an odd lot, as they recognize peptides bound with low affinity by MHC molecules[9], peptides carrying post-translational modifications[10], antigens poorly processed in the thymus[11], peptides binding in an unusual register within the MHC molecules' grooves (B. Stadinski, personal communication) or TCR–peptide-MHC interactions that adopt unusual angles[12]. Finally, there are likely several inflammatory diseases masquerading as autoimmune, in that they do not entail activation of lymphocytes by cognate autoantigen. In some cases, there may just be a loss of feedback controls on lymphocyte proliferation[13]. In other instances, apparent organ-specific manifestations that evoke a targeted autoimmune process result from local particularities of nonspecific effector systems[14]. In this vein, might the manifestations of systemic lupus erythematosus be explained by inappropriate interferon responses to immune complexes that do not have an autoimmune specificity in themselves and would be ignored in nonsusceptible individuals? Likewise, is there a true initiating autoantigen in rheumatoid arthritis, or are runaway inflammatory cytokine responses primordial?

There are surprisingly few autoimmune diseases

Tolerance systems are also likely to be multiply redundant, which results in a relative paucity of targets. Even when T_{reg} cells are compromised owing to *FOXP3* mutations in individuals with IPEX, the disease is relatively slow and progressive[15]. Even in *Aire*$^{-/-}$*Foxp3*$^{-/-}$ mice, many organs remain unaffected[4]. Redundancy is certainly at play here: ectopic antigen expression in the thymus is substantially amplified by Aire but does exist in its absence, and T_{reg} cells are the best studied but not the only lymphocyte population with inhibitory functions. There are various explanations for the limited molecular and anatomical scope of any one disease.

Some limitations reflect the target tissue itself. Anatomical barriers can delay or restrict lymphocytic infiltration or the presentation of autoantigens, and refractory states may even repulse inflammatory infiltrates. An intriguing but unexplained phenomenon has been repeatedly observed in NOD mice: despite circulating pools of activated autoreactive T cells that overrun many pancreatic islets, one always observes a fraction of completely untouched islets, and it is unclear how these islets escape attack. Conversely, the general preponderance of endocrine organs as autoimmune targets likely results from the local concentrations of secreted products, which are much higher than in the lymphocyte-tolerizing compartments.

Other restrictions to autoimmunity are tied to 'choices' made by the immune system. The most obvious of these preferences lies in the MHC, as the restricted binding of peptides by any one of the hypervariable MHC-I or MHC-II molecules clearly helps define the choice of targets. This selection is reflected in the towering dominance of the MHC in determining genetic susceptibility to autoimmune disease, and is experimentally best exemplified by the range of autoimmune targets seen in MHC-congenic mice on the NOD genetic background, as different introduced haplotypes sometimes bring distinct autoimmune targets[16]. (However, there is an unsettling element to this simple explanation: as the range of epitopes bound by any one MHC allele is fairly extensive, why is the target restriction so tight?) Other choices may include a time element. For instance, attack of endocrine islets in NOD mice can be prevented by immunization with an irrelevant immunogenic peptide[17], or by the exocrine pancreatitis that results from *Cd28* or *Aire* deficiencies[18–20]. Whichever autoimmune attack comes first seems to win.

It may be that our perception of immunological tolerance is distorted because the tolerance mechanisms we have so far identified are preferentially those that relatively easily go awry. Certain mechanisms may break down more readily because of genetic features. For example, the *FOXP3* gene is located on the X chromosome, so any mutation need only be hemizygous to manifest as IPEX in males. Other tolerance modes may be fragile because they have one or more nonredundant elements. Aire may be the only protein that drives expression of certain peripheral-tissue antigens in the thymus; insulin might well be one such antigen[21]. Still other tolerance mechanisms may break down more frequently because they protect tissues that are especially at risk owing to some physiological feature such as frequent exposure to mechanical stress (such as the joints) or infectious insults (such as the lungs and skin). It is easy to make the mistake of assuming that the tolerance modes that are highlighted over and over again are the most important ones. In fact, one might argue the opposite: we have not yet uncovered the sturdiest edifices keeping autoimmune disease in check because they are backed up by a multiplicity of redundancies or because rapid lethality ensues if they are genetically compromised.

