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Scientific background 2025

Immune tolerance
The identification of regulatory T cells and FOXP3

The immune system is a marvel of intricate checks and balances, enabling robust defences against infections while, in most cases, avoiding destructive responses against the body's own tissues. How is this balance maintained? This question has puzzled immunologists for more than a century. Through a combination of insightful observations and carefully designed experiments, this year's Laureates of the Nobel Prize in Physiology or Medicine, Mary E. Brunkow, Fred Ramsdell and Shimon Sakaguchi, made discoveries that provided critical answers. By defining CD4⁺CD25⁺FOXP3⁺ regulatory T cells (Treg cells) and their importance in the control of self-reactive responses, their work decisively launched the field of Treg cell-mediated peripheral immune tolerance. The story is one of scientific curiosity, persistent investigations and critical discoveries that have revolutionized the understanding of immune regulation, with relevance for self-tolerance, autoimmunity and tumour evasion.

A robust immune system is critical for our survival and health. Without it, we would be highly vulnerable, as we are constantly exposed to microbes in our environment. The immediate response following an infection is provided by the innate immune system, while the adaptive immune system requires a few days to be mobilised. A hallmark of the adaptive immune system is its ability to "remember" pathogens we have been exposed to previously, so we can respond quicker and more efficiently the next time we encounter the same agent. Immunological memory is mediated by our T cells and B cells, which recognise foreign structures (antigens). The recognition is mediated by antigen receptors called T cell receptors (TCRs) and B cell receptors (BCRs/membrane-bound antibodies), which are generated during lymphocyte development. Each lymphocyte has its own unique antigen receptor and collectively, this provides our immune systems with an enormous capacity to recognise any potential foreign structure we may encounter during our lifetime.

How is antigen recognition achieved?

The question of how we can make so many different antigen receptors was a mystery prior to the 1980s. It was clear that there was not enough space in the genome for a "one gene-one receptor" explanation. The mechanisms that finally explained how highly diverse BCR repertoires are generated came from Susumu Tonegawa, who received the Nobel Prize in Physiology or Medicine in 1987. He demonstrated that a large set of germline-encoded genes, the so-called *variable* (V), *diversity* (D), and *joining* (J) genes, are assembled in a combinatorial manner when B cells are formed, resulting in a unique pair of heavy (VDJ) and light (VJ) chains that make up the functional receptor in each cell (Tonegawa 1983). This remarkable set of findings was soon followed by the identification of genomic loci encoding the TCR V, D, and J genes, by the groups of Tak Mak at the University of Toronto and Mark Davis at Stanford University (Hedrick et al. 1984; Yanagi et al. 1984). Like BCRs, TCRs are formed by recombination of V,

D and J genes, theoretically giving rise to more than 10^{15} distinct receptors in each individual. TCRs consist of either an alpha (α) chain paired with a beta (β) chain (**Figure 1**), or a gamma (γ) chain paired with a delta (δ) chain, yielding either αβ T cells or a γδ T cells. Most of our T cells are αβ T cells, including the regula-tory T cells that are central to this year's Nobel Prize in Physiology or Medicine.

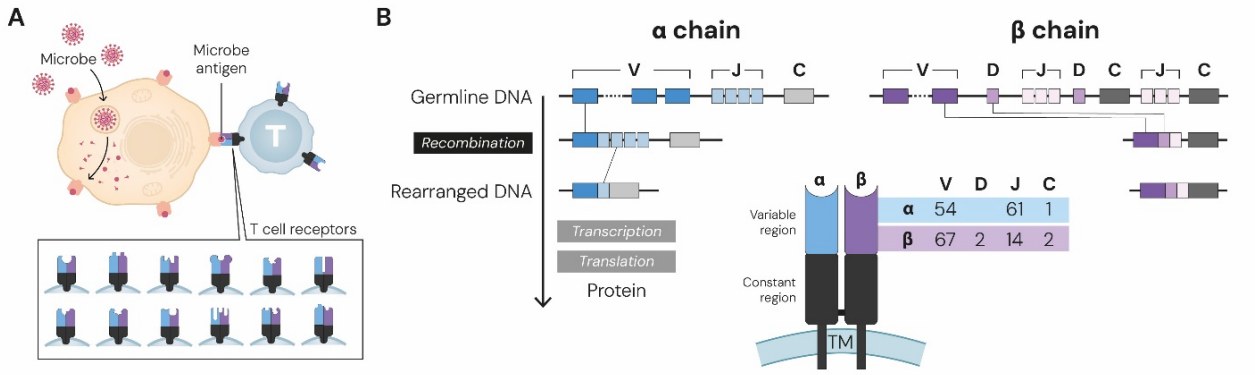

Figure 1. A. T cells recognise microbial peptides through their TCRs, which bind peptide fragments presented by MHC molecules. This interaction can trigger T cell activation. Each T cell expresses a unique TCR, generating highly diverse T cell repertoires capable of recognizing an almost unlimited number of foreign peptides. **B.** TCR diversity arises from the combinatorial assembly of multiple germline-encoded genes, together with insertion and/or trimming of non-templated nucleotides at the V-J junction of the α–chain and the V-D and D-J junctions of the β-chain. The human TCRα locus contains 54 V genes, 61 J genes and 1 constant (C) gene and the TCRβ-locus contains roughly 67 V genes, 2 D genes, 14 J genes, and 2 constant genes. The immense diversity generated by V(D)J recombination inevitably results in some degree of self-reactivity within the repertoire.

