

Non-genetic Causes of Th17/Treg Imbalance and its Impact on Systematic Autoimmune Diseases

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Abstract:

Autoimmune is the situation that immune system loses self-tolerance and attacks tissues, causing inflammatory responses. Th17/Treg imbalance is the case that quantity or efficacy of pro-inflammatory helper T cell Th17 is greater than suppressive regulatory T cell, which widely observed in autoimmune diseases. Research on Th17/Treg imbalance initialized from discovering similarity between Th17 and Treg development and activation of their critical transcriptional regulator ROR γ t and Foxp3. In decades of studies, researchers have successfully identified risk factors and possible reasons inducing Th17/Treg imbalance, including infection, diet, environment and gut microbiota etc. Moreover, several trials of treatment based on knowledge of Th17/Treg imbalance have been successful. However, though several explanations leading to imbalance have risen, detailed signalling pathways and protein mechanisms have remained unknown due to complexity of experimental proof. This review summarizes current researching progress on understanding how Th17 and Treg matures and compares their similarity in differentiation. Moreover, this review explains the role of Th17 and Treg in immune system, and analyzes the detrimental result and reasons for Th17/Treg imbalance, using some systematic autoimmune diseases as examples. Research in Th17/Treg explains why systematic autoimmune diseases and other types of hypersensitivity can prolong excluding genetic deficiency, and discovers optimal targets to ameliorate symptoms. Future studies should explain in detail mechanism which external factors can cause imbalance and why imbalance still generates albeit sufficient conditions for Treg differentiation possess.

Keywords: Treg imbalance; Autoimmune diseases; Immune system regulation.

1. Introduction

The immune system composed by innate and adaptive immunity acts as the defense of human body against foreign pathogens. In innate immunity, antigen presenting cells like dendritic cells (DC) activate T cells via MHC-TCR and co-stimulatory receptor interactions, and determine the types of T cell differentiation by MHC type and secretion of cytokines. T cell composes the cell immunity in adaptive immune system, and divides into three types according to their surface receptor, which are CD4⁺ helper T cells, CD8⁺ effector T cells, and CD4⁺ Foxp3⁺ regulatory T cells (Tregs). Activated helper T cells can differentiate into specific subsets depending on their microenvironment and mediate immune response by secreting particular cytokines. The well-studied T helper cells include Th1, Th2 and Th17. However, Tregs in immune system show repressive behavior which modifies and limit immune response by inhibitory receptor interaction via PD-1 or CTLA-4 and repressive cytokines like TGF- β and IL-10. Tregs play a major role in peripheral tolerance and recov-

ery after infection, while preventing overlarge immune response, which causes chronic and destructive inflammation.

Immune tolerance is the mechanism that recognizes and prevents attack against self-antigen. However, due to gene susceptibility or environmental factors, defective tolerance can develop into autoimmunity. During autoimmunity, the microenvironment of immune system is occupied predominantly by inflammatory cytokines and autoimmune factors. Nonetheless, Treg function in autoimmune circumstances is quite limited, and the reason is currently considered complex and still under debate. One of the theories explaining Treg dysfunction is Th17/Treg imbalance.

Th17 is a recently described subset of pro-inflammatory helper T cells, which is defined by its secretion of IL-17. Current study indicates that Th17 development is distinct from Th1 and Th2 lineage, but shows reciprocal pathway with Tregs. Therefore, Th17 and Treg under normal immune response are in balance while the deficiency in this balance can be a factor of autoimmunity and chronic

inflammation. The Th17/Treg theory provides a basis explaining and understanding the reason for prolonged autoimmune response and insufficient regulation by Tregs.

This review will compare the developmental pathway of Th17 and Tregs, with examples of Th17/Treg imbalance in some representative systematic autoimmune diseases, and suggests how the non-genetic factors of alter this balance. Eventually, this paper will discuss some potential treatment based on specific target and their perspective.

2. Treg and Th17 Development and Functions

2.1 Functions of Treg

Tregs are significant modifiers of immune homeostasis, since it has been shown to suppress a wide range of cell types, including helper T cells, B cells, various types of macrophages, antigen presenting cells including DCs during infection. Besides, Tregs express immunosuppressive cytokines, including IL-10, TGF- β and IL-35, preventing maturation of lymphocytes.

