Re-defining the immune peace: The enduring legacy of three physiologists in peripheral immune tolerance

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INTRODUCTION

It was a voyage dedicated to finding nature's splendor. This serene journey was shared with a life partner and pet dogs. They travelled together along a secluded trail deep within the grand Yellowstone National Park. Those who are fond of nature and wildlife can deeply appreciate the bracing surge of Sir Ramsdell's experience on that untamed trail. Yet, that memorable excursion did not culminate simply in the tranquillity of unburdened exploration; it ended, instead, with an astonishing revelation. When his wife's sharp yell echoed through the forest, Ramsdell initially feared that she had encountered a Grizzly Bear. In reality, hundreds of congratulatory messages flooded her phone, exclaimed Laura O' Neil. The 65-year-old hiker had, in fact, ascended the pinnacle of global recognition: the 2025 Nobel Prize in Physiology or Medicine, an honour he shared with two distinguished researchers: Mary Brunkow of the Institute for Systems Biology in Seattle and Shimon Sakaguchi of Osaka University in Japan.

Mary E. Brunkow, a 64-year-old Princeton University alumna, received a phone call from an unknown number with a Swedish area code. She thought it was spam, switched off her phone, and went to sleep. Her husband's decisive action woke her, and the subsequent events are now legendary.

The journey began in the 1980s with Shimon Sakaguchi, who then struggled to secure funding for research. The journey was not smooth indeed. The discovery of a novel T cell population that can combat autoimmunity elucidates the mechanisms of Peripheral Immune Tolerance. Peripheral tolerance is the immune system's second line of defence, acting in the body's tissues to control self-reactive T cells that escape elimination during central tolerance in the thymus. The scientific community at the time was somewhat hesitant to accept the new concept of Peripheral Immune Tolerance. However, work by Brunkow and Ramsdell, published in the 1990s and early 2000s, showed how these novel T cells function.

For decades, immunologists weren't certain why some immune cells functioned as they should and why others went rogue and attacked the body's own tissues, resulting in autoimmune conditions. Earlier, it was believed that self-reactive T cells are eliminated by negative selection in the thymus, a process known as central immune tolerance, a crucial step in the adaptive immune system (Kappler 1987). Later, it was observed that some self-reactive T cells evade thymic deletion, prompting researchers to consider the existence of an additional mechanism that suppresses these cells. In 1987, the concept of co-stimulation emerged. It stated that T cells are not activated by simply "seeing" an enemy. They need a second confirmation signal. In the absence of this co-stimulatory signal, the T cell enters an anergic state. This prevents T cells from accidentally attacking healthy body tissue. CD28 on T cells mediates this co-stimulatory signal. CD80 or CD86 on antigen-presenting cells binds to CD28, and, ultimately, T cells receive the signal to attack. Another twist was introduced with the discovery of CTLA-4 by Allison (Nobel Prize in Physiology or Medicine, 2018). CTLA-4 is structurally similar to CD28 and binds to the identical ligands (CD80/CD86). However, instead of activating the cell, CTLA4 sends a negative signal. It acts as a brake, preventing the immune response from becoming uncontrolled.

Sakaguchi's experiment using a murine thymectomy model reconfirmed that, in addition to central immune tolerance, other mechanisms contribute to the prevention of autoimmunity (Figure 1A & B). Sakaguchi discovered a new class of T cells, known as T regulatory cells (Tregs), characterised by the expression of a novel cell-surface protein. In the 1980s, Sakaguchi removed the thymus of the three-day-old mice, resulting in the development of autoimmune disease. Injected isolated T cells from genetically identical mice conferred protection from autoimmunity, despite the lack of a thymus. First, he thought it might be a T helper cell (CD4+), which he injected. However, the event was the suppression of the immune system, rather than its activation, which is the mechanism of T helper cells.

