

Reprogramming Regulatory T cells to Promote Tolerance in Allergic Diseases

Düzenleyici T Hücrelerinin Alerjik Hastalıklarda Toleransı Teşvik Etmesi İçin Yeniden Programlanması

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INTRODUCTION

Allergic diseases, including allergic rhinosinusitis, asthma, eczema, and food allergies, are chronic inflammatory disorders affecting large segments of the population, both children and adults, that have come into prominence in recent decades by virtue of changes in life style and living conditions (1). They exact considerable morbidity and result in substantial financial costs incurred by affected individuals and their healthcare systems. Allergic diseases are characterized by a T helper type 2 (TH2)-type immune response against environmental allergens, foods and drugs. This inflammatory response mobilizes innate immune components, including innate lymphoid cells type 2 (ILC2) mast cells, basophils, eosinophils, as well as adaptive immunity including T helper type 2 (TH2) cells and B cells switched to the production of immunoglobulin type E (IgE) (2-6). These pathways promote disease in the face of immune regulatory mechanisms that normally operate to maintain

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Öz

Alerjik hastalıklar, ağır morbidite riskine neden olabilen oldukça yaygın bozukluklardır. Normalde, kendi kendine yeten ve minimal zarar veren immün cevapları sağlayan bu immün efektör ve tolerojenik mekanizmalar arasında ince bir denge varken, bu denge alerjik bozukluklarda başarısız olur. Bağışıklık homeostazını korumak ve aşırı veya dokuya zarar veren bağışıklık tepkilerini engellemek için hareket eden düzenleyici T (Treg) hücrelerinin bu bozukluklarındaki rolü özellikle ilgi çekicidir. Bu derlemede, kronik alerjik inflamasyonun Treg hücrelerini nasıl dengesiz hale getirebildiğini ve hastalıklarının kötüleştirilen patojenik T yardımcı hücre fenotiplerinin kazanılmasını nasıl uyarabileceğini araştırdık. Treg hücrelerinin yeniden programlanmasında yer alan hücre ve moleküler mekanizmaları ile kalıcı toleransı yeniden oluşturmak için yeniden programlanmanın tersine çevrilebileceği terapötik seçenekleri tanımladık.

Anahtar Kelimeler: Besin alerjisi, Düzenleyici T hücreleri, Foxp3, İnterlökin 4, Tip 2 T Yardımcı hücreler.

Abstract

Allergic diseases are highly prevalent disorders that exact a heavy toll of morbidity. Whereas a fine balance is normally struck between immune effector and tolerogenic mechanisms to ensure effective but self-contained and minimally damaging immune responses, this balance fails in those disorders. Of particular interest is the role in these disorders of regulatory T (Treg) cells, which act to maintain immune homeostasis and ward off exuberant or tissue damaging immune responses. In this review, we explore how chronic allergic inflammation can destabilize Treg cells and promote their acquisition of pathogenic T helper cell phenotypes that aggravate disease. We describe cellular and molecular mechanisms involved in such re-programming of Treg cells and therapeutic opportunities by which reprogramming can be reversed to reestablish enduring tolerance.

Keywords: Food Allergy, Regulatory T cells, Foxp3, Interleukin 4, T Helper cells type 2.

tolerance to allergens, most prominent of which are regulatory T (Treg) cells that act to enforce tolerance to allergens and prevent allergic disorders (7-9). Recent findings in the field have elucidated key cellular and molecular events involved in tolerance breakdown in these disorders and pointed to potentially novel approaches to the re-establishment of tolerance in allergic diseases.

Regulatory T cells: Function and heterogeneity.

Regulatory T (Treg) cells that express the transcription factor forkhead box P3 (FOXP3). Treg cells actively maintain peripheral tolerance to self-antigens and also control the magnitude and selectivity of immune responses to foreign antigens, thus preventing potentially tissue damaging exuberant immune response (10-12). FOXP3 is a master-switch transcription factor controlling Treg cell differentiation and enabling their regulatory phenotype (13). Deficiency of functional Treg cells due to mutations in FOXP3 gene in humans and its orthologue Foxp3 in

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mice leads to severe inflammatory and autoimmune disease, reflective of the primacy of Treg cells in controlling immune responses to self and foreign antigens in the periphery (14, 15).

