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Benchmark

The history and promise of Treg cells

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The seminal discoveries that established the role of regulatory T cells in immunological tolerance were recognized by this year's Nobel Prize in Physiology or Medicine. We present here the unfolding of the Treg story, the players involved at various stages, and the explosive growth of knowledge about this fascinating cell population.

The 2025 Nobel Prize in Physiology or Medicine was awarded to Mary E. Brunkow, Fred Ramsdell, and Shimon Sakaguchi "for their discoveries concerning peripheral immune tolerance"—more specifically, for their groundbreaking work on FoxP3$^+$CD4$^+$ regulatory T cells, now more commonly known as "Treg cells." Nobel Prizes are seldom awarded for cell types, and immunologists are delighted at this well-deserved and long-overdue recognition of one of the immune system's cardinal cellular players. By now, we know that Treg cells control most types of immune reactions, including allergic, autoimmune, inflammatory, anti-microbe, and anti-tumor responses, and that Treg cells can directly impact most cell types of the innate and adaptive arms of the immune system. The critical importance of FoxP3$^+$ Treg cells among the dizzying array of immunocyte subsets is illustrated by the life-threatening lymphoproliferative diseases of immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) patients and *scurfy* mice, which lack this cell type. The therapeutic potential of Treg cells is also being tested with new targeting and engineering approaches for the treatment of cancer, autoimmunity, and inflammatory conditions. As is perhaps increasingly the case, the requirement that the Nobel Prize be awarded to a maximum of three nominees can mean, unfortunately, that major contributors to the genesis and evolution of the chosen research area have not been celebrated. The goal of this piece is to salute the accomplishments of the three Nobel laureates and to highlight other seminal contributions to the Treg field.

Epoch 1: Suppressor phenomena

The need for, and existence of, T cells that operate in *trans* to regulate the activities of other immunocytes has been recognized for decades. In the 1970s, this field was driven by Gershon and colleagues at Yale,[1] but the knowledge base and technological options at the time were inadequate to permit investigators to generate data robust enough to convince the immunology community of the importance—even the actual existence—of such cells. During the 1980s and '90s, work on a number of animal models established that tolerance to self-antigens involves thymus-derived CD4$^+$ T cells: for example, in studies on oophoritis in neonatally thymectomized mice by Sakaguchi in Nishizuka's lab[2]; for mice or birds hosting a heterotypic thymic epithelium graft, in a collaboration between the Le Douarin and Coutinho groups[3,4]; and for congenitally athymic rats supplemented with various T cell subsets by Powrie in Mason's lab.[5] An important practical advance during this period was the recognition of CD25, the high-affinity-conferring subunit of the interleukin (IL)-2 receptor, as a marker permitting enrichment of T cells with suppressive functions: by Hall in Drosch's lab, studying a cyclosporin-A-treated heart allograft model,[6] and by Sakaguchi's team, complementing athymic nude mice with effector plus suppressor T cell populations.[7] Also very useful was the development of a convenient *in vitro* T cell suppression assay by Shevach's group.[8] Despite these advances, the field remained somewhat hazy at the end of the 1990s, dependent on partially purified, inadequately characterized materials and rather complex experimental systems.

The breakthrough: FoxP3 determines Treg cells

This all changed in the early 2000s, with the breakthrough discovery of FoxP3. Working at Celltech, Brunkow, a molecular geneticist; Ramsdell, an immunologist; and colleagues identified the gene underlying the murine *scurfy* mutation as *Foxp3*, which encodes a zinc-finger transcription factor (TF) of the forkhead family.[9] *Scurfy* mice exhibit severe lymphoproliferation, multi-organ leukocyte infiltration, and overproduction of cytokines. Exploiting this information, the same team,[10] as well as Ochs and colleagues,[11] determined that IPEX, a human syndrome with striking similarities to the *scurfy* phenotype, is also rooted in a mutation in *FOXP3*. Simultaneously, Chatila et al. independently documented *FOXP3* mutations in IPEX patients[12]—a discovery often overlooked because this group called the TF-encoding gene *JM2* rather than *FOXP3* and termed the human disease resulting from its mutation XLAAD rather than IPEX. But none of these reports recognized the link between FoxP3 and Treg cells. Rather, the early follow-up studies focused on FoxP3 as a transcriptional repressor that can control effector T cell activation and differentiation.[13–15]

