© SEP 2026 | IRE Journals | Volume 10 Issue 3 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV10I3-1723380

Regulatory T Cells in Immune Homeostasis: Mechanisms of Peripheral Tolerance and Immunoregulation

AUGUSTINE CHIMDINDU IGWE[1], NKIRUKA ROSE UKIBE[2], SAMUEL CHUKWUEMEKA MELUDU[3]

[1,2,3]*Department and Institute: Faculty of Medical Laboratory Science, Nnamdi Azikiwe University*

Abstract- ***Regulatory T cells (Tregs) are specialized subsets of T lymphocytes that maintain immune tolerance, suppress excessive immune responses, and preserve immune homeostasis. These cells are essential for preventing autoimmunity, controlling inflammation, and modulating immune responses during infection, cancer, and transplantation. The transcription factor Forkhead box P3 (FOXP3) serves as the master regulator of Treg development and function, while cytokines such as interleukin-10 (IL-10), transforming growth factor-beta (TGF-β), and interleukin-35 (IL-35) mediate their suppressive activities. Recent advances have revealed substantial heterogeneity among Treg populations, including thymus-derived and peripheral Tregs, tissue-resident Tregs, and memory-like Tregs. Dysregulation of Treg number or function contributes significantly to autoimmune diseases, chronic inflammatory conditions, infections, and tumor progression. Furthermore, emerging therapeutic approaches targeting Tregs, including low-dose IL-2 therapy and chimeric antigen receptor Treg (CAR-Treg) therapy, are gaining clinical importance. This review discusses the biology, classification, molecular mechanisms, suppressive pathways, and clinical significance of Tregs in immune homeostasis and disease.***

Keywords: Regulatory T cells, FOXP3, immune tolerance, immune homeostasis, cytokines, IL-10, TGF-β, CTLA-4, immunoregulation

I. INTRODUCTION

The vertebrate immune system has evolved sophisticated mechanisms capable of discriminating between harmful pathogens and harmless self-antigens. Effective immunity requires a delicate balance between immune activation and immune suppression. While immune effector mechanisms are essential for protection against infections and malignancies, uncontrolled activation can lead to autoimmunity, chronic inflammation, tissue destruction, and immunopathology. Maintenance of this balance depends heavily on specialized immunoregulatory networks, among which regulatory T cells (Tregs) represent the most important cellular mediators of peripheral immune tolerance.

The concept of immune suppression by specialized lymphocytes emerged more than five decades ago when Gershon and Kondo proposed the existence of suppressor T cells capable of downregulating immune responses [1]. Although the suppressor T-cell hypothesis was initially controversial due to the absence of definitive molecular markers, advances in immunology eventually validated the existence of a distinct regulatory T-cell lineage. A major breakthrough occurred in 1995 when Sakaguchi and colleagues identified a subset of $CD4^+$ T lymphocytes expressing high levels of CD25 that prevented autoimmune disease development in mice [2]. Subsequent discovery of the transcription factor Forkhead box P3 (FOXP3) established the molecular identity of Tregs and transformed understanding of immune regulation.

FOXP3 functions as the master regulator of Treg lineage commitment, differentiation, stability, and suppressive activity. Mutations in FOXP3 result in severe autoimmune disorders such as Immune Dysregulation Polyendocrinopathy Enteropathy X-linked syndrome (IPEX), highlighting its indispensable role in maintaining self-tolerance [3]. Since this discovery, extensive research has demonstrated that Tregs regulate virtually every aspect of adaptive and innate immunity.

Regulatory T cells suppress immune responses through multiple mechanisms, including secretion of anti-inflammatory cytokines such as interleukin-10 (IL-10), transforming growth factor-beta (TGF-β), and interleukin-35 (IL-35); modulation of antigen-presenting cell function; metabolic disruption of effector lymphocytes; cytolytic elimination of

activated immune cells; and expression of inhibitory receptors including CTLA-4, PD-1, TIGIT, and LAG-3 [4,5].

Recent advances in high-dimensional flow cytometry, single-cell RNA sequencing, spatial transcriptomics, epigenomics, and systems immunology have revealed remarkable heterogeneity among Treg populations. It is now recognized that Tregs comprise multiple subsets with distinct developmental origins, phenotypic characteristics, tissue localization patterns, metabolic programs, and functional specializations. These include thymus-derived Tregs (tTregs), peripheral Tregs (pTregs), induced Tregs (iTregs), memory Tregs, follicular regulatory T cells (Tfr), and tissue-resident Tregs located in adipose tissue, skin, intestine, lungs, and tumors [6].

The biological significance of Tregs extends beyond maintenance of self-tolerance. These cells influence tissue repair, wound healing, metabolic homeostasis, pregnancy, transplantation tolerance, microbial symbiosis, and tumor progression. Dysregulation of Treg function has been implicated in numerous diseases, including rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, inflammatory bowel disease, type 1 diabetes mellitus, psoriasis, asthma, chronic viral infections, tuberculosis, and cancer [7].

Because of their central role in immune regulation, Tregs have emerged as attractive therapeutic targets. Strategies aimed at enhancing Treg activity are being investigated for treatment of autoimmune and inflammatory diseases, whereas depletion or functional inhibition of Tregs is being explored in cancer immunotherapy. Novel approaches such as low-dose IL-2 therapy, adoptive Treg transfer, antigen-specific Treg expansion, genome editing, and chimeric antigen receptor regulatory T-cell (CAR-Treg) therapy are advancing rapidly toward clinical application [8].

II. DEVELOPMENT, DIFFERENTIATION AND CLASSIFICATION OF REGULATORY T CELLS

2.1 Historical Evolution of Regulatory T Cell Biology

The understanding of regulatory T cells has undergone remarkable evolution over the past several decades. Initial studies focused on suppressor T cells whose existence remained controversial due to the lack of specific molecular markers. The identification of $CD4^+CD25^+$ T cells capable of suppressing autoimmunity provided the first convincing evidence of a dedicated immunoregulatory lineage [2].

The subsequent discovery that mutations in FOXP3 caused severe autoimmune disease in both humans and mice definitively established Tregs as a distinct T-cell lineage. Since then, advances in molecular immunology have expanded the concept of Tregs from a homogeneous population into a highly diverse family of immunoregulatory cells adapted to specific immunological niches.

2.2 Thymus-Derived Regulatory T Cells

Thymus-derived regulatory T cells (tTregs) develop within the thymic microenvironment during T-cell maturation. Unlike conventional T cells that undergo positive and negative selection, tTregs emerge from thymocytes that recognize self-antigens with intermediate affinity.

This process is orchestrated by thymic epithelial cells and dendritic cells presenting self-peptides via major histocompatibility complex molecules. Strong self-reactivity leads to clonal deletion, whereas moderate self-reactivity promotes FOXP3 induction and Treg differentiation [9].

IL-2 signaling through STAT5 activation represents a critical requirement for thymic Treg development. Deficiencies in IL-2, CD25, or STAT5 profoundly impair Treg generation and result in severe autoimmune pathology.

2.3 Peripheral Regulatory T Cells (pTregs)

Peripheral regulatory T cells (pTregs) are generated extrathymically from naïve conventional $CD4^+$ T lymphocytes in peripheral tissues under tolerogenic conditions. Unlike thymus-derived Tregs, which primarily recognize self-antigens, pTregs are particularly important for maintaining tolerance toward environmental antigens, food proteins,

commensal microbiota, allergens, and fetal antigens during pregnancy [10,11].

The differentiation of pTregs requires a complex interplay between antigen presentation, cytokine signaling, and metabolic cues. Transforming growth factor-beta (TGF-β) is considered the principal cytokine driving peripheral FOXP3 induction. TGF-β activates SMAD2 and SMAD3 signaling pathways, which directly promote transcription of the FOXP3 gene [12,13]. Interleukin-2 (IL-2) further stabilizes FOXP3 expression through STAT5 activation, enhancing Treg survival and suppressive activity [14]. Specialized tolerogenic dendritic cells contribute significantly to pTreg generation. Intestinal $CD103^+$ dendritic cells produce retinoic acid, a metabolite of vitamin A that synergizes with TGF-β to promote FOXP3 induction while suppressing differentiation of pro-inflammatory Th17 cells [15]. This mechanism is particularly important in the gastrointestinal tract, where immune tolerance must coexist with exposure to trillions of commensal microorganisms.

Emerging evidence suggests that gut microbiota profoundly influence pTreg development. Certain bacterial species, particularly members of the genera *Clostridium*, *Bacteroides*, and *Faecalibacterium*, produce short-chain fatty acids such as butyrate and propionate that promote FOXP3 expression through epigenetic modifications [16,17]. These findings highlight the intimate relationship between host immunity and microbial ecology.

Defective pTreg generation has been implicated in inflammatory bowel disease, food allergy, asthma, and several autoimmune disorders, emphasizing their indispensable role in peripheral immune tolerance [18].

2.4 Induced Regulatory T Cells (iTregs)

Induced regulatory T cells (iTregs) represent a population of regulatory cells generated from naïve $CD4^+$ T cells following antigen stimulation in the presence of TGF-β and IL-2. These cells express FOXP3 and possess suppressive functions similar to those of naturally occurring Tregs [19].

Experimental generation of iTregs has attracted considerable interest because these cells can be expanded ex vivo and potentially utilized for adoptive immunotherapy. In animal models, iTregs have demonstrated efficacy in suppressing autoimmune diseases, preventing transplant rejection, and reducing inflammatory responses [20].

However, important differences exist between iTregs and thymus-derived Tregs. One major distinction concerns lineage stability. Naturally occurring Tregs possess a highly demethylated Treg-specific demethylated region (TSDR) within the FOXP3 locus, ensuring stable FOXP3 expression even under inflammatory conditions. In contrast, iTregs often exhibit incomplete TSDR demethylation, rendering them more susceptible to phenotypic instability and conversion into inflammatory effector cells [21,22].

Recent studies utilizing CRISPR-mediated gene editing, epigenetic reprogramming, and synthetic biology approaches aim to improve the stability and therapeutic potential of iTregs for clinical applications [23].

2.5 $CD8^+$ Regulatory T Cells

Although $CD4^+FOXP3^+$ Tregs constitute the dominant regulatory population, accumulating evidence indicates that $CD8^+$ T cells can also acquire potent immunosuppressive functions. $CD8^+$ regulatory T cells represent a heterogeneous population characterized by expression of molecules such as FOXP3, CD122, CD103, CTLA-4, PD-1, and LAG-3 [24].

$CD8^+$ Tregs suppress immune responses through several mechanisms including:

- Secretion of IL-10 and TGF-β
- Cytolytic killing of activated immune cells
- Inhibition of antigen-presenting cells
- Modulation of dendritic cell maturation
- Suppression of autoreactive T-cell responses

These cells have been implicated in transplantation tolerance, autoimmune disease control, and suppression of graft-versus-host disease [25].

Despite increasing interest, the developmental pathways and lineage-defining transcription factors of $CD8^+$ Tregs remain incompletely understood compared with classical $CD4^+FOXP3^+$ Tregs.

© SEP 2026 | IRE Journals | Volume 10 Issue 3 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV10I3-1723380

2.6 Tissue-Resident Regulatory T Cells

One of the most significant advances in contemporary immunology has been the recognition that Tregs adapt to specific tissue environments and acquire specialized functions beyond classical immune suppression [26,27].

These tissue-resident Tregs exhibit unique transcriptional programs shaped by local environmental signals.

Adipose Tissue Tregs

Adipose-resident Tregs constitute a highly specialized population expressing high levels of the transcription factor PPAR-γ. They regulate adipose tissue inflammation, glucose homeostasis, and insulin sensitivity [28].

Obesity is associated with a reduction in adipose Treg frequency, contributing to chronic low-grade inflammation and insulin resistance. Restoration of adipose Tregs improves metabolic outcomes in experimental models [29].

Skin Tregs

Skin-resident Tregs participate in wound healing, hair follicle regeneration, and maintenance of cutaneous tolerance. These cells interact closely with epithelial stem cells and influence tissue repair processes [30].

Intestinal Tregs

The intestine contains one of the largest Treg populations in the body. Gut-resident Tregs prevent excessive immune responses toward dietary antigens and commensal microorganisms while maintaining mucosal integrity [31].

Lung Tregs

Pulmonary Tregs contribute to airway tolerance and limit tissue damage during respiratory infections. They play important roles in asthma, allergic diseases, and recovery following viral pneumonia [32].

Tumor-Resident Tregs

Tumor-associated Tregs represent one of the most immunosuppressive Treg populations. These cells accumulate within tumor microenvironments and express elevated levels of CTLA-4, TIGIT, ICOS, PD-1, and CCR8, thereby suppressing anti-tumor immunity [33].

Their presence often correlates with poor clinical prognosis in numerous cancers including breast, ovarian, lung, and colorectal malignancies [34].

Figure 1. Development and Differentiation of Regulatory T Cells

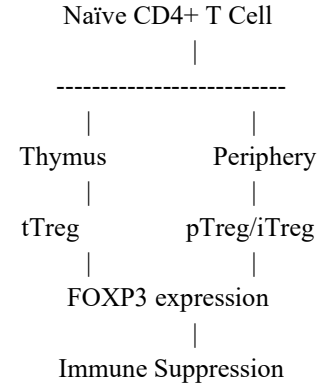

Figure 1. Development and differentiation pathways of regulatory T cells (Tregs).

Illustration showing thymus-derived Tregs (tTregs), peripheral Tregs (pTregs), induced Tregs (iTregs), FOXP3 induction, and the roles of IL-2 and TGF-β in Treg differentiation and maintenance.

III. FOXP3 AND REGULATORY T CELL IDENTITY

3.1 Discovery and Biological Importance of FOXP3

The discovery of FOXP3 represented a landmark event in immunology. FOXP3 belongs to the forkhead/winged-helix family of transcription factors and functions as the master regulator of Treg development and suppressive function [35].

The critical role of FOXP3 was first demonstrated through studies of the scurfy mouse, which develops fatal systemic autoimmunity due to mutations in the Foxp3 gene [36]. Similar mutations in humans cause Immune Dysregulation Polyendocrinopathy Enteropathy X-linked (IPEX) syndrome, characterized by severe autoimmune disease early in life [37].

These observations established FOXP3 as indispensable for immune tolerance.

3.2 Molecular Structure of FOXP3

Human FOXP3 is located on chromosome Xp11.23 and encodes a protein consisting of four major domains:

1. N-terminal repressor domain
2. Zinc-finger domain
3. Leucine zipper domain
4. Forkhead DNA-binding domain

These domains enable FOXP3 to interact with numerous transcription factors including NFAT, RUNX1, STAT5, GATA3, and AP-1, forming extensive transcriptional regulatory networks [38].

FOXP3 directly controls the expression of more than 700 genes involved in immune regulation, metabolism, migration, and cellular survival.

3.3 Transcriptional Regulation of FOXP3

FOXP3 expression is regulated through multiple signaling pathways.

IL-2/STAT5 Pathway

IL-2 signaling represents one of the strongest positive regulators of FOXP3 transcription. Binding of IL-2 to CD25 activates JAK1/JAK3 and STAT5, which directly bind the FOXP3 promoter and enhance transcription [39].

TGF-β/SMAD Pathway

TGF-β induces FOXP3 expression through activation of SMAD2 and SMAD3 transcription factors. These proteins interact with conserved non-coding sequences within the FOXP3 locus to promote stable expression [40].

