

Review Article

Treg cells: a potential regulator for IL-22 expression?

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Abstract: Interleukin-22 (IL-22) is a IL-10 family cytokine member and is mainly produced by innate lymphoid cells (ILCs), Th17 cells, and Th22 cells. Previous studies have indicated that IL-23 and several transcription factors, including STAT3, ROR γ t, and the AhR are important stimulus. Recently, there is emerging evidence that Tregs can regulate IL-22 expression. In the review, we discuss the updated advancement on Tregs function and its regulatory role on IL-22 expression.

Keywords: Interleukin-22, regulatory (Treg) cells, T helper cells, innate immune cells

Introduction

IL-22 is a member of the IL-10 family, mainly produced by T cells and natural killer (NK) cells and represents an effector cytokine of the Th17 lineage [1-4]. The regulation of IL-22 expression consists of cytokine-mediated regulation such as IL-23 and transcriptional control including STAT3, ROR γ t, and the AhR [5]. Here, we focus on the regulatory role of T regulatory (Treg) cells in IL-22 expression.

IL-22

The IL-22-IL-22R pathway

IL-22 belongs to the IL-10 cytokine family and is produced by special immune cell populations, including Th22, Th1, and Th17 cells, classical and non-classical (NK-22) NK cells, NKT cells, and lymphoid tissue inducer cells [3]. IL-22 binds to a heterodimeric receptor consisting of IL-22R1 and IL-10R2. IL-22R1, the ligand binding subunit, is expressed by a variety of non-immune tissues: skin, lung, kidney, and pancreas [6, 7]. Thus, IL-22 functions as a signaling mediator that can connect lymphocytes and epithelial cells. There is also a soluble IL-22R called IL-22-binding protein (IL-22BP) [6, 7]. The

binding of IL-22 to IL-22BP is of 20- to 1000-fold higher affinity compared to its binding to the membrane bound IL-22R1 [8].

IL-22 binding to IL-22R complex induces a cascade of downstream signaling pathways [5]. Initial studies utilizing a murine kidney cell line revealed that IL-22R ligation induced phosphorylation of STAT3, and to a lesser extent, STAT5 [4], while other studies observed phosphorylation of STAT1, STAT3, and STAT5 in a human kidney cell line [9]. Further analysis has concluded that IL-22 signaling utilizes Jak1 and Tyk2 to propagate downstream phosphorylation signals, including several MAPK pathways (ERK1/2, MEK1/2, JNK, and p38 kinase), and STAT1, STAT3, and STAT5 [10].

The regulation of IL-22 expression

IL-23 is an important stimulus for induction of IL-22 expression in both innate and adaptive immune cells. The importance of IL-23 in the induction of IL-22 *in vivo* is evident in several models [11-13]. IL-23 has also been found to be essential in the terminal differentiation of Th17 cells, aiding in their expansion and effector functions [14]. The ability of IL-23 to enhance Th17 cell expansion appears to be linked to

Tregs regulate IL-22

IL-22 expression, as increased expansion was only observed in IL-22⁺Th17 cells but not in IL-17A⁺Th17 cells [2]. Several transcription factors, including STAT3, ROR γ t, and AhR have also been found to be essential in the regulation of IL-22 in multiple cell lineages [15-17]. In addition to expression, several functional properties of IL-22 can be regulated by different inflammatory cytokines such as IL-17A, 17-F and TNF- α in a synergistic or inhibitory manner [11, 18, 19].

The biological effect of IL-22

IL-22-IL-22R signaling in epithelial cells results in expression of genes involved in antimicrobial host defense including S100 proteins, defensins, Lipocalin 2, and RegIII-family proteins [20, 21]. IL-22 also induces inflammatory molecules such as chemokines and cytokines including IL-6 [22, 23]. In addition, IL-22 has an important function in tissue repair *via* induction of epithelial cell proliferation and survival [6, 22, 23]. By inducing such genes and by enhancing epithelial barrier function, IL-22 plays an important role in promoting resistance to extracellular pathogens [21-23].

During an inflammatory response, IL-22 can act to either promote or protect from inflammation. IL-22 prevents tissue destruction in several mouse models. In the intestine, IL-22 prevents tissue destruction in a murine model of inflammatory bowel diseases (IBD) [24, 25] and in a mouse model of *graft versus* host disease [26]. In a Concanavalin A-induced hepatitis model, IL-22 protects from liver injury by enhancing the growth and survival of hepatocytes [27-29]. In contrast, IL-22 can promote pathological inflammatory responses in the skin and intestine in mouse models, and the concentration of IL-22 is increased in a variety of human diseases including psoriasis [21, 22], rheumatoid arthritis and others [29, 30]. Furthermore, excessive and aberrant IL-22 results in colon cancer development, as exemplified by mice lacking IL-22-binding protein (IL-22BP) [31].