Interactions between Treg and other immune cells are species-specific. Most well-researched aspect is Treg's interaction with DC. Recent studies have concluded three crosstalk pathways that inhibits DC's function, and indirectly suppress the maturation of helper T cells: CD28/CTLA-4/B7 pathway, PD-L1/PD-1 feedback loop and potential LAG-3/TCR/MHC-II feedback loop [1] (figure 1.). Here, CTLA-4, PD-1/PD-L1 and LAG-3 are three major types of inhibitory receptors that are highly expressed on the surface of Tregs.

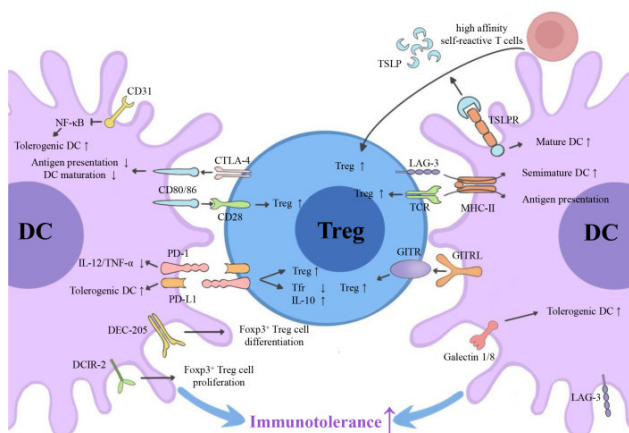


Fig. 1 Receptor binding and effects induced by Tregs [1].

CTLA-4 is the receptor that specifically interacts with CD80/86 B7 receptors to compete with CD80/86-CD28 interaction, which promotes helper T cells' maturation. Besides, CTLA-4-B7 interaction induces trans-endocytosis of CD80/86 in Treg, further inhibiting co-stimulation

between CD80/86 and CD28. PD-1/PD-L1 pathway is another pivotal immunosuppressive pathway that is similar to CTLA-4, but PD-1 and PD-L1 are simultaneously expressed on Treg and DC and interacts mutually. PD-L1+ DCs can maintain proliferation of Tregs and Tregs inversely inhibit function of DCs, reflected in decrease in IL-6, IL-12 and TNF- α secretion from DCs. While LAG-3 is the receptor expressed by Tregs that binds to MHC-II, similar to TCR, and potentially LAG-3-MHC-II interaction performs inhibitory effect on DCs by interference with TCR-MHC-II interaction, and trans-endocytosis of MHC-II [1].

In addition, Tregs can induce inhibition of effector T cells (CD8+, Teff) indirectly when interacting with DC, by secretion of cytokines or substances that essential for Teff maturation. For example, the IDO expression induced by CTLA-4/B7 interaction can induce modulation of tryptophan catabolism, inducing the turnover of tryptophan which is essential for Teff maturation to kynurenine. Besides, Tregs express inhibitory molecule TIGIT to bind with CD155 on DCs, which induces suppressive cytokine IL-10, hence inhibits inflammatory response of Teffs, especially Th1 and Th17 induced response [2].

2.2 Regulation of Development and Maintenance Of Tregs

Tregs are developed from CD4+ T cells but distinctive from other CD4+ T cells like Ths or follicular helper T cells (Tfh) by its presentation of Foxp3 and CD25(IL-2R α), which is the key regulator of Treg development. Foxp3 is the regulator that suppresses the function of NFAT and NF κ B signal pathways, [3] which leads to differentiation to conventional T cells (Tconv) and the transcriptional activator of CTLA-4, PD-1, IL-2R and other suppressive receptors. Therefore, whether Foxp3 gene is expressed is the key to Treg differentiation. Based on how Foxp3 expression is induced, Tregs can be classified into three types: tTregs, pTregs, and iTregs.

tTregs are developed via positive and negative selection in thymus gland, depending on TCR-MHC-II interaction and TCR signalling. tTregs development is hypothesized in a two-step pattern, which are affinity selection and signal activation. In the first step, the high affinity of TCR interaction with self-antigen induces upregulation of CD25 expression, whilst is not upon the threshold which induces elimination of autoreactive T cells. In second step, the association between cytokines IL-2, with less degree of IL-7 and IL-15, to common gamma-chain (γ c) cytokine receptors like CD25 triggers expression of Foxp3. Recent studies supposed STAT5 is the main regulator controls this step, while its mechanism is still under debate [4].