Treg cells comprise different subsets with suppressive capacity able to inhibit the initiation and development of allergic diseases and allergic patients seem to exhibit a specific impairment in the generation or homeostasis of regulatory T cells. Based on their developmental or functional differences, Treg cells are categorized into two main populations: naturally occurring (nTreg) cells that are generated in thymus and peripherally induced Treg (iTreg) cells generated in peripheral lymphoid tissues from non-Treg cells CD4⁺ precursor (16, 17). The latter are derived upon the engagement of the T cell receptor (TCR) of naïve CD4⁺ cells by antigen-presenting cells found in specialized niches in mucosal tissues that produce transforming growth factor-beta (TGF- β) and retinoic acid, including CD11c+CD103⁺ dendritic cells in the gut, and alveolar and interstitial macrophages in the lungs (18-22). It is now clear that both nTreg and iTreg act to cooperatively regulate peripheral tolerance (23, 24). Although the long-term stability and lineage commitment of iTreg cells are the subject of debate (25, 26), they appear to be mainly responsible for limiting de novo immune responses especially to innocuous non-self-antigens. Fate mapping analysis showed that under chronic inflammatory states iTreg cells are not stable and tend to acquire T helper cell-like effector phenotypes and down-regulate or completely lose FOXP3 expression (herein called ex-Treg cells) (27). Of note, recent evidences indicate the contribution of ex-Treg cells to the acceleration of inflammatory disease where transferring of ex-Treg cells promote inflammatory responses to a new healthy host (28).

In the field of allergy, the current approach to induce tolerance centers on allergen-specific immunotherapy (AIT), which involve the administration of increasing doses of the causative allergen to induce a state of allergen-specific non-responsiveness that may evolve upon extended therapy to a state of immune tolerance (29). Although many clinical trials have demonstrated the efficacy of AIT, the therapy itself requires a long period of time to achieve optimal efficacy and is marred by relatively high frequencies of disease relapse, especially in the treatment of food allergies. Thus, there is an acute need to develop novel efficacious strategies that aim to restore durable tolerance to allergens. In this review we detail the mechanisms

that promote the reprogramming of allergen-specific Treg cells towards pathogenic effector T cell-like phenotypes in inflammatory allergic diseases, and potential therapeutic strategies to reverse such immune deviation in favor of durable allergen-specific immune tolerance.

Transcriptional and biological alteration of Treg cells in allergic disease.

Classically the differentiation of T helper (TH) cell subsets is controlled by master transcription factors. Thus, the differentiation of the effector TH1, TH2 or TH17 subsets requires the expression of the TH cell lineage committing transcription factors T-bet, GATA-3 and ROR- γ t, respectively, whereas Treg cells require FOXP3 expression (30). Treg cells might further differentiate and gain functional specialization based on their environment. The emerging paradigm of “paired differentiation” between Treg and effector T cells indicates that Treg cells acquire transcriptional program of specific effector T cells they suppress (31). Sharing transcriptional regulation allows Treg cells to adapt to the local environment and mediate suppression of the specific type of inflammation, including TH1 and TH17 cell responses (32, 33). Similarly, in the course of the TH2 cell type immune responses, Treg cells acquire the transcriptional machinery of TH2 cells, such as IRF4, to efficiently restrain the corresponding type of the immune response (34). The acquisition by Treg cells of a partial or an “aborted” forms of the transcriptional programs of the target TH cells, acquiring the relevant transcriptional factors and chemokine receptor expression but not effector cytokine production, enables them to coordinately regulate the tissue TH cell response. However, in the context of intense and sustained inflammation, Treg cell may become fully subverted to the TH cell effector cell type they are trying to suppress (28, 35, 36). Sustained TH cell polarizing cytokine signaling subvert Treg cell identity in part by antagonizing FOXP3 expression by transcriptional and epigenetic mechanisms (37, 38).

