Immune Tolerance

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Immune Tolerance

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Preface

The evolution of multicellular organisms has been influenced by their interactions with invading pathogens. One strategy multicellular organisms employ in dealing with infection is tolerance in its literal meaning of "endurance" or "fortitude" toward infectious agents. In this context, tolerance is the ability of a host to "live with the enemy" (i.e., to bear a toll of pathogen load without reducing it, while avoiding a critical detriment to host fitness). Another strategy is avoidance of microbial pathogens and parasites guided by sensory organs (Medzhitov et al. 2012). The most studied strategy, however, is the host resistance that invokes a variety of immunologic mechanisms to effectively eliminate or sequester the infectious agent or reduce its load. Immune responses against infectious agents elicit inflammation to facilitate their elimination and to restore tissue homeostasis. Yet the benefits of resistance to pathogens are constrained by temporal or lasting immune-mediated impairment in host function. Therefore, implicit in the resistance strategy are numerous mechanisms of negative regulation of immune-mediated inflammation. Foremost among these mechanisms are those that prevent deleterious immune responses against the host.

The possibility of the immune system turning against "self" was recognized by Paul Ehrlich, who over a century ago called this scenario "horror autotoxicus" and was the first to postulate the existence of "certain contrivances" (mechanisms) that prevent immunity against "the organism's own elements." To paraphrase Charlie Janeway's words, the immune system must enable effective responses against "infectious non-self" and its discrimination from "non-infectious self." Multiple mechanisms that operate in a cell-autonomous manner in the immune system and that manifest as a result of intercellular communications ensure restraint of deleterious immune responses to "self," and constitute immunological or immune tolerance ("immune" or "immunological tolerance" are used in this volume interchangeably). In this context, "self" encompasses not only antigens encoded by the host genome, but also foreign ones the organism's immune system is continuously exposed to. The latter are encoded by the genomes of commensal microorganisms or are of nonmicrobial origin, such as food and environmental antigens.

In the innate immune system the problem of "self–non-self" discrimination is addressed through the specificity of evolutionarily selected, germline encoded pattern recognition receptors (exemplified by toll-like receptors [TLRs]) for conserved molecular features in microorganisms, like lipopolysaccharide (LPS), lipoteichoic acid, or flagellin (Janeway 1989). In addition to certain structural properties, spatial segregation and tightly controlled expression levels of innate immune sensors of nucleic acids, the presence of nucleases, and accessory molecules afford discrimination of microbial and host-derived DNA and RNA (Barton and Kagan 2009). The emergence of the adaptive immune system, with its essentially unlimited antigen-recognition capability afforded by receptors generated somatically in anticipation of future encounters with yet unknown pathogens, brought a demand for novel mechanisms enforcing immune tolerance.

The elimination of differentiating self-reactive lymphocytes, one of the central tenets of Burnet and Lederberg's clonal selection theory proposed largely on a theoretical basis, along with the notion of fetal and neonatal tolerance put forward by Owen, Billingham, Brent, and Medawar appeared to settle the issue when first proposed in the 1940s and 1950s (see Schwartz, this volume). However, the discovery of self-MHC restriction of T-cell recognition, and a requirement for a certain degree of self-reactivity of antigen receptors of T cells and likely B cells for their differentiation and peripheral

survival, presented the conundrum of control of weakly self-reactive T and B cells that escape clonal elimination. Subsequent work by many groups has shown that this control is enforced by a requirement for antigen-presenting cells (APCs) to display antigens together with inducible ligands for co-stimulatory receptors, whose expression is driven either directly or indirectly by TLRs or other immune sensor signaling, in order for lymphocytes to differentiate into effector cells (Janeway 1989). Induction of a hyporesponsive or nonresponsive state in mature self-reactive lymphocytes or their elimination commences upon chronic exposure to an antigen in the absence of co-stimulatory signals. Physical elimination or functional inactivation of self-reactive lymphocyte clones during their differentiation in the primary lymphoid organs or in the periphery is complemented by numerous cell-intrinsic mechanisms of tuning the thresholds of antigen and cytokine receptor triggering to limit lymphocyte activation. Although the majority of these mechanisms can be considered manifestations of negative feedback regulation common in biology, a distinct mechanism of immune tolerance mediated by regulatory T cells, a dedicated lineage of lymphocytes acting *in trans* to limit immune-mediated inflammation in the context of autoimmunity, cancer, infection, tissue injury, metabolic dysfunction, and commensalism, appears unique.

