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The Yin and Yang of Interleukin-21 in Allergy, Autoimmunity and Cancer

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Summary

IL-21 is a type I cytokine that like IL-2, IL-4, IL-7, IL-9, and IL-15 shares the common cytokine receptor γ chain, γ_c . IL-21 is produced by activated CD4⁺ T cells, NKT cells, and Th17 cells, and has pleiotropic actions on a range of lymphoid lineages. IL-21 regulates immunoglobulin production and drives B-cell terminal differentiation into plasma cells, cooperatively expands CD8⁺ T cells and drives Th17 differentiation, has inhibitory effects on antigen-presentation by dendritic cells, and can be pro-apoptotic for B and NK cells. Moreover, IL-21 has potent anti-tumor effects and is implicated in the development of autoimmune diseases. Regulating IL-21 actions in vivo therefore has clinical potential for a range of diseases and is an area of active investigation.

Introduction

IL-21 is the most recently discovered member of the family of cytokines whose receptors share the common cytokine receptor γ chain, γ_c [1**]. γ_c is also a component of the receptors for IL-2, IL-4, IL-7, IL-9, and IL-15, and when mutated results in X-linked severe combined immunodeficiency (XSCID) in humans [1**,2]. The simultaneous inactivation of IL-4 and IL-21 signaling in this disease is responsible for the B cell defect in XSCID [3]. IL-21 production was originally thought to be restricted to CD4⁺ T cells, but it is now clear that IL-21 also is produced by Th17 cells and by NKT cells [1**], indicating roles for IL-21 in innate as well as adaptive immune responses. Although IL-21 production is restricted to lymphoid populations, it acts on a range of both lymphoid and non-lymphoid cells, indicating broad functions [1**]. In the past few years, it has become clear that not only does IL-21 regulate normal lymphoid development and function, but it also serves critical roles in inflammatory responses, allergy, and autoimmunity, and it has anti-tumor actions. We herein review the basic biology and signaling by IL-21 and discuss new insights regarding the function of IL-21 in pathogenic processes and therapeutic approaches for manipulating in vivo levels of IL-21 in allergy, autoimmunity, and cancer.

Regulation of expression of IL-21 and IL-21R and mechanisms of signal transduction

The *IL21* gene is adjacent to the *IL2* gene, and both genes are potently induced in T cells following TCR stimulation in a fashion that is dependent on a calcium signal and NFAT sites

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[4]. The IL-21 receptor is expressed on CD4⁺ and CD8⁺ T cells, B cells, NK cells, dendritic cells, macrophages, and keratinocytes [1**]. IL-21R is more highly expressed on B than T cells, but its expression within both lineages increases following activation via the antigen receptor [1**,5]. In T cells, this process involves the induction and dephosphorylation of Sp1 [5]. The expression of IL-21R on multiple lineages corresponds to the broad actions mediated by the cytokine [1**]. IL-21 acts through a receptor comprising IL-21R and γ_c , with Jak1 and Jak3 being rapidly activated and in turn activating Stat3 and to a lesser extent Stat1, Stat5a, and Stat5b [1**,6*]. Tyrosine 510 in the cytoplasmic domain of IL-21R mediates activation of Stat3 and Stat5, and IL-21 also activates PI 3-kinase/Akt and MAP kinase pathways, which together with Jak-STAT pathways contribute to IL-21 signaling [6*].

IL-21 regulation of B cell function

IL-21 plays a central role in the proliferation and survival of B cells and in their differentiation to immunoglobulin (Ig) producing cells [1**,3]. Although IL-21R knockout (KO) and wild type (WT) mice have indistinguishable immature and mature B cell populations [3], IL-21 affects survival and proliferation of mature B cells in vitro, depending on co-stimulatory signals [7–9]. Specifically, although IL-21 augments B-cell proliferation induced by BCR signals or signaling via CD40, IL-21 is pro-apoptotic, alone or when combined with activators of toll-like receptor signaling. These in vitro data suggest that in an immune response, if IL-21 signals without a specific antigen or in the presence of a non-specific polyclonal signal, potentially autoreactive B cells are deleted, whereas IL-21 acting in the context of a specific antigen and T-cell interaction instead induces expansion of responding cells (Figure 1).