However, pTregs activation is different from tTregs, and

its mechanism is not fully understood currently. pTregs are derived from CD4⁺ Foxp3⁻ T cells, or Tconv. Apart from essential IL-2 and IL-15 cytokines, transition from Tconv to pTreg requires additional TGF- β signal and retinoic acid. Additionally, recent researches about inflammatory bowel diseases illustrated pTreg activation can be associated with microbiota in mesenteric lymph nodes, and intestine mucosa.

2.3 Development and Function of Th17 and Similarity to Treg

Interleukin-17-producing cell (Th17) is a proinflammatory CD4⁺ T cell that plays a crucial role in regulating immune responses by mediating activation of other cell types. Like other CD4⁺ helper T cells, Th17 specifically expresses pro-inflammatory cytokines, including the major type IL-17A and IL-17F, which are two close related subsets of IL-17 family. Apart from IL-17, Th17 can also express TNF- α , IL-22 and IL-26, which mediates non-specific immune response, while partly protecting specific tissues during inflammation, acting as a suppressive cytokine, for example, IL-22 can protect hepatocytes during acute liver inflammation.

Development of Th17, although is a pro-inflammatory helper T cells, is distinct from Th1 and Th2 cells, shows some similarities with pTregs whilst their functions are opposite. For example, Th17 maturation requires TGF- β , which is also an essential cytokine that triggers development of pTregs. However, the pivotal cytokine that induces Th17 maturation is IL-6, which shows dichotomy to Treg. IL-6, opposite to IL-2 is able to inhibit TGF- β -induced Treg development by inhibiting expression of Foxp3. Other than TGF- β and IL-6, maintenance of Th17 also requires IL-21, IL-23 and IL-1 β , which can also inhibit expression of Foxp3 induced by TGF- β . IL-21 is the cytokine that is not only expressed by Th17 but is essential for generation of Th17, and loss of IL-21 is verified inhibiting for Th17 differentiation. IL-23 is the cytokine binding with heterodimeric receptor complex consisting of IL-12R β 1 and IL-23R. IL-23 is essential for IL-17 production, and survival and proliferation of Th17, causing pro-inflammatory response IL-23 loss-of-function experiment in mice indicating the relationship to prevention of experimental autoimmune encephalomyelitis, collagen-induced arthritis, and inflammatory bowel disease (IBD) [5]. TGF- β , IL-6, IL-21 stimulate Th17 activation in a sequential pattern. In the early development of Th17, TGF- β and IL-6 simultaneously cause the expression of definitive transcription factor retinoic acid receptor-related orphan receptor gamma-t (ROR γ t), which leads to activation of TH17-associated genes, such as cytokines Il17a, Il17f, and Il23r, or other transcriptional factors and inhibit IFN- γ and

IL-4 cytokines expression thus determining naïve CD4⁺ T cell development into Th17 but not Th1 or Th2. Meanwhile, TGF- β specifies lineage development into Th17 by inhibiting expression of Foxp3. Then, IL-6 induces IL-21 activation, further amplifying Th17 activation and maintenance signal, in a manner similar to IL-6 [6].

Comparatively, IL-23 is relatively a co-stimulative cytokine that normally acts together with IL-6. For example, in human cells, IL-6 is insufficient for ROR γ t ortholog, RORC, but requires IL-23 and IL-1 β . Moreover, IL-23 is crucial for transcription factor signal transducer and activator of transcription 3 (STAT3) signal pathway for Th17 activation. STAT3 can bind with Th17 promoter and activate Th-17 related transcription, and is essential for ROR γ t expression [6].