A critical transcription factor promoting the TH2 response in Treg cells is GATA3, which is normally expressed at a low, steady level in different populations of Treg cells and plays a broader role in maintaining their homeostasis (39, 40). GATA3-deficient Treg cells exhibit attenuated expression of FOXP3 and are deficit in controlling TH1, TH2, and TH17 responses (39, 40), whereas in severe and intense form of TH2-driven inflammatory responses the expression of GATA3 is elevated in Treg cells and promote an

acquisition of TH2-type phenotypes by those cells (41, 42). In the presence of IL-4, the induced GATA3 directly binds to a regulatory region in the FOXP3 gene promoter and represses FOXP3 transactivation and the Treg cell suppression capacity (43).

Pathogenic TH2 cell-like Treg cell reprogramming in allergic diseases.

Increasing evidence indicates that Treg cell lineage commitment may not be irreversible, as downregulation of Foxp3 and consecutive expression of effector TH cytokines by Treg cells has been noted by several groups (28, 42, 44-46). In particular, IL-4R-driven signaling pathways antagonize Foxp3 expression and overcome Treg cells stability and suppressive activities in the course of various TH2-type inflammatory diseases, including asthma and food allergy (42, 45, 47, 48). Food allergy development is associated with impaired generation of food allergen-specific iTreg cells (9). We have demonstrated that those food allergen-specific Treg cells that do develop are prone to acquire a pathogenic skewed TH2-like phenotype, resulting in increased GATA-3 expression and IL-4 secretion, in a STAT6 dependent manner (42, 49). The converted Treg cells are dysfunctional and lacking in suppressor function. In addition, in an acute murine model of food allergy we demonstrated that disease-susceptible mice, with a Tyrosine to Phenylalanine substitution at amino acid residue 709 of IL-4R alpha (IL-4R α) chain that augments STAT6 activation (*Il4ra*^{F709}), exhibited impaired generation, stability and function of mucosal allergen-specific iTreg cells (42). Those allergen-specific Treg cells that were generated exhibited a TH2-cell-like phenotype evidenced by highly increased expression of IRF4 and GATA3 (two master-switch TH2 cells transcription factors) and production of TH2-type cytokines by mucosal Treg cells. The TH2-like iTreg cells were not able to control innate lymphoid cells type 2 (ILC2) activation, mast cell activation and expansion and the effector TH2 cell immune response, thus amplifying and perpetuating the food allergic response (Figure 1) (42, 50). Importantly, Treg cell-specific deletion of *Il4* and *Il13* in these mice normalized their airway inflammatory responses in both models and reversed Treg cell TH2 cell-like reprogramming (42). Thus, the aggravated food allergic phenotype manifested by *Il4ra*^{F709} mice reflects in large part the destabilization of iTreg cells and their conversion to TH2-cell like effector cells.

In addition to their susceptibility to the induction of food allergy, *Il4ra*^{F709} mice also exhibit grossly

exaggerated allergic airway inflammatory responses allergen sensitization followed by allergen inhalation challenge (Figure 1) (47, 51). This phenotype recapitulates the human TH2-high asthma endotype, associated with heightened attributes of TH2 cell inflammation in the airways including airway eosinophilia and TH2 cytokine production. Of note, and the similar to the food allergy model, deletion of *Il4* and *Il13* in Treg cells protected *Il4ra*^{F709} mice from exaggerated allergic airway inflammation following allergen sensitization and challenge (47). These results suggest that TH2 reprogramming of Treg cells into TH2 cell-like cells may be an important mechanism in diverse allergic disorders associated with strong TH2 skewing.

IL-4-driven reprogramming of Treg cells into TH17-like cells as a mechanism of generating mixed TH2-TH17 inflammation

While exaggerated IL-4R/STAT6 signaling directs Th2 cell-like reprogramming of Treg cells to promote Th2 cell-high allergic disease attributes, a second mechanism involving a different branch of IL-4R signaling has recently been implicated in directing

