separate elements like cuirass, breastplate, brassard, and helmet. The connections between these elements might be more vulnerable to a sword of therapy than originally thought.

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Not-So-Negative Selection

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It once seemed clear that negative selection of self-specific T cells in the thymus was the major mechanism of central tolerance. But recent studies, including Legoux et al. (2015) in this issue of *Immunity*, show that this is not always the case.

It's hard to think of a subject over which more ink has been spilled in immunology than the dilemma of how organisms fortunate enough to have an adaptive immune system distinguish self from non-self. This has been debated almost since the field began, when Ehrlich mused over how antisera, with their diverse specificities, could somehow avoid what he called "horror autotoxis," or self reactivity (Silverstein, 2001). Fast forward to the 1950s, and with the clonal selection theory, Burnet proposed that self-specific lymphocytes were disposed of, or clonally deleted (Burnet, 1957). Decades later, Nossal found evidence that specific B cells could also be inactivated, at least in vitro, which he referred to as "clonal anergy" (Nossal and Pike, 1980). So there seemed to be at least two competing models for how organisms dealt with self-specific lymphocytes: either during their maturation, also known as central tolerance, or in the periphery, known as peripheral tolerance. In the T cell world, clonal deletion became known as "negative selection," to distinguish it from "positive selection," which is the process by which T cells are selected to be able to recognize peptide antigens in the context of one's own major histocompatibility complex (MHC) molecules. Late in the 1980s, a series of dramatic papers, based on either endogenous superantigen effects or T cell receptor transgenic mice, showed the wholesale deletion of self-specific T cells in the thymus, basically expunging any thoughts of anergy being a viable option for central tolerance (Goodnow and Ohashi, 2013). Interestingly, at the same time, Goodnow and colleagues showed equally dramatic evidence of clonal anergy in an immunoglobulin transgenic system (Goodnow et al., 1989), but this didn't seem to have any influence on the T cell community. Instead, the issue seemed to be settled that, at least with respect to thymocyte maturation, it was all about negative selection getting rid of all, or almost all, of the "dangerous" T cells. But even then, cracks began appearing in that certainty. First, it was demonstrated that self antigens, at least in mouse models, could trigger autoimmunity, but most thought these were the exception rather than the rule. Another warning sign emerged when Jensen et al. showed that, whereas work with TCR transgenics had showed that γδ T cells specific for a minor histocompatibility gene were negatively selected in the presence of that molecule in vivo, analysis using a tetramer reagent in wild-type mice found no evidence of negative selection (Jensen et al., 2008). Although there had been hints of transgenic artifacts before, this was the first indication that things could go seriously wrong in terms of the earlier interpretation. More recently, my own group, as well as others, has found that self-specific T cells are quite abundant in the periphery of healthy individuals, human or mouse, although when self versus non-self can be compared directly with the same tetrameric reagents, as in the case of the male antigen H-Y (or, SmcY), there is, at least in the human case, a significant (3×) reduction in the

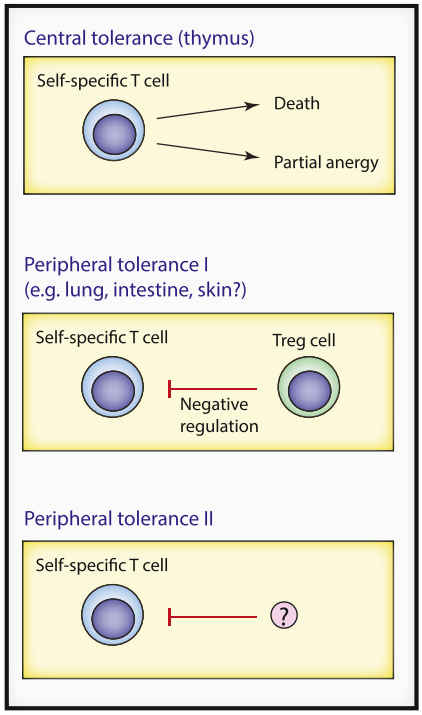

Figure 1. Distinct Zones of T Cell Tolerance
Generalizing from the results of Legoux et al. and others, there appear to be at least three distinct zones where T cell tolerance to antigens expressed in those areas manifests itself in different ways. Central tolerance, which involves thymocytes encountering peptides derived from self molecules in the thymus, seems to deal with self-specific αβ T cells by either inducing cell death or negative selection or by a type of anergy, where T cells emerge in the periphery with a higher threshold for activation. In the second zone, illustrated by the lung and intestinal data, there is no apparent negative selection, but Treg cell numbers are elevated, and these cells seem to be the main enforcers of tolerance. In the third zone, none of these three mechanisms seems to be operative, and thus are seemingly immunologically ignorant.