PI3K-AKT-mTOR Pathway

Excessive activation of the PI3K-AKT-mTOR pathway negatively affects FOXP3 stability and Treg differentiation. Consequently, pharmacological inhibition of mTOR using rapamycin promotes Treg expansion and function [41].

3.4 Epigenetic Regulation of FOXP3 and Treg Stability

While FOXP3 is the master transcription factor of regulatory T cells, its expression alone is insufficient to guarantee stable suppressive activity. The long-term maintenance of Treg identity depends heavily on epigenetic mechanisms that regulate chromatin accessibility and transcriptional programming. Epigenetic regulation encompasses DNA methylation, histone modifications, chromatin remodeling, and non-coding RNA-mediated regulation, all of which contribute to the establishment and maintenance of the Treg lineage [42,43].

A hallmark of stable Tregs is the demethylation of the Treg-specific demethylated region (TSDR), also known as conserved non-coding sequence 2 (CNS2), within the FOXP3 locus. Complete demethylation of this region permits sustained FOXP3 expression even in inflammatory environments, whereas incomplete demethylation is associated with unstable Treg phenotypes [44,45].

Histone modifications further contribute to FOXP3 regulation. Histone acetylation generally promotes transcriptional activity by relaxing chromatin structure, whereas histone deacetylation represses gene expression. Histone acetyltransferases such as p300 enhance FOXP3 activity through acetylation, while histone deacetylases (HDACs) can negatively regulate FOXP3 stability [46].

MicroRNAs also play important roles in Treg biology. miR-155 enhances Treg survival by increasing responsiveness to IL-2, whereas miR-146a regulates inflammatory signaling pathways and contributes to Treg suppressive function [47,48]. Dysregulation of these microRNAs has been implicated in autoimmune diseases and chronic inflammatory disorders.

Recent advances in epigenomic profiling have revealed that Tregs possess a unique chromatin landscape distinct from conventional T cells. This epigenetic architecture facilitates rapid expression of immunosuppressive genes while repressing inflammatory transcriptional programs [49]. Understanding these epigenetic mechanisms has opened new opportunities for therapeutic manipulation of Treg stability in autoimmune diseases and transplantation.

3.5 Treg Plasticity and Lineage Instability

Although Tregs were once considered terminally differentiated and phenotypically stable, accumulating evidence demonstrates that they exhibit considerable functional plasticity. Under certain inflammatory conditions, Tregs may lose FOXP3 expression and acquire characteristics of effector T-cell subsets such as Th1, Th17, or T follicular helper (Tfh) cells [50,51]. Inflammatory cytokines including IL-6, IL-1β, TNF-α, and IL-23 promote Treg instability by interfering with FOXP3 expression and activating alternative transcriptional programs. For example, IL-6 induces STAT3 activation, favoring differentiation toward Th17 cells and antagonizing FOXP3-mediated suppression [52].

Th1-like Tregs expressing T-bet and IFN-γ have been observed in autoimmune diseases such as multiple sclerosis and rheumatoid arthritis [53]. Similarly, Th17-like Tregs expressing RORγt and IL-17 have been identified in inflammatory bowel disease and psoriasis.

Despite these observations, plasticity is not always detrimental. In some tissues, acquisition of lineage-associated transcription factors enables Tregs to specialize in suppressing corresponding effector responses. For example, T-bet-expressing Tregs preferentially suppress Th1-mediated inflammation, whereas IRF4-expressing Tregs effectively control Th2 responses [54,55].

The dual nature of Treg plasticity highlights the complexity of immune regulation and presents both challenges and opportunities for immunotherapy.

Table 1. Major phenotypic markers and functional molecules expressed by regulatory T cells.

Marker	Function
FOXP3	Master transcription factor controlling Treg development
CD25	High-affinity IL-2 receptor α-chain
CTLA-4	Inhibits T-cell activation
TIGIT	Suppresses immune responses
PD-1	Regulates T-cell exhaustion and suppression
CD39	Converts ATP to AMP
CD73	Converts AMP to adenosine
GITR	Modulates Treg survival
Helios	Associated with thymic Tregs
Neuropilin-1	Treg stability and migration

IV. MECHANISMS OF REGULATORY T CELL-MEDIATED IMMUNOSUPPRESSION

Regulatory T cells employ multiple complementary mechanisms to suppress immune responses and maintain immune homeostasis. These mechanisms can be broadly categorized into cytokine-mediated suppression, inhibitory receptor signaling, metabolic disruption, cytolytic activity, and modulation of antigen-presenting cells [56,57].

The diversity of suppressive pathways reflects the need for Tregs to function across various tissues and immunological contexts.

4.1 Cytokine-Mediated Suppression

Interleukin-10 (IL-10)
IL-10 is one of the most important anti-inflammatory cytokines produced by Tregs. It suppresses activation of macrophages, dendritic cells, and effector T cells while inhibiting production of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-12 [58]. Studies in IL-10-deficient mice demonstrate severe intestinal inflammation, highlighting the crucial role of IL-10 in maintaining mucosal tolerance [59]. In humans, impaired IL-10 signaling has been associated with inflammatory bowel disease and autoimmune pathology.

Treg-derived IL-10 is particularly important in barrier tissues such as the intestine, lungs, and skin, where continuous exposure to environmental antigens requires tightly regulated immune responses.

Transforming Growth Factor-β (TGF-β)
TGF-β is a multifunctional cytokine with profound immunoregulatory properties. It suppresses T-cell proliferation, inhibits inflammatory cytokine production, promotes tissue repair, and induces differentiation of peripheral Tregs [60].

The immunosuppressive effects of TGF-β are mediated through activation of SMAD-dependent signaling pathways that regulate transcription of numerous target genes. In addition to controlling adaptive immunity, TGF-β contributes to maintenance of epithelial barrier integrity and wound healing.

Dysregulation of TGF-β signaling has been implicated in autoimmune diseases, fibrosis, cancer progression, and chronic inflammatory disorders.

Interleukin-35 (IL-35)
IL-35 is a relatively recently identified cytokine belonging to the IL-12 family. It is composed of the p35 and EBI3 subunits and is predominantly produced by regulatory T cells [61].

IL-35 suppresses proliferation of effector T cells and promotes the generation of induced suppressive populations termed iTr35 cells. Experimental studies demonstrate potent anti-inflammatory effects of IL-35 in autoimmune diseases including multiple sclerosis, rheumatoid arthritis, and inflammatory bowel disease [62].

Because of its unique immunosuppressive properties, IL-35 has emerged as a promising therapeutic target for inflammatory disorders.

4.2 CTLA-4-Mediated Suppression
Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is one of the most important inhibitory molecules expressed by Tregs. CTLA-4 competes with CD28 for binding to CD80 and CD86 on antigen-presenting cells, thereby preventing effective co-stimulation of conventional T cells [63].

CTLA-4 possesses significantly higher affinity for CD80/CD86 than CD28, allowing Tregs to efficiently inhibit T-cell activation. Furthermore, CTLA-4 mediates trans-endocytosis of CD80/CD86 molecules from dendritic cell surfaces, effectively reducing their capacity to activate effector T cells [64].

Genetic deficiencies of CTLA-4 result in severe immune dysregulation and autoimmunity, underscoring its essential role in immune tolerance.
The importance of CTLA-4 is further illustrated by cancer immunotherapy. Blockade of CTLA-4 with monoclonal antibodies such as ipilimumab enhances anti-tumor immunity but may induce autoimmune toxicities due to disruption of Treg-mediated suppression [65].

4.3 PD-1, TIGIT, LAG-3 and Additional Inhibitory Receptors
Beyond CTLA-4, Tregs express numerous inhibitory receptors that contribute to their suppressive function.
Programmed Cell Death Protein-1 (PD-1)

PD-1 negatively regulates T-cell activation by inhibiting TCR signaling pathways. Engagement of PD-1 with PD-L1 or PD-L2 suppresses proliferation, cytokine production, and survival of effector T cells [66].

Within tumors, PD-1-expressing Tregs contribute significantly to immune suppression and tumor progression.

TIGIT

T-cell immunoreceptor with Ig and ITIM domains (TIGIT) is highly expressed on activated Tregs. TIGIT signaling enhances Treg suppressive function and promotes tolerogenic dendritic cell phenotypes [67].

LAG-3

Lymphocyte activation gene-3 (LAG-3) interacts with MHC class II molecules and suppresses activation of antigen-presenting cells. Treg-derived LAG-3 contributes to immune tolerance in autoimmunity and transplantation [68].

Collectively, these inhibitory pathways form an integrated network that enables Tregs to regulate diverse immune responses.

4.4 Metabolic Disruption as a Suppressive Mechanism

An increasingly recognized mechanism of Treg-mediated suppression involves metabolic competition and regulation.

Tregs constitutively express high levels of CD25, allowing them to efficiently consume IL-2 from the local environment. This deprives conventional T cells of a critical growth factor, thereby limiting their expansion [69].

Tregs also express the ectoenzymes CD39 and CD73, which convert extracellular ATP into adenosine. Adenosine subsequently binds A2A receptors on effector immune cells and suppresses inflammatory responses [70].

Additional metabolic mechanisms include:

- Tryptophan depletion through induction of indoleamine 2,3-dioxygenase (IDO)
- Modulation of glucose metabolism
- Suppression of amino acid availability
- Regulation of mitochondrial function

These metabolic pathways contribute substantially to the suppressive microenvironment generated by Tregs.

4.5 Cytolytic Mechanisms

Certain Treg populations directly eliminate target cells through cytotoxic pathways involving perforin and granzyme B [71].

Granzyme-mediated killing has been observed against:

- Activated T cells
- B lymphocytes
- Natural killer cells
- Dendritic cells

This mechanism appears particularly important in tumor microenvironments and sites of chronic inflammation where rapid suppression of activated immune cells is required.

Figure 2. Major suppressive mechanisms employed by regulatory T cells.

Include:

- IL-10 secretion
- TGF-β secretion
- IL-35 secretion
- CTLA-4-mediated inhibition
- CD39/CD73 adenosine production
- IL-2 consumption
- Granzyme B/perforin killing
- Dendritic cell suppression

V. IMMUNOMETABOLISM OF REGULATORY T CELLS

Over the last decade, immunometabolism has emerged as one of the most rapidly evolving fields in immunology. Immunometabolism refers to the intricate relationship between cellular metabolic pathways and immune cell function. Regulatory T cells possess a unique metabolic profile that distinguishes them from conventional effector T cells and contributes significantly to their suppressive capacity, survival, lineage stability, and adaptation to diverse tissue microenvironments [72,73].

Unlike activated effector T cells, which predominantly rely on aerobic glycolysis (the Warburg effect) to support rapid proliferation and cytokine production, Tregs preferentially utilize oxidative phosphorylation (OXPHOS), fatty acid oxidation (FAO), and mitochondrial respiration for energy generation. This metabolic specialization allows Tregs to function effectively in nutrient-deprived, hypoxic, and inflammatory environments such as tumors,

chronically inflamed tissues, and sites of infection ([74,75].

Recent evidence indicates that metabolic pathways are not merely passive energy sources but actively regulate Treg differentiation, stability, and suppressive function through integration with signaling networks such as mTOR, AMPK, HIF-1α, PI3K-AKT, and FoxO transcription factors. Consequently, metabolic dysregulation can profoundly influence immune homeostasis and contribute to autoimmune diseases, cancer progression, and chronic inflammation.

5.1 Metabolic Distinctions Between Effector T Cells and Regulatory T Cells

Activation of naïve T lymphocytes induces profound metabolic reprogramming. Effector T cells rapidly switch from oxidative metabolism to glycolysis, even under aerobic conditions. This metabolic shift supports rapid ATP generation and provides biosynthetic intermediates necessary for proliferation and cytokine production [76].

In contrast, Tregs preferentially depend on:

- Oxidative phosphorylation (OXPHOS)
- Fatty acid oxidation (FAO)
- Mitochondrial respiration
- Lipid metabolism

This metabolic phenotype promotes long-term survival, functional stability, and suppressive activity. Foxp3 itself contributes to metabolic programming by suppressing glycolytic pathways while enhancing oxidative metabolism. Angelin et al. (2017) demonstrated that FOXP3 directly reprograms T-cell metabolism toward mitochondrial respiration, thereby reinforcing the regulatory phenotype [77].

This metabolic distinction enables Tregs to thrive in environments where glucose availability is limited, such as tumors and chronically inflamed tissues.

5.2 Oxidative Phosphorylation and Mitochondrial Function

Oxidative phosphorylation serves as the primary source of ATP generation in resting and activated Tregs. Mitochondrial electron transport chains convert reducing equivalents derived from fatty acid oxidation and the tricarboxylic acid (TCA) cycle into ATP through oxidative phosphorylation.

Mitochondrial integrity is essential for:

- Treg survival
- FOXP3 stability
- Cytokine production
- Suppressive activity

Genetic disruption of mitochondrial respiratory complexes results in impaired Treg function and severe autoimmune pathology [78].

Recent studies have demonstrated that mitochondrial fitness regulates expression of suppressive molecules including:

- CTLA-4
- ICOS
- TIGIT
- CD39
- CD73

Mitochondrial dysfunction also promotes oxidative stress, which may destabilize FOXP3 expression and contribute to Treg plasticity [79].

Importantly, tissue-resident Tregs often exhibit enhanced mitochondrial capacity compared with circulating Tregs, reflecting adaptation to local environmental demands.

5.3 Fatty Acid Oxidation and Lipid Metabolism

Fatty acid oxidation (FAO) constitutes a major energy source for Tregs. During FAO, fatty acids are transported into mitochondria and metabolized through β-oxidation to generate acetyl-CoA, which enters the TCA cycle.

Several studies have demonstrated that Tregs exhibit increased expression of genes involved in:

- Lipid uptake
- Fatty acid transport
- β-oxidation
- Mitochondrial respiration

Key molecules include:

- Carnitine palmitoyltransferase-1A (CPT1A)
- CD36

- FABP5
- PPAR-γ

Activation of FAO promotes Treg differentiation and enhances suppressive activity, whereas inhibition of lipid metabolism impairs Treg function (Michalek et al., 2011).

In adipose tissue, Tregs express exceptionally high levels of PPAR-γ, a transcription factor regulating lipid metabolism. PPAR-γ signaling enables adipose-resident Tregs to adapt to lipid-rich environments and maintain metabolic homeostasis [80].

Recent evidence suggests that tumor-infiltrating Tregs exploit lipid metabolism more effectively than conventional T cells, allowing them to survive within nutrient-restricted tumor microenvironments [81].

5.4 The mTOR Pathway in Regulatory T Cells
The mechanistic target of rapamycin (mTOR) serves as a master regulator of cellular metabolism, growth, and differentiation.

mTORC1 \rightarrow Protein\ Synthesis + Cell\ Growth + Glycolysis

The mTOR signaling pathway consists of two complexes:

- mTORC1
- mTORC2

These complexes integrate signals from:

- Nutrients
- Growth factors
- Cytokines
- Energy availability

Moderate mTOR activity is necessary for Treg activation and proliferation; however, excessive mTOR activation favors effector T-cell differentiation and suppresses FOXP3 expression [82].

Pharmacological inhibition of mTOR using rapamycin selectively promotes Treg expansion while suppressing conventional T-cell responses. This property has made rapamycin an important agent in transplantation and autoimmune disease therapy.