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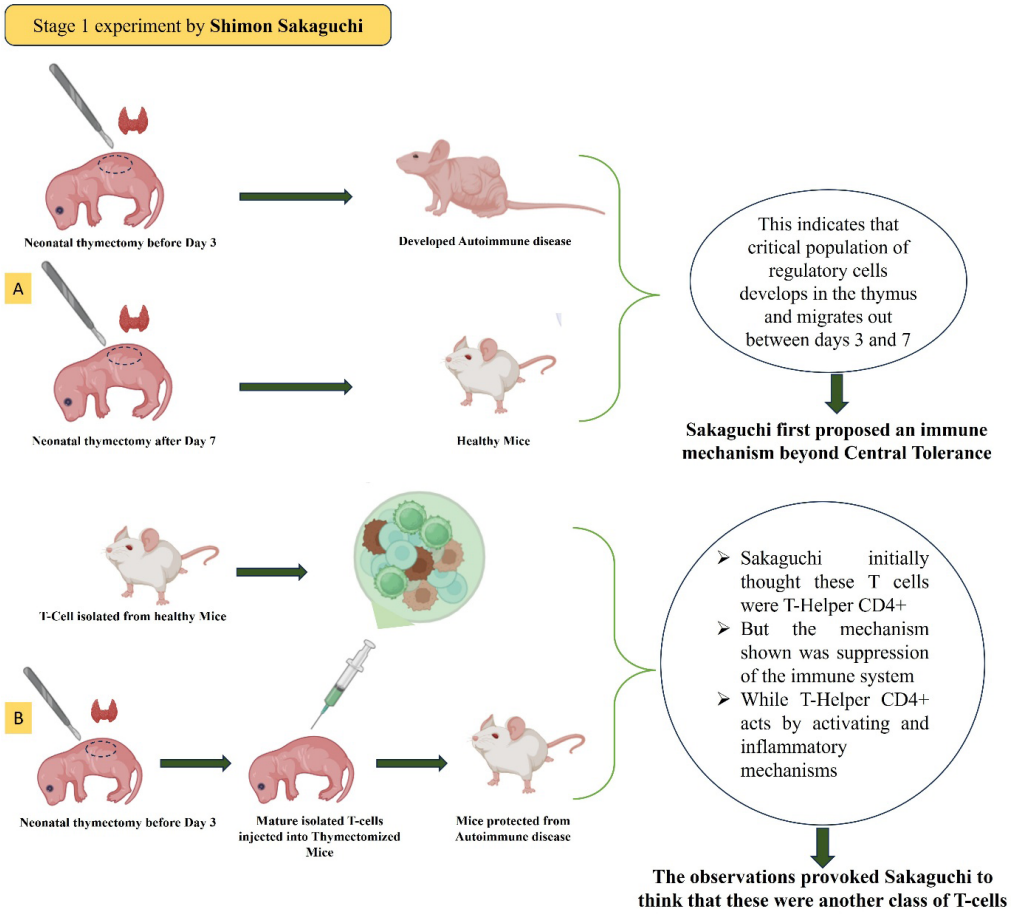

Figure 1: (A & B) The diagram illustrates Shimon Sakaguchi's foundational experiments on Tregs. Final assembly performed in Microsoft PowerPoint 2021 using BioRender icons.

Sakaguchi suspected that these T cells were different, but what were they?

The mystery was solved in 1995 with the publication of Sakaguchi's article in The Journal of Immunology (Figure 2). The new class of T cells is defined by the expression of CD4 and the novel protein CD25+, the α-chain of the IL-2 receptor. CD25 actually triggers peripheral immune tolerance by suppressing overactive immune cells. In brief, Sakaguchi's experiments demonstrated that neonatal thymectomy before day 3 eliminated the wave of T cells that would give rise to natural thymic Tregs, resulting in multi-organ autoimmunity, and that the adoptive transfer of CD4\+CD25\+ cells prevents disease. These experiments identified a distinct CD4\+CD25\+ population with suppressive activity, later termed as Tregs.

These Tregs function as suppressors within the peripheral immune tolerance mechanism. Tregs suppress immune activity by releasing powerful inhibitory signals, such as IL-10 and TGF-β, which inhibit the growth of nearby Effector T cells and APCs, ultimately suppressing their ability to produce inflammatory molecules. The recently discovered IL-35 also contributes to the downregulation of inflammation and induces other T cells to become more suppressive. Tregs starve the dangerous effector T cells by saturating and hogging the supply of IL-2, which is a key nutrient for all T cells. The metabolic suppression of effector T cells is facilitated by Tregs' capacity to generate adenosine. Finally, effector T-cell paralysis occurs through the direct transfer of cAMP by Tregs via gap junctions. This is how, through diverse molecular and cellular strategies, Tregs suppress other effector T cells that might attack the immune system.

The idea that Regulatory Tregs are all the same simple suppressors is outdated. Treg heterogeneity and tissue specialization are key to understanding the nuance of peripheral immune tolerance and how we maintain tissue homeostasis. The heterogeneous Treg population, including thymus-derived Tregs (tTregs), maintains systemic self-tolerance, whereas peripherally derived Tregs (pTregs) mediate localized responses. The most exciting aspect of Treg heterogeneity is tissue specialization. Tregs resident in non-lymphoid tissues (such as fat, muscle, and gut) adopt distinct phenotypes, acquire specific surface markers, and perform specialized functions that extend beyond simple suppression of inflammation. This high degree of specialization ensures precise, context-dependent control over immunity and non-immune functions, reinforcing peripheral tolerance and maintaining tissue homeostasis.