3. Non-genetic Factors Contribute to Th17/Treg Imbalance

The Th17/Treg imbalance is a status the quantity or efficacy of Th17 in tissue is far greater than Treg, causing the loss of homeostasis and self-tolerance and inducing inflammatory diseases. However, in real cases, Th17/Treg imbalance situation is not fully generated by genetic Treg dysfunction like IPEX, and most autoimmune cases are gradually generated, by risk factors like age, daily manners, and living environment. These risk factors have complex effect on cellular events that controls differentiation preference between Th17 and Treg.

3.1 Abnormal Posttranslational Modification (PTMs) of Foxp3:

Foxp3 is the central transcription regulator that controls differentiation into Treg, thus its efficacy affects stability of Treg suppressive function stability. However, recent studies have found a relationship between Foxp3 modification and generation of autoimmune diseases due to Foxp3 function's alternation.

PTMs on Foxp3 include phosphorylation, O-GlcNAcylation, acetylation, ubiquitination, and methylation and their reversed process. PTMs are controlled by external signals and expression of corresponding enzymes, while negatively or positively regulates Foxp3 subcellular localization, binding affinity of DNA, and association with other proteins.

A recent study has discovered between dysfunction of Foxp3 and ubiquitination induced by infection, which causes multiple autoimmune diseases. For example, an increased concentration of chemokine CCL3 in serum induced by infection results in increased degradation of Foxp3 mediated by K48-linked polyubiquitination. This process is mediated by the activation of the protein kinase B (PKB) α /Akt1 pathway and potentially induces psoriasis

[7].

Correlation between acetylation/deacetylation and dysfunction of Treg is also a well-researched field. For example, acetylation of K250 and K252 of Foxp3 by histone acetyltransferase p300 disrupts Foxp3 coiled-coil dimerization by perturbs electrostatic interaction between K252, Q234 and E238. Therefore, this dimer relaxation causes loss of binding activity with DNA, thus negatively regulating Treg activity. p300 acetylation can be repressed by TGF- β , supposing its activity is downregulated by TGF- β [8]. However, positive effect of acetylation on the activity of Foxp3 was also reported. In 2009, Jorg van Loosdregt et. al. found evidence that acetylation by P300 can prevent Foxp3 from degradation by proteasome, thus up-regulating Foxp3 activity, generating an opposite result [9].

The most recent research progress is identification of importance of O-GlcNAcylation in Foxp3 stability. Bing Liu et. al. discovered role of O-GlcNAcylation in stabilization of FOXP3 and activation STAT5, while knock-out of O-GlcNAcylation strongly impaired lineage stability and effector function, and ultimately fatal autoimmunity in mice [10].

Although correlation between PTM on specific amino acid and reasons for Foxp3 dysfunction is well-studied, signalling network and detailed mechanism are still not fully known. However, recent studies have found these abnormal PTMs of Foxp3 can be induced by microenvironment generated by autoimmune disease and microbiota.

3.2 Diet, Metabolism and Metabolic Signalling

Differentiation of Treg and Th17 depends on several metabolic signalling, while the most significant signalling pathway that promotes Treg maintenance is LKB1/AMPK (AMP-activated protein kinase), comparatively, PI3K/AKT/mTOR pathway antagonizes induction of Treg but promotes activation of effector T cells.

PI3K/AKT/mTOR signalling lineage is stimulated by upstream TCR and IL-2 signalling, but also insulin. mTOR is an unconventional serine-threonine kinase that has two types of complex, mTORC1, and mTORC2. mTORC1 controls cell homeostasis, synthesis of protein and metabolism of lipid and glucose, while mTORC2 influences the differentiation of CD4+ T cells. During maturation of Th17, mTORC1 activates S6K2, a kinase that induces expression of Th17 transcription factor ROR γ t, while inhibiting tuberous sclerosis 1 (TSC1), which correlates to suppressive function of Treg. Besides, mTORC2 phosphorylates Akt, which inhibits transcription factor FOXO1/3a which promotes expression of Foxp3. Therefore, both mTORC1 and mTORC2 inhibit the expression of

Foxp3 but activate expression of ROR γ t. In fact, Tregs perform hyporepresentation of this pathway [11]. However, LKB/AMPK is quite the opposite. AMPK is the AMP kinase that senses cellular stress and inhibits mTOR pathway, thus promoting proliferation of Tregs [12].