Recent studies indicate that balanced mTOR activity is essential for maintaining Treg stability and preventing lineage instability.

5.5 AMPK Signaling and Energy Homeostasis
AMP-activated protein kinase (AMPK) functions as a cellular energy sensor that becomes activated during conditions of low energy availability.

AMPK \rightarrow Fatty\ Acid\ Oxidation + Oxidative\ Phosphorylation

Activation of AMPK promotes:

- Fatty acid oxidation
- Mitochondrial biogenesis
- Energy conservation
- Treg differentiation

AMPK opposes mTOR signaling and facilitates metabolic conditions favorable for Treg development. Experimental activation of AMPK enhances FOXP3 expression and suppressive activity, whereas AMPK deficiency impairs Treg generation [83].

The interplay between AMPK and mTOR constitutes a central regulatory axis controlling Treg metabolism.

5.6 HIF-1α and Hypoxic Adaptation
Many tissues experience periods of reduced oxygen availability. Tumors, inflamed tissues, and sites of infection are particularly hypoxic environments.

Hypoxia-inducible factor-1 alpha (HIF-1α) serves as the primary transcriptional regulator of cellular responses to hypoxia.

HIF-1α influences Treg biology through multiple mechanisms:

- Regulation of glycolysis
- Control of FOXP3 stability
- Adaptation to hypoxic tissues
- Modulation of inflammatory responses

The role of HIF-1α remains complex. Some studies indicate that HIF-1α destabilizes FOXP3 and promotes Th17 differentiation, whereas others

demonstrate that HIF-1α facilitates Treg adaptation within hypoxic tumors [84,85].

These findings suggest context-dependent effects of HIF-1α on Treg biology.

5.7 Metabolic Adaptation of Tregs in the Tumor Microenvironment

The tumor microenvironment represents one of the most metabolically challenging environments encountered by immune cells.

Characteristics include:

- Low glucose availability
- Hypoxia
- Elevated lactate concentrations
- Amino acid depletion
- Oxidative stress

While effector T cells often become metabolically exhausted under these conditions, Tregs possess remarkable metabolic flexibility that allows them to survive and function effectively [86].

Tumor-associated Tregs utilize:

- Fatty acid oxidation
- Lactate metabolism
- Enhanced mitochondrial respiration
- Cholesterol metabolism

This metabolic adaptation contributes significantly to tumor immune evasion.

Recent studies have identified metabolic targets including:

- CD36
- FABP5
- PPAR signaling
- Lactate transporters

as potential strategies for selectively disrupting tumor-associated Tregs while preserving systemic immune tolerance.

5.8 Metabolism and Treg Stability

Emerging evidence suggests that metabolic pathways directly influence Treg lineage stability.

Stable Tregs are characterized by:

- High mitochondrial fitness
- Enhanced FAO
- Controlled glycolysis
- Sustained oxidative phosphorylation

Conversely, excessive glycolysis and metabolic stress promote:

- Reduced FOXP3 expression
- Treg plasticity
- Conversion toward inflammatory phenotypes

Metabolic control therefore represents an additional layer of regulation governing immune homeostasis.

Advances in metabolomics and single-cell technologies continue to reveal previously unrecognized links between metabolism and Treg function, offering exciting opportunities for therapeutic intervention.

Table 2. Role of regulatory T cells in major autoimmune diseases.

Disease	Treg Abnormality	Clinical Consequence
Rheumatoid arthritis	Reduced suppressive function	Chronic inflammation
SLE	Decreased Treg numbers	Autoantibody production
Multiple sclerosis	Defective Treg regulation	CNS inflammation
Type 1 diabetes	Reduced Treg-mediated tolerance	β-cell destruction
IBD	Impaired FOXP3 activity	Intestinal inflammation

VI. REGULATORY T CELLS IN AUTOIMMUNE DISEASES

6.1 Overview

Autoimmune diseases arise when immune tolerance mechanisms fail, resulting in immune-mediated

destruction of self-tissues. Because Tregs are central mediators of peripheral tolerance, defects in their number, function, migration, stability, or suppressive capacity contribute significantly to autoimmune disease pathogenesis [87].

Studies across multiple autoimmune disorders have demonstrated:

- Reduced Treg frequency
- Impaired suppressive activity
- FOXP3 instability
- Altered cytokine production
- Defective tissue homing

These abnormalities permit expansion of autoreactive lymphocytes and perpetuation of chronic inflammation.

Consequently, restoration of Treg function has emerged as a major therapeutic goal in modern immunology.

6.2 Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by persistent synovial inflammation and progressive joint destruction.

Patients with RA frequently exhibit:

- Impaired Treg suppressive function
- Increased Th17/Treg ratio
- Elevated inflammatory cytokines
- Reduced immune tolerance

Although Treg numbers may not always be decreased, their functional capacity is often compromised by inflammatory mediators such as TNF-α and IL-6 [88]. The imbalance between Th17 cells and Tregs is now recognized as a central pathogenic mechanism in RA.

Low-dose IL-2 therapy and adoptive Treg transfer are currently being investigated as novel therapeutic approaches for restoring immune balance in rheumatoid arthritis.

6.3 Type 1 Diabetes Mellitus

Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by immune-mediated destruction of insulin-producing pancreatic β-cells. Both genetic susceptibility and environmental triggers contribute to disease development; however, loss of immune tolerance remains the fundamental pathogenic mechanism [89].

Numerous studies have demonstrated abnormalities in Treg number and function among patients with T1DM. Although circulating Treg frequencies may appear normal in some individuals, their suppressive capacity is often significantly impaired [90]. Defective IL-2 signaling represents a major contributor to Treg dysfunction in T1DM. Reduced responsiveness to IL-2 impairs STAT5 activation, compromises FOXP3 stability, and diminishes suppressive activity [91].

Within pancreatic islets, insufficient Treg-mediated suppression permits activation of autoreactive $CD4^+$ and $CD8^+$ T cells that target β-cell antigens such as insulin, GAD65, and IA-2. The resulting inflammatory milieu promotes progressive β-cell destruction and eventual insulin deficiency.

Experimental studies have demonstrated that adoptive transfer of Tregs can prevent diabetes development in non-obese diabetic (NOD) mice, highlighting the therapeutic potential of Treg-based interventions [92]. Clinical trials involving low-dose IL-2 therapy and ex vivo expanded autologous Tregs have shown encouraging results in preserving residual β-cell function [93].

6.4 Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease characterized by production of autoantibodies against nuclear antigens, immune complex deposition, and widespread tissue inflammation.

A growing body of evidence indicates that Treg abnormalities contribute significantly to SLE pathogenesis. Patients frequently exhibit reduced numbers of circulating Tregs, impaired suppressive function, and altered expression of FOXP3 [94]. Simultaneously, expansion of follicular helper T cells (Tfh) promotes excessive B-cell activation and autoantibody production.

The imbalance between Tregs and Tfh cells is increasingly recognized as a critical determinant of

disease activity. Reduced Treg-mediated suppression permits uncontrolled activation of autoreactive B cells, resulting in production of anti-dsDNA, anti-Sm, and other pathogenic autoantibodies.
Inflammatory cytokines such as IFN-α, IL-6, and IL-21 further destabilize Treg populations and perpetuate immune dysregulation [95].

Recent therapeutic approaches targeting Treg restoration, including low-dose IL-2 administration, have demonstrated promising efficacy in SLE by selectively expanding functional Treg populations and reducing disease activity scores [96].

6.5 Multiple Sclerosis
Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system characterized by inflammatory demyelination and neurodegeneration.
Tregs play essential roles in maintaining immune tolerance to central nervous system antigens. In patients with MS, Tregs exhibit impaired suppressive function despite relatively normal frequencies in peripheral blood [97]. This functional deficiency permits activation of autoreactive T cells targeting myelin proteins such as myelin basic protein and proteolipid protein.

The pathogenic process is further exacerbated by expansion of Th1 and Th17 cells, which secrete pro-inflammatory cytokines including IFN-γ, TNF-α, and IL-17. Numerous studies have demonstrated an altered Th17/Treg balance in active MS lesions [98].

Within the central nervous system, Tregs contribute not only to suppression of inflammation but also to tissue repair and remyelination. Emerging evidence suggests that Tregs interact directly with oligodendrocyte precursor cells and promote regeneration of damaged myelin [99].

Current disease-modifying therapies for MS may exert part of their efficacy through enhancement of Treg function and restoration of immune tolerance.

6.6 Inflammatory Bowel Disease
Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, results from inappropriate immune responses against intestinal microbiota in genetically susceptible individuals.

The gastrointestinal tract contains one of the largest Treg populations in the human body. These cells are essential for maintaining tolerance toward dietary antigens and commensal microorganisms [100].

Defects in Treg generation, stability, or suppressive activity contribute significantly to IBD pathogenesis. Experimental models have demonstrated that transfer of $CD4^+CD25^+$ Tregs prevents development of colitis, whereas depletion of Tregs induces severe intestinal inflammation [101].

Several mechanisms contribute to Treg dysfunction in IBD:

- Reduced IL-10 production
- Impaired TGF-β signaling
- Increased IL-6 and IL-23 activity
- Enhanced Th17 differentiation
- Microbiome dysbiosis

The importance of IL-10 is particularly evident from observations that genetic deficiencies in IL-10 or its receptor cause severe early-onset enterocolitis in humans [102].

Therapeutic strategies aimed at enhancing Treg function, including microbiome-based interventions, low-dose IL-2 therapy, and adoptive Treg transfer, are currently under active investigation.

6.7 Psoriasis
Psoriasis is a chronic immune-mediated inflammatory skin disease characterized by keratinocyte hyperproliferation and infiltration of activated immune cells.

Accumulating evidence suggests that impaired Treg function contributes substantially to disease pathogenesis. Patients with psoriasis often exhibit increased numbers of circulating Tregs; however, these cells display reduced suppressive capacity and impaired control of inflammatory responses [103].

The cytokine milieu within psoriatic lesions favors differentiation of Th17 cells while destabilizing Tregs.

Elevated concentrations of IL-6, IL-23, TNF-α, and IL-17 promote chronic inflammation and impair immune regulation.

Recent studies have identified plasticity of Tregs as an important mechanism in psoriasis. Some Tregs acquire Th17-like characteristics and begin producing IL-17, thereby contributing directly to disease progression [104].

Biological therapies targeting TNF-α, IL-17, and IL-23 may partially restore immune balance by correcting abnormalities in Treg function.

6.8 Autoimmune Hepatitis

Autoimmune hepatitis (AIH) is characterized by chronic immune-mediated destruction of hepatocytes, leading to progressive liver inflammation, fibrosis, and cirrhosis.

Multiple studies have demonstrated reduced frequencies of functional Tregs in patients with AIH [105]. Defective Treg-mediated suppression permits activation of autoreactive lymphocytes directed against hepatic antigens.

Furthermore, inflammatory cytokines within the liver microenvironment may impair FOXP3 stability and reduce Treg survival. Reduced production of IL-10 and TGF-β further contributes to loss of immune tolerance.

The liver normally maintains a highly tolerogenic immune environment. Consequently, disruption of Treg-mediated regulation plays a particularly important role in hepatic autoimmunity.

Emerging therapeutic strategies seek to restore liver tolerance through expansion of autologous Tregs and enhancement of antigen-specific regulatory responses.

6.9 Common Mechanisms of Treg Dysfunction in Autoimmune Diseases

Although autoimmune diseases affect diverse organs and tissues, several common abnormalities in Treg biology have been identified:

Quantitative Defects

Some diseases exhibit reduced numbers of circulating or tissue-resident Tregs, limiting suppressive capacity.

Functional Defects

Many autoimmune disorders are characterized by impaired suppressive activity despite apparently normal Treg numbers.

FOXP3 Instability

Inflammatory cytokines may destabilize FOXP3 expression, resulting in reduced suppressive function and increased plasticity.

Altered Cytokine Signaling

Defective IL-2 signaling represents a recurring abnormality across multiple autoimmune diseases.

Th17/Treg Imbalance

Expansion of pathogenic Th17 cells combined with reduced Treg activity is a hallmark of numerous autoimmune conditions.

Defective Tissue Homing

Impaired migration of Tregs to target tissues may limit local immune regulation despite normal circulating populations.

Understanding these shared mechanisms provides important opportunities for development of broadly applicable Treg-based therapies.

Figure 3. Metabolic pathways regulating Treg function.

Show:

- Fatty acid oxidation (FAO)
- Oxidative phosphorylation (OXPHOS)
- mTOR signaling
- AMPK signaling
- HIF-1α
- Lipid uptake via CD36
- Adenosine pathway

VII. REGULATORY T CELLS IN CANCER

7.1 Introduction

While Tregs are indispensable for maintaining immune tolerance and preventing autoimmunity, their immunosuppressive properties become detrimental in

cancer. Tumors exploit Treg-mediated suppression to evade immune surveillance and establish an immunosuppressive microenvironment that favors tumor growth and metastasis [106,107].

Indeed, tumor-associated Tregs represent one of the most significant barriers to effective anti-tumor immunity. Elevated infiltration of Tregs has been associated with poor prognosis in numerous malignancies including:

- Breast cancer
- Ovarian cancer
- Lung cancer
- Pancreatic cancer
- Gastric cancer
- Hepatocellular carcinoma
- Melanoma

Tumor-resident Tregs often exhibit enhanced suppressive capacity compared with circulating Tregs, reflecting adaptation to the unique metabolic and inflammatory conditions of the tumor microenvironment.

7.2 Recruitment of Tregs to the Tumor Microenvironment

Tumors actively recruit Tregs through production of chemokines that attract regulatory cells from the circulation.

Important chemokine pathways include:

- CCL22–CCR4
- CCL17–CCR4
- CCL28–CCR10
- CXCL12–CXCR4

Curiel et al. (2004) demonstrated that ovarian tumors produce CCL22, which selectively recruits CCR4-expressing Tregs into tumor tissues. Increased Treg infiltration correlated strongly with reduced survival [108].

Once recruited, Tregs accumulate within tumors and contribute to establishment of profound local immunosuppression.

7.3 Expansion and Activation of Tumor-Associated Tregs

Tumor microenvironments provide numerous factors that support Treg expansion and activation, including:

- TGF-β
- IL-10
- VEGF
- Indoleamine 2,3-dioxygenase (IDO)
- Adenosine
- Lactic acid

These mediators promote proliferation, survival, and suppressive activity of Tregs while simultaneously inhibiting effector T-cell responses.

Tumor-associated Tregs frequently express high levels of:

- CTLA-4
- PD-1
- TIGIT
- ICOS
- CCR8
- CD39
- CD73

which collectively enhance their suppressive function.

7.4 Mechanisms of Treg-Mediated Tumor Immune Suppression

Regulatory T cells (Tregs), primarily characterized by the transcription factor FOXP3, contribute to tumor immune evasion through multiple overlapping suppressive mechanisms that affect both innate and adaptive immune responses. In the tumor microenvironment (TME), Tregs suppress cytotoxic T lymphocytes (CTLs), dendritic cells (DCs), natural killer (NK) cells, and effector $CD4^+$ T helper subsets, thereby facilitating tumor progression and metastasis [109,110].