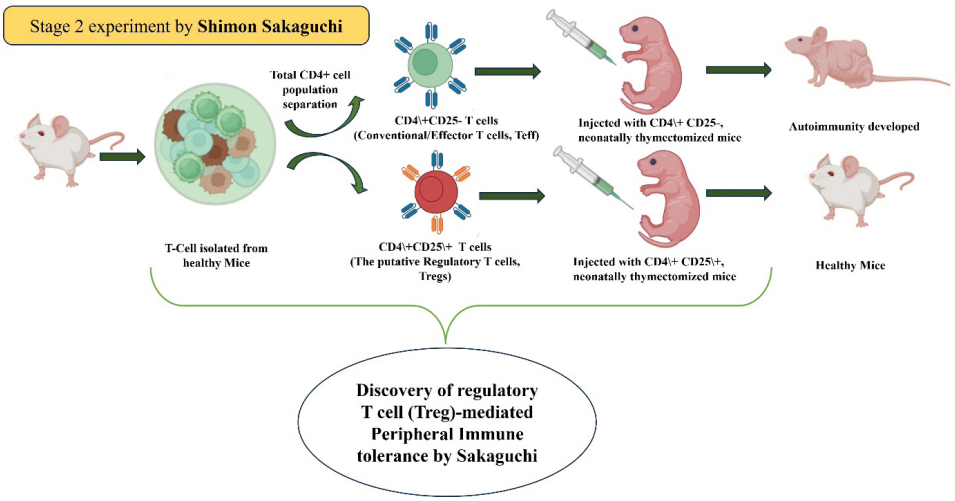

Figure 2: Illustration of Shimon Sakaguchi's stage 2 experiment showing that CD4\+CD25\+ cells are essential for preventing autoimmunity. Final assembly performed in Microsoft PowerPoint 2021 using BioRender icons.

The story could have ended here, but the researchers' skepticism had already prepared two other scientists in another corner of the world. The research initiated by Sakaguchi was finally resolved by independent discoveries by Mary Brunkow and Ramsdell in 2001 (Figure 3). Those discoveries, particularly that of the *FOXP3* gene mutation, were found to be the cause of autoimmune diseases in Scurfy mice.

The male mice developed autoimmunity, but the females did not. This amount of evidence was sufficient to prompt Brunkow and Ramsdell to focus on the X chromosome sequences. Although it was challenging to sequence the large X chromosome at the time, they developed innovative sequencing tools. They were fortunate enough to sequence a short segment of the X chromosome, consisting of approximately 500,000 nucleotides, among the 170 million nucleotides of the X chromosome. After years of dedication and hard work, they finally identified the gene of interest for the scurfy mutation. They named the previously unnamed gene FOXP3 because of its similarity to previously known forkhead box (*FOX*) genes. Their work was published in Nature Genetics, claiming that this *FOXP3* mutation is responsible for IPEX disease (autoimmune), a condition linked to the X chromosome. With Brunkow and Ramsdell's findings, Sakaguchi again began to recall the missing puzzles. The question was: Does *FOXP3* regulate these Tregs to control the immune system, and does its mutation lead to autoimmunity? Yes, with other researchers, Sakaguchi finally solved the puzzle by retroviral transfer of *FOXP3,* which converts conventional T cells into Tregs (Figure 4). The *FOXP3* gene regulates Treg cell function, providing insight into why, as hominins, we have not been constantly plagued by autoimmune diseases. These Tregs prevent other T cells from attacking their own body tissue, which is a key event in peripheral immune tolerance. FOXP3 is the master transcriptional regulator of Treg cell programming. During inflammatory stress, sustained FOXP3 expression in Tregs is required to convert proinflammatory signals into anti-inflammatory ones, thereby providing stability to Tregs. Epigenetic, metabolic, and transcriptional factors are strictly monitored and run the Treg stability program. Demethylation of the Treg-specific demethylated region provides stability; conversely, transcription factors such as STAT5, NFAT, and Runx bind to the promoter or enhancer regions of the Treg-specific gene and coordinate *FOXP3* expression. Taken together, the two pivotal discoveries: the discovery of Tregs with cell surface markers CD4\+CD25\+ and the identification of *FOXP3 by* Shimon Sakaguchi (Osaka University, Osaka, Japan), Mary E. Brunkow (Institute for Systems Biology, Seattle, USA), and Fred Ramsdell (Sonoma Biotherapeutics, San Francisco, USA) respectively established that the loss of a single cell population, Tregs, due to a defect in the *foxp3/FOXP3* gene locus, is enough to trigger the complete breakdown of immune tolerance and cause autoimmunity in both mice and humans.