The breakthrough that galvanized the field did not come until two years later, in 2003, when the Ramsdell, Rudensky, and Sakaguchi groups published studies within months of each other showing that the devastating immunological aberrations of *scurfy* mice develop because their defective *Foxp3* gene leads to loss of the $CD25^+CD4^+$ regulatory T cell compartment. The Sakaguchi team showed that *Foxp3* transcripts are expressed specifically in $CD25^+CD4^+$ T cells and that retroviral transduction of *Foxp3* endows $FoxP3^-CD4^+$ conventional T cells with a regulatory phenotype.[16] Ramsdell and colleagues added that while FoxP3-deficient *scurfy* mice lack $CD25^+CD4^+$ regulatory T cells, FoxP3-overexpressing transgenic mice are better able to control the autoinflammatory disease characteristic of CTLA4 deficiency.[17] In arguably the most complete study, Rudensky and colleagues additionally engineered a FoxP3-deficient mouse strain, reporting that these mice lack $CD25^+CD4^+$ regulatory T cells; then produced $FoxP3^+/FoxP3^-$ mixed bone-marrow chimeras, establishing that FoxP3 expression intrinsic to hematopoietic cells is required for the differentiation and accumulation of $CD4^+CD25^+$ regulatory T cells; and then adoptively transferred $CD4^+CD25^+$ T cells into *scurfy* mice, thereby correcting their lymphoproliferative disease.[18]

The definition of FoxP3 as the molecular identifier of regulatory T cells quickly revolutionized the field. It finally anchored the "suppressor cell" notion, clarified Treg lineage determination, and enabled a staggering array of experimental handles to purify, quantitate, profile, ablate, perturb, trace, edit, or engineer Treg cells.

Epoch 2: After FoxP3

The following two decades saw an explosion of activity and considerable progress, from understanding the mechanics of Treg cell and FoxP3 function to devising potential therapeutics that harness these functionalities, also arriving at the conclusion that Treg cells exert broader functions than peripheral tolerance. Many groups contributed to this work, particularly Rudensky and colleagues. We highlight some key themes within this blossoming of knowledge.

How do Treg cells operate? Their non-redundant role in immunological homeostasis was established by IPEX and *scurfy* pathology in the seminal 2003 papers, but it was unclear how these diseases really come about, and how Treg cells relate to perturbed self-tolerance. Although it was generally considered that the absence of Treg cells unleashed self-reactive T effector (Teff) cells, this conclusion was experimentally demonstrated by the Rudensky lab only a few years later.[19] *In vitro* studies showed that bystander suppression could keep a T cell response in check, without obligatory recognition of the same antigen by Teff and Treg cells.[20–22] On the other hand, Treg function *in vivo* is highly enhanced by recognition of a specific antigen.[22–24] Beyond Teff cells, it soon became apparent that many other immunocyte types could be controlled by Treg cells. In the Treg ablation model pioneered by the Rudensky lab,[25] natural killer and dendritic cells were the first cells to respond to Treg loss, long before T cells did. The T follicular regulatory (Tfr) subset controls B cells and affinity maturation of immunoglobulins in the germinal centers,[26,27] and there are also counter-intuitively-positive effects of Treg cells in shaping the spectrum of $CD8^+$ T cells reactive to viral antigens.[28] In keeping with these diverse roles, Treg cells leverage transcriptional programs that are controlled by the same TFs as those employed by the cells they regulate.[29–31] Thus, the impact of Treg cells on immunological function is now recognized to be far broader than the maintenance of self-tolerance.

Treg cells undergo differentiation in response to T cell receptor (TCR) engagement at fundamentally different sites and times. In the thymus, exposure to self-antigens drives differentiation (or selection)[32–34] and results in a self-focused pool of thymic (t)Treg cells. In peripheral lymphoid organs, the von Boehmer and Lafaille groups showed that foreign antigens can elicit, under particular circumstances, the conversion of conventional T (Tconv) cells into $FoxP3^+$ peripheral (p)Tregs,[35,36] yielding a non-self-focused pool of pTreg cells. Indeed, pTreg cells play an important role in tolerance to the environment at organismal borders,[37] maintaining peaceful co-existence with microbes and food antigens. The generation and population set points of these barrier Treg cells involve both cognate recognition of foreign antigens and the influence of microbe-generated metabolites.[38–48]

Treg cells employ a diverse armamentarium in their regulatory activities,[49,50] including inhibitory cytokines (transforming growth factor β [TGF-β], IL-10, and IL-35) and small molecules (cAMP and adenosine via CD39/73), trogocytosis, and stripping of ligands from antigen-presenting cells (APCs) (by CTLA-4), but perhaps most importantly, their high-affinity IL-2 receptors deprive neighboring cells of this crucial growth factor. In fact, since IL-2 is so central to their differentiation, trophic control, and localization,[51–55] one might argue that the relation to IL-2 is as important as FoxP3 is in defining a Treg cell.

How does FoxP3 work?