One of the central mechanisms involves cytokine-mediated suppression, particularly through secretion of IL-10, TGF-β, and IL-35, which inhibit T cell proliferation and cytokine production while promoting immune tolerance [111]. Tregs also consume IL-2 via high CD25 expression, effectively depriving effector T cells of this critical growth factor, leading to apoptosis or anergy of anti-tumor T cells [112].

Another key mechanism is cytotoxic granzyme/perforin-mediated killing, where Tregs directly eliminate effector T cells and antigen-presenting cells (Granzyme B-dependent pathway), thereby reducing antigen presentation and immune activation within the TME [113].

Tregs further express immune checkpoint molecules such as CTLA-4, PD-1, LAG-3, and TIGIT, which downregulate co-stimulatory signals on dendritic cells and suppress T cell priming in lymphoid organs and tumor sites [114,115]. CTLA-4-mediated trans-endocytosis of CD80/CD86 is particularly important in suppressing early T cell activation.

Additionally, Tregs modulate antigen-presenting cell function by inducing tolerogenic DC phenotypes and metabolic reprogramming of the TME, thereby reinforcing immunosuppressive circuits [116].

7.5 Tregs and Immune Checkpoint Therapy

Immune checkpoint inhibitors (ICIs) targeting CTLA-4, PD-1, and PD-L1 have revolutionized cancer therapy, but Tregs play a critical role in modulating therapeutic outcomes.

Anti-CTLA-4 therapy (e.g., ipilimumab) not only blocks inhibitory signaling on effector T cells but may also deplete intratumoral Tregs via Fcγ receptor-mediated ADCC, depending on antibody isotype and tumor context [117,118].

Similarly, PD-1/PD-L1 blockade can indirectly reduce Treg-mediated suppression by restoring exhausted $CD8^+$ T cell function. However, paradoxically, PD-1 signaling is also involved in maintaining Treg stability; thus, blockade may sometimes enhance Treg proliferation in certain tumor settings [119].

Emerging evidence suggests that the ratio of effector T cells to Tregs ($CD8^+$/$FOXP3^+$ ratio) is a stronger predictor of response to checkpoint therapy than absolute Treg abundance alone [120].

Combination strategies targeting both checkpoints and Tregs are being actively investigated to overcome adaptive resistance mechanisms mediated by immunosuppressive Treg expansion.

7.6 Prognostic Significance of Tumor Tregs

The prognostic role of Tregs in cancer is highly context-dependent and varies across tumor types.

In many solid tumors such as ovarian, hepatocellular, and pancreatic cancers, high $FOXP3^+$ Treg infiltration correlates with poor prognosis, increased metastasis, and reduced overall survival [121,122].

However, in certain malignancies such as colorectal cancer, Tregs may paradoxically associate with improved outcomes, likely due to their role in suppressing pro-tumor inflammatory responses driven by IL-17 and Th17 cells [123].

Meta-analyses suggest that tumor localization of Tregs (intratumoral vs stromal) and their functional phenotype (activated vs resting) are critical determinants of prognostic impact [124].

Thus, FOXP3 expression alone is insufficient as a universal prognostic marker without spatial and functional context.

7.7 Metabolic Adaptation of Tumor Tregs

Tregs exhibit remarkable metabolic plasticity that enables their survival and suppressive function within the nutrient-deprived and hypoxic tumor microenvironment.

Unlike effector T cells that rely heavily on glycolysis, Tregs preferentially utilize fatty acid oxidation (FAO) and oxidative phosphorylation (OXPHOS) for energy production [125,126]. This metabolic preference allows them to persist in glucose-depleted tumor niches.

Tumor-derived lactate, hypoxia, and adenosine further enhance Treg stability by promoting FOXP3 expression through HIF-1α-dependent pathways. Additionally, lipid uptake via CD36 has been shown to enhance Treg suppressive capacity in tumors [127]. mTOR signaling plays a central role in metabolic programming; inhibition of mTOR enhances Treg differentiation while suppressing effector T cell

activity, further tipping the immune balance toward tolerance [128].
These metabolic adaptations make Tregs highly resilient and a major barrier to effective anti-tumor immunity.

7.8 Strategies for Targeting Tregs in Cancer
Several therapeutic strategies are being developed to selectively target Tregs in cancer without inducing systemic autoimmunity.

1. Depletion strategies
   - Anti-CD25 monoclonal antibodies (e.g., daclizumab) target IL-2 receptor-expressing Tregs, though specificity remains a challenge due to CD25 expression on activated effector T cells [129].

2. Checkpoint targeting
   - CTLA-4 blockade may deplete intratumoral Tregs via Fc-dependent mechanisms [130].

3. Metabolic disruption
   - Targeting lipid metabolism (FAO inhibitors) or adenosine signaling (CD39/CD73 axis) impairs Treg function in the TME [131].

4. Chemokine receptor blockade
   - CCR4 and CCR8 inhibitors reduce Treg trafficking into tumors [132].

5. Low-dose chemotherapy
   - Agents such as cyclophosphamide selectively deplete Tregs at low doses while sparing effector T cells.

6. Vaccination strategies
   - Tumor antigen vaccines combined with Treg depletion improve anti-tumor immune activation.

Overall, combination therapies targeting multiple Treg pathways are considered the most promising approach.

Table 3. Current therapeutic strategies targeting Tregs in cancer.

Strategy	Target	Mechanism
Anti-CD25 antibodies	CD25	Treg depletion
Anti-CTLA-4	CTLA-4	Checkpoint blockade
Anti-CCR4	CCR4	Prevents tumor recruitment
Anti-CCR8	CCR8	Tumor Treg depletion
CD39/CD73 inhibitors	Adenosine pathway	Reverses suppression
FAO inhibitors	Lipid metabolism	Reduces Treg fitness
Cyclophosphamide	Tregs	Selective depletion

7.9 Tregs in Hematologic Malignancies
In hematological cancers such as leukemia, lymphoma, and multiple myeloma, Tregs play a crucial role in immune evasion and disease progression.

In chronic lymphocytic leukemia (CLL), elevated Treg frequencies correlate with disease stage and impaired immune surveillance [133]. Similarly, in acute myeloid leukemia (AML), Tregs suppress anti-leukemic T cell responses and contribute to chemoresistance.

In Hodgkin lymphoma, the tumor microenvironment is highly enriched with $FOXP3^+$ Tregs, which suppress cytotoxic responses and promote immune privilege [134].

Multiple myeloma also exhibits increased Treg activity, which correlates with bone marrow immunosuppression and disease progression [135].
Importantly, Treg modulation is being explored as an adjunct to bone marrow transplantation and CAR-T cell therapies to enhance treatment efficacy.

7.10 Tregs in Solid Tumors: Breast, Prostate, Lung, Colorectal, and Liver Cancers

Breast Cancer
Tregs are frequently enriched in triple-negative and HER2-positive breast cancers. High FOXP3 infiltration is associated with aggressive disease and poor survival, particularly in basal-like subtypes [136].

Prostate Cancer
In prostate cancer, Tregs accumulate in tumor tissue and draining lymph nodes, suppressing anti-tumor immunity and contributing to disease progression and recurrence [137].

Lung Cancer
Non-small cell lung cancer (NSCLC) exhibits high Treg infiltration, which correlates with immune checkpoint expression and poor response to immunotherapy in some cases [138].

Colorectal Cancer
As previously noted, Tregs can have dual roles. While they suppress anti-tumor immunity, they may also reduce inflammation-driven tumorigenesis, making their prognostic role complex [139].

Hepatocellular Carcinoma (HCC)
HCC is characterized by strong immunosuppressive networks, with Tregs contributing significantly to tumor immune escape via TGF-β-rich hepatic microenvironment [140].

Across all these malignancies, the balance between effector T cells and Tregs remains a critical determinant of tumor progression and therapeutic response.

VIII. REGULATORY T CELLS IN INFECTIOUS DISEASES

Tregs are also central regulators of immune responses in chronic infectious diseases, where they maintain immune homeostasis but can also facilitate pathogen persistence.

8.1 HIV Infection
In HIV infection, Tregs accumulate in lymphoid tissues and peripheral blood. While they limit chronic immune activation and inflammation, they also suppress HIV-specific $CD8^+$ T cell responses, contributing to viral persistence [141,142].

The balance between immune control and viral latency is therefore critically influenced by Treg frequency and function.

8.2 Tuberculosis (TB)
In Mycobacterium tuberculosis infection, Tregs suppress Th1 responses required for bacterial clearance. Elevated Treg levels correlate with active disease and poor granuloma containment [143].

However, Tregs also limit tissue damage caused by excessive inflammation, indicating a dual protective-pathogenic role.

8.3 Hepatitis B and C Viruses (HBV/HCV)
In chronic HBV and HCV infections, Tregs suppress antiviral T cell responses, facilitating viral persistence and chronic liver inflammation. Increased $FOXP3^+$ T cells have been observed in both peripheral blood and hepatic tissue [144].

In HCV, Tregs may contribute to progression toward cirrhosis by limiting effective viral clearance.

8.4 COVID-19 (SARS-CoV-2)
During COVID-19 infection, Treg dynamics are highly variable. In severe disease, reduced Treg function contributes to cytokine storm and hyperinflammation, whereas in recovery phases, Tregs aid in resolving immune-mediated tissue damage [145].

Immunomodulatory therapies targeting Treg balance are being explored to manage post-COVID immune dysregulation.

8.5 Malaria
In Plasmodium infection, Tregs suppress effector immune responses, facilitating parasite survival and chronic infection. However, they also prevent severe

immunopathology associated with cerebral malaria [146].

Thus, Tregs act as critical regulators of disease severity versus pathogen clearance.

Table 4. Regulatory T cells in infectious diseases.

Disease	Effect of Tregs
HIV	Suppress antiviral immunity and reduce inflammation
Tuberculosis	Inhibit Th1 responses
HBV	Promote viral persistence
HCV	Facilitate chronic infection
COVID-19	Limit cytokine storm
Malaria	Reduce immunopathology

IX. TRANSLATIONAL AND CLINICAL APPLICATIONS OF REGULATORY T CELLS IN DISEASE MANAGEMENT

The growing understanding of regulatory T cells (Tregs) in cancer and infectious diseases has shifted attention toward their translational value as diagnostic, prognostic, and therapeutic targets. Tregs are now recognized not only as immunological regulators but also as biomarker candidates and therapeutic modulators in precision medicine [147,116].

9.1 Tregs as Diagnostic and Prognostic Biomarkers

$FOXP3^+$ Treg frequency in peripheral blood and tumor tissues has been widely investigated as a biomarker of disease progression. Elevated circulating Tregs often reflect systemic immune suppression, while tumor-infiltrating Tregs indicate local immune escape mechanisms.

In oncology, the $CD8^+/FOXP3^+$ ratio is increasingly considered a superior prognostic marker compared to absolute FOXP3 expression alone [148]. A low ratio generally indicates poor immune surveillance and worse clinical outcomes.

In infectious diseases, Treg frequency correlates with disease chronicity, as seen in HIV, HBV, and tuberculosis, where persistent elevation is associated with impaired pathogen clearance [149,150].

9.2 Tregs in Predictive Immunotherapy Biomarker Panels

Checkpoint blockade therapy response is influenced by baseline Treg infiltration and functional state. High intratumoral Tregs often predict reduced response to PD-1/PD-L1 monotherapy, but may respond better to combination strategies involving CTLA-4 blockade or metabolic inhibitors [118].

Multiparametric biomarker panels incorporating:

- FOXP3
- CTLA-4
- CD25
- IL-10
- TGF-β
- CD39/CD73 axis

are emerging as robust predictors of immunotherapy outcomes [134].

These panels allow stratification of patients into immunologically “hot” or “cold” tumors for tailored therapy.

9.3 Therapeutic Targeting of Tregs in Clinical Practice

Although Tregs are essential for immune tolerance, their suppression in cancer is being actively explored. Current translational approaches include:

(i) Immune checkpoint modulation

CTLA-4 blockade remains the most clinically validated strategy for partial Treg depletion in tumors [117].

(ii) Metabolic targeting
Inhibition of lipid metabolism (FAO pathways) and adenosine signaling (CD39/CD73 axis) selectively weakens tumor-resident Tregs [151].

(iii) Chemokine blockade
CCR4 and CCR8 antagonists prevent Treg recruitment into tumors, enhancing effector T cell infiltration [33].

(iv) Combination immunotherapy
Combining checkpoint inhibitors with chemotherapy, vaccines, or metabolic drugs enhances tumor regression by simultaneously activating effector T cells and reducing Treg suppression.

However, systemic Treg depletion remains risky due to the potential induction of autoimmune toxicity, requiring carefully balanced therapeutic design [152].

9.4 Tregs in Personalized and Precision Medicine
The heterogeneity of Treg function across diseases highlights their role in precision immunology. Tumor-specific Tregs differ phenotypically and metabolically from peripheral Tregs, making them potential targets for selective immunomodulation [116].

Advances in single-cell RNA sequencing have revealed distinct Treg subsets within tumors, including:

- Effector Tregs (high suppressive activity)
- Memory-like Tregs
- Tissue-resident Tregs

This heterogeneity supports personalized targeting strategies rather than global immune suppression.

Figure 4. Translational applications of Tregs in modern medicine.

Illustrate:

- Diagnostic biomarkers
- Prognostic biomarkers
- Checkpoint immunotherapy
- CAR-Treg therapy
- Low-dose IL-2 therapy
- Precision medicine
- Cell-based therapy

X. METHODS (LITERATURE SEARCH STRATEGY AND METHODOLOGICAL APPROACH)

This review employed a structured narrative literature search approach to comprehensively synthesize current knowledge on regulatory T cells (Tregs) in immune homeostasis, cancer, autoimmune diseases, infectious diseases, and immunometabolism. The methodology was designed to ensure broad coverage of relevant peer-reviewed evidence while maintaining reproducibility and transparency.

10.1 Databases Searched
A systematic electronic search of the following databases was conducted:

- PubMed/MEDLINE
- Scopus
- Web of Science Core Collection
- Google Scholar (for supplementary and citation-tracking purposes)

These databases were selected due to their extensive coverage of biomedical, immunological, and clinical research literature.

10.2 Search Strategy and Keywords
The literature search was performed using combinations of controlled vocabulary (MeSH terms where applicable) and free-text keywords. The following core search terms were used, along with Boolean operators (AND, OR):

- “regulatory T cells” OR “Tregs”
- “FOXP3”
- “immune tolerance”
- “immune homeostasis”
- “tumor microenvironment”
- “immune checkpoint” OR “CTLA-4” OR “PD-1”
- “immunometabolism”
- “autoimmune disease”
- “cancer immunology”
- “infectious diseases” AND “Treg”
- “cytokines” AND “IL-10” OR “TGF-β” OR “IL-35”
- “T cell plasticity”
- “metabolic reprogramming” AND “T cells”

Additional keyword combinations were used iteratively to expand coverage of specific disease contexts such as cancer, HIV infection, tuberculosis, hepatitis B and C, malaria, and COVID-19.

10.3 Time Frame of Literature Search
The search included studies published between January 2000 and June 2026, capturing both foundational immunological discoveries and recent advances in high-throughput single-cell technologies, immunometabolism, and cancer immunotherapy.