PI3K/AKT/mTOR pathway facilitates glycolysis, which is preferred by effector T cells and helper T cells, and responds with excess nutrients like sugar, amino acid and fatty acids. Therefore, high-calorie diet is a key factor that causes Treg/Th17 imbalance and multiple studies have confirmed that low-calorie diet can ameliorate autoimmune diseases via aforementioned mechanism, for example, IBD [13]. Besides, low-calorie diet is able to generate a low ATP environment, which triggers activation of LKB/AMPK pathway and hence induces proliferation of Treg.

3.3 Gut Microbiota

Correlation between gut microbiota and autoimmune diseases like IBD is a recently popular research topic. Besides, evidences indicate that specific gut microbiota and their metabolites impact Th17/Treg balance maintenance mutually.

Gut microbiota is required for maintaining expansion and differentiation of Th17 and Tregs, via immune response to specific cell component. Some species can activate Th17, for example, Segmented Filamentous Bacteria (SFB). SFB promotes secretion of IL-1 β and IL-23 by APCs via production of self-antigen and serum amyloid A isoforms recognized by APCs [14].

However, some bacteria can promote Treg development and inhibit Th17 development. For example, some strains of *Bacteroides fragilis* (*B. fragilis*) presents polysaccharide A (PSA), which is recognized by pattern recognition receptors on CD4+ T cells and APCs, especially TLR1, TLR2 and NOD2, and is required for adaptation of CD4+ T cells to become Tregs. Besides, PSA on *B. fragilis* can suppress development of Th17 via suppression of IL-1 β secretion. Bacteria with similar functions include *Clostridia* which induces secretion of TGF- β , and *L. reuteri* which is indicated to have modulating effect on some autoimmune diseases [14].

Metabolites produced by gut microbiota can also affect homeostasis of Th17 and Tregs. Short-chain fatty Acids (SCFA), a group of single-chain fatty acids with lengths less than 6 carbon chains, is a class of metabolites produced by fermentation by microbiota. SCFA acts as a significant signalling molecule that modulates immune behaviour but also acts as histone deacetylase inhibitor (HDAC) that promotes Foxp3 transcription in a concentration-dependent manner. HDAC removes acetyl groups from specific amino acids on chromatin, which locally tightens Foxp3 gene and inhibits transcription. Besides,

HDAC possesses PTM activity of Foxp3, as aforementioned, deacetylation of Foxp3 can potentially make Foxp3 more susceptible to degradation by proteasome. High concentration of specific SCFA like butyrate and propionate shows strong inhibition effect thus promoting Treg/Th17 balance by enhancing Treg maturation [15]. Balance of microbiota is essential for immune homeostasis. Several studies indicate the relationship between imbalanced microbiota and inflammatory diseases like IBD and Crohn's disease. Besides, fecal transplantation which complements microbiota has shown therapeutic effect on improvement of those diseases, which is also evidence that emphasizing importance of microbiota to immune system. Besides, microbiota theory can explain how age issue generates multiple autoimmune diseases since microbiota component is correlated to age.

4. Th17/Treg Imbalance in Systematic Autoimmune Diseases and Cell Therapy

Systematic autoimmune diseases are caused by wrongly recognition to self-antigen while causing inflammation and tissue damage around the body. Expansion of inflammatory response correlates to activation of DCs and effector T cells, which secretes pro-inflammatory cytokines. Treg is the central modulator of inflammation, which suppress overresponse of immune system; however, in autoimmune cases, effector T cell response overwhelms suppressive effect of Tregs. Therefore, Th17/Treg imbalance can be a phenomenon that sustains autoimmunity and is widely observed in multiple autoimmune diseases.

Rheumatoid arthritis (RA) is the systematic autoimmune disease that is characterized by progressively destructive joint inflammation, destruction of articular cartilage, and bone and synovial hyperplasia. RA is a well-studied autoimmune disease that has been confirmed to correlate with over-proliferation of Th17. Th17 secretes cytokine IL-17 and IL-23, causing inflammatory response in joint. As for Tregs, recent research found evidence indicating Treg activity is inhibited in RA conditions. Ineffective Treg function can attribute to overexpression of IL-6 and hypoxia environment created by overresponse by pro-inflammatory T cells. The latter issue will induce hypoxia-inducible factor-1 α (HIF-1 α) pathway, which promotes Th17 proliferation and degradation of Foxp3 via ubiquitination [16].

