Advances of the interleukin-21 signaling pathway in immunity and angiogenesis (Review)

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Abstract. Interleukin-21 (IL-21) and its receptor (IL-21R) are broadly expressed on human B cells, activated T cells and other myeloid cells. IL-21 cooperates with IL-6 and transforming growth factor-β to regulate T-cell differentiation. IL-21-mediated human B cell and dendritic cells differentiation requires signal transducer and activator of transcription 3 (STAT3), and also induces B-cell apoptosis dependents on the Toll-like receptor signal. Recently, *in vitro* and *in vivo* experiments showed that IL-21/IL-21R regulate angiogenesis through STAT3. IL-21 signaling pathways are complex due to its cooperation with other transcriptional factors, such as interferon regulatory factor 4 and granulocyte-macrophage colony-stimulating factor. The Janus kinase-STAT pathway has been the most extensively studied. With the increase in the understanding of IL-21 biology in the context of each specific disease or pathological condition, IL-21 could be a new therapeutic target for immune-related disease.

Contents

1. Introduction

Interleukin-21 (IL-21) and its receptor (IL-21R) were identified in 2000 (1). IL-21 is primarily produced by cluster of differentiation 4$^+$ (CD4$^+$) cells and natural killer cells, while IL-21R is broadly expressed on human B cells, activated T cells and other myeloid cells (2,3). IL-21 is a pleiotrophic cytokine that is composed of four α-helical bundles. IL-21R shares the common cytokines receptor γ chain (γc) with the IL-2 family cytokines, such as IL-4, IL-7, IL-9 and IL-15 (4). In addition, IL-21R has a distinct α chain, and contains six tyrosine residues in the cytoplasmic domain (3,5). This specific IL-21R structure differentiates IL-21R from IL-2R. IL-21 exerts its effect on a broad range of cell types. Increasing evidence shows that IL-21 potently regulates innate and adaptive immune response (6-8). Furthermore, the role of IL-21 in angiogenesis has also been studied (9,10). In the present review, the recent advances regarding the role of IL-21 in immune cells and angiogenesis are discussed.

2. Function of IL-21 on immune cells

Although IL-21 is not required for CD4$^+$ T-cell development, it contributes to the functional differentiation of several subsets (11,12), such as T helper 2 (Th2) cells (13,14), Th17 (15,16) and follicular helper T (Tfh) cells (17,18). Th17 and Tfh cells can be generated in the absence of IL-21/IL-21R (16), indicating an IL-21-independent pathway for their development. IL-21 is produced by the Th17 cells, and transforming growth factor-β (TGF-β) and IL-6 can activate Th17 cells even in the absence of IL-21 (19,20). IL-21 regulates the transcription factors B-cell lymphoma 6 (BCL-6) and MAF, which are important to the transcriptional programme of the Tfh cells (21,22). IL-6 can induce Tfh-cell differentiation via its induction of IL-21 production. The number of Treg cells is increased in IL-21- and IL-6-knockout mice, and TGF-β signaling enhances the generation of Treg cells in the absence of either IL-21 or IL-6 (23,24). Thus, IL-21 appears to have a complementary role in regulating CD4$^+$ T-cell differentiation.

B-cell expression of IL-21R notably exceeds that of T cells. A large number of studies confirm that IL-21 involved in the regulation of both B cell proliferation and maturation. IL-21 can stimulate B cells proliferation and differentiation in the context of a co-stimulatory T-cell signal. IL-21-mediated human B-cell differentiation requires signal transducer and activator of transcription 3 (STAT3), and cannot be compensated by alternative signaling pathways (25). The effect of IL-21 can be augmented by IL-2 or IL-10, and

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IL-21 induces IL-10 in human B cells and interacts with TGF-β (26,27). In particular, IL-21 promotes B cells differentiation to Ig-producing plasma through its induction of B lymphocyte-induced maturation protein-1 (28), which is a transcription factor critical for plasma cell formation. Notably, IL-21 also induces B cell apoptosis either in the absence of a T-cell signal or in the activation of a Toll-like receptor signal (29). The pro-apoptotic activity of IL-21 results from the induction of BCL-2, which is a pro-apoptotic protein.

IL-21 has broad actions on T and B cells, but its innate immunity is poorly understood. IL-21 has a potent inhibitory effect on granulocyte-macrophage colony-stimulating factor (GM-CSF)-induced dendritic cells (DCs) (30). IL-21 induces apoptosis of conventional DCs (cDCs) via STAT3 and inhibiting Bim, and this effect is prevented by GM-CSF, which partially opposes the biological action by these cytokines. Furthermore, the number of STAT3 sites was reduced in the presence of GM-CSF when DCs were treated with IL-21, and GM-CSF primarily activates STAT5 instead of STAT3 and inhibits Bim (31). These findings suggest that IL-21-induced STAT3-dependent apoptosis of DCs provides a mechanism for alleviating the immune response, and IL-21 has a cross-negative regulation with GM-CSF.