10.4 Inclusion Criteria
Studies were included if they met the following criteria:

- Published in peer-reviewed journals
- Written in English
- Focused on regulatory T cell biology, function, or clinical relevance
- Included human studies, animal models, or in vitro experimental data
- Addressed at least one of the following domains:
    - Immune homeostasis and tolerance
    - Autoimmune diseases
    - Cancer immunology and tumor microenvironment
    - Infectious diseases (viral, bacterial, parasitic)
    - Immunometabolism or T cell signaling pathways
- Original research articles, systematic reviews, meta-analyses, and clinical trial reports were considered eligible

10.5 Exclusion Criteria
Studies were excluded if they met any of the following conditions:

- Non-peer-reviewed publications, editorials, or opinion pieces without primary data
- Conference abstracts without full-text availability
- Articles not written in English
- Studies unrelated to Treg biology or immune regulation
- Duplicate publications across databases
- Animal studies with no translational or immunological relevance to Treg function

10.6 Study Selection Process
Titles and abstracts retrieved from the database search were screened for relevance to regulatory T cell biology and immunological function. Full-text articles were then assessed for eligibility based on the inclusion and exclusion criteria. Relevant studies were selected to ensure balanced representation across basic science, translational research, and clinical immunology.

Reference lists of selected articles were also manually screened to identify additional relevant studies not captured in the initial database search (snowballing technique).

10.7 Data Extraction and Synthesis
Key information extracted from selected studies included:

- Study design and experimental model
- Treg subsets analyzed (tTregs, pTregs, iTregs, tumor Tregs, etc.)
- Molecular pathways (FOXP3 regulation, cytokine signaling, metabolic pathways)
- Disease context (autoimmune diseases, cancer, infections)
- Major findings related to Treg function and immunoregulation

Extracted data were synthesized thematically rather than statistically, consistent with a narrative review design. Emphasis was placed on integrating mechanistic insights with clinical relevance.

10.8 Quality Assessment Approach
Although a formal risk-of-bias scoring system was not applied due to the narrative nature of this review, preference was given to:

- High-impact peer-reviewed journals
- Recent publications with strong experimental or clinical validation
- Landmark studies in Treg biology and immunology

Where conflicting evidence existed, findings were interpreted in the context of study design, sample size, and experimental methodology.

10.9 Reporting Framework

This review was conducted in accordance with established principles for narrative literature reviews. While a full PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram was not mandatory for this narrative format, PRISMA principles were used as a guiding framework to enhance transparency in literature identification, screening, and selection.

XI. CHALLENGES AND LIMITATIONS IN REGULATORY T CELL RESEARCH AND TRANSLATION

Despite major advances in understanding regulatory T cell (Treg) biology, several challenges continue to limit full clinical translation of experimental findings into therapeutic applications.

11.1 Heterogeneity of Treg Populations

One of the major limitations in Treg research is the extensive heterogeneity within Treg subsets. Classical $FOXP3^{+}CD4^{+}$ Tregs coexist with multiple specialized populations including tissue-resident Tregs, follicular regulatory T cells (Tfr), memory-like Tregs, and tumor-infiltrating Tregs. These subsets differ significantly in phenotype, function, and metabolic programming, making it difficult to define universal biomarkers or therapeutic targets.

11.2 Lack of Specificity in Treg Targeting

Many molecules used to identify or target Tregs, such as CD25, CTLA-4, and FOXP3, are also expressed on activated effector T cells. This overlap complicates selective therapeutic targeting, particularly in cancer immunotherapy where systemic Treg depletion may lead to immune-related adverse events and autoimmunity.

11.3 Functional Plasticity and Instability

Tregs exhibit significant plasticity under inflammatory conditions. Exposure to cytokines such as IL-6, IL-1β, and IL-23 can induce conversion into Th1- or Th17-like effector cells. This instability undermines therapeutic strategies aimed at expanding or transferring stable Treg populations.

11.4 Limited Standardization of Experimental Approaches

Differences in experimental models, flow cytometry gating strategies, cytokine assays, and animal models lead to variability in reported findings. Lack of standardization reduces reproducibility and complicates cross-study comparisons.

11.5 Translational Gaps Between Preclinical and Clinical Studies

Although Treg-based therapies show strong efficacy in animal models, clinical translation has been inconsistent. Differences in immune complexity, tumor heterogeneity, and metabolic environments between humans and experimental models contribute to this gap.

11.6 Tumor Microenvironment Complexity

In cancer, the immunosuppressive tumor microenvironment involves multiple overlapping pathways beyond Tregs, including myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages, and metabolic suppression. This redundancy limits the effectiveness of strategies targeting Tregs alone.

XII. FUTURE PERSPECTIVES AND EMERGING DIRECTIONS

The rapidly evolving field of regulatory T cell biology continues to open new avenues for therapeutic innovation and mechanistic understanding.

12.1 Single-Cell and Spatial Multi-Omics Profiling

Future studies will increasingly rely on single-cell RNA sequencing, spatial transcriptomics, and epigenomic mapping to define precise Treg subpopulations within tissues. These approaches will enable high-resolution mapping of Treg heterogeneity in cancer, infection, and autoimmune diseases.

12.2 Precision Immunotherapy Targeting Treg Subsets

Rather than global Treg depletion, future therapies will focus on selectively targeting pathogenic Treg

subsets (e.g., $CCR8^+$ tumor Tregs) while preserving homeostatic Tregs essential for immune tolerance.

12.3 Metabolic Reprogramming as a Therapeutic Strategy

Targeting metabolic pathways such as fatty acid oxidation, cholesterol metabolism, and adenosine signaling (CD39/CD73 axis) represents a promising strategy to modulate Treg function in tumors without inducing systemic autoimmunity.

12.4 Microbiome–Treg Interactions

Emerging evidence highlights the gut microbiome as a key regulator of peripheral Treg differentiation. Future interventions may involve microbiota engineering, probiotics, or microbial metabolites (e.g., short-chain fatty acids) to enhance or suppress Treg activity depending on disease context.

12.5 Engineered Treg Therapies

Advances in synthetic biology and gene editing are enabling development of:

- CAR-Tregs (chimeric antigen receptor regulatory T cells)
- CRISPR-modified Tregs with enhanced stability
- Antigen-specific Tregs for transplantation tolerance

These approaches may provide highly targeted and durable immune regulation.

12.6 Biomarker Development and Liquid Biopsy Approaches

Non-invasive biomarkers such as circulating $FOXP3^+$ Tregs, cytokine signatures, and exosomal Treg markers are expected to improve disease monitoring, prognosis, and therapeutic stratification.

Table 5. Emerging research directions in regulatory T-cell biology.

Emerging Area	Potential Impact
Single-cell sequencing	Identification of novel Treg subsets
Spatial transcriptomics	Tissue-specific Treg mapping
CAR-Tregs	Precision immune regulation
CRISPR-edited Tregs	Enhanced stability
Microbiome-Treg interactions	Novel therapeutics
Liquid biopsy biomarkers	Non-invasive monitoring

XIII. CLINICAL AND HEALTH POLICY IMPLICATIONS

The expanding knowledge of Treg biology has significant implications for clinical practice and health policy development.

13.1 Clinical Implications in Disease Management

Tregs represent both therapeutic targets and therapeutic tools across multiple disease categories:

- Autoimmune diseases: Strategies to enhance Treg function (low-dose IL-2, adoptive Treg transfer) are emerging as promising therapies.
- Cancer: Treg depletion or functional inhibition is a major component of immunotherapy strategies.
- Infectious diseases: Modulation of Tregs may help balance pathogen clearance and immunopathology.

13.2 Personalized and Precision Medicine

Assessment of Treg frequency, phenotype, and functional status can guide personalized treatment strategies, particularly in oncology and chronic infections. The $CD8^+/FOXP3^+$ ratio and cytokine profiles may serve as predictive biomarkers for therapy response.

13.3 Implications for Immunotherapy Policy

Immune checkpoint inhibitors have demonstrated that manipulation of immune tolerance pathways can achieve durable clinical responses. However,

variability in Treg dynamics highlights the need for regulatory frameworks that support:

- Biomarker-driven patient selection
- Combination immunotherapy strategies
- Monitoring of immune-related adverse events

13.4 Public Health and Translational Impact

In infectious diseases such as HIV, tuberculosis, and hepatitis, Treg modulation may influence disease transmission dynamics, chronic infection rates, and long-term immune recovery. This underscores the importance of integrating immunological insights into public health strategies.

13.5 Ethical Considerations

Manipulation of immune tolerance raises ethical concerns, particularly regarding:

- Risk of autoimmunity from Treg depletion
- Long-term effects of immune reprogramming
- Gene-edited cellular therapies

Careful regulatory oversight is required as these therapies transition into clinical practice.

Figure 5. Central role of regulatory T cells in immune homeostasis, cancer, autoimmunity, infectious diseases, and therapeutic applications.

This should be your graphical summary figure showing:

- Immune Homeostasis
- Autoimmunity
- Cancer
- Infectious Diseases
- Immunometabolism
- Precision Medicine
- Therapeutic Targeting

all linked through a central $FOXP3^+$ Regulatory T Cell.

XIV. CONCLUSION

Regulatory T cells represent a central immunological hub connecting cancer biology, infectious disease progression, immune tolerance, and therapeutic response. Their dual role—as protectors of immune homeostasis and facilitators of immune evasion—makes them critical determinants of disease outcomes. In cancer, Tregs suppress anti-tumor immunity through cytokine secretion, checkpoint signaling, metabolic adaptation, and direct cytotoxic suppression. In infectious diseases, they balance pathogen clearance with prevention of immunopathology. Their dynamic nature positions them as both therapeutic targets and prognostic biomarkers in modern immunology.

A deeper mechanistic understanding of Treg biology will continue to shape next-generation immunotherapies and precision medicine strategies.

REFERENCES

[1] R. K. Gershon and K. Kondo, “Infectious immunological tolerance,” *Immunology*, vol. 21, no. 6, pp. 903–914, 1971.

[2] S. Sakaguchi, N. Sakaguchi, M. Asano, M. Itoh, and M. Toda, “Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α-chains (CD25): Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases,” *The Journal of Immunology*, vol. 155, no. 3, pp. 1151–1164, 1995.

[3] C. L. Bennett, J. Christie, F. Ramsdell, M. E. Brunkow, P. J. Ferguson, L. Whitesell, T. E. Kelly, F. T. Saulsbury, P. F. Chance, and H. D. Ochs, “The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3,” *Nature Genetics*, vol. 27, no. 1, pp. 20–21, 2001. Nature

[4] D. A. A. Vignali, L. W. Collison, and C. J. Workman, “How regulatory T cells work,” *Nature Reviews Immunology*, vol. 8, no. 7, pp. 523–532, 2008. Nature

[5] S. Dikiy and A. Y. Rudensky, “Principles of regulatory T cell function,” *Immunity*, vol. 56, no. 2, pp. 240–255, 2023. ScienceDirect

[6] A. Kaminski, F. T. Hager, L. Kopplin, F. Ticconi, A. Leufgen, E. Vendelova, L. Rüttger, G. Gasteiger, V. Cerovic, W. Kastenmüller, O. Pabst, and M. Ugur, “Resident regulatory T cells reflect the immune history of individual lymph nodes,” *Science Immunology*, vol. 8, no. 89, Art. no. eadj5789, 2023. Science

[7] P. Grover, P. N. Goel, and M. I. Greene, "Regulatory T Cells: Regulation of Identity and Function," *Frontiers in Immunology*, vol. 12, Art. no. 750542, 2021. Frontiers

[8] M. Arjomandnejad, A. L. Kopec, and A. M. Keeler, "CAR-T Regulatory (CAR-Treg) Cells: Engineering and Applications," *Biomedicines*, vol. 10, no. 2, Art. no. 287, 2022. MDPI

[9] F. Shirafkan, L. Hensel, and K. Rattay, "Immune tolerance and the prevention of autoimmune diseases essentially depend on thymic tissue homeostasis," *Frontiers in Immunology*, vol. 15, Art. no. 1339714, 2024. Frontiers

[10] S. Z. Josefowicz, L. F. Lu, and A. Y. Rudensky, "Regulatory T cells: mechanisms of differentiation and function," *Annual Review of Immunology*, vol. 30, pp. 531–564, 2012. Annual Reviews

[11] A. M. Bilate and J. J. Lafaille, "Induced $CD4^{+}Foxp3^{+}$ regulatory T cells in immune tolerance," *Annual Review of Immunology*, vol. 30, pp. 733–758, 2012. Annual Reviews

[12] W. Chen, W. Jin, N. Hardegen, K.-J. Lei, L. Li, N. Marinos, G. McGrady, and S. M. Wahl, "Conversion of peripheral $CD4^{+}CD25^{-}$ naive T cells to $CD4^{+}CD25^{+}$ regulatory T cells by TGF-β induction of transcription factor Foxp3," *The Journal of Experimental Medicine*, vol. 198, no. 12, pp. 1875–1886, 2003. JEM

[13] W. Chen, "TGF-β Regulation of T Cells," *Annual Review of Immunology*, vol. 41, pp. 483–512, 2023. Annual Reviews

[14] T. R. Malek and I. Castro, "Interleukin-2 receptor signaling: At the interface between tolerance and immunity," *Immunity*, vol. 33, no. 2, pp. 153–165, 2010. ScienceDirect

[15] J. L. Coombes, K. R. R. Siddiqui, C. V. Arancibia-Cárcamo, J. Hall, C.-M. Sun, Y. Belkaid, and F. Powrie, "A functionally specialized population of mucosal $CD103^{+}$ dendritic cells induces $Foxp3^{+}$ regulatory T cells via a TGF-β- and retinoic acid-dependent mechanism," *Journal of Experimental Medicine*, vol. 204, no. 8, pp. 1757–1764, 2007. JEM

[16] Y. Furusawa, Y. Obata, S. Fukuda, T. A. Endo, G. Nakato, D. Takahashi, Y. Nakanishi, C. Uetake, K. Kato, T. Kato, M. Takahashi, N. N. Fukuda, S. Murakami, E. Miyauchi, S. Hino, K. Atarashi, S. Onawa, Y. Fujimura, T. Lockett, J. M. Clarke, D. L. Topping, M. Tomita, S. Hori, O. Ohara, T. Morita, H. Koseki, J. Kikuchi, K. Honda, K. Hase, and H. Ohno, "Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells," *Nature*, vol. 504, no. 7480, pp. 446–450, 2013. Nature

[17] N. Arpaia, C. Campbell, X. Fan, S. Dikiy, J. van der Veeken, P. deRoos, H. Liu, J. R. Cross, K. Pfeffer, P. J. Coffer, and A. Y. Rudensky, "Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation," *Nature*, vol. 504, no. 7480, pp. 451–455, 2013. Nature

[18] S. Z. Josefowicz, L. F. Lu, and A. Y. Rudensky, "Regulatory T cells: mechanisms of differentiation and function," *Annual Review of Immunology*, vol. 30, pp. 531–564, 2012. Annual Reviews

[19] W. Chen, W. Jin, N. Hardegen, K.-J. Lei, L. Li, N. Marinos, G. McGrady, and S. M. Wahl, "Conversion of peripheral $CD4^{+}CD25^{-}$ naive T cells to $CD4^{+}CD25^{+}$ regulatory T cells by TGF-β induction of transcription factor Foxp3," *The Journal of Experimental Medicine*, vol. 198, no. 12, pp. 1875–1886, 2003. JEM

[20] J. A. Bluestone and Q. Tang, "Treg cells—the next frontier of cell therapy," *Science*, vol. 362, no. 6411, pp. 154–155, 2018. Science