Work performed in the 1970s demonstrated that αβ T cells can be divided into subsets that play complementary roles in the immune response (Gershon and Kondo 1970; Reinherz et al. 1979). The two main types are $CD4^+$ T helper cells, which provide critical supportive functions for both the cellular and humoral arms of the immune system, and $CD8^+$ cytotoxic T cells, which recognise and eliminate infected cells and tumour cells. Both types of T cells recognise antigens presented by Major Histocompatibility Complex (MHC) molecules present. The gene clusters encoding the MHC molecules had been discovered during the 1940s and 1950s, first in mice by George Snell (Snell 1948), and a decade later, the corresponding human genes encoding the Human Leukocyte Antigen (HLA) molecules were described by Jean Dausset (Dausset 1958), earning them the 1980 Nobel Prize in Physiology or Medicine together with Baruj Benacerraf.

The MHC molecules were discovered by their capacity to induce graft rejection following transplantation, but their physiological function(s) remained an enigma. Critical findings were provided by Peter Doherty and Rolf Zinkernagel in the 1970s when they demonstrated that T cells only respond to viruses in the context of MHC molecules, referred to as MHC restriction (Zinkernagel 1974; Zinkernagel and Doherty 1974, 1975), a finding, which earned them the Nobel Prize in Physiology or Medicine in 1996. This intriguing finding was further explained by experiments performed by Alain Townsend

Fact box. Clusters of differentiation (CD) nomenclature
The CD system was established at the First International Workshop on Human Leucocyte Differentiation Antigens, organized by the IUIS-WHO Nomenclature Subcommittee in 1984 (Bernard and Boumsell 1984). Since then, over 400 CD markers have been defined. Thanks to the development of increasingly sophisticated techniques for single cell analyses, we now know that $CD4^+$ and $CD8^+$ T cells can be divided into many different subtypes and differentiation states.

who demonstrated that $CD8^+$ cytotoxic T cells recognise peptides presented by MHC class I molecules, while $CD4^+$ T helper cells recognise peptides presented by MHC class II molecules (Townsend, Gotch, and Davey 1985; Townsend et al. 1986), Emil R. Unanue (Babbitt et al. 1985), with structural studies performed by Pamela Bjorkman and Don Wiley (Bjorkman et al. 1987).

While the generation of vast BCR and TCR diversity maximises the capacity of our immune systems to recognize foreign antigens, it comes at a cost, as some receptors inadvertently recognise self-antigens – a phenomenon that Paul Ehrlich, 1908 Nobel Prize Laureate, described as "*horror autotoxicus*". It would turn out to be a major challenge to unravel the molecular basis for severe autoimmune manifestations, but from the work of Ehrlich and others, we know that clinicians and researchers already in the early 1900s recognised the risks associated with a powerful immune system. They realised that elucidating the cellular and molecular mechanisms underlying immune regulation would be crucial for understanding immune-mediated diseases and for the development of treatments against these diseases.

How is immune tolerance achieved?

So, how is immune tolerance achieved and maintained? The first experiments to address this question were performed by Ray Owen, who published his findings in *Science* in 1945 (Owen 1945). He studied calves with different blood groups that shared a common placental circulation and developed chimerism. He unexpectedly observed that they did not elicit an immune response to each other's blood group antigens after birth, providing an important clue. Around the same time, Sir Frank Macfarlane Burnet wrote: "*If, in embryonic life, expendable cells from a genetically different race are implanted and established, no antibody response should develop against the foreign antigen when the animal takes on an independent existence*" (Burnet and Fenner 1949). Burnet thus predicted that immune tolerance must be acquired during embryonic or early life, although he was possibly already aware of Owen's observations.

In 1953, Peter B. Medawar and co-workers at University College, University of London published results from experiments in which foetuses of one inbred mouse strain were inoculated with cells from a second strain (Billingham, Brent, and Medawar 1953). In these experiments, skin grafts from the second strain were accepted after birth of the mice, but grafts from a third unrelated mouse strain were rejected. They proposed that this mechanism was due to "actively acquired tolerance". The same year, the Czecho-slovakian scientist Milan Hašek reported findings consistent to those of Owen when studying eggs wherein the vascular systems of chick embryos had been joined, a procedure known as parabiosis (Hašek 1953). These studies were published in a local scientific journal in Russian and were overlooked for a long time.

Soon thereafter, Burnet published an influential paper on clonal selection (Burnet 1957), proposing that selection occurred at the cellular level rather than, as commonly believed at the time, at the antibody level. He expanded these ideas, which mainly related to positive selection of immune cells (Burnet 1959), and in 1960, the Nobel Prize in Physiology or Medicine was awarded to Sir Frank Macfarlane Burnet and Peter B. Medawar for their "*discovery of acquired immunological tolerance*". Burnet later amended his theory to include negative selection, i.e., the removal of undesired T cells from the immune repertoire as a mechanism for acquired immune tolerance to self (Burnet 1962; Burnet 1969).

