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The late 1980s were marked by the end of the suppressor CD8+ T cell era that had reigned for over a decade in the world of immunology. Although the concept of cell-mediated suppression of immune responses reemerged 15 years later as CD4+ regulatory T cells, the CD8+ suppressor T cells, which were associated with elusive Ag-specific suppressor factors and MHC restriction by molecules in the “nonexistent” I-J subregion, have never recovered (1–3). But in the mid-1980s, circa 1985–86, CD8+ T suppressors, although on the ropes, still captured the imagination of many investigators. It was in this environment that Marc Jenkins joined the Schwartz laboratory in an effort to examine T cell suppression using a robust in vivo tolerance-inducing regimen to study suppressor cell development. The work published by Marc Jenkins and Ron Schwartz in 1987 (4), which has been selected for the Pillars of Immunology series, demonstrates once again that “chance favors the prepared mind” (Louis Pasteur). This single paper, although it did not fulfill the original promise of solving the suppressor cell “problem,” opened the field of T cell anergy and provided a framework for understanding the field of T cell costimulation.

The tolerance system, developed by Steve Miller and Henry Claman and employed by Jenkins when he joined the graduate program in Colorado (5, 6), involved the treatment of mice with ethylene carbodiimide (ECDI)2-fixed, Ag-pulsed spleen cells to induce a profound state of unresponsiveness in vivo (7–9). Logically, at the time Jenkins and Schwartz “assumed” that the in vivo basis of tolerance must be related to suppressor T cells. Thus, the investigators built on extensive experience with T cell fine Ag specificity (10) to study the phenomenon of ECDI-fixed, Ag-pulsed, APC-induced tolerance in vitro. The goal was to “convert” Ag-specific T cell clones derived in the Schwartz lab into suppressor cells as a means toward understanding suppressor cell function and specificity (M. Jenkins, personal communication).

The investigators used I-Ek-restricted B10.A CD4+ T cell clones specific for pigeon cytochrome c (PCC) residues 81–104 as responder cells in their in vitro studies. Although it is difficult to appreciate now, it is important to remember that this work took place in the early days of the molecular characterization of TCR-peptide/MHC interactions. The precise antigenic epitope was not known for most proteins recognized by T cell clones, but Schwartz’s laboratory had just defined one of the first peptide determinants in PCC and identified the residues interacting with the MHC and those interacting with the TCR (10). This was instrumental in establishing the rigorous experimental model necessary to be certain that the T cell unresponsiveness induced by Ag-coupled splenocytes was Ag specific and MHC restricted. The surprising result of these initial experiments was that the T cell clones that were incubated with PCC peptide 81–104-pulsed, ECDI-fixed splenocytes became completely unresponsive to subsequent restimulation with untreated APCs plus peptide, although they remained viable and proliferated in response to IL-2 (4). The induction of unresponsiveness by Ag-coupled splenocytes depended on ECDI-treated APCs, but not ECDI-treated T cells, arguing against a role for suppressor T cells in the altered T cell clone responses. Furthermore, experiments with APCs of distinct MHC haplotypes and irrelevant peptides demonstrated that only the ECDI-treated APCs bearing the same peptide-MHC complex that induced proliferation of these same T cell clones under normal conditions induced T cell clone unresponsiveness in vitro. Thus, ECDI-treated, Ag-coupled APCs induced T cell unresponsiveness that was cell intrinsic, had the same Ag and MHC requirements as the productive proliferative response, and did not depend on suppressor T cells. Moreover, detailed examination under the microscope by Helen Quill (also in the Schwartz laboratory), who had observed a similar state of unresponsiveness when T cell clones were cultured with Ag and MHC molecules in planar membranes (11), led to the conclusion that the cells had indeed “seen” Ag, as the cloned cells got larger and changed morphology. This was the first visualization of what was to become known as “clonal anergy.”

These data implied that stimulation of T cells by their cognate Ag alone was necessary but not sufficient to induce proliferation and that other “accessory” signals were required, as previously suggested by Weiss et al. (12) and Martin et al. (13). Additionally, the investigators reached another key conclusion, namely, that missing key accessory signal(s) resulted in profound and long-lasting T cell unresponsiveness (4, 11). Thus, for the first time, it was suggested that the Bretscher and Cohn two-signal model proposed for B cells (14) applied to T cell activation and showed that the so-called “second signal” was key to avoiding cell inactivation. Demonstration that the second signal could be provided in trans by APCs and that it required cell-cell contact (15) further supported the existence of costimulatory interactions involving cell surface molecules on T cells and APCs. Although many adhesion molecules appeared to enhance T cell proliferation or function, previous studies by
several groups led to the identification of CD28 as the quintessential costimulatory molecule for T cells. Indeed, CD28 signals alone did not activate T cells, but they induced T cell proliferation and IL-2 production in conjunction with TCR engagement (16). Furthermore, CD28 signaling alone could bypass the need for accessory cells for mitogenic T cell activation (17) and, conversely, blocking CD28 hampered T cell activation by APCs plus Ag (18). The key observation of T cell anergy, ultimately shown to be a consequence of deficient CD28-mediated costimulation, went beyond the importance of costimulation in T cell activation but rather created the concept that, absent costimulation, immune responses were actively terminated. Thus, the Jenkins-Schwartz experiments led to wide acceptance of the concept that signal 1 leads to tolerance and signal 1 plus 2 leads to activation. It should be noted, however, that recent results suggest that this is probably not entirely correct. The complete absence of signal 2 typically leads to no response, and tolerance is really an imbalance of a weak signal 2 that cannot overcome strong inhibitory signals (such as those from CTLA-4 or regulatory T cells (Tregs)). Thus, despite the modification of current dogma, the concepts established by this paper have been instrumental in guiding both basic and clinical research targeting the CD28/B7 and other costimulatory pathways as targets of tolerogenic immunotherapy in humans in transplantation and autoimmunity (16, 19, 20).