[21] S. Floess, J. Freyer, C. Siewert, U. Baron, S. Olek, J. Polansky, K. Schlawe, H.-D. Chang, T. Bopp, E. Schmitt, S. Klein-Hessling, E. Serfling, and A. Hamann, "Epigenetic control of the foxp3 locus in regulatory T cells," *PLoS Biology*, vol. 5, no. 2, Art. no. e38, 2007. PLOS

[22] J. K. Polansky, K. Kretschmer, J. Freyer, S. Floess, A. Garbe, U. Baron, S. Olek, A. Hamann, and H. von Boehmer, "DNA methylation controls Foxp3 gene expression," *European Journal of Immunology*, vol. 40, no. 7, pp. 1835–1844, 2010. Wiley

[23] S. Dikiy and A. Y. Rudensky, "Principles of regulatory T cell function," *Immunity*, vol. 56, no. 2, pp. 240–255, 2023. ScienceDirect

[24] M. R. Vieyra-Lobato, J. Vela-Ojeda, L. Montiel-Cervantes, R. López-Santiago, and M. C. Moreno-Lafont, "Description of $CD8^+$ Regulatory T Lymphocytes and Their Specific Intervention in Graft-versus-Host and Infectious Diseases, Autoimmunity, and Cancer," *Journal of Immunology Research*, vol. 2018, Art. ID 3758713, 2018. Wiley

[25] H. Jiang and L. Chess, "Regulation of immune responses by T cells," *New England Journal of Medicine*, vol. 354, no. 11, pp. 1166–1176, 2006. NEJM

[26] M. Panduro, C. Benoist, and D. Mathis, "Tissue Tregs," *Annual Review of Immunology*, vol. 34, pp. 609–633, 2016. Annual Reviews

[27] A. Kaminski, F. T. Hager, L. Kopplin, F. Ticconi, A. Leufgen, E. Vendelova, L. Rüttger, G. Gasteiger, V. Cerovic, W. Kastenmüller, O. Pabst, and M. Ugur, "Resident regulatory T cells reflect the immune history of individual lymph nodes," *Science Immunology*, vol. 8, no. 89, Art. no. eadj5789, 2023. Science

[28] M. Feuerer, L. Herrero, D. Cipolletta, A. Naaz, J. Wong, A. Nayer, J. Lee, A. B. Goldfine, C. Benoist, S. Shoelson, and D. Mathis, "Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters," *Nature Medicine*, vol. 15, no. 8, pp. 930–939, 2009. Nature

[29] D. Cipolletta, M. Feuerer, A. Li, N. Kamei, J. Lee, S. E. Shoelson, C. Benoist, and D. Mathis, "PPAR-γ is a major driver of the accumulation and phenotype of adipose tissue Treg cells," *Nature*, vol. 486, no. 7404, pp. 549–553, 2012. Nature

[30] N. Ali, B. Zirak, R. S. Rodriguez, M. L. Pauli, H.-A. Truong, K. Lai, R. Ahn, K. Corbin, M. M. Lowe, T. C. Scharschmidt, K. Taravati, M. R. Tan, R. R. Ricardo-Gonzalez, A. Nosbaum, M. Bertolini, W. Liao, F. O. Nestle, R. Paus, and M. D. Rosenblum, "Regulatory T Cells in Skin Facilitate Epithelial Stem Cell Differentiation," *Cell*, vol. 169, no. 6, pp. 1119–1129.e11, 2017. ScienceDirect

[31] E. Sefik, N. Geva-Zatorsky, S. Oh, L. Konnikova, D. Zemmour, A. M. McGuire, D. Burzyn, A. Ortiz-Lopez, M. Lobera, J. Yang, S. Ghosh, A. Earl, S. B. Snapper, R. Jupp, D. Kasper, D. Mathis, C. Benoist, and R. J. Xavier, "Individual intestinal symbionts induce a distinct population of $ROR\gamma^+$ regulatory T cells," *Science*, vol. 349, no. 6251, pp. 993–997, 2015. Science

[32] N. Arpaia, J. A. Green, B. Moltedo, A. Arvey, S. Hemmers, S. Yuan, P. M. Treuting, and A. Y. Rudensky, "A Distinct Function of Regulatory T Cells in Tissue Protection," *Cell*, vol. 162, no. 5, pp. 1078–1089, 2015. ScienceDirect

[33] G. Plitas, C. Konopacki, K. Wu, P. D. Bos, M. Morrow, E. V. Putintseva, D. M. Chudakov, and A. Y. Rudensky, "Regulatory T Cells Exhibit Distinct Features in Human Breast Cancer," *Immunity*, vol. 45, no. 5, pp. 1122–1134, 2016. ScienceDirect

[34] J. B. Wing, A. Tanaka, and S. Sakaguchi, "Human $FOXP3^+$ Regulatory T Cell Heterogeneity and Function in Autoimmunity and Cancer," *Immunity*, vol. 50, no. 2, pp. 302–316, 2019. ScienceDirect

[35] S. Hori, T. Nomura, and S. Sakaguchi, "Control of regulatory T cell development by the transcription factor Foxp3," *Science*, vol. 299, no. 5609, pp. 1057–1061, 2003. Science

[36] M. E. Brunkow, E. W. Jeffery, K. A. Hjerrild, B. Paeper, L. B. Clark, S.-A. Yasayko, J. E. Wilkinson, D. Galas, S. F. Ziegler, and F. Ramsdell, "Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse," *Nature Genetics*, vol. 27, no. 1, pp. 68–73, 2001. Nature

[37] C. L. Bennett, J. Christie, F. Ramsdell, M. E. Brunkow, P. J. Ferguson, L. Whitesell, T. E. Kelly, F. T. Saulsbury, P. F. Chance, and H. D. Ochs, "The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3," *Nature Genetics*, vol. 27, no. 1, pp. 20–21, 2001. Nature

[38] D. Rudra, P. deRoos, A. Chaudhry, R. E. Niec, A. Arvey, R. M. Samstein, C. Leslie, S. A.

Shaffer, D. R. Goodlett, and A. Y. Rudensky, "Transcription factor Foxp3 and its protein partners form a complex regulatory network," *Nature Immunology*, vol. 13, no. 10, pp. 1010–1019, 2012. Nature

[39] M. A. Burchill, J. Yang, C. Vogtenhuber, B. R. Blazar, and M. A. Farrar, "IL-2 receptor β-dependent STAT5 activation is required for the development of Foxp3$^+$ regulatory T cells," *The Journal of Immunology*, vol. 178, no. 1, pp. 280–290, 2007. Journal of Immunology

[40] W. Chen, W. Jin, N. Hardegen, K.-J. Lei, L. Li, N. Marinos, G. McGrady, and S. M. Wahl, "Conversion of peripheral CD4$^+$CD25$^-$ naive T cells to CD4$^+$CD25$^+$ regulatory T cells by TGF-β induction of transcription factor Foxp3," *Journal of Experimental Medicine*, vol. 198, no. 12, pp. 1875–1886, 2003. JEM

[41] G. M. Delgoffe, T. P. Kole, Y. Zheng, P. E. Zarek, K. L. Matthews, B. Xiao, P. F. Worley, S. C. Kozma, J. D. Powell, and T. J. Curiel, "The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment," *Immunity*, vol. 30, no. 6, pp. 832–844, 2009. ScienceDirect

[42] N. Ohkura, M. Hamaguchi, H. Morikawa, K. Sugimura, A. Tanaka, Y. Ito, M. Osaki, Y. Tanaka, R. Yamashita, N. Nakano, J. Huehn, H. J. Fehling, T. Sparwasser, K. Nakai, and S. Sakaguchi, "T Cell Receptor Stimulation-Induced Epigenetic Changes and Foxp3 Expression Are Independent and Complementary Events Required for Regulatory T Cell Development," *Immunity*, vol. 37, no. 5, pp. 785–799, 2013. ScienceDirect

[43] R. M. Samstein, A. Arvey, S. Z. Josefowicz, X. Peng, A. Reynolds, R. Sandstrom, S. Neph, P. Sabo, J. M. Kim, W. Liao, M. O. Li, C. Leslie, J. A. Stamatoyannopoulos, and A. Y. Rudensky, "Foxp3 Exploits a Pre-existent Enhancer Landscape for Regulatory T Cell Lineage Specification," *Cell*, vol. 151, no. 1, pp. 153–166, 2012. ScienceDirect

[44] S. Floess, J. Freyer, C. Siewert, U. Baron, S. Olek, J. Polansky, K. Schlawe, H.-D. Chang, T. Bopp, E. Schmitt, S. Klein-Hessling, E. Serfling, and A. Hamann, "Epigenetic control of the Foxp3 locus in regulatory T cells," *PLoS Biology*, vol. 5, no. 2, Art. no. e38, 2007. PLOS

[45] J. K. Polansky, K. Kretschmer, J. Freyer, S. Floess, A. Garbe, U. Baron, S. Olek, A. Hamann, and H. von Boehmer, "DNA methylation controls Foxp3 gene expression," *European Journal of Immunology*, vol. 40, no. 7, pp. 1835–1844, 2010. Wiley

[46] J. van Loosdregt, Y. Vercoulen, T. Guichelaar, Y. Y. J. Gent, J. M. Beekman, O. van Beekum, A. B. Brenkman, D. J. Hijnen, T. Mutis, E. Kalkhoven, B. J. Prakken, P. J. Coffer, and R. A. W. van Lier, "Regulation of Treg functionality by acetylation-mediated Foxp3 protein stabilization," *Blood*, vol. 115, no. 5, pp. 965–974, 2010. Blood

[47] L. Lu, J. Barbi, F. Pan, J. Yu, S. Parish, S. Chowdhury, Y. Wu, Y. Zheng, D. Pardoll, and F. Pan, "The forkhead transcription factor Foxp3 promotes DNA binding of NFAT by cooperative interaction," *Cell*, vol. 139, no. 5, pp. 949–962, 2009.

[48] S. Kohlhaas, O. A. Garden, C. Scudamore, M. Turner, K. Okkenhaug, and E. Vigorito, "Cutting Edge: The Foxp3 target miR-155 contributes to the development of regulatory T cells," *The Journal of Immunology*, vol. 182, no. 5, pp. 2578–2582, 2009. Journal of Immunology

[49] N. Ohkura, Y. Kitagawa, and S. Sakaguchi, "Development and maintenance of regulatory T cells," *Immunity*, vol. 52, no. 1, pp. 30–41, 2020.

[50] X. Zhou, S. L. Bailey-Bucktrout, L. T. Jeker, and J. A. Bluestone, "Plasticity of CD4$^+$ FoxP3$^+$ T cells," *Current Opinion in Immunology*, vol. 21, no. 3, pp. 281–285, 2009. ScienceDirect

[51] S. Hori, "FOXP3 as a master regulator of Treg cells," *Nature Reviews Immunology*, vol. 21, no. 10, pp. 618–619, 2021. Nature

[52] E. Bettelli, Y. Carrier, W. Gao, T. Korn, T. B. Strom, M. Oukka, H. L. Weiner, and V. K. Kuchroo, "Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells," *Nature*, vol. 441, no. 7090, pp. 235–238, 2006. Nature

[53] M. Dominguez-Villar and D. A. Hafler, "Regulatory T cells in autoimmune disease," *Nature Immunology*, vol. 19, no. 7, pp. 665–673, 2018. Nature

[54] M. A. Koch, G. Tucker-Heard, N. R. Perdue, J. R. Killebrew, K. B. Urdahl, and D. J. Campbell, "The transcription factor T-bet controls regulatory T cell homeostasis and function during type 1 inflammation," *Nature Immunology*, vol. 10, no. 6, pp. 595–602, 2009. Nature

[55] Y. Zheng, A. Chaudhry, A. Kas, P. deRoos, J. M. Kim, T.-T. Chu, L. Corcoran, P. Treuting, U. Klein, and A. Y. Rudensky, "Regulatory T-cell suppressor program co-opts transcription factor IRF4 to control TH2 responses," *Nature*, vol. 458, no. 7236, pp. 351–356, 2009. Nature

[56] D. A. A. Vignali, L. W. Collison, and C. J. Workman, "How regulatory T cells work," *Nature Reviews Immunology*, vol. 8, no. 7, pp. 523–532, 2008. Nature

[57] S. Dikiy and A. Y. Rudensky, "Principles of regulatory T cell function," *Immunity*, vol. 56, no. 2, pp. 240–255, 2023. ScienceDirect

[58] D. A. A. Vignali, L. W. Collison, and C. J. Workman, "How regulatory T cells work," *Nature Reviews Immunology*, vol. 8, no. 7, pp. 523–532, 2008. Nature

[59] R. Kühn, J. Löhler, D. Rennick, K. Rajewsky, and W. Müller, "Interleukin-10-deficient mice develop chronic enterocolitis," *Cell*, vol. 75, no. 2, pp. 263–274, 1993. ScienceDirect

[60] W. Chen, "TGF-β Regulation of T Cells," *Annual Review of Immunology*, vol. 41, pp. 483–512, 2023. Annual Reviews

[61] L. W. Collison, C. J. Workman, T. T. Kuo, K. Boyd, Y. Wang, K. M. Vignali, R. Cross, D. Sehy, R. S. Blumberg, and D. A. A. Vignali, "The inhibitory cytokine IL-35 contributes to regulatory T-cell function," *Nature*, vol. 450, no. 7169, pp. 566–569, 2007. Nature

[62] C. Ye, H. Yano, C. J. Workman, and D. A. A. Vignali, "Interleukin-35: Structure, Function and Its Impact on Immune-Related Diseases," *Journal of Interferon & Cytokine Research*, vol. 41, no. 11, pp. 391–406, 2021. Mary Ann Liebert

[63] L. S. K. Walker and D. M. Sansom, "The emerging role of CTLA4 as a cell-extrinsic regulator of T cell responses," *Nature Reviews Immunology*, vol. 11, no. 12, pp. 852–863, 2011. Nature

[64] O. S. Qureshi, Y. Zheng, K. Nakamura, K. Attridge, C. Manzotti, E. M. Schmidt, J. Baker, L. E. Jeffery, S. Kaur, Z. Briggs, T. Z. Hou, C. E. Futter, G. Anderson, L. S. K. Walker, and D. M. Sansom, "Trans-endocytosis of CD80 and CD86: A molecular basis for the cell-extrinsic function of CTLA-4," *Science*, vol. 332, no. 6029, pp. 600–603, 2011. Science

[65] N. Sobhani, D. R. Tardiel-Cyril, A. Davtyan, D. Generali, R. Roudi, and Y. Li, "CTLA-4 in Regulatory T Cells for Cancer Immunotherapy," *Cancers*, vol. 13, no. 6, Art. no. 1440, 2021. MDPI

[66] "The diverse functions of the PD1 inhibitory pathway," *Nature Reviews Immunology*, vol. 18, no. 3, pp. 153–167. Nature

[67] N. Joller, E. Lozano, P. R. Burkett, B. Patel, S. Xiao, C. Zhu, J. Xia, T. G. Tan, E. Sefik, V. Yajnik, A. H. Sharpe, F. J. Quintana, D. Mathis, C. Benoist, and V. K. Kuchroo, "Treg cells expressing the coinhibitory molecule TIGIT selectively inhibit proinflammatory Th1 and Th17 cell responses," *Immunity*, vol. 40, no. 4, pp. 569–581, 2014. ScienceDirect