Notably, the function of the thymus (and hence also the existence of T cells) had remained obscure until Jacques Miller, then at the Chester Beatty Research Institute in London, demonstrated that neonatal thymectomy in mice resulted in severe immune deficiency and the absence of specific lymphocyte subsets (Miller 1961b, 1961a). More than 20 years later, Philippa Marrack and John W. Kappler, then at the National Jewish Center for Immunology and Respiratory Medicine in Denver, USA, and Harald von Boehmer and co-workers at the Basel Institute for Immunology in Switzerland, provided experimental evidence that self-reactive T cells are eliminated by negative selection in the thymus (Kappler, Roehm, and Marrack 1987) (Kisielow et al. 1988).

In the following years, investigators sought to understand the molecular basis of central tolerance, where self-reactive T cells are deleted in the thymus. An important step was the identification of the AIRE (autoimmune regulator) gene by two consortia, one led by Leena Peltonen (Finnish-German Apeced Consortium 1997) using a large cohort of patients with the rare disorder autoimmune polyendocrine syndrome type 1 (APS-1) – also named autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) – collected by the Finnish paediatrician Jaakko Perheentupa (Perheentupa 1996), and the other by the team of Nobuyoshi Shimizu (Nagamine *et al.* 1997).

Mark Anderson, Christophe Benoist and Diane Mathis were the first to explain how AIRE, a transcription factor, enables the elimination of self-reactive T-cells in the thymus *(*Anderson *et al.* 2002). They showed that AIRE activates the expression of tissue-specific antigens in medullary thymic epithelial cells (mTECs), thereby allowing newly formed T cells to be tested for potential self-reactivity. In the absence of significant self-reactivity, the T cells are left intact and exit the thymus to enter the circulation. In contrast, strongly self-reactive T cells undergo elimination through apoptosis.

The elimination of self-reactive, potentially harmful T cells from the naïve T cell repertoire became known as *central tolerance*, a fundamental pillar of the adaptive immune system. For many years, central tolerance was believed to be the sole mechanism preventing T cells from reacting against our own tissues. Although AIRE-mediated negative selection is robust, it is not absolute and some self-reactive T cells escape deletion in the thymus and enter the circulation. This led researchers to speculate that additional mechanisms must exist to contain these cells. A major question was whether a specialised suppressive T cell subset existed, which we will return to below, but researchers also wished to understand immune tolerance mechanisms in a broader sense, including those involving other immune cells.

Peripheral immune mechanisms in play

One model concerned the concept of co-stimulation, which suggested that additional cell-to-cell interactions, beyond TCR-MHC/peptide binding, were required to activate T cells. Early work by Mark Jenkins and Ron Schwartz at the National Institutes of Health, Bethesda, USA, demonstrated that TCR engagement in the absence of co-stimulation caused T cells to become unresponsive, or anergic (Jenkins and Schwartz 1987). This led to the identification of CD28 on T cells by the laboratories of Craig Thompson (Turka et al. 1990) and James Allison (Gross, St John, and Allison 1990), and its interaction with CD80 or CD86 on antigen-presenting cells (APCs) to mediate a positive co-stimulatory signal (Linsley, Clark, and Ledbetter 1990). Allison was awarded the Nobel Prize in Physiology or Medicine in 2018, for his discovery that

antibodies against the negative co-stimulatory molecule, CTLA4 (CD152), which also binds CD80 and CD86, can active the T cell response against tumours. Many investigators subsequently contributed to the field of co-stimulation, as reviewed in Chen et al. (Chen and Flies 2013), firmly establishing this extra layer of control.

Another related line of investigation focused on a class of APCs called dendritic cells (DCs), which play a major role in activating naïve T cells during immune priming. In the early 2000s, one of the Laureates of the Nobel Prize in Physiology or Medicine 2011, Ralph Steinman, showed that the targeting of antigen to DCs in the absence of co-stimulation induced T cell tolerance (Hawiger et al. 2001). He named these cells tolerogenic DCs (Steinman, Hawiger, and Nussenzweig 2003). In even earlier work addressing B cell-mediated tolerance, Christopher Goodnow and Antony Basten used BCR transgenic mice to demonstrate the concept of B cell clonal anergy (Goodnow et al. 1988). Regulatory B cells that produce IL-10, an important cytokine for dampening immune responses, have also been described. This is a growing and intriguing field, as comprehensively reviewed (Lund et al. 2005). Thus, there are multiple mechanisms of peripheral immune tolerance in place to safeguard us against undesired self-reactivity. One of the most important mechanisms involves regulatory T cells and we will now return to T cell-mediated peripheral tolerance against self, the topic of this year's Nobel Prize in Physiology or Medicine.

Suppressor T cells: a detour

In the 1970s, the concept of "suppressor T cells" had emerged, based on experiments showing that certain T cell populations could suppress immune responses *in vitro* and *in vivo* (Gershon and Kondo 1970). However, the field was plagued by inconsistent methodologies, a lack of specific markers, and the inability to definitively distinguish these cells from other T cell subsets. Suppressor cells were thought to be a $CD8^+$ T cells subset, and the I-J locus in mice was reported to encode functionally important determinants of suppressor T cells. With the emergence of more accurate gene sequencing technologies, it became clear that the I-J locus did not exist (Kronenberg et al. 1983). As a result, enthusiasm waned, and the notion of suppressor T cells faced growing scepticism until the field eventually collapsed (Keating and Cambrosio 1997). However, some of the reported results were likely correct even though they were not sufficiently conclusive.