Treg cells

Cells targeted by Tregs

Tregs, a subset of CD4⁺ T cells is characterized by the expression of the IL-2 receptor α -chain (CD25) [32-34]. CD25, however, is also

expressed on activated T cells and therefore cannot serve as a Treg-specific marker molecule. Recently, the forkhead family transcription factor (Foxp3) has been described as a highly specific intracellular marker molecule for Treg [35, 36]. Tregs regulate the activation and expansion of CD4⁺ T cells lineage, via expression of forkhead box P3 and/or their capacity to produce cytokines such as transforming growth factor (TGF)- β , IL-10, and IL-35 [37-40]. IL-10 antagonizes pro-inflammatory cytokines such as IL-6 and might also be an antagonist to the inflammatory IL-17. Additionally, IL-10 negatively regulates Th17 cell differentiation [41]. Moreover, besides CD4⁺ T and CD8⁺ T cells, also other cell types such as B-cells, dendritic cells (DCs), monocytes, mast cells, osteoblasts, NK cells and NK T cells were identified as target cell populations for Tregs [42-45].

Tregs function

Tregs can suppress the immune response of CD4⁺ and CD8⁺ T cells [46]. The suppressive functions are deficient or reduced in Foxp3 deficient mice and the human autoimmune diseases including autoimmune diabetes, autoimmune proliferative syndrome (APS) type II, multiple sclerosis (MS), *graft versus* host disease, autoimmune hepatitis and rheumatoid arthritis (RA) [47-49]. On the other hand, increased proportions of functionally active CD4⁺CD25⁺ Treg have been described in the synovial fluid of RA patients [47]. So far, conflicting data have been reported and no overall consensus has been reached. One of the major reasons for such controversial observations is the pleiotropic function of Tregs.

A recent focus may point to a new role for Tregs in the perpetuation of inflammatory processes, rather than in the suppression thereof [50-53]. A stable expression of Foxp3 is required for Treg differentiation and for their suppressor function, proliferative potential and metabolic fitness [36, 54-56]. The transcriptional repressive effects of Foxp3 protein render Treg cells incapable of producing certain key cytokines such as interleukin-2 (IL-2). Therefore, Treg cells require an exogenous supply of these cytokines for their peripheral maintenance [57]. Suppression by Treg cells depends strongly on the local cytokine milieu and the proximity of Treg cells to effector cells during an immune response [34, 58]. Tregs cells could lose their

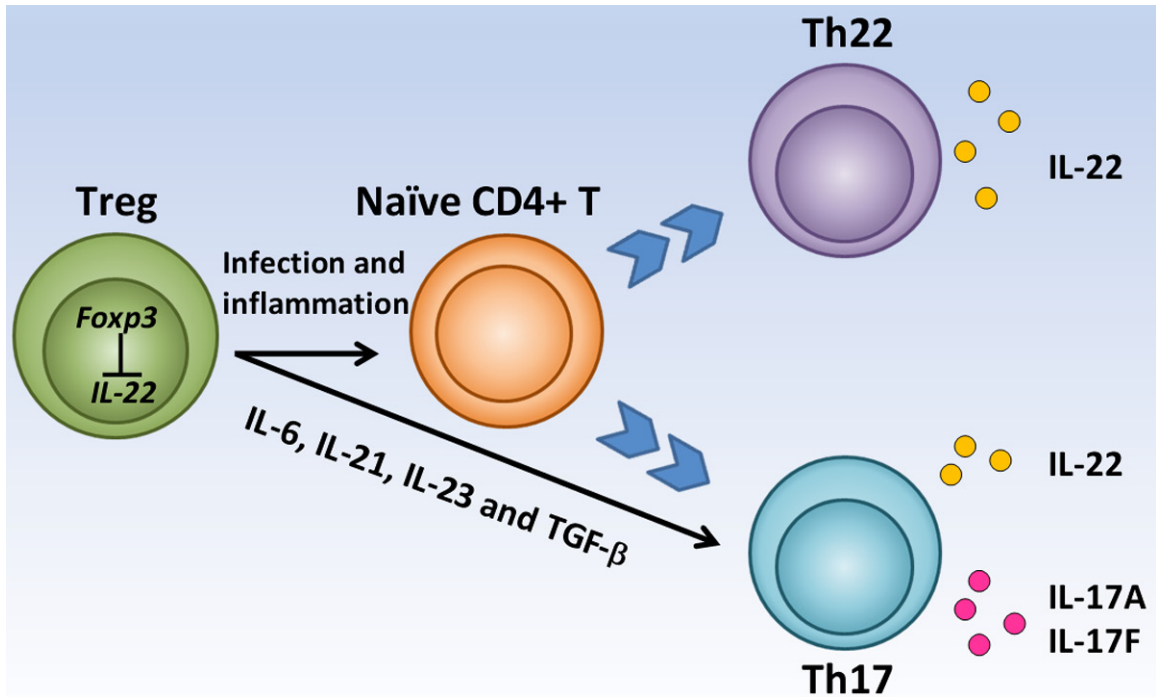


Figure 1. The regulation of Treg cells on IL-22 expression. *Foxp3* suppresses *IL-22* directly in Treg. Treg promotes naïve $CD4^+$ cells to become Th22 and Th17 in the inflammatory conditions and then release IL-22, IL-17A and IL-17F. Treg also boosts the production of IL-22 in Th17.

suppressive functions [59, 60], because they may not effectively suppress by cytokine competition when cytokines are abundant during an infection. Moreover, during acute inflammation, Tregs cells promote Th17 cell differentiation leading to up-regulated the expression of pro-inflammatory IL-17A, IL-17F and IL-22 [61]. So Treg cells may have broader roles in immunity than just the previously recognized suppressor functions. Further studies on the function properties and the mechanism of action of these Treg are needed.