[68] C.-T. Huang, C. J. Workman, D. Flies, X. Pan, A. L. Marson, G. Zhou, E. L. Hipkiss, S. Ravi, J. Kowalski, H. I. Levitsky, J. D. Powell, D. M. Pardoll, and C. G. Drake, "Role of LAG-3 in regulatory T cells," *Immunity*, vol. 21, no. 4, pp. 503–513, 2004. ScienceDirect

[69] P. Pandiyan, L. Zheng, S. Ishihara, J. Reed, and M. J. Lenardo, "$CD4^+CD25^+Foxp3^+$ regulatory T cells induce cytokine deprivation-mediated apoptosis of effector $CD4^+$ T cells," *Nature Immunology*, vol. 8, no. 12, pp. 1353–1362, 2007. Nature

[70] S. Deaglio, K. M. Dwyer, W. Gao, D. Friedman, A. Usheva, A. Erat, J.-F. Chen, K. Enjyoji, J. Linden, M. Oukka, V. K. Kuchroo, T. B. Strom, and S. C. Robson, "Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression," *The Journal of*

Experimental Medicine, vol. 204, no. 6, pp. 1257–1265, 2007. JEM

[71] W. J. Grossman, J. W. Verbsky, W. Barchet, and M. Colonna, "Human T regulatory cells can use the perforin pathway to cause autologous target cell death," *Immunity*, vol. 21, no. 4, pp. 589–601, 2004. ScienceDirect

[72] R. D. Michalek, V. A. Gerriets, S. R. Jacobs, A. N. Macintyre, N. J. MacIver, E. F. Mason, S. A. Sullivan, A. G. Nichols, and J. C. Rathmell, "Cutting Edge: Distinct Glycolytic and Lipid Oxidative Metabolic Programs Are Essential for Effector and Regulatory $CD4^+$ T Cell Subsets," *The Journal of Immunology*, vol. 186, no. 6, pp. 3299–3303, 2011. Journal of Immunology

[73] A. Angelin, L. Gil-de-Gómez, S. Dahiya, J. Jiao, L. Guo, M. H. Levine, Z. Wang, W. J. Quinn III, P. K. Kopinski, L. Wang, T. Akimova, Y. Liu, T. R. Bhatti, R. Han, B. L. Laskin, J. A. Baur, I. A. Blair, D. C. Wallace, W. W. Hancock, and U. H. Beier, "Foxp3 Reprograms T Cell Metabolism to Function in Low-Glucose, High-Lactate Environments," *Cell Metabolism*, vol. 25, no. 6, pp. 1282–1293.e7, 2017. ScienceDirect

[74] D. Howie, S. P. Cobbold, E. Adams, A. Ten Bokum, A. S. Necula, W. Zhang, H. Huang, D. J. Roberts, B. Thomas, S. S. Hester, M. Mayr, Y. Chernajovsky, H. Waldmann, and S. P. Cobbold, "Foxp3 drives oxidative phosphorylation and protection from lipotoxicity," *The Journal of Clinical Investigation*, vol. 127, no. 11, pp. 4188–4202, 2017. JCI

[75] N. M. Chapman, M. R. Boothby, and H. Chi, "Metabolic coordination of T cell quiescence and activation," *Nature Reviews Immunology*, vol. 20, no. 1, pp. 55–70, 2020. Nature

[76] E. L. Pearce, M. C. Poffenberger, C.-H. Chang, and R. G. Jones, "Fueling immunity: Insights into metabolism and lymphocyte function," *Science*, vol. 342, no. 6155, Art. no. 1242454, 2013. Science

[77] A. Angelin, L. Gil-de-Gómez, S. Dahiya, J. Jiao, L. Guo, M. H. Levine, Z. Wang, W. J. Quinn III, P. K. Kopinski, L. Wang, T. Akimova, Y. Liu, T. R. Bhatti, R. Han, B. L. Laskin, J. A. Baur, I. A. Blair, D. C. Wallace, W. W. Hancock, and U. H. Beier, "Foxp3 Reprograms T Cell Metabolism to Function in Low-Glucose, High-Lactate Environments," *Cell Metabolism*, vol. 25, no. 6, pp. 1282–1293.e7, 2017. ScienceDirect

[78] S. E. Weinberg, B. D. Singer, and E. M. Steinert, "Mitochondrial complex III is essential for suppressive function of regulatory T cells," *Nature*, vol. 565, no. 7740, pp. 495–499, 2019. Nature

[79] N. M. Chapman, M. R. Boothby, and H. Chi, "Metabolic coordination of T cell quiescence and activation," *Nature Reviews Immunology*, vol. 20, no. 1, pp. 55–70, 2020. Nature

[80] D. Cipolletta, M. Feuerer, A. Li, N. Kamei, J. Lee, S. E. Shoelson, C. Benoist, and D. Mathis, "PPAR-γ is a major driver of the accumulation and phenotype of adipose tissue Treg cells," *Nature*, vol. 486, no. 7404, pp. 549–553, 2012. Nature

[81] S. A. Lim, J. Wei, T.-L. M. Nguyen, H. Shi, W. Su, G. Palacios, Y. Dhungana, N. M. Chapman, L. Long, J. Saravia, P. Vogel, and H. Chi, "Lipid signalling enforces functional specialization of Treg cells in tumours," *Nature*, vol. 591, no. 7849, pp. 306–311, 2021. Nature

[82] G. M. Delgoffe, T. P. Kole, Y. Zheng, P. E. Zarek, K. L. Matthews, B. Xiao, P. F. Worley, S. C. Kozma, J. D. Powell, and T. J. Curiel, "The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment," *Immunity*, vol. 30, no. 6, pp. 832–844, 2009. ScienceDirect

[83] M. M. Mihaylova and R. J. Shaw, "The AMPK signalling pathway coordinates cell growth, autophagy and metabolism," *Nature Cell Biology*, vol. 13, no. 9, pp. 1016–1023, 2011. Nature

[84] E. V. Dang, J. Barbi, H.-Y. Yang, D. Jinasena, H. Yu, Y. Zheng, Z. Bordman, J. Fu, Y. Kim, H.-R. Yen, W. Luo, K. Zeller, L. Shimoda, S. L. Topalian, G. L. Semenza, C. V. Dang, and D. M. Pardoll, "Control of TH17/Treg Balance by Hypoxia-Inducible Factor 1," *Cell*, vol. 146, no. 5, pp. 772–784, 2011. ScienceDirect

[85] E. T. Clambey, E. N. McNamee, J. A. Westrich, L. E. Glover, E. L. Campbell, P. Jedlicka, E. F. de Zoeten, J. C. Cambier, K. R. Stenmark, S. P. Colgan, and H. K. Eltzschig, "Hypoxia-inducible factor-1 alpha–dependent induction of FoxP3 drives regulatory T-cell abundance and function during inflammatory hypoxia of the mucosa," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 41, pp. E2784–E2793, 2012. PNAS

[86] R. Zappasodi, S. Budhu, M. D. Hellmann, M. A. Postow, and T. Merghoub, "Non-conventional Inhibitory CD4^{+}Foxp3^{-} PD-1hi T Cells as a Biomarker of Immune Checkpoint Blockade Activity," *Cancer Cell*, vol. 39, no. 3, pp. 310–321, 2021. ScienceDirect

[87] S. Sakaguchi, N. Mikami, J. B. Wing, A. Tanaka, K. Ichiyama, and N. Ohkura, "Regulatory T cells and human disease," *Annual Review of Immunology*, vol. 38, pp. 541–566, 2020. Annual Reviews

[88] J. Nie, Y.-Y. Li, S.-G. Zheng, A. Tsun, and B. Li, "FOXP3^{+} regulatory T cells and their roles in immune regulation," *Clinical and Experimental Immunology*, vol. 172, no. 1, pp. 1–9, 2013. Wiley

[89] J. A. Bluestone, C. R. Mackay, J. J. O'Shea, and B. Stockinger, "The functional plasticity of T cell subsets," *Nature Reviews Immunology*, vol. 10, no. 9, pp. 594–602, 2010. Nature

[90] M. Long, A. J. Adler, Z. Yao, C. S. Bonder, and X. Zhang, "CD4^{+}CD25^{+}Foxp3^{+} regulatory T cells promote tumor progression by suppressing antitumor immune responses," *Cancer Research*, vol. 70, no. 5, pp. 185–192, 2010.

[91] J. A. Todd, "Etiology of type 1 diabetes," *Immunity*, vol. 32, no. 4, pp. 457–467, 2010. ScienceDirect

[92] Q. Tang and J. A. Bluestone, "The Foxp3^{+} regulatory T cell: a jack of all trades, master of regulation," *Nature Immunology*, vol. 9, no. 3, pp. 239–244, 2008. Nature

[93] M. Rosenzwajg, E. Languille, H. Debiec, J. Hygino, E. Dériaud, L. Moumne, M. Bonyhadi, G. Afonso, R. Bacchetta, and D. Klatzmann, "Transient regulatory T cells: a therapeutic approach for autoimmune diseases," *Journal of Translational Medicine*, vol. 13, Art. no. 201, 2015. Springer

[94] M. Miyara and S. Sakaguchi, "Natural regulatory T cells: mechanisms of suppression," *Trends in Molecular Medicine*, vol. 11, no. 8, pp. 367–372, 2005. ScienceDirect

[95] D. Comte, M. P. Karampetsou, and G. C. Tsokos, "T cells as a therapeutic target in autoimmune disease," *Current Opinion in Immunology*, vol. 43, pp. 84–90, 2016. ScienceDirect

[96] J. He, X. Zhang, Y. Wei, X. Sun, Y. Chen, J. Deng, Y. Jin, Y. Gan, X. Hu, R. Jia, M. Zhang, Y. Shen, B. Wang, N. Li, L. Lv, Y. Li, X. Zhang, and Z. Li, "Low-dose interleukin-2 treatment selectively modulates CD4^{+} T cell subsets in patients with systemic lupus erythematosus," *Nature Medicine*, vol. 22, no. 9, pp. 991–993, 2016. Nature

[97] V. Viglietta, C. Baecher-Allan, H. L. Weiner, and D. A. Hafler, "Loss of functional suppression by CD4^{+}CD25^{+} regulatory T cells in patients with multiple sclerosis," *The Journal of Experimental Medicine*, vol. 199, no. 7, pp. 971–979, 2004. JEM

[98] M. Kleinewietfeld and D. A. Hafler, "The emerging role of regulatory T cells in multiple sclerosis," *Immunological Reviews*, vol. 259, no. 1, pp. 116–128, 2014. Wiley

[99] Y. Dombrowski, T. O'Hagan, M. Dittmer, R. Penalva, S. R. Mayoral, P. Bankhead, S. Fleville, G. Eleftheriadis, C. Zhao, M. Naughton, R. Hassan, J. Moffat, J. Falconer, A. Boyd, P. Hamilton, I. V. Allen, A. Kissenpfennig, P. N. Moynagh, E. Evergren, B. Perbal, A. C. Williams, R. J. Ingram, J. R. Chan, R. J. M. Franklin, and D. C. Fitzgerald, "Regulatory T cells promote myelin regeneration in the central nervous system," *Nature Neuroscience*, vol. 20, no. 5, pp. 674–680, 2017. Nature

[100] S. Z. Josefowicz, L. F. Lu, and A. Y. Rudensky, "Regulatory T cells: mechanisms of differentiation and function," *Annual Review of Immunology*, vol. 30, pp. 531–564, 2012. Annual Reviews

[101] S. Read, V. Malmström, and F. Powrie, "Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of $CD25^+CD4^+$ regulatory cells that control intestinal inflammation," *The Journal of Experimental Medicine*, vol. 192, no. 2, pp. 295–302, 2000. JEM

[102] E.-O. Glocker, D. Kotlarz, C. Klein, N. Shah, and B. Grimbacher, "IL-10 and IL-10 receptor defects in humans," *Annals of the New York Academy of Sciences*, vol. 1166, pp. 18–34, 2009. Wiley

[103] H. Sugiyama, R. Gyulai, E. Toichi, E. Garaczi, S. Shimada, S. R. Stevens, T. S. McCormick, and K. D. Cooper, "Dysfunctional blood and target tissue $CD4^+$CD25^high regulatory T cells in psoriasis: Mechanism underlying unrestrained pathogenic effector T cell proliferation," *The Journal of Immunology*, vol. 174, no. 1, pp. 164–173, 2005. Journal of Immunology

[104] H. J. Bovenschen, P. C. van de Kerkhof, P. E. J. van Erp, R. Woestenenk, I. Joosten, and H. J. P. M. Koenen, "$Foxp3^+$ regulatory T cells of psoriasis patients easily differentiate into IL-17A-producing cells and are found in lesional skin," *Journal of Investigative Dermatology*, vol. 131, no. 9, pp. 1853–1860, 2011. Nature

[105] M. S. Longhi, M. J. Hussain, R. R. Mitry, S. K. Arora, G. Mieli-Vergani, D. Vergani, and Y. Ma, "Functional study of $CD4^+CD25^+$ regulatory T cells in health and autoimmune hepatitis," *The Journal of Immunology*, vol. 173, no. 11, pp. 6569–6577, 2004. Journal of Immunology

[106] J. B. Wing, A. Tanaka, and S. Sakaguchi, "Human $FOXP3^+$ Regulatory T Cell Heterogeneity and Function in Autoimmunity and Cancer," *Immunity*, vol. 50, no. 2, pp. 302–316, 2019. ScienceDirect

[107] Y. Togashi, K. Shitara, and H. Nishikawa, "Regulatory T cells in cancer immunosuppression—implications for anticancer therapy," *Nature Reviews Clinical Oncology*, vol. 16, no. 6, pp. 356–371, 2019. Nature

[108] T. J. Curiel, G. Coukos, L. Zou, X. Alvarez, P. Cheng, P. Mottram, M. Evdemon-Hogan, J. R. Conejo-Garcia, L. Zhang, M. Burow, Y. Zhu, S. Wei, I. Kryczek, B. Daniel, A. Gordon, L. Myers, A. Lackner, M. L. Disis, K. L. Knutson, L. Chen, and W. Zou, "Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival," *Nature Medicine*, vol. 10, no. 9, pp. 942–949, 2004. Nature

[109] S. Sakaguchi, T. Yamaguchi, T. Nomura, and M. Ono, "Regulatory T cells and immune tolerance," *Cell*, vol. 133, no. 5, pp. 775–787, 2008. ScienceDirect

[110] S. Z. Josefowicz, L. F. Lu, and A. Y. Rudensky, "Regulatory T cells: mechanisms of differentiation and function," *Annual Review of Immunology*, vol. 30, pp. 531–564, 2012. Annual Reviews

[111] D. A. A. Vignali, L. W. Collison, and C. J. Workman, "How regulatory T cells work," *Nature Reviews Immunology*, vol. 8, no. 7, pp. 523–532, 2008. Nature

[112] E. M. Shevach, "Mechanisms of $foxp3^+$ T regulatory cell-mediated suppression," *Immunity*, vol. 30, no. 5, pp. 636–645, 2009. ScienceDirect

[113] D. C. Gondek, L.-F. Lu, S. A. Quezada, S. Sakaguchi, and R. J. Noelle, "Cutting edge: Contact-mediated suppression by $CD4^+CD25^+$ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism," *The Journal of Immunology*, vol. 174, no. 4, pp. 1783–1786, 2005. Journal of Immunology

[114] K. Wing, Y. Onishi, P. Prieto-Martin, T. Yamaguchi, M. Miyara, Z. Fehervari, T. Nomura, and S. Sakaguchi, "CTLA-4 Control over $Foxp3^+$ Regulatory T Cell Function," *Science*, vol. 322, no. 5899, pp. 271–275, 2008. Science

[115] O. S. Qureshi, Y. Zheng, K. Nakamura, K. Attridge, C. Manzotti, E. M. Schmidt, J. Baker, L. E. Jeffery, S. Kaur, Z. Briggs, T. Z. Hou, C. E. Futter, G. Anderson, L. S. K. Walker, and D. M. Sansom, "Trans-endocytosis of CD80 and CD86: A molecular basis for the cell-extrinsic function of CTLA-4," *Science*, vol. 332, no. 6029, pp. 600–603, 2011. Science

[116] G. Plitas and A. Y. Rudensky, "Regulatory T Cells in Cancer," *Annual Review of Cancer Biology*, vol. 1, pp. 459–477, 2016.