The regulatory T cell renaissance

The field of immune regulation gained new traction in the late 1980s and 1990s with Shimon Sakaguchi and colleagues in Japan playing a pivotal role. As early as 1969, Yasuaki Nishizuka and Teruyo Sakakura demonstrated that removal of the thymus from newborn mice day 3 postpartum led to spontaneous development of ovarian dysgenesis due to autoimmune ovarian insufficiency along with various other autoimmune diseases including gastritis and thyroiditis (Nishizuka and Sakakura 1969). In 1973, William J. Penhale extended these observations by showing that neonatal thymectomy of rats induced autoimmune thyroiditis with the development of thyroid autoantibodies (Penhale *et al.* 1973). He later demonstrated that the thyroiditis could be prevented if lymphocytes from healthy animals were injected into the thymectomized rats (Penhale *et al.* 1976).

After completing his medical studies at Kyoto University, Shimon Sakaguchi joined Nishizuka's laboratory at the Aichi Cancer Center Research Institute. In 1982, they provided evidence consistent with Penhale's work in rats, that the autoimmune process in thymectomized mice could be abrogated by transferring a specific fraction of immune cells

from normal mice, characterized by expressing the surface marker CD5 (Lyt-1) and low levels of CD45RB (Sakaguchi, Takahashi, and Nishizuka 1982). Notably, these studies were performed using allosera to classify cells, before monoclonal antibodies directed against distinct cell surface markers became available. Thus, the toolbox available for detailed molecular examination of cell phenotypes was limited, but this would soon change.

Because the question of immune tolerance is fundamental to our understanding of immunology, it attracted much interest, and many accomplished scientists joined the research field. With the increasing availability of monoclonal antibodies against cell surface antigens, Fiona Powrie and Don Mason at the University of Oxford demonstrated that $CD4^+$ T helper lymphocytes expressing high levels of CD45RB induced a severe wasting disease, characterized by inflammatory infiltrates in liver, lung, stomach, thyroid and pancreas, when injected into athymic mice. In contrast, injection of the $CD4^+$ lymphocyte subset expressing low levels of CD45RB protected the animals from disease (Powrie and Mason 1990). They concluded that functional heterogeneity and specialization within the peripheral $CD4^+$ T cell population could account for mechanisms of immune regulation.

The first breakthrough – defining the critical subpopulation of T cells

Sakaguchi continued his diligent work to understand immune regulation. He aimed to further fractionate the $CD5^+/CD45RB^{low}$ T cells he had described in his earlier publication (Sakaguchi, Takahashi, and Nishizuka 1982) by separating the cell population based on additional surface markers. In a landmark 1995 study, Sakaguchi and co-workers showed that $CD4^+$ T lymphocytes expressing the surface marker CD25, the alpha chain of the IL-2 receptor, possessed immune regulatory functions (Sakaguchi *et al.* 1995). When Balb/c athymic nude (nu/nu) mice were injected with $CD4^+$ cells from spleen and lymph nodes depleted of $CD25^+$ T cells from heterozygous Balb/c nu/+ mice, they developed histologically and serologically evident autoimmune diseases, including thyroiditis, gastritis, insulitis, adrenalitis, and polyarthritis. However, re-constitution with $CD4^+CD25^+$ T cells within a limited period after transfer of $CD4^+CD25^-$ T cells prevented the development of autoimmunity (**Figure 2**). These findings indicated that the $CD4^+CD25^+$ T

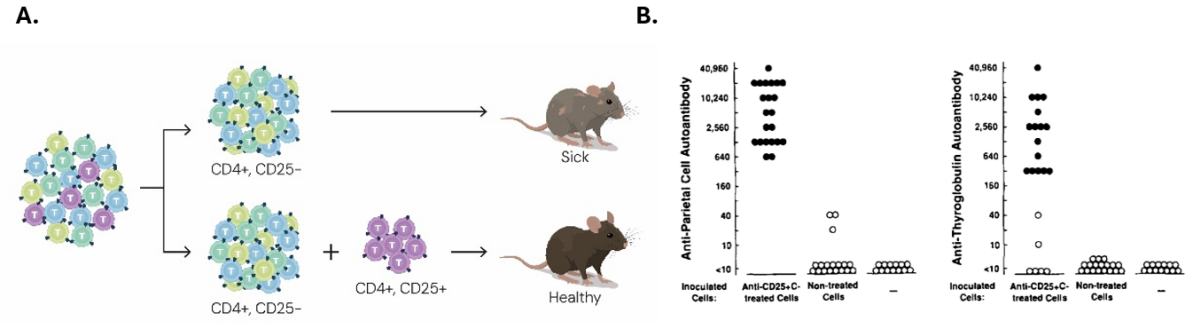

Figure 2. A. Schematic overview of Sakaguchi' cell separation experiment. **B.** Results from Sakaguchi *et al.* 1995 showing Balb/c nu/nu mice injected with spleen and lymph node cells from heterozygous Balb/c nu/+ mice that had been treated with anti-CD25 plus complement (Anti-CD25+C-treated cells), treated with complement alone (Non-treated Cells), or left untreated (-). Autoantibody titers against parietal cells and thyroglobulin, features of autoimmune gastritis and thyroiditis, respectively, are shown.

cells were essential for maintaining immune self-tolerance.