Since the original framing of the problem of "self–non-self" discrimination by Ehrlich and its theoretical and mechanistic elaboration by Owen, Medawar, Brent, Billingham, Burnet, Lederberg, Janeway, and other investigators, major progress has been made in our understanding of the genetic, molecular, and cellular bases of immune tolerance. Many of the past and present advances and new challenges are discussed in this volume.

Immune Tolerance covers topics that range from a history of the field, to mechanisms of tolerance induction, to factors that underlie the breakdown of tolerance, to strategies to restore it in clinical settings. It seems a ripe moment to assemble and contemplate these different perspectives: Early phenomenological studies have given way to sophisticated mechanistic dissections of animal models at the cellular, molecular, and genetic levels; it is now time to integrate the resulting body of knowledge into a set of crucial principles that can facilitate our attempts at clinical extrapolation. It is time to evolve from a reductionist to a systemic level of comprehension.

Schwartz's leadoff essay provides a historical overview of how our understanding of immunological tolerance has developed over the last 70 years, beginning with the classical observations from Owen on dizygotic twin cattle, Hâsek on parabiotic chickens, and Billingham and coworkers on neonatal mice. Leaps in understanding came with developments such as cloning of the MHC, TCR, and BCR genes; the discovery of superantigens encoded by endogenous retroviruses; generation of TCR/BCR-transgenic mice; substantiation of the importance of dominant tolerance in multiple systems; and identification of crucial molecular drivers, including Foxp3, Aire, and a repertoire of positively and negatively acting co-stimulatory molecules and cytokines. The picture that has emerged portrays a complex net of overlapping tolerance mechanisms organized around critical checkpoints, a portrait that challenges us to rationalize the heterogeneity and high and growing frequency of autoimmune diseases.

Tolerance mechanisms employed by the three major classes of lymphocytes—T, B, and NK cells—are then discussed, revealing interesting commonalities and divergences. Xing and Hogquist describe the major modes of central and peripheral T-cell tolerance induction, and what is currently known about their molecular underpinnings. Thymically, the strength of a thymocyte TCR's engagement of MHC-peptide complexes is the major (though not only) determinant of its fate. Clonal deletion and clonal diversion to another lineage (e.g., the Treg cell lineage) are the major mechanisms of purging self-reactive thymocytes. Extrathymically, regulatory T cells, anergy, and, debatably, tolerogenic dendritic cells take over as the most important means of enforcing T-cell tolerance. The major type of regulatory T cell, $Foxp3^+$ $CD4^+$ Tregs, is examined in more detail by Benoist and Mathis, who present a more nuanced view of their biology than originally proposed and highlight their phenotypic and functional diversity. Cleverly engineered mouse models

and transcriptome analyses have led to the realization that there are specific subpopulations of Treg cells optimized to control the different "flavors" of immune responses (e.g., Th1 vs. Th2 vs. Th17) or even certain nonimmunological processes. Lewis and Reizis weigh the role of dendritic cells (DCs) in tolerance induction, emphasizing, on the one hand, that diverse subsets of DCs perform a diverse set of functions in T-cell homeostasis or T-cell responses and, on the other hand, that there is so far inadequate evidence to argue convincingly for a particular tolerogenic DC subset or state, or even for a required role in maintaining tolerance. They make the case that, as a consequence, DCs are more likely to have utility in breaking than in remaking tolerance in clinical settings.