Contributions of the microbiome

If autoimmune diseases represent such diverse breaches of the tolerance levees, where does the contribution of the microbiome fit in? Chervonsky[22] reviews the strong arguments for a major influence of environmental agents, microbial flora in particular, on autoimmune deviation: in humans, there are geographic gradients of susceptibility, autoimmune diseases being far more prevalent at higher latitudes; in animal models, there are effects of infections or other manipulations of microbial exposure (for example, germ-free conditions)[23]. Appropriately, Chervonsky also refutes the oft-repeated, but fallacious, notion that incomplete penetrance of autoimmune disease in monozygotic twins must reflect environmental effects. A simple genetically imparted probability does not need environmental triggers, and may merely reflect stochastic elements such as immune repertoire generation, noisy gene expression or epigenetic fluctuations. Indeed, all biology is probabilistic, being rooted in the mass-action law of molecular interactions. Chervonsky also distinguishes autoimmune contexts where the microbiome seems to have no effect ("pure autoimmunity") from diseases where microbes have important potentiating or preventing influences. A range of mechanisms can subtend the latter cases, as the commensal gut flora is known to generically influence the development and composition of the immune system, and microbial infection can reveal loopholes in tolerance by unmasking, through tissue damage, a previously shielded self antigen or by amplifying, through molecular mimicry, a minor self-reactive lymphocyte clone. Infections can also break equilibria that maintain effective self tolerance, perhaps by activating innate pathogen-sensing pathways that disrupt tuned signaling pathways, or by compromising the balance between effector and regulatory cells. These considerations also lead one to question what actually constitutes self. Beyond our genomic self, should we not include microbes in this definition? This notion certainly seems appropriate for retroviral elements that long ago integrated into the human genome, or for the quasi-obligate symbiotic flora that has also coevolved with humans for thousands of years and is passed on from mother to child.

Therapeutic implications

Of late, we have been both surprised at and disappointed with the performance of some of the immunomodulatory strategies tested in human autoimmune disease settings. An example is recent findings on rituximab, an antibody that recognizes the B lineage–specific cell-surface molecule CD20 and thereby specifically targets B cells[24]. Surprisingly, rituximab had an impressive beneficial effect in patients with multiple sclerosis[25,26], a disease not generally thought to have a critical dependence on B cells. On the other hand, its lack of impact in patients with systemic lupus erythematosus[25,26] was disappointing, given that this disorder has often been touted as a paradigm antibody-mediated autoimmune disease. These unexpected outcomes highlight several issues.

First, we may know a lot less then we think we do. Our view of human autoimmune disease is heavily colored by results on murine autoimmune disease models. Obviously, models may not always be predictive of human disease, and, of course, this is especially true when the models have been heavily manipulated—whether it be with drugs (for example, adjuvants) or through genetics (for example, transgenics). Steinman[27] argues that clinical trials themselves are the best means to evaluate the importance of a particular immune system cell or molecule in a given disease context. This seems a dangerous thought-mode, however. Palmer and Weaver[28] review just how complex it can be to unravel T helper responses, in particular T_H-17 responses. Some other factors are almost certainly at play. Interventions may be made at variable points in the course of pathogenesis when different autoimmune diseases are treated with the same drug (for example, multiple sclerosis was treated with anti–IL-12p40 at a rather late stage, in the relapsing state). Drug target(s) can be complex (for example, anti–IL-12p40 recognizes both IL-12 and IL-23, which could have very different influences). Different therapeutic reagents used to treat the same disorder can have quite dissimilar pharmacological properties (for example, at least part of the explanation for the early notion that targeting the tumor necrosis factor pathway is far superior to targeting the IL-1 pathway might be the relatively short half-life of the IL-1 receptor antagonist anakinra). In short, lack of an effect may not tell us much, and comparisons across drugs or across diseases can be uninterpretable.

Second, "an autoimmune disease is an autoimmune disease is an autoimmune disease…" does not hold. With variable disease etiologies and courses of pathogenesis, and, especially, considering the different modes of tolerance breakdown involved, we cannot expect that a given therapeutic strategy will successfully intervene in all, or even most, autoimmune disorders. Worse, given our current state of ignorance concerning human immune disease mechanisms, we are even unable to accurately predict, for a drug that is successful in one autoimmune setting, to which one or two other contexts it might best be extrapolated. An excellent example of this issue was Steinman's citation that drugs targeting the tumor necrosis factor pathway had an unanticipated exacerbating effect on multiple sclerosis. Taking up the imagery of the dike system, we should not expect that reinforcing a dike near The Hague in southwest Holland would save Groningen in the northeast from inundation through a nearby leak.

It might be instructive to consider the parallels between cancers and autoimmune diseases, both of which represent a constellation of related, yet quite distinct, pathologies. Cancers are, at root, diseases of runaway cell division, involving defects in a basic set of signaling networks; however, precisely which molecules and pathways harbor the initiating defect, and which particular tissues are targeted, differ in different cancers. Early cancer therapies usually entailed broadly active antiproliferative strategies (for example, chemotherapy or irradiation), with their potentially widespread side effects. More recent strategies are much more precise in their targeting. For example, imatinib (Gleevec) is specific for a designated protein kinase. However, this precision means that, in general, successful drugs may be extrapolatable to at most a few other types of cancer. Similarly, as autoimmune diseases are rooted in inappropriate immune responses that also lead to runaway cell division, early therapies (for example, cyclosporine) were broadly acting drugs that aimed to stifle cell proliferation. An important goal has been to devise more precise reagents, targeting a molecule or cell crucial to a particular autoimmune disease. But we must not expect, then, that these more specific reagents will be applicable to any and every autoimmune pathology.

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1. von Boehmer, H. & Melchers, F. Checkpoints in lymphocyte development and autoimmune disease. *Nat. Immunol.* **11**, 14–20 (2010).
2. Mueller, D.L. The problem of peripheral self-peptide–MHC recognition: mechanisms maintaining peripheral tolerance. **11**, 21–27 (2010).