This discovery provided robust evidence for the existence of a regulatory $CD4^+CD25^+$ T cell subset capable of dampening immune responses, a finding that was soon supported by results from the laboratory of Ethan Shevach at the National Institutes of Health, Bethesda, USA (Thornton and Shevach 1998) and others. The identification of $CD4^+CD25^+$ T cells marked a breakthrough in immunology and laid the foundation for further in-depth investigations. The term regulatory T cell had been occasionally used in the 1970s and 1980s (Baker 1975; Chaouat *et al*. 1982). However, it was only after Sakaguchi defined the $CD4^+CD25^+$ subset as regulatory T cells (Sakaguchi 2000) that the term Treg cells gained wide acceptance in the scientific community. Sakaguchi and co-workers demonstrated that the majority of the regulatory T cells originated in the thymus (Itoh *et al*. 1999). They also identified CTLA-4 as an additional marker of these cells, simultaneously with Powrie's group, in back-to-back publications (Read, Malmström, and Powrie 2000; Takahashi et al. 2000).

In parallel investigations in transplantation biology, Bruce Hall and Susan Dorsch working in Sydney, Australia, described a lymphocyte subset with inhibitory function in Cyclosporin A-treated rat recipients of heart allografts. Hall and Dorsch described these as $CD4^+$ T cell (Hall, Jelbart, and Dorsch 1984; Hall *et al*. 1985) and later reported that they also expressed $CD25^+$ (Hall et al. 1990). These results provided important information for the field, but Hall and Dorch did not investigate whether the cells they had identified played a role in self-tolerance under normal physiological conditions.

As techniques developed and interest in immune regulation increased, researchers sought to better understand the origins, mechanisms and the defining characteristics of the cells identified by Sakaguchi. While CD25 proved to be a useful marker, it was also expressed, albeit at lower levels, on conventional activated effector T cells (Leonard *et al*. 1982; Uchiyama, Broder, and Waldmann 1981; Uchiyama *et al*. 1981), which limited its specificity for identifying the regulatory T cell subset. Experimental studies demonstrated that these cells could suppress the proliferation and cytokine production of conventional T cells through several mechanisms: cell-cell contact-dependent interactions, secretion of suppressive cytokines such as IL-10, IL-35 and TGFβ, and sequestration of IL-2, thereby depriving other T-cells of a critical growth factor (Goldmann *et al*. 2024). While the capacity of $CD4^+CD25^+$ T cells to maintain tolerance and prevent autoimmunity was known, scepticism remained and a definitive molecular marker was still missing, hampering progress in the field. This would change dramatically with the identification of a mechanism that selectively controls the development and function of the regulatory T cell subset.

The second breakthrough – The scurfy mouse and the discovery of FOXP3

At the Department of Energy's Oak Ridge National Laboratory in Tennessee, as part of the Manhattan Project during the 1940s, researchers studied the effects of radiation on mice. Laboratory personnel noted a spontaneous mutant, which they named scurfy. This strain developed a serious autoimmune disease, and breeding studies revealed that the mutation was lethal if carried by males, while female mice were unaffected. Thus, it was concluded that the mutation responsible for the autoimmune phenotype was located on the X-chromosome (Russell, Russell, and Gower 1959).

The severe multisystem autoimmune phenotype of the scurfy mouse stimulated the attention of Mary Brunkow and Fred Ramsdell at Celltech Chiroscience Inc., in Bothell, WA, US. In an ambitious, curiosity-driven project, they set out to identify the scurfy mutation using the positional cloning techniques available at the time. After meticulous efforts, they narrowed the candidate region on the X-chromosome to 500,000 bases. From this segment they isolated 11 large DNA fragments as bacterial artificial chromosome clones, focusing their attention on 4 of them (**Figure 3**). Random shotgun sequencing revealed that the regions contained approximately 20 genes. They sequenced each gene one after the other and compared them to the corresponding genes in humans and in seven other mouse strain.

Only when the final gene was examined did Brunkow and Ramsdell identify a two-base pair insertion resulting in a frame shift and a premature stop codon. This gene had not been previously described and was absent from the available databases at the time. Because of its homology with other forkhead/winged-helix genes, Brunkow and Ramsdell named the gene Forkhead box P3 (*Foxp3*) (Brunkow *et al.* 2001).