percentage of T cells with this specificity in males in comparison to females (Su et al., 2013; Yu et al., 2015). In addition, compared to foreign antigen-specific T cells, a collection of self-antigen-specific T cells were refractory to antigen-dependent stimulation, although both could be stimulated by anti-CD3 plus anti-CD28 (Yu et al., 2015).

In this issue of *Immunity*, Legoux et al. (2015) enter the fray, taking a very clever tack and asking whether tissue-specific expression has an impact on negative selection. By taking advantage of the many available transgenics expressing cre in specific tissues for the purposes of making conditional knockouts, they looked for and found a dominant class II MHC (I-A^b) T cell epitope in the cre protein and then assayed the fraction of T cells specific for cre by using a tetramer reagent in the various cre-expressing transgenes. In the case of a ubiquitously expressing cre construct, they found a significant degree of deletion, approximately 60% in both the thymus and periphery, very similar to recent work analyzing the Y-chromosome-encoded SmcY antigen in males versus in females, and not at all like the super-efficient deletion of roughly 99% of specific T cells in the transgenic systems previously. Even more remarkably, when they examined mice expressing cre in a tissue-specific context—lung, intestine, and pancreas—they could find no evidence of negative selection at all. They also investigated whether there was a role for cre-specific regulatory T (Treg) cells and found that in the intestinal- and lung-specific cre mice, but not in the ubiquitously expressed and pancreas-specific cre mice, there was an elevation of Treg-cell-phenotype $CD4^+$ T cells that were stained with the cre:I-A^b tetramer. In the lung and intestinal cases, the authors noted an inhibition of cre-specific T cell responses. To determine whether Treg cells were responsible for this inhibition, they employed an elegant tetracycline inducible method to selectively kill Treg cells, and when they did this, T cell reactivity was largely restored in those cre mice. Legoux et al. suggest that this type of Treg cell control is not important in the case of pancreas expression of antigens because this organ is not part of the front line of immune defense, unlike the lungs and intestine, which are constantly having to distinguish self from dangerous foreign entities. This role of Treg cells is consistent with the previous results of Sakaguchi (Maeda et al., 2014), who found that T cells specific for MART-1, a melanocyte-specific antigen, are present in the peripheral blood of healthy human beings but held in check by Treg cells.

There is also an important contrast between the ubiquitously expressed cre mice and those dependent on Treg cell control of tolerance, in that the former seems to be an intrinsic form of cell autonomous anergy, whereas repeated immunization with cre plus adjuvant of the lung and intestinal cre-expressing mice can produce a response, indicating that this form of tolerance is somewhat shaky. The authors suggest that this vulnerability to the breaking of tolerance to tissue-specific antigens could be why autoimmunity typically appears as a tissue-specific disease. Legoux et al. also point out that this suggests that effective therapeutic vaccines against tumor antigens in cancer is at least of possible benefit, as it has been in some cases, although these vaccines have generally been too rarely successful to be a stand-alone treatment.