3. Wing, K. & Sakaguchi, S. Regulatory T cells exert checks and balances on self tolerance and autoimmunity. *Nat. Immunol.* **11**, 7–13 (2010).
4. Chen, Z., Benoist, C. & Mathis, D. How defects in central tolerance impinge on a deficiency in regulatory T cells. *Proc. Natl. Acad. Sci. USA* **102**, 14735–14740 (2005).
5. Nagamine, K. *et al.* Positional cloning of the *APECED* gene. *Nat. Genet.* **17**, 393–398 (1997).
6. Finnish-German APECED Consortium. An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. *Nat. Genet.* **17**, 399–403 (1997).
7. Klein, L., Klugmann, M., Nave, K.-A., Tuohy, V.K. & Kyewski, B. Shaping of the autoreactive T-cell repertoire by a splice variant of self protein expressed in thymic epithelial cells. *Nat. Med.* **6**, 56–61 (2000).
8. Anderson, A.C. *et al.* High frequency of autoreactive myelin proteolipid protein-specific T cells in the periphery of naive mice: mechanisms of selection of the self-reactive repertoire. *J. Exp. Med.* **191**, 761–770 (2000).
9. Liu, G.Y. *et al.* Low avidity recognition of self-antigen by T cells permits escape from central tolerance. *Immunity* **3**, 407–415 (1995).
10. Anderton, S.M. Post-translational modifications of self antigens: implications for autoimmunity. *Curr. Opin. Immunol.* **16**, 753–758 (2004).
11. Gammon, G. & Sercarz, E. How some T cells escape tolerance induction. *Nature* **342**, 183–185 (1989).
12. Wucherpfennig, K.W., Call, M.J., Deng, L. & Mariuzza, R. Structural alterations in peptide-MHC recognition by self-reactive T cell receptors. *Curr. Opin. Immunol.* (published online, doi:10.1016/j.coi.2009.07.008 (19 August 2009).
13. Mingueneau, M. *et al.* Loss of the LAT adaptor converts antigen-responsive T cells into pathogenic effectors that function independently of the T cell receptor. *Immunity* **31**, 197–208 (2009).
14. Binstadt, B.A. *et al.* The same systemic autoimmune disease provokes arthritis and endocarditis via distinct mechanisms. *Proc. Natl. Acad. Sci. USA* **106**, 16758–16763 (2009).
15. Wildin, R.S., Smyk-Pearson, S. & Filipovich, A.H. Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. *J. Med. Genet.* **39**, 537–545 (2002).
16. Rasooly, L., Burek, C.L. & Rose, N.R. Iodine-induced autoimmune thyroiditis in NOD-H-2h4 mice. *Clin. Immunol. Immunopathol.* **81**, 287–292 (1996).
17. Vaysburd, M., Lock, C. & McDevitt, H. Prevention of insulin-dependent diabetes mellitus in nonobese diabetic mice by immunogenic but not by tolerated peptides. *J. Exp. Med.* **182**, 897–902 (1995).
18. Jiang, W., Anderson, M.S., Bronson, R., Mathis, D. & Benoist, C. Modifier loci condition autoimmunity provoked by Aire deficiency. *J. Exp. Med.* **202**, 805–815 (2005).
19. Niki, S. *et al.* Alteration of intra-pancreatic target-organ specificity by abrogation of Aire in NOD mice. *J. Clin. Invest.* **116**, 1292–1301 (2006).
20. Meagher, C. *et al.* Spontaneous development of a pancreatic exocrine disease in CD28-deficient NOD mice. *J. Immunol.* **180**, 7793–7803 (2008).
21. Fan, Y. *et al.* Thymus-specific deletion of insulin induces autoimmune diabetes. *EMBO J.* **28**, 2812–2824 (2009).
22. Chervonsky, A.V. Influence of microbial environment on autoimmunity. *Nat. Immunol.* **11**, 28–35 (2010).
23. Bach, J.F. The effect of infections on susceptibility to autoimmune and allergic diseases. *N. Engl. J. Med.* **347**, 911–920 (2002).
24. Martin, F. & Chan, A.C. B cell immunobiology in disease: evolving concepts from the clinic. *Annu. Rev. Immunol.* **24**, 467–496 (2006).
25. Leandro, M.J. & de la Torre, I. Translational mini-review series on B cell-directed therapies: the pathogenic role of B cells in autoantibody-associated autoimmune diseases–lessons from B cell-depletion therapy. *Clin. Exp. Immunol.* **157**, 191–197 (2009).
26. Dorner, T., Radbruch, A. & Burmester, G.R. B-cell-directed therapies for autoimmune disease. *Nat. Rev. Rheumatol.* **5**, 433–441 (2009).
27. Steinman, L. Mixed results with modulation of T_H-17 cells in human autoimmune diseases. *Nat. Immunol.* **11**, 41–44 (2010).
28. Palmer, M.T. & Weaver, C.T. Autoimmunity: increasing suspects in the $CD4^+$ T cell lineup. **11**, 36–40 (2010).