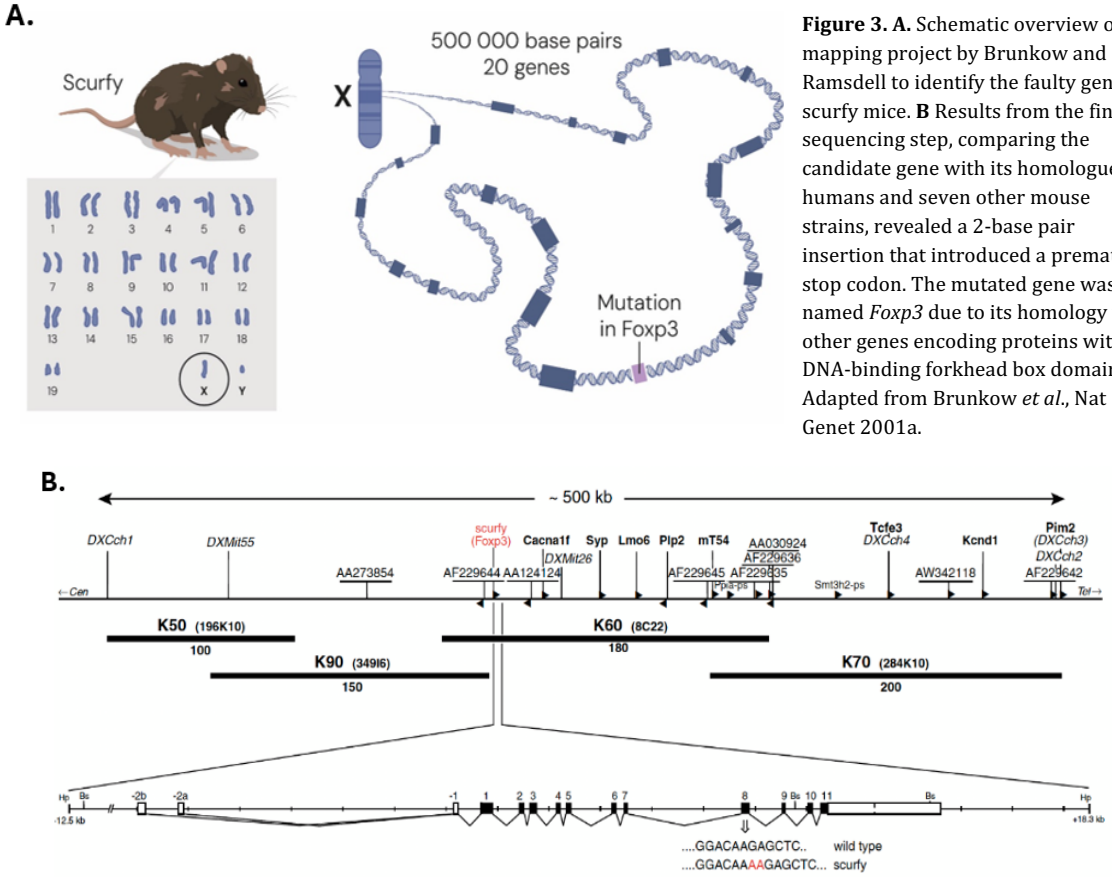

Figure 3. A. Schematic overview of the mapping project by Brunkow and Ramsdell to identify the faulty gene in scurfy mice. **B** Results from the final sequencing step, comparing the candidate gene with its homologues in humans and seven other mouse strains, revealed a 2-base pair insertion that introduced a premature stop codon. The mutated gene was named *Foxp3* due to its homology with other genes encoding proteins with a DNA-binding forkhead box domain. Adapted from Brunkow *et al.*, Nat Genet 2001a.

Brunkow and Ramsdell did not stop there. To formally prove that the mutation was responsible for the scurfy phenotype, they performed a series of genetic rescue experiments. They generated five transgenic mouse lines carrying the *Foxp3* gene, each with a different copy number, and individually crossed them with scurfy mice. Through these elegant and definitive experiments, the team demonstrated that wild-type Foxp3 rescued male scurfy mice from disease (**Table 1**) (Brunkow *et al*. 2001).

Table 1 • Scurfin transgene rescues scurfy disease

Tg line	Tg copy no.	Genotype of males (no. with scurfy disease) wt non-Tg	*sf* non-Tg	wt Tg	*sf* Tg
1292L	3	1 (0)	2 (2)	2 (0)	2 (0)
1292H	9	1 (0)	1 (1)	6 (0)	4 (0)
2826	~16	0	1 (1)	4 (0)	1 (0)
2827	~70	0	2 (2)	0	3 (0)
2828	~45	1 (0)	0	8 (0)	4 (0)
	Totals	3 (0)	6 (6)	20 (0)	14 (0)

For each of the five transgenic lines, the transgene was crossed onto the *sf* mutant background; resulting male progeny were genotyped with respect to *sf* mutation (wt or *sf*) and transgene (non-Tg or Tg) status. The number of animals with scurfy disease within each genotypic class was ascertained (in parentheses). The last column demonstrates that the presence of the transgene, from any of the five lines, prevents disease in genotypically *sf* animals. Tg, transgene; wt, wild type.

Table 1. Wild type *FOXP3* rescues male scurfy mice from disease. From Brunkow *et al*., Nat Genet 2001.

Immediately after their discovery, Brunkow and Ramsdell turned their attention to a rare inherited human disease known as IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome or XLAAD (X-linked autoimmunity-allergic dysregulation) syndrome. This condition is characterised by fatal autoimmunity in young boys unless they receive an allogenic stem cell transplantation. In 2001, in collaborations with Hans Ochs at the Department of Pediatrics, University of Washington, Seattle, USA and Robert Wildin at the Oregon Health Sciences University, Portland, USA and their teams, Brunkow and Ramsdell demonstrated that mutations in *FOXP3,* the human counterpart to the gene mutated in the scurfy mouse, were responsible for IPEX (Bennett *et al*. 2001; Wildin *et al*. 2001).