Historically, it is important to acknowledge that in addition to defining the core properties of T cell anergy and setting the stage for the explosion of the costimulation field, the landmark paper by Jenkins and Schwartz reopened the field of regulation—moving it from a notion of extrinsic regulation to the notion that the molecular mediators and interactions that sealed the T cell fate could function in an intrinsic manner. This led to the biochemical and molecular characterization of CD28 and other important costimulators, but it also set the stage for the defining of negative regulators such as CTLA-4 and mediators important for T cell apoptosis or survival like Bcl-xL. More generally, it helped shape the understanding that T cells had additional outcomes apart from either fully functioning in the absence of suppression or being inhibited by extrinsic factors or cells, including becoming anergic and undergoing apoptosis.

In some ways, the direct contribution of this *Pillars of Immunology* article, the first description of an Ag-specific form of T cell anergy, has been less transformative to the field. In fact, to this day many laboratories refer to this type of unresponsiveness as the "Jenkins and Schwartz" kind of anergy because many of the characteristics of the process, such as the ability of IL-2 to reverse the effect, are unique to the in vitro study of T cell clones. In fact, the absence of IL-2 production and IL-2 reversibility was part of the core definition posited by Schwartz in those early years. In recent times, the term has been expanded and is now widely used to describe any form of T cell unresponsiveness. Clonal anergy can be induced in vitro in activated cells, chiefly T cell clones, usually by a strong TCR engagement with Ag or anti-CD3 mAbs without costimulation and results in a profound proliferative defect (4, 11, 18). CD28/B7 interactions are essential to prevent clonal anergy, although it is not known whether it is a direct effect of CD28 signaling or an indirect effect of CD28 on IL-2 production and cell cycle progression (21). The biochemical basis of clonal anergy is not fully elucidated, although Mueller and colleagues have shown a clear role for activation of the calcium/NFAT pathway, in the absence of MAPK signals (22), that is believed to participate in the induction and maintenance of clonal anergy. Other intracellular molecules such as ubiquitin ligases, mTOR antagonists, and molecules regulating the Ras pathway have been described as potential anergy factors (23–26). Intriguingly, it is still not clear whether clonal anergy has any in vivo relevance to tolerance to self-Ags and the prevention of autoimmunity. Conversely, adaptive tolerance (coined by Schwartz), induced in vivo by the prolonged stimulation of naïve T cells by Ag in the relative deficiency of costimulation or in the presence of negative regulators such as CTLA-4, has become an accepted pathway of immune regulation (27–29). In this case, unresponsiveness usually occurs after vigorous but transient proliferation, and its maintenance requires the persistence of Ag (30, 31). In contrast to clonal anergy, adaptive tolerance is associated with a proximal signaling block and cannot be reversed by exogenous IL-2 (32). Finally, another form of anergy, termed "clonal exhaustion," has recently been described that may follow a transient response that includes proliferation and effector cell generation but culminates in the development of unresponsiveness. Although this phenomenon resembles previous forms of tolerance in some respects, it depends on engagement of the programmed death-1 pathway and is commonly seen in protracted activation settings such as viral infections (33). It is interesting that this effect has been observed in in vivo models of Ag-coupled, fixed APC therapy (34), bringing the field full circle.

Finally, in an ironic twist of fate, the anergic state and regulation by CD28 signals are two major characteristics of CD4+CD25+Foxp3+ Tregs, closing the loop of the original premises of the Jenkins and Schwartz paper. Although not completely elucidated, the biochemical and cellular aspects of Treg unresponsiveness share features with both clonal anergy and adaptive tolerance and are closely associated with the suppressive function of these cells (35, 36). In this regard, the tight linkage between anergy and regulatory T cells suggests a potential linkage during Treg development. Furthermore, CD28 plays a dual role in Treg biology, because CD28 is necessary for Treg development and homeostasis, but strong CD28 signals can reverse Treg anergy and impair their suppressive function (37). Thus, the work of Jenkins and Schwartz paved the way for fundamental discoveries that have shaped the world of immune regulation since its original publication in 1987.

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Disclosures

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References