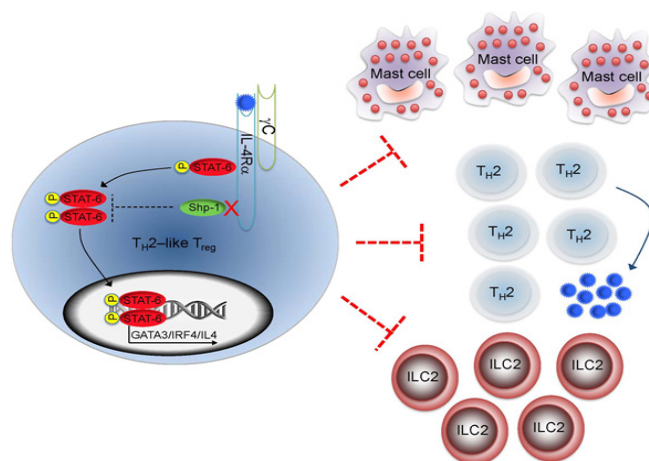


Figure 1. Exaggerated signaling via the IL-4R/STAT6 axis results in the acquisition by Treg cells of a pathogenic TH2 cell-like phenotype. Mutagenesis of immune-tyrosine inhibitory motif (ITIM) at position Y709 of mouse IL-4R α chain results in the loss of the binding of the phosphotyrosine phosphatase SHP1 to the IL-4R α chain, leading to the exaggerated activation of STAT6 upon the binding of its cytokine ligands IL-4 or IL-13. Intense IL-4R/STAT6 signaling results in increased GATA3 and IRF4 expression in Treg cells and their own expression of the TH2 cell cytokines IL-4 and IL-13. These TH2 cell-like pathogenic Treg cells are unable to suppress the activation of mast cells, innate lymphocyte cells type 2 (ILC2) or TH2 cells, leading to a dysregulated allergic inflammatory response.

mixed TH2-TH17 inflammation by directing Treg cell reprogramming into TH17 cell-like cells (47). This mechanism involves a common glutamine to arginine substitution at amino acid residue 576 of human IL-4R α chain (IL-4R α Q576R). The Q576R polymorphism highly prevalent in populations of African descent, including African Americans, and also found at lower frequencies in other populations and ethnicities. It has been associated with asthma, especially with asthma severity and symptomatology (52-54). It has also been associated with eczema and atopy.

The mechanisms by which the Q576R polymorphism exacerbate allergic airway inflammation have been clarified in studies on a mouse model that took advantage of the conservation of the Q576 residue in the murine receptor to introduce the R576 substitution by germline knock-in mutagenesis. Mice bearing the R576 residue in their IL-4R α exhibited exaggerated allergic airway inflammation with intense tissue eosinophilia as compared to control mice harboring the wild-type Q576 bearing IL-4R α (55). We have recently dissected the mechanisms by which the Q576R substitution induced severe airway inflammation by showing that the IL-4R α R576 variant enables recruitment of the adaptor GRB2 to IL-4R α at the adjacent Y575 residue. GRB2 recruitment establishes a new branch of IL-4R signaling that acts in parallel to the classical IL-4R-mediated STAT6 activation to activate the MAPK pathway, leading to IL-4 (and IL-13)-induced IL-6 production by T cells and other immune cells (Figure 2) (47). In the presence of TGF- β -produced by antigen presenting cells normally promoting iTreg cell differentiation such as lung macrophages (18), the aberrant IL-4-induced production of IL-6 directs TGF- β -dependent iTreg cell differentiation toward the TH17 cell-like lineage. Concurrent STAT6 activation by IL-4R α and STAT3 activation by autocrine and paracrine produced IL-6 also drive hyper activation of the eotaxin promoter in lung macrophages and dendritic cells, leading to greatly increased recruitment of eosinophils into the airways. Thus, signaling via the IL-4R α -R576 variant acts to promote a unique mixed TH2/TH17-type inflammation in the airways that has been associated in human subjects with recalcitrant, steroid resistant asthma. Of note, intervention strategies that target IL-6/IL-6R signaling in Treg cells, such as deletion of *Il6ra* in Treg cells or treatment of mutant mice with an IL-6-specific mAb, and those that target the skewing of Treg cells to TH17 effector cell, such as deletion of *Rorc* in Treg cells, protected against