Th17/Treg imbalance is also evident in systemic lupus erythematosus, an autoimmune disease that caused by autoantibodies that attack self-proteins, especially proteins in nucleus and generates inflammation around the body. In SLE cases, cytokine IL-2 production is compromised, and CD25 expression is low, which cause insufficient Treg

cell activation. However, the reason why IL-2 secretion is insufficient is needed to be explain and unexpected results that indicate Foxp3+ Treg level can be higher or equal in some SLE cases is required to be reconcile [17].

Inflammatory Bowel diseases (IBD) including Crohn's disease and ulcerative colitis are also correlated to Th17/Treg imbalance. Pathogenesis of IBD shows strong correlation to intestinal flora, which metabolites and surface protein can alter Th17/Treg differentiation as mentioned before [5]. Besides, IBD can also be generated from environment issues, resulting in reduce in Treg number. Other autoimmune diseases that are found Th17/Treg imbalance include multiple sclerosis, type 1 diabetes, Hashimoto's thyroiditis, myasthenia gravis etc. due to genetic or non-genetic factors.

Novel treatment that applying engineered Treg cell to ameliorate Th17/Treg imbalance have achieved success in clinical trial. However, this therapy can be unstable because autoimmune environment can possibly influence Treg stability that repress Treg activity. Therefore, sustaining of environment favoring Treg maintenance is required. For example, in SLE, defective IL-2 production may reduce Treg efficacy even after Treg therapy; hence, supplement of IL-2 is essential for treatment. Besides, in IBD, replenish of beneficial flora is also crucial for Treg maintenance. Sustaining of Treg-favored environment requires analysis of comprehensive factors like aforementioned that can impact Th17/Treg balance to ensure success in Treg cell therapy.

5. Conclusion

Autoimmune diseases generation is caused by dysregulation of immune system due to abnormal activation of helper T cells that triggers inflammatory response or dysfunction of suppressive Tregs. Since former studies discovered the similarity in development between Tregs and Th17, researchers suppose Th17/Treg imbalance generated by biased differentiation towards Th17 can be a factor that explains why Tregs have limited efficacy during autoimmunity.

Th17/Treg imbalance is not always caused by genetic issue like mutation of specific receptors that relates to essential functions of Tregs, but also other factors like age, diet, manner, environment and infection history, which favors Th17 development. Correlation of these factors and CD4+ differentiation pathway is illustrated in signaling pathway, protein modifications, metabolic control and microbiota environment. These factors can indicate potential targets for ameliorating autoimmune symptoms, provide further research guidance on explaining how autoimmune diseases generate and sustains, and emphasize the impor-

tance of healthy lifestyle in guarding against autoimmune diseases.

However, factors contribute to Th17/Treg imbalance is complicated, and other cellular mechanisms like Treg instability, Treg plasticity and Treg/Th17 interchanging are not fully covered in this review. Besides, researches on Th17/Treg imbalance usually uses knock-out method, which examines the effect of existence of an enzyme or a substance on Foxp3 expression and Treg development. Hence, this experiment method does not explain the detailed mechanism in molecular level like impact on protein interaction. Moreover, Th17/Treg cannot explain the individual difference in risk of develop autoimmunity, since autoimmune diseases are generated from a complex of issues.

Current treatment of autoimmune diseases focuses on blocking cytokines or DCs response, but these therapeutic methods have high risk of immune over-repression that results in susceptibility from infection, and prominent side effect that causes mental impair. Nowadays, emergence of cellular treatment method that transplant Tregs to limit inflammation brought by overwhelmed Th17 effect and promote immune homeostasis is widely studied and applied, but cannot prevent proliferation of pro-inflammatory T cells. In the future, by utilizing possessing theory of Treg development, modification in CD4+ T cell development pathway that alters preferential towards Treg can be a method that can sustain immune homeostasis and control progression of autoimmune diseases.

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