[117] T. R. Simpson, F. Li, W. Montalvo-Ortiz, M. A. Sepulveda, K. Bergerhoff, F. Arce, C. Roddie, J. Y. Henry, H. Yagita, J. D. Wolchok, K. S. Peggs, and S. A. Quezada, "Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma," *Journal of Experimental Medicine*, vol. 210, no. 9, pp. 1695–1710, 2013. JEM

[118] P. Sharma and J. P. Allison, "The future of immune checkpoint therapy," *Science*, vol. 348, no. 6230, pp. 56–61, 2015. Science

[119] K. E. Pauken, M. A. Sammons, P. M. Odorizzi, S. Manne, J. Godec, O. Khan, A. M. Drake, Z. Chen, D. R. Sen, M. Kurachi, R. A. Barnitz, C. Bartman, B. Bengsch, A. C. Huang, J. M. Schenkel, G. Vahedi, W. N. Haining, S. L. Berger, and E. J. Wherry, "Epigenetic stability of exhausted T cells limits durability of reinvigoration by PD-1 blockade," *Science*, vol. 354, no. 6316, pp. 1160–1165, 2016. Science

[120] P. C. Tumeh, C. L. Harview, J. H. Yearley, I. P. Shintaku, E. J. M. Taylor, L. Robert, B. Chmielowski, M. Spasic, G. Henry, V. Ciobanu, A. N. West, M. Carmona, C. Kivork, E. Seja, G. Cherry, A. J. Gutierrez, T. R. Grogan, C. Mateus, G. Tomasic, J. A. Glaspy, R. O. Emerson, H. Robins, R. H. Pierce, D. A. Elashoff, C. Robert, and A. Ribas, "PD-1 blockade induces responses by inhibiting adaptive immune resistance," *Nature*, vol. 515, no. 7528, pp. 568–571, 2014. Nature

[121] T. J. Curiel, G. Coukos, L. Zou, X. Alvarez, P. Cheng, P. Mottram, M. Evdemon-Hogan, J. R. Conejo-Garcia, L. Zhang, M. Burow, Y. Zhu, S. Wei, I. Kryczek, B. Daniel, A. Gordon, L. Myers, A. Lackner, M. L. Disis, K. L. Knutson, L. Chen, and W. Zou, "Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival," *Nature Medicine*, vol. 10, no. 9, pp. 942–949, 2004. Nature

[122] B. Shang, Y. Liu, S.-J. Jiang, and Y. Liu, "Prognostic value of tumor-infiltrating FoxP3$^+$ regulatory T cells in cancers: a systematic review and meta-analysis," *Scientific Reports*, vol. 5, Art. no. 15179, 2015. Nature

[123] P. Salama, M. Phillips, F. Grieu, M. Morris, N. Zeps, D. Joseph, C. Platell, and B. Iacopetta, "Tumor-infiltrating FOXP3$^+$ T regulatory cells show strong prognostic significance in colorectal cancer," *Journal of Clinical Oncology*, vol. 27, no. 2, pp. 186–192, 2009. ASCO

[124] W. H. Fridman, F. Pagès, C. Sautès-Fridman, and J. Galon, "The immune contexture in human tumours: impact on clinical outcome," *Nature Reviews Cancer*, vol. 12, no. 4, pp. 298–306, 2012. Nature

[125] R. D. Michalek, V. A. Gerriets, S. R. Jacobs, A. N. Macintyre, N. J. MacIver, E. F. Mason, S. A. Sullivan, A. G. Nichols, and J. C. Rathmell, "Cutting Edge: Distinct Glycolytic and Lipid Oxidative Metabolic Programs Are Essential for Effector and Regulatory CD4$^+$ T Cell Subsets," *The Journal of Immunology*, vol. 186, no. 6, pp. 3299–3303, 2011. Journal of Immunology

[126] U. H. Beier, A. Angelin, T. Akimova, L. Wang, Y. Liu, H. Xiao, M. A. Koike, S. A. Hancock, T. R. Bhatti, R. Han, J. Jiao, S. C. Veasey, C. A. Sims, J. A. Baur, D. C. Wallace, and W. W. Hancock, "Essential role of mitochondrial energy metabolism in Foxp3$^+$ T-regulatory cell function and allograft survival," *FASEB Journal*, vol. 29, no. 6, pp. 2315–2326, 2015. FASEB

[127] Pacella, C. Procaccini, C. Focaccetti, S. Miacci, E. Timperi, D. Faicchia, M. Severa, F. Rizzo, E. M. Coccia, F. Bonacina, N. Mitro, G. D. Norata, G. Rossetti, V. Ranzani, M. Pagani, E. Giorda, Y. Wei, G. Matarese, V. Barnaba, and S. Piconese, "Fatty acid metabolism complements glycolysis in the selective regulatory T cell expansion during tumor growth," *Proceedings of the National Academy of Sciences of the United States of America (PNAS)*, vol. 115, no. 28, pp. E6546–E6555, 2018. PNAS

[128] G. M. Delgoffe, T. P. Kole, Y. Zheng, P. E. Zarek, K. L. Matthews, B. Xiao, P. F. Worley, S. C. Kozma, J. D. Powell, and T. J. Curiel,

"The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment," *Immunity*, vol. 30, no. 6, pp. 832–844, 2009. ScienceDirect

[129] S. Onizuka, I. Tawara, J. Shimizu, S. Sakaguchi, T. Fujita, and E. Nakayama, "Tumor rejection by in vivo administration of anti-CD25 (interleukin-2 receptor α) monoclonal antibody," *Cancer Research*, vol. 59, no. 13, pp. 3128–3133, 1999.

[130] T. R. Simpson, F. Li, W. Montalvo-Ortiz, M. A. Sepulveda, K. Bergerhoff, F. Arce, C. Roddie, J. Y. Henry, H. Yagita, J. D. Wolchok, K. S. Peggs, and S. A. Quezada, "Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma," *The Journal of Experimental Medicine*, vol. 210, no. 9, pp. 1695–1710, 2013. JEM

[131] B. Allard, M. S. Longhi, S. C. Robson, and J. Stagg, "The ectonucleotidases CD39 and CD73: Novel checkpoint inhibitor targets," *Immunological Reviews*, vol. 276, no. 1, pp. 121–144, 2017. Wiley

[132] G. Plitas, C. Konopacki, K. Wu, P. D. Bos, M. Morrow, E. V. Putintseva, D. M. Chudakov, and A. Y. Rudensky, "Regulatory T Cells Exhibit Distinct Features in Human Breast Cancer," *Immunity*, vol. 45, no. 5, pp. 1122–1134, 2016. ScienceDirect

[133] Giannopoulos, M. Schmitt, P. Wlasiuk, J. Chen, A. Bojarska-Junak, M. Kowal, A. Dmoszyńska, and J. Rolinski, "The high frequency of T regulatory cells in patients with B-cell chronic lymphocytic leukemia is diminished through treatment with thalidomide," *Leukemia*, vol. 22, no. 1, pp. 222–224, 2009.

[134] N. A. Marshall, L. E. Christie, L. R. Munro, D. J. Culligan, P. W. Johnston, R. N. Barker, and M. A. Vickers, "Immunosuppressive regulatory T cells are abundant in the reactive lymphocytes of Hodgkin lymphoma," *Blood*, vol. 103, no. 5, pp. 1755–1762, 2004. Blood

[135] R. H. Prabhala, P. Neri, J. E. Bae, P. Tassone, M. A. Shammas, C. K. Allam, J. F. Daley, D. Chauhan, E. Blanchard, H. S. Thatte, K. C. Anderson, and N. C. Munshi, "Dysfunctional T regulatory cells in multiple myeloma," *Blood*, vol. 107, no. 1, pp. 301–304, 2006. Blood

[136] S. Ladoire, L. Arnould, G. Mignot, L. Apetoh, C. Rébé, F. Martin, B. Chauffert, and F. Ghiringhelli, "Presence of Foxp3 expression in tumor-infiltrating lymphocytes is associated with better prognosis in breast cancer: a rationale for immune-based therapies," *Cancer Research*, vol. 71, no. 8, pp. 3076–3087, 2011. AACR

[137] S. Karanika, T. Karantanos, L. Li, P. G. Corn, and T. C. Thompson, "Systematic review and meta-analysis of the prognostic significance of PD-L1 expression and tumor-infiltrating lymphocytes in genitourinary malignancies," *European Urology*, vol. 68, no. 4, pp. 701–710, 2015. ScienceDirect

[138] B. Shang, Y. Liu, S.-J. Jiang, and Y. Liu, "Prognostic value of tumor-infiltrating FoxP3$^+$ regulatory T cells in cancers: a systematic review and meta-analysis," *Scientific Reports*, vol. 5, Art. no. 15179, 2015. Nature

[139] P. Salama, M. Phillips, F. Grieu, M. Morris, N. Zeps, D. Joseph, C. Platell, and B. Iacopetta, "Tumor-infiltrating FOXP3$^+$ T regulatory cells show strong prognostic significance in colorectal cancer," *Journal of Clinical Oncology*, vol. 27, no. 2, pp. 186–192, 2009. ASCO

[140] C.-X. Li, C.-C. Ling, Y. Shao, A. Xu, X.-C. Li, K. T.-P. Ng, X. B. Liu, Y.-Y. Ma, X. Qi, H. Liu, J. Liu, W. H. O. Yeung, C. M. Lo, K. Man, and Y. Ma, "CXCL10/CXCR3 signaling mobilizes regulatory T cells to promote hepatocellular carcinoma progression," *Journal of Hepatology*, vol. 62, no. 4, pp. 950–959, 2015. ScienceDirect

[141] E. M. Aandahl, J. Michaëlsson, W. J. Moretto, F. M. Hecht, and D. F. Nixon, "Human CD4$^+$CD25$^+$ regulatory T cells control T-cell responses to human immunodeficiency virus and cytomegalovirus antigens," *Journal of Virology*, vol. 78, no. 5, pp. 2454–2459, 2004. ASM

[142] A. L. Kinter, R. Horak, M. Sion, L. Riggin, J. McNally, Y. Lin, R. Jackson, A. O'Shea, G. Roby, C. Kovacs, M. Connors, and A. S. Fauci, "CD25$^+$ regulatory T cells isolated from HIV-

infected individuals suppress the cytolytic and nonlytic antiviral activity of HIV-specific CD8⁺ T cells in vitro," *AIDS Research and Human Retroviruses*, vol. 23, no. 3, pp. 438–450, 2007. Mary Ann Liebert

[143] N. Seddiki, B. Santner-Nanan, J. Martinson, J. Zaunders, S. Sasson, A. Landay, M. Solomon, W. Selby, S. I. Alexander, R. Nanan, A. Kelleher, and B. F. de St. Groth, "Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells," *The Journal of Experimental Medicine*, vol. 203, no. 7, pp. 1693–1700, 2006. JEM

[144] O. Franzese, P. T. F. Kennedy, A. J. Gehring, J. Gotto, R. Williams, M. K. Maini, and A. Bertoletti, "Modulation of the CD8⁺ T-cell response by CD4⁺CD25⁺ regulatory T cells in patients with hepatitis B virus infection," *Journal of Virology*, vol. 79, no. 6, pp. 3322–3328, 2005. ASM

[145] E. Stephen-Victor and M. Das, "Regulatory T Cells: The Master Regulators of Immune Responses," *Frontiers in Immunology*, vol. 11, Art. no. 598, 2020. Frontiers

[146] G. Minigo, T. Woodberry, K. A. Piera, E. Salwati, E. Tjitra, E. Kenangalem, R. N. Price, C. R. Engwerda, N. M. Anstey, and M. Plebanski, "Parasite-dependent expansion of TNF receptor II-positive regulatory T cells with enhanced suppressive activity in adults with severe malaria," *PLoS Pathogens*, vol. 5, no. 4, Art. no. e1000402, 2009. PLOS

[147] S. Sakaguchi, M. Miyara, C. M. Costantino, and D. A. Hafler, "FOXP3⁺ regulatory T cells in the human immune system," *Nature Reviews Immunology*, vol. 10, no. 7, pp. 490–500, 2010. Nature

[148] P. C. Tumeh, C. L. Harview, J. H. Yearley, I. P. Shintaku, E. J. M. Taylor, L. Robert, B. Chmielowski, M. Spasic, G. Henry, V. Ciobanu, A. N. West, M. Carmona, C. Kivork, E. Seja, G. Cherry, A. J. Gutierrez, T. R. Grogan, C. Mateus, G. Tomasic, J. A. Glaspy, R. O. Emerson, H. Robins, R. H. Pierce, D. A. Elashoff, C. Robert, and A. Ribas, "PD-1 blockade induces responses by inhibiting adaptive immune resistance," *Nature*, vol. 515, no. 7528, pp. 568–571, 2014. Nature

[149] E. M. Aandahl, J. Michaëlsson, W. J. Moretto, F. M. Hecht, and D. F. Nixon, "Human CD4⁺CD25⁺ regulatory T cells control T-cell responses to human immunodeficiency virus and cytomegalovirus antigens," *Journal of Virology*, vol. 78, no. 5, pp. 2454–2459, 2004. ASM

[150] N. Seddiki, B. Santner-Nanan, J. Martinson, J. Zaunders, S. Sasson, A. Landay, M. Solomon, W. Selby, S. I. Alexander, R. Nanan, A. Kelleher, and B. F. de St. Groth, "Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells," *The Journal of Experimental Medicine*, vol. 203, no. 7, pp. 1693–1700, 2006. JEM

[151] Pacella, C. Procaccini, C. Focaccetti, S. Miacci, E. Timperi, D. Faicchia, M. Severa, F. Rizzo, E. M. Coccia, F. Bonacina, N. Mitro, G. D. Norata, G. Rossetti, V. Ranzani, M. Pagani, E. Giorda, Y. Wei, G. Matarese, V. Barnaba, and S. Piconese, "Fatty acid metabolism complements glycolysis in the selective regulatory T cell expansion during tumor growth," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 115, no. 28, pp. E6546–E6555, 2018. PNAS

[152] S. Z. Josefowicz, L. F. Lu, and A. Y. Rudensky, "Regulatory T cells: mechanisms of differentiation and function," *Annual Review of Immunology*, vol. 30, pp. 531–564, 2012. Annual Reviews