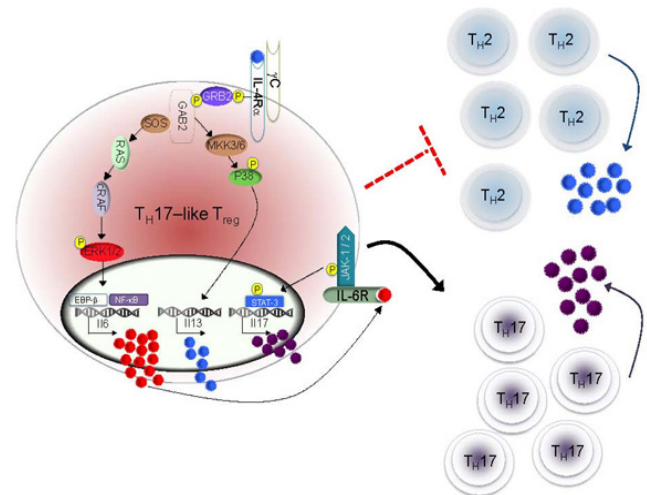


Figure 2. iTreg cell reprogramming towards a TH17 cell-like fate by the asthma-promoting IL-4R α -Q576R variant. Schematic representation of pathways mediating iTreg cell reprogramming into TH17-like cells by the IL4R variant Q576R. The glutamine to arginine substitution at position 576 of IL-4R α results in the recruitment of the signal transduction adaptor protein Growth factor receptor-bound protein 2 (GRB2) upon receptor activation. GRB2 activates downstream extracellular-signal-regulated kinases, which drive IL-6 production by activating the transcription factors NF- κ B and C/EBP- β . It also activates p38 MAP kinase which promotes IL-13 production. Newly formed antigen-specific iTreg cells are thus destabilized, leading to exaggerated TH2 and TH17 cell responses that foster severe airway inflammation.

exaggerated airway inflammation, suppressed the TH17 cell skewing and normalized the Treg cell responses. In contrast, treatment with an IL-17 or eotaxin-specific mAbs only partially protected against airway inflammation and failed to prevent iTreg cell reprogramming toward the TH17 cell fate (47). Therefore, the efficacy of intervention strategies in airway inflammation associated with the IL-4R α -R576 variant hinges on stabilizing Treg cell responses rather than targeting end products of inflammation.

Recent technological advances in lineage tracing allow us to trace the conversion of Treg cells into ex-Treg cells (27). In IL-4R α R576 mutant mice we further demonstrated that at the site of the inflammation, the ex-Treg cells constitute a fraction of ~30% of TH17 effector infiltrates, whereas in wild-type counterparts this number is as low as ~5%, indicating the pathogenic contribution of plastic Treg cells in the severity of disease.

Treg cell programming into TH17-like cells in autoimmunity

IL-6-dependent subversion of Treg cells into TH17-like cells has a critical role in the pathogenesis of other diseases rather than asthma. In a murine model of arthritis, Komatsu et al. has shown that TH17 cells with arthritogenic and autoreactive properties arise from FOXP3^{3+T} cells, in an IL-6 dependent manner, emphasizing the critical pathological role of IL-6 in destabilization of Treg cells and skewing toward TH17 cells fate in different inflammatory contexts (28).

Unstable Treg cells are particularly enriched in the CD25^{low} Treg population. Adoptive transfer of autoreactive, antigen-experienced CD25^{low}FOXP3³⁺ T cells into mice followed by secondary immunization with collagen accelerated the onset and increased the severity of arthritis and was associated with the loss of FOXP3 expression in the majority of transferred T cells, indicating the contribution of ex-Treg in the pathogenesis of autoimmune and inflammatory disorders.

In addition, IL-6 acts to downregulate Foxp3 expression via downregulation of the co-repressor Eos expression in Treg cells, leading to loss of FOXP3 expression and rapid reprogramming of Treg cells (56). Eos is a member of the Ikaros family of transcription factors that forms a complex with FOXP3, and is required for FOXP3 to inhibit its downstream target genes and maintain the suppressive Treg cell phenotype (57). Thus, targeting the IL-6 axis is potentially an effective approach to increasing Treg cell lineage stability in allergic and other inflammatory conditions.