The regulation of Treg cells on IL-22 expression

IL-22 as a direct target gene of FOXP3

The IL-22 genomic locus is part of a small gene cluster comprising IFN- γ , IL-26, and IL-22. The IL-22 gene lies on the minus strand of the human genome and encodes for 5 exons [62]. Andreas et al has analyzed the Foxp3 transcription factor binding sites (TFBSs) in a human T-cell line by genomic tiling microarray (ChIP-on-chip), and observed that the down-regulation of IL-22 in stimulated Jurkat-Foxp3 (J-Foxp3) T cells was accompanied by Foxp3 binding only

under stimulated conditions [63]. A reproducible Foxp3 binding site can be found in proximity (3646 bp) to the IL-22 TSS. In the middle of the ChIP region there was a perfectly matching Foxp3 consensus site with the following genomic coordinates: chr12 66937190 – 66937197 (hg18 reference assembly). Genomic site-specific real-time PCR of this binding region confirmed about 5-fold ChIP enrichment. A prerequisite for a direct Foxp3 target gene is the occurrence, suggesting IL-22 is a direct target gene of Foxp3 [63].

Treg cells regulate the expression of IL-22 on the transcriptional level

Consistent with the down-regulation of IL-22 in stimulated J-Foxp3 T cells, the decreased expression of IL-22 in Foxp3⁺ Treg cells has also been observed compared to conventional naïve Foxp3⁻ T cells [63]. Recent studies reported a close relationship between CD4⁺Foxp3⁺ Tregs and proinflammatory IL-17-producing Th17 expressing the lineage-specific transcription factor ROR γ t [56, 64]. It has been shown that IL-17 secreting Foxp3⁺ T cells that express ROR γ t share features of conventional ROR γ t⁺Th17 cells. However, ROR γ t⁺Foxp3⁺

Tregs mostly fail to secrete IL-22 after PMA/ionomycin stimulation [51]. Foxp3 TFBS in the IL-22 promoter restrains RORyt⁺Foxp3⁺ T cells to produce IL-22 on the transcriptional level [63].

Treg cells regulate the expression of IL-22 in CD4⁺ T cells

Despite the decreased expression of IL-22 in Foxp3⁺ Treg cells, it has been found that Treg cells can promote naïve T cell differentiation, to some extent, depending on inflammatory environment and the local cytokine milieu. In mouse mode of infection with an oral *Candida albicans*, Foxp3⁺ Treg cells can powerfully promote the transition of naïve CD4⁺ T cells to responding CD4⁺ cells (Tresp). Tresp cells markedly produce IL-22 [61]. Th17 cells are developed from naïve CD4⁺ T cells under the influence of a network of inflammatory cytokines, including IL-1, IL-6, IL-21 and TGF- β . Th17 cells produce pro-inflammatory cytokines IL-17A, IL-17F and IL-22 and the expression of the retinoic acid orphan receptor-related transcription factor (RORC). In the same study, the full differentiation program of Th17 cells promoted by the presence of Treg cells boosted the production of IL-22, in addition to IL-17A and IL-17F. Treg cells did not suppress, but actually promoted IL-17A, IL-22-dependent clearance of fungi during acute *C. albicans* infection [61]. These findings demonstrated plastic regulation of FOXP3⁺ Treg cells on IL-22 expression (**Figure 1**).

Perspective

IL-22 is a critical cytokine in a number of immune processes and plays an important role in barrier surfaces as well as in the development and pathogenesis of autoimmunity. IL-22 is produced by T cells and innate immune cells including CD4⁺ T and CD8⁺ T cells, NK cells and NK T cells. Differentiation of these cells is, at least, partially regulated by Treg cells. Therefore, there is the presence of possibility that Treg cells might regulate the expression of IL-22. Despite the decreased expression of IL-22 in Foxp3⁺ Treg cells, Treg cells also induce the secretion of IL-22 from CD4⁺ T cells during acute inflammation. The evidence might be useful to further study on the exact association of Treg cells with IL-22 expression in immunity and infection. However, many questions remained to be answered. For example, Treg

cells regulate the IL-22 expression on these cells: directly or indirectly? With a synergistic or inhibitory manner?

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Disclosure of conflict of interest

The authors declare no competing interests.

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