A team led by Chatila and co-workers at Washington University School of Medicine in St. Louis, USA also studied patients with IPEX/XLAAD. They focused on two transcription factors, Tcfe3 and JM2, the latter being an alternative name for FOXP3. Sequence analysis of the exons of these genes resulted in the identification of mutations in the *JM2* gene in the affected patients (Chatila *et al*. 2000). The findings by Chatila *et al*. provided important insight, but they did not constitute formal experimental evidence for a causative role for the *FOXP3* mutation in immune dysregulation. As noted above, such evidence was established by Brunkow and Ramsdell using the scurfy mouse strain, underscoring the value of combining experimental animal models with clinical studies.

Making the connection and defining the Treg lineage

Following the two pivotal discoveries – the isolation of a regulatory T cell subset with a $CD4^+CD25^+$ cell surface signature (Sakaguchi *et al*. 1995) and the identification of Foxp3 (Brunkow *et al*. 2001) – Shimon Sakaguchi's team rapidly established the connection by demonstrating that *Foxp3* was selectively expressed in $CD4^+CD25^+$ T lymphocytes and that retroviral transfer of *Foxp3* converted conventional $CD4^+$ T cells to Treg cells (Hori, Nomura, and Sakaguchi 2003). Independently and soon thereafter, Ramsdell's group showed that Treg cells were absent in the scurfy mice and that mice overexpressing *Foxp3* exhibited an increased number of Treg cells (Khattri *et al*. 2003). At the same time, Alexander Rudensky at the Howard Hughes Medical Institute and University of Washington, generated *Foxp3*-deficient mice. In a paper published alongside the study from Ramsdell, Rudensky's team reported that *Foxp3*-deficient mice displayed a phenotype strikingly similar to the scurfy strain (Fontenot, Gavin, and Rudensky 2003). Rudensky's group subsequently showed that depletion of *Foxp3* in the T cell compartment alone was sufficient to induce an early onset lymphoproliferative syndrome and autoimmunity, similar to that found in Foxp3-deficient mice (Fontenot *et al*. 2005).

Taken together, the critical findings made by this year's Nobel Laureates have made it clear that the absence of one cell type, Treg cells, controlled by one single gene locus, *foxp3*/*FOXP3*, is sufficient to break tolerance and cause "*horror autotoxicus*" in both mouse and man. This groundbreaking discovery has

shed light on a fundamental mechanism for body homeostasis and laid the foundation for a highly dynamic and expanding research area.

How should Treg cells be positioned in our understanding of immune tolerance as described in the brief science history above? They are certainly a part of the Nobel Prize-awarded concept of acquired tolerance, and to a certain extent also part of central (thymic) tolerance, the prevailing dogma for more than 50 years. However, Treg cells stand out as conceptually different as they acquire their tolerizing power centrally (during positive selection in the thymus), but they execute their functions in peripheral tissues; and they can exert their power during the entire life span by controlling other immune cells. Most importantly, the discoveries offered a molecular handle on Treg cells, which propelled the field of peripheral immune regulation forward to the highly active research area it is today. It is now possible to characterize Treg cells in detail and to explore different strategies to harness or inhibit their activities to treat a range of diseases.

Treg cell characteristics and nuances

As a transcription factor, Foxp3 orchestrates the expression of a large set of genes that characterize Treg cell development and functional properties, including their suppressive capacity. Mice and humans lacking a functional *Foxp3/FOXP3* gene, as observed in the scurfy mouse strain and clinically, in the IPEX syndrome, develop fatal multiorgan autoimmunity, underscoring the fundamental role of Treg cells in immune regulation.

With the identification of *Foxp3*, researchers were able to use flow cytometry and genetic tools to precisely identify and study Treg cells. This facilitated the identification of additional subsets of Treg cells, such as peripherally induced Treg cells (pTregs), which develop in tissues under certain circumstances. It is also possible to generate so-called induced Treg cells (iTregs) from conventional $CD4^+$ T cells *in vitro* by providing the appropriate cytokine stimulation, typically a high dose of IL-2 and TGFβ; however, iTregs generally express *FOXP3* less stably than thymus-derived Treg cells (tTregs).

As the field matured, studies showed that human Treg cells exhibit more complexity compared to their murine counterparts. For example, low levels of FOXP3 can be detected in activated conventional human $CD4^+$ T cells (Gavin et al. 2006; Walker et al. 2003). How Treg cells are selected in the thymus, including if there is an affinity threshold that dictates whether T cells are clonally deleted or selected into the Treg cell repertoire, or if avidity or other contextual factors influence the outcome, remain topics of interest (Cozzo Picca *et al*. 2011; Lee et al. 2012).

Treg studies on the horizon

Treg cell populations with distinct properties have been shown to arise from different developmental pathways (Owen *et al*. 2019), a question that warrants further investigation. How Treg cells behave in different disease contexts is also a question that is under intense investigation. For example, Treg cells have been investigated in the context of ongoing autoinflammatory diseases (Hu *et al*. 2021), with results suggesting that Treg cells present in inflamed environments are more heavily dependent on Foxp3 to maintain their function than are Treg cells outside of such environments (Jager *et al*. 2025).