Finally, in recognition of their three decades of work in developing the immune peace, these three received the Nobel Prize in Physiology or Medicine in 2025. The Nobel Prize-winning work provided a fundamental biological and molecular roadmap, identifying Tregs and their master switch, FOXP3, which underpins these cutting-edge therapeutic approaches and accelerates their translation from the lab to the clinic. While multiple Treg-targeted strategies, including metabolic modulators and the novel checkpoint inhibitors, are currently being explored, cell-based therapies such as TCR-Tregs and CAR-Tregs have garnered particular attention. Following extensive preclinical development, the first clinical trial of adoptive Treg transfer was successfully conducted in patients with Graft-versus-Host Disease (GVHD). This pioneering success quickly paved the way for allogeneic adoptive transfer to be implemented in a range of challenging conditions, including Crohn's disease, stem cell transplantation, and Type 1 diabetes, ushering in an era of targeted immune peace. The advent of CAR-Tregs promises

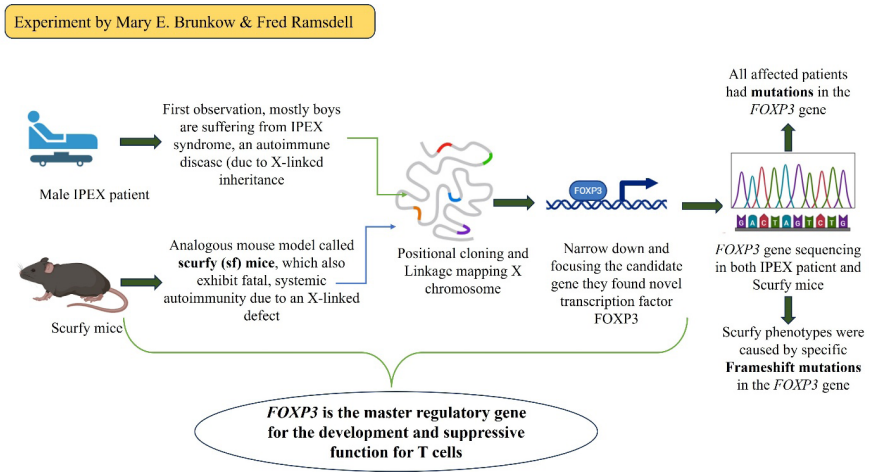

Figure 3: Illustration of Mary E. Brunkow and Fred Ramsdell's experiments showing that the *FOXP3* gene is responsible for IPEX syndrome in humans and the "Scurfy" phenotype in mice, respectively. Final assembly performed in Microsoft PowerPoint 2021 using BioRender icons.

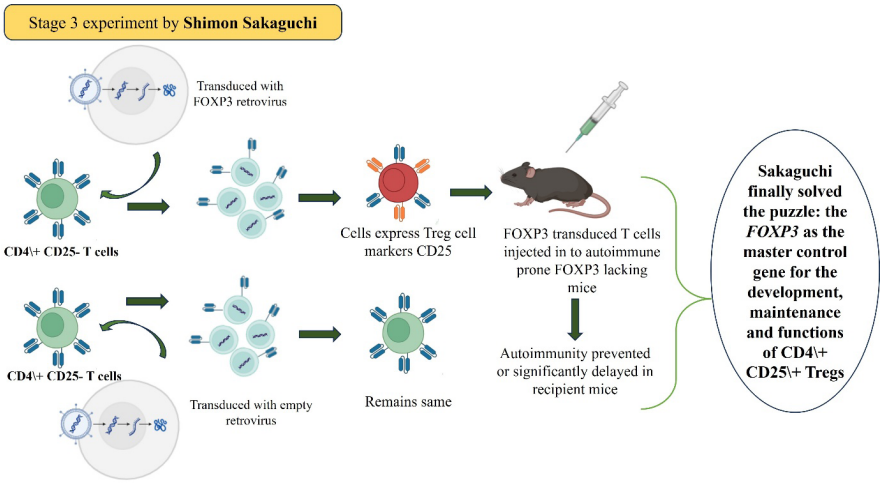

Figure 4: Functional characterization of the *Foxp3* gene by Sakaguchi and colleagues. The diagram illustrates how the introduction of the *Foxp3* gene into effector T cells induces a regulatory phenotype, demonstrating that Foxp3 is the master control switch for Treg function. Final assembly performed in Microsoft PowerPoint 2021 using BioRender icons.

a future in which immune disorders can be treated with unparalleled specificity, offering the potential for long-term remission in conditions that previously required lifelong medications.

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PEER-REVIEWED CERTIFICATION

During the review of this manuscript, a double-blind peer-review policy has been followed. The author(s) of this manuscript received review comments from a minimum of two peer-reviewers. Author(s) submitted revised manuscript as per the comments of the assigned reviewers. On the basis of revision(s) done by the author(s) and compliance to the Reviewers' comments on the manuscript, Editor(s) has approved the revised manuscript for final publication.