3. Signaling by IL-21

IL-21 regulates the innate and adaptive immune responses via heterodimers of the IL-21R and the common cytokine receptor γc1. IL-21 signals via the Janus kinase (JAK)-STAT signaling pathway (25,26), the mitogen-activated protein kinase signaling pathway and the phosphoinositide 3-kinase-AKT signaling pathway (2). Of these, the JAK-STAT pathway has been the most extensively studied. In T cells, IL-21 activates STAT3 more than STAT1 and STAT5. STAT1 and STAT3 have partially opposing roles in IL-21 signaling. RNA-sequence analysis showed that STAT1 and STAT3 are critical for IL-21-mediated gene regulation, including Tbx21 and interferon γ (32). Notably, IL-21-induced expression of suppressor of cytokine signaling 3 (Socs3) and Socs1 are decreased in $Stat3^{-/-}$ cells (33). SOCS3 and SOCS1 can negatively regulate STAT protein phosphorylation, and this may in part explain the opposing roles of STAT1 and STAT3 in IL-21 function in $CD4^{+}$ T cells. In cDCs, IL-21 induces IL-1β production via a STAT3 dependent and nuclear factor-κB independent pathway. Furthermore, this processing in cDCs does not require caspase-1 or caspase-8, but depends on IL-21-mediated death (34). IL-21 can induce the expression of PR domain containing 1, with ZNF domain in multiple B lymphoma cell lines, and IL-21 induces STAT3 binding also bound interferon regulatory factor 4 (IRF4) *in vivo* (35,36), and $Irf4^{-/-}$ mice showed impaired IL-21 induced Tfh cells differentiation (37). These results reveal broad cooperative gene regulation by STAT3 and IRF4. In T cells, numerous target genes of IL-21 are regulated through basic leucine zipper transcription factor, ATF-like, JUN, IRF4 and STAT3 (37,38). Notably, these transcription factors are also potential targets through which IL-21 signaling may be regulated. Our recent study reported that IL-21 activated STAT3 in HUVECs exposed to ischemia conditions; however, there were no significant changes in STAT1, AKT1 or extracellular-signal-regulated kinase 1/2 (ERK1/2) phosphorylation at any time point following IL-21 treatment (9).

4. IL-21 and angiogenesis

It has been shown that IL-21R exists in endothelial cells (ECs), which is a key process in the formation of new blood vessels during angiogenesis. IL-21 treatment decreases EC proliferation and sprouting *in vitro*. Furthermore, in a tumor mouse model, IL-21 inhibited tumor angiogenesis *in vivo* and decreased angiogenesis vascular endothelial growth factor A and its receptors (10). Another study demonstrated conflicting results, in which genetic ablation of IL-21 in $Apc^{min/+}$ mice reduced STAT3 activation and diminished cytokines, including IL-6 and tumor necrosis factor-α, and decreased angiogenesis in the lesions (8).

In our recent study of a mouse model with surgical hindlimb ischemia (HLI), the IL-21R levels were higher in the EC-enriched fraction isolated from ischemic hindlimb muscle. Furthermore, HUVECs showed 10-fold IL-21R expression following hypoxia and serum starvation *in vitro*. IL-21 treatment increased cell viability, decreased cell apoptosis and augmented tube formation in HUVECs under ischemic conditions. Knockout IL-21R resulted in less perfusion recovery following HLI *in vivo*. In particular, the activated STAT3 pathway and increase in the BCL-2/BCL-2-associated X protein ratio were involved in the *in vitro* and *in vivo* experiments (9). These results suggest that the elevated IL-21R levels in EC in ischemia muscle are adaptive.

5. Potential therapeutic effect of IL-21

Numerous studies have shown that IL-21 has therapeutic effects in animal models of a wide range of diseases [including cancer (12), immunity-deficient disease (39), type 1 diabetes (40) and inflammatory bowel disease (41)] and various clinical trials are underway (42).

An investigation regarding the association between IL-21 levels and myocardial function following acute myocardial showed that plasma IL-21 concentration correlated significantly with left ventricular end-systolic volume index, and multivariate analysis suggested that IL-21 was an independent predictor of remodeling. Furthermore, IL-21 was also significantly associated with higher tissue inhibitor of metalloproteinases-4 (TIMP-4) concentrations and lower MMP-9 concentrations (43). A previous experiment demonstrated that IL-21R was expressed on cardiac fibroblasts (44), and whether IL-21 may directly stimulate MMP/TIMP release within the myocardium is unknown and merits further study.

6. Conclusion

IL-21 has been implicated in broad immunological processes since its discovery in 2000. IL-21 regulates at least 3 pathways (STAT3, ERK1/2 and AKT-1), which can either enhance cell survival or pro-apoptosis in different cell lines. IL-21 signaling pathways are complex due to their cooperation with other transcriptional factors. With the improvement of our

understanding in IL-21 biology regarding each specific disease or pathological condition, IL-21 could be a new therapeutic target for immune relative disease.

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