The role of follicular Treg cells in protection against TH2-type inflammatory responses.

A so-called subset of “follicular regulatory T (Tfr) cells” has been identified to express CXCR5 in a Bcl6 dependent manner and localize in the germinal centers in mice and in humans (58, 59). In addition to controlling the germinal center reactions, Tfr cells are essential in suppression of TH2-driven inflammatory responses. Mice whose Treg cells are selectively deficient in Bcl6 failed to suppress the TH2-mediated inflammation in a model of allergic airway disease (60). Further, adoptive transfer of Bcl6^{-/-} Treg cells promotes spontaneous TH2-type inflammation in recipient that emphasize the indispensable role of Bcl6 in maintaining of Treg lineage homeostasis in TH2-driven allergic responses. Intriguingly, TGFβ and retinoic acid present in the mucosal surfaces induce miR-10a, a microRNA that targets Bcl6, so

maintaining Treg cell stability and preventing Treg cell programming (61).

Epigenetic changes of the non-coding gene sequences of FOXP3 gene during inflammation.

Following Treg cell differentiation, continued expression of FOXP3 is imperative for the maintenance of the Treg cell suppressive functions (62, 63). Stable FOXP3 expression is accompanied by epigenetic modulation of the Treg-specific demethylated region (TSDR), an evolutionary conserved CpG-rich non-coding sequence within the first intron of the FOXP3 gene locus (64). Ex-Treg cells developed during the inflammatory responses were noted to exhibit increased methylation levels of the evolutionarily conserved non-coding sequence CNS2, located in the TSDR, that directly correlated with loss of FOXP3 expression, instability of Treg cells, especially iTreg cells (28, 47). Interestingly, an increased demethylation level of FOXP3-TSDR occurs during the resolution phase of the inflammation (65). Demethylated CpG island of CNS2 serve as binding sites for Runx and CREB/ATF and Ets-1 complexes (66-68), Suggesting that recruitment of these TCR-response elements to the demethylated FOXP3 CNS2 facilitates the maintenance of the active state of the FOXP3 locus and, therefore, Treg cell lineage stability. In the same vein, we have shown that Treg cells isolated from IL-4RαQ576R mice with allergic airway disease exhibit decreased methylation of the FOXP3 CNS2 enhancer region, indicative of decreased Treg cell phenotypic stability.

Besides CNS2, two other conserved non-coding regions in the FOXP3 promoter region termed CNS1 and CNS3 are critical for optimal induction or maintenance of FOXP3 expression in iTreg or nTreg cells. CNS3 acts as a “pioneer” element to facilitate FOXP3 induction during thymic and peripheral Treg differentiation by recruiting c-Rel (68). In contrast, CNS1, which contains a TGF-β–NFAT binding site, is dispensable for nTreg cell differentiation, but has a prominent role in iTreg cell generation in gut-associated lymphoid tissues (68). Enzymes that regulate the demethylation of CNS elements, such as the ten-eleven translocation (Tet) enzymes Tet2/Tet3, which oxidize 5-methylcytosine (5mC) to 5-hydroxymethylcytosine and other oxidized methylcytosines intermediates in DNA demethylation, are also involved in maintaining FOXP3 promoter stability in Treg cells. Their deficiency results in compromised FOXP3 epigenetic stability and the destabilization of Treg cells. Vitamin C potentiates

Tet activity and acts through Tet2/Tet3 to increase the stability of Foxp3 expression in TGF- β -induced Treg cells, suggesting a potential role for small molecule activators like vitamin C in increasing iTreg cell stability (69).

CONCLUSION

Recent evidence indicates the association of the acquisition of iTreg cells of an effector T cell-like program with allergic inflammation and its severity. We have shown that pro-asthmatic IL-4R alpha chain polymorphisms promote food allergy or distinct asthma endotypes (a mixed TH2-TH17 cell inflammation) by reprogramming Treg cells differentiation toward the respective TH cell program. Blocking the IL-4R or IL-6R pathways may prevent the subversion of Treg cells into effector T cells fates and provide personalized therapeutic approaches for tolerance re-establishment in food allergies, asthma and other allergic disorders.

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