To what extent do these principles translate to B and NK cells? Pelanda and Torres argue that editing of BCR genes in the bone marrow is the major mechanism for purging the B-cell repertoire of self-reactive specificities. In contrast to the situation with T cells, and despite being highlighted in certain of the early BCR-transgenic models, clonal deletion appears to be a less-employed fallback mechanism; anergy, another mechanism emphasized in early BCR-transgenic studies, may also be a less frequent option than originally thought. Tolerization of NK cells, given that they are an element of the innate immune system, entails a different set of issues, including the fact that they express a repertoire of defining cell-surface receptors whose genes do not rearrange. As Jaeger and Vivier point out, the phenomenon of NK-cell tolerance and its mechanisms are at a very early stage of exploration, but it is an important topic given potential ramifications in tumor immunology. An emerging concept is "adaptive tolerance," reflecting constant NK-cell sensing of their microenvironment, but the molecular mechanisms of such a process remain undefined. Nonetheless, certain features of "adaptive tolerance" seem reminiscent of mechanisms employed by T and B lymphocytes, notably induced hyporesponsiveness and sequential adjustments of NK-cell receptor repertoires.

Despite the extensive net of mechanisms enforcing immunological tolerance, it breaks down in many ways and quite frequently. More than 80 different autoimmune disorders have been described, and in many developed countries 7%–10% of the population has an autoimmune disease. It is commonly thought that genetic and environmental factors are the two major determinants of disease penetrance. However, there is growing appreciation that stochastic events (quasi-random rearrangement of BCR and TCR gene segments) and epigenetic processes (e.g., DNA methylation) can also play a role. It has been recognized for decades that most autoimmune disorders have a strong heritable component. A few appear to be essentially monogenic, notably IPEX (immune dysregulation–polyendocrinopathy–enteropathy–X-linked), APS1 (autoimmune polyendocrine syndrome type 1), and ALPS (autoimmune lymphoproliferative syndrome); the relevant genes are now known and their identity and function has yielded critical insights into mechanisms of immunological tolerance. But the large majority of autoimmune diseases are polygenic. HLA/MHC loci are by far the most impactful genetic elements; in addition, a multiplicity of interacting, weakly acting loci show up in most genetic dissections. Yet, in general, the totality of HLA/MHC and non-HLA/MHC influences adds up to much less than the calculated genetic inheritance. One goal of the contribution from Goris and Liston is to arrive at an explanation for this "missing" or "hidden" heritability. Another important point made in their essay is that genetic analyses on a number of autoimmune diseases point to several common pathways (e.g., IL-2/IL-2R, PTPN22, co-stimulatory molecules). Indeed, some of these pathways are already being targeted therapeutically.

There has been a recent burst of interest in the impact of the microbiome—in particular the gut microbiome—on the induction and breakdown of immunological tolerance. Again, it has been known for decades that microbes can exert such an influence. What has changed is our appreciation of the potential scale of the microbiome impact: Individuals are constituted of greater than 10 times more microbial than human cells, which express greater than 100 times more microbial than human genes. So, in this light, what is actually self and what non-self? What has also changed is that technological improvements in DNA and RNA sequencing, gene-expression profiling, polychromatic flow cytometry, and animal husbandry have permitted the association of particular microbes with

molecularly defined effects on particular lymphoid cell subsets, leading to changes in the incidence or course of particular autoimmune diseases. Chervonsky surveys this new landscape, focusing on how microbes can enhance or dampen autoimmunity in different contexts. He explores how innate versus adaptive versus bridging immune elements come into play, and weighs whether influences on autoimmunity primarily reflect disturbances of "specific lineages" of microbes or of "balanced signals" they engender. The essay by Nussbaum and Locksley entertains the novel idea that microbe-induced pathology may often reflect an unsuccessful attempt at commensalism: the host and invader cells communicate back and forth, but fail to achieve homeostasis. A tenet of this notion is that the immune system recognizes and promotes the installation of microbes that are beneficial for one reason or other and, conversely, that microbes generate signals to elicit a nurturing niche. Both sides of this interplay promise that mining the microbiome will yield a treasure trove of new immunomodulatory molecules.

Last, Bluestone and Bour-Jordan tackle potential clinical applications. How do we match our current understanding of the diverse mechanisms of tolerance induction with our knowledge of how tolerance breaks down in different autoimmune diseases to develop a strategy to contain them? A panoply of therapeutic strategies encompassing systemic immunotherapies, antigen-specific approaches, more general immunomodulation of co-stimulatory or trafficking pathways, and cellular therapies are reviewed. There have been some successes. New targets with considerable potential are in the pipeline. But we need new ways to extrapolate the impressive growth of mechanistic insights to quicker and more effective clinical realization.

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