Efforts are also dedicated towards delineating regulatory networks that control Treg cell differentiation. Transcriptional events upstream of, or concurrent with, *FOXP3* expression were shown to influence human Treg-cell development (Hill *et al*. 2007). Sakaguchi's team has also identified non-coding genetic elements that control FOXP3 expression, influencing Treg cell development

and maintenance (Kawakami *et al*. 2021). In efforts to improve current protocols for *in vitro* production of iTreg cells for clinical use, Sakaguchi has delineated repressor complexes that negatively regulate FOXP3 expression, which can be targeted for improved iTreg production (Chen *et al*. 2025).

Overall, the impact of Treg cells ranges from maintaining self-tolerance and preventing autoimmunity, to avoiding foetal rejection during pregnancy, controlling chronic inflammation, and regulating immune responses in infectious disease to prevent excessive activity that causes more harm than protection.

Approaches to target Treg cells in the clinic

The therapeutic potential of Treg cells remains largely untapped. While Treg cell therapeutics have not yet reached the clinic, numerous strategies to modulate their activity are under investigation, with some already being evaluated in clinical trials. Targeting Treg cells holds significant therapeutic potential for treating autoimmune diseases and allergies, as well as to diminish the risk of transplant rejection. Conversely, strategies to enhance anti-tumour immunity by eliminating or deactivating tumour-infiltrating Treg cells are ongoing. Work is also in progress to delineate the basis for immune tolerance to commensal microorganisms where both Treg cells and tolerogenic antigen-presenting cells play roles, as reviewed (Brown and Rudensky 2023).

At present, there are more than 200 clinical trials involving regulatory T cells with the aim to treat common diseases such as asthma, inflammatory bowel disease and skin-related conditions, or to improve outcomes following organ transplantation or treat cancer (www.clinicaltrials.gov).

The approaches to harness the potential of Treg cells in clinical settings include:

- Expansion of polyclonal Treg cells *in vitro* using for example, IL-2 stimulation, followed by reperfusion (Bender *et al*. 2024).
- Chimeric antigen receptor T regulatory cells (CAR-Tregs), in which Treg cells are engineered to express a chimeric receptor consisting of a targeting antibody domain. The aim of CAR-Tregs is to suppress inflammation by targeting regulatory activity to specific anatomical sites (Wardell *et al*. 2025).
- T cell receptor Treg (TCR-Tregs) cells are engineered to express a specific TCR for diseases where the pathogenic epitope is known, e.g. celiac disease (Porret *et al*. 2025).
- *In vivo* expansion of Treg cells using either low dose IL-2 or the IL-2-receptor agonist Rezpegaldesleukin. The latter was used in a randomized controlled trial that demonstrated efficacy in patients with moderate to severe atopic dermatitis (Silverberg *et al*. 2024).
- The identification of CCR8 on tumour-infiltrating Treg cells (Kidani *et al*. 2022; Plitas *et al*. 2016) has stimulated the development of monoclonal antibodies designed to target and deplete tumour-associated Treg cells and clinical trials are underway (Roider *et al*. 2024; Wen et al. 2025).

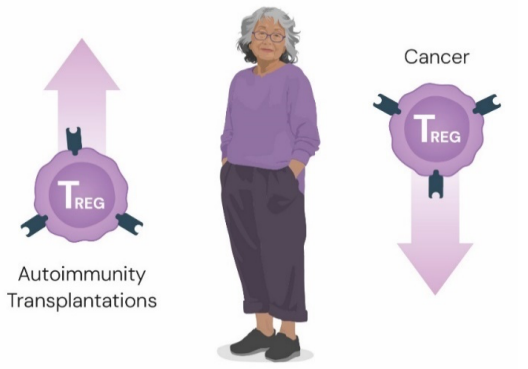

Figure 4. Approaches to harness or interfere with Treg cells in the treatment of disease.

Summary

The history behind the identification of regulatory T cells and FOXP3 and their role in immune tolerance by this year's Nobel Laureates is a testament to the power of scientific perseverance and the importance of integrating clinical observations with basic research. From the early studies of immunological tolerance, and the controversial suppressor T cell hypothesis, to the molecular definition of Treg cells via $CD4^+CD25^+$ and FOXP3, led to the expansive field that has unveiled fundamental principles of immune regulation. As our understanding of Treg cells deepens, so does the potential to harness their power for therapeutic benefit—protecting us from the twin perils of autoimmunity and immunopathology, while ensuring immune system homeostasis.

Professor Gunilla Karlsson Hedestam, Ph.D. (gunilla.karlsson.hedestam@ki.se), Member of the Nobel Committee, and Professor Olle Kämpe, M.D., Ph.D. (olle.kampe@ki.se), Chair of the Nobel Committee

For additional information on this year's Nobel Prize: www.nobelprize.org

The Nobel Assembly, consisting of 50 professors at Karolinska Institutet, awards the Nobel Prize in Physiology or Medicine. Its Nobel Committee evaluates the nominations. Since 1901 the Nobel Prize has been awarded to scientists who have made the most important discoveries for the benefit of humankind.

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