The dependence of Treg cells on FoxP3 was initially portrayed as the influence of a "master regulator," a pioneer TF that opens chromatin regions and sets the transcriptional program of a cell lineage. But a more nuanced view eventually emerged. Transduction experiments showed that FoxP3 can elicit, directly or indirectly, only a fraction of the transcriptional signature typical of Treg cells.[56,57] Samstein et al. showed that, during Treg differentiation, FoxP3 exploits a pre-existing chromatin landscape rather than molding one *ab initio*.[58] Accordingly, and in a surprising counterpoint to the key 2003 papers, the Chatila and Rudensky groups discovered the existence of "Treg wannabes," Treg-like cells that differentiate in FoxP3-deficient mice and humans.[59–61] More recently, acute elimination of FoxP3 using DEGRON strategies had surprisingly limited effects on already established Treg cells.[62,63] Interestingly, the DEGRON experiments help close the loop with the origin story of Treg cells: it has always been puzzling that neonatal thymectomy induces autoimmunity only when performed during a narrow time window. The recent results support the observation of a unique pool of Treg cells that differentiate shortly after birth and cannot be replaced by adult-generated Treg cells.[64]

How FoxP3 functions as a TF is still incompletely understood. FoxP3 was initially considered a repressor (especially of *Il2* expression, which it does antagonize), but it remains in debate just how much it operates as a transcriptional

activator versus repressor, directly or indirectly, alone or in the context of other cofactors.[65–67] Even answering the apparently simple question of how FoxP3 binds DNA has proven vexing. Structural biology experiments have successively proposed differing FoxP3 structures, and it remains unclear today which of these structures and motifs actually operate in the Treg nucleus.[68,69]

Location, location

A peculiar function of Treg cells involves maternal-fetal tolerance. In particular, Treg cells suppress responses against paternal histocompatibility antigens. Pregnancy induces a marked expansion of maternal Treg cells, the depletion of which leads to rejection of the fetus, while adoptive transfer of Treg cells restores tolerance.[70,71] Reduced Treg frequency or function is linked to recurrent miscarriages.[72] The Rudensky lab showed that an evolutionarily acquired enhancer of FoxP3 expression, CNS1, enables extrathymic pTreg differentiation at the fetal-maternal interface, suggesting that fetal tolerance might be a primordial function of FoxP3 in placental mammals[73]—although the pro-regenerative FoxP3$^+$ T cells found in zebrafish[74] may temper this interpretation.

In cancer, tumor-infiltrating Tregs have a substantive impact on the prognosis and response to immunotherapy,[75,76] most intuitively by inhibiting productive responses and promoting T cell dysfunction. Treg cells are elevated in the peripheral blood of cancer patients, and increased intra-tumoral Treg cell frequency correlates with reduced survival for multiple solid tumors,[77–79] albeit not always. Acute depletion studies demonstrated that Treg cells represent a major barrier to anti-tumor immunity in multiple mouse models of cancer.[80–82] Treg cell expression of CCR4 and CCR8 facilitates their intra-tumoral recruitment, a finding that has promoted the development of therapeutics to limit Treg cell infiltration.[83,84] Tumors create a challenging environment for all cell types, including Treg cells.[85] Transcriptional studies reveal specific tumor Treg signatures, generally similar to those of tissue Treg cells but with particular features and fragilities,[82,84,86,87] and the specific requirement for FoxP3 noted earlier.[63] Treg cells also adapt to the tissue microenvironment by utilizing unique metabolic pathways.[88] Responsiveness to checkpoint blockade immunotherapy can be dependent on the degree of Treg cell fragility and dominance.

Another extension of Treg cell function into non-immunological realms is represented by so-called "tissue Tregs," found in organs such as adipose tissue, skin, lung, or sterilely injured muscle.[89–92] These cells not only have many of the canonical Treg transcriptional features, including FoxP3 dependence, but they also adapt to their tissular context. The non-immunological functions they control vary widely—hair growth, insulin sensitivity, myocyte repair, and lung fibrosis—but often involve some impact on inflammation and an interaction with specialized stem or progenitor cells in those tissues.[90,91] Tissue Treg cells facilitate the smooth resolution of inflammation after tissue injury, proper repair and regeneration of the tissue subsequently, and the avoidance of fibrosis. These functions may be those most evolutionarily conserved, as suggested by the activities of FoxP3$^+$ T cells in zebrafish.[74] Tissue Treg cells and their direct control of non-immunological tissue homeostasis exemplify the spread of knowledge on Treg cells beyond their classical immune suppressive role.

Coda

The different epochs of Treg cells have thus spanned from enticing but nebulous phenomena to molecularly driven explorations over the last two decades, and it is the transition between the two that the Nobel committee has justly recognized. What will the next phase bring? Clarification of some of the remaining questions, and the hope that the many types of Treg-related therapeutic applications—Treg cell therapy, Treg cell engineering, Treg-inducing vaccines, and CAR-Treg cells, among others—will have the promised impact on cancer and autoimmune disease.

DECLARATION OF INTERESTS

C.B. is an advisory board member of Repertoire and Actus; D.A.A.V. is a cofounder or scientific advisory board member of Novasenta, Trishula, Werewolf, Apeximmune, and T7/Imreg Bio. R.A.F. is an advisory board member of Odyssey and TR1X Bio; D.M. is co-founder or scientific advisory board member of Trex Bio and Zag.

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