Although these results involve only one antigen, and thus their generality needs to be confirmed with other examples, the results regarding central tolerance do correspond well with other reports (Yu et al., 2015, Su et al., 2013). But assuming there is corroboration with other antigens for the peripheral tolerance results, this paper sheds important new light on the variety of tolerance mechanisms in play with respect to T cells. This is illustrated in Figure 1, where there are at least three distinct immunological environments for T cell tolerance. The first is a central tolerance zone—the thymus in the case of T cells, where T cells mature and encounter many, but not all, potential antigens in the context of their own MHC repertoire. This is facilitated by the Aire protein, which seems engaged in expressing a large number of proteins for the purpose of being presented to maturing thymocytes (as reviewed by Goodnow and Ohashi, 2013). The consequences of self recognition in this central zone are either the induction of apoptotic death e.g., negative selection, or a type of anergy, which makes those T cells more difficult to stimulate in the periphery. A second tolerance zone is postulated by Legoux et al. to be the extensive mucosal immune compartments of the lung and intestine where many immunologically significant encounters take place with microbes. In this zone, they see no evidence for negative selection for the cre protein, but instead see a heightened number of Treg cells and a direct role for those cells in suppressing responses. It would interesting to know whether this is also true for lymphocytes in the skin, another tissue that has extensive contact with microbes, but is not mucosal. A third distinct environment is represented by the results for pancreatic expression, where there

seems to be no consequences for cre-specific T cells expression there. This seems to be a state of immunological ignorance, and a puzzle as to just how one protects against autoimmunity in such cases. Perhaps the answer is that there is yet another, unknown mechanism promoting tolerance in organs of this type.

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Pyroptosis: Caspase-11 Unlocks the Gates of Death

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How inflammatory caspases trigger pyroptotic cell death is mostly unexplained. In this issue of *Immunity*, Núñez and colleagues report that caspase-11 cleaves the transmembrane channel pannexin-1, causing an efflux of cellular ATP that promotes a P2X7 receptor-dependent pyroptosis.

The term pyroptosis was coined to describe a proinflammatory form of cell death (Bergsbaken et al., 2009). It is derived from the Greek roots *pyro*, meaning fire, and *ptosis*, which denotes falling and matches the terms used for other forms of programmed cell death. MLKL pseudokinase phosphorylation or loss of mitochondrial integrity, which are hallmarks of necrotic or apoptotic cell death respectively, are not required for pyroptosis. Instead, this pathway is defined by the activation of inflammatory caspases, cell swelling, and rapid destabilization of plasma membrane integrity. This results in the release of cellular content including danger-associated molecular patterns (DAMPs) and cytokines that mount a robust inflammatory response. Pyroptosis can also contribute to the clearance of intracellular bacteria; by disrupting infected cells it can release pathogens, making them susceptible to phagocytosis, and killing by neutrophils (Jorgensen and Miao, 2015).

Initiation of pyroptosis requires at least one member of the inflammatory caspases, a family of proteases including caspase-1 and caspase-11 in mice and caspase-1, caspase-4, and caspase-5 in humans. These enzymes resemble the initiator caspases involved in apoptosis but are unable to process the many substrates associated with initiation and execution of apoptotic cell death; in fact, only a few inflammatory caspases substrates have been described so far.

Inflammatory caspases are activated within high molecular weight complexes known as inflammasomes. Caspase-1 inflammasomes typically assemble upon oligomerization of a scaffold protein that directly or indirectly senses activating stress signals or pathogen signatures. Beyond their role in promoting pyroptosis, caspase-1 inflammasomes are well known for their involvement in the maturation of the pro-inflammatory cytokines IL-1β and IL-18, an activity that cannot be fulfilled directly by caspase-11. The caspase-11 inflammasome, also known as the non-canonical inflammasome, is formed upon cytosolic exposure of lipopolysaccharide (LPS), a bacterial component that binds and directly activates mouse caspase-11, as well as the two human paralogues caspase-4 and caspase-5. Activation of the caspase-11 inflammasome has been described as the main pathway involved in LPS-induced lethality in mice, suggesting that pyroptosis might contribute to inflammatory syndromes in vivo.

Because the proteolytic activity of the inflammatory caspases is required to initiate pyroptosis, it is likely that the cleavage of at least one of their substrate promotes cell death. Yet, little is known on the proteolytic activities that might initiate the cascade of events associated with pyroptotic cell death.

A study in this issue of *Immunity* (Yang et al., 2015) sheds new light on the initial steps of pyroptosis. The groups of Gabriel Núñez and Quin Liu describe the cleavage of pannexin-1 as a pyroptosis-initiating event.

Pannexin-1 is a plasma membrane channel widely expressed in diverse tissues. It forms pore channels that allow the passage of small molecules such as ions and nucleotides between