The abilities of IL-21 to promote the differentiation of Ig-producing plasma cells [7,10] and regulate specific levels of IgE [3,11] have important implications for B-cell mediated autoimmunity and allergy. Naive IL-21R KO mice have diminished serum IgG1 and elevated serum IgE, and immunization of these mice with T-dependent antigens leads to reduced antigen-specific IgG1 and elevated antigen-specific IgE [3]. The IL-21-mediated downregulation of IgE may result from IL-21-induced expression of the pro-apoptotic Bcl-2-modifying factor, Bmf, in IgE-expressing B cells [12] or the induction of inhibitor of differentiation-2 (Id2) in B cells, leading to suppression of class-switch recombination (CSR) to IgE [13*]. The downregulation of IgE by IL-21 has implications for controlling IgE-mediated allergic responses.

The ability of IL-21 to regulate B-cell function and Blimp-1-dependent plasma cell differentiation (Figure 1) also has therapeutic implications [7,10]. Corresponding to reduced Ig production in IL-21R KO mice and elevated Ig in IL-21 transgenic mice [7], mice constitutively expressing IL-21 have increased plasma cell numbers. In vitro stimulation of naive or memory B cells with IL-21 in conjunction with either BCR or CD40 ligand signals results in the accumulation of Ig-secreting plasma B cells [7,10]. Although IL-21 and IL-4 cooperatively augment Ig-producing cells in vivo [3], these two cytokines have opposing effects on Blimp-1 induction in vitro [10,14] as well as on CSR leading to augmented production of IgE [13*]. IL-4-mediated suppression of IL-21-induced Blimp-1 expression is more potent in naive than memory B cells [14], and memory B cells show augmented response to antigen when IL-21 is present. A distinctive role of IL-21 in B cell biology was underscored in an in vitro CD4⁺ T cell: B cell collaboration model wherein inhibiting IL-21 but not other cytokines greatly diminished plasma cell differentiation and Ig production [15].

IL-21 effects in allergy and asthma

The ability of IL-21 to downregulate IgE production implied that IL-21 might diminish the severity of allergy and asthma. Indeed, in an ovalbumin-induced mouse model of allergic rhinitis, administering IL-21 during the initial antigen challenge significantly reduced allergic

symptoms, with diminished antigen-specific serum IgE and reduced Th2 cytokines (IL-4, IL-5, and IL-13) in the nasal tissue, and decreased IL-4-induced levels of eotaxin-1 and eotaxin-2 in nasal fibroblasts, leading to suppressed eosinophil migration into the nasal tissue [16]. Analogous to the local effects of IL-21 in this allergic rhinitis model, systemic administration of IL-21 blocked antigen-induced anaphylaxis in a mouse food allergy model [13*]. This inhibition was accompanied by the induction of Id2 and corresponding diminished IgE CSR and antigen-specific IgE. The relationship between IL-21-induced prevention of CSR and anaphylaxis was demonstrated by showing that IL-21 could not block anaphylaxis in Id2-deficient mice [13*]. Despite these inhibitory effects of IL-21, in a model of airway inflammation, IL-21R KO mice unexpectedly had less antigen-induced airway hyper-responsiveness than WT littermates, even though they had elevated antigen-specific serum IgE levels [17], suggesting that IgE-mediated mast cell activation is just one component of cytokine involvement in airway inflammation.

IL-21 effects on CD4⁺ T cells

IL-21 is produced in vitro by Th1 [18], Th2 [19], and Th17 [20**,21**,22**] populations of CD4⁺ T cells as well as by NKT cells [23]. Although in vitro differentiation experiments revealed no difference in Th1 vs. Th2 polarization of WT vs. IL-21R KO CD4⁺ T cells [3], IL-21R KO CD4⁺ T cells have a reduced ability to differentiate into Th17 cells [20**,21**]. Infection of IL-21R KO mice with *S. mansoni* [24] or *H. polygyrus* [17] parasites resulted in reduced Th2 responses in vivo, even though the levels of Th1 or Th2 cytokines measured ex vivo were similar. Thus, priming in the absence of IL-21 may alter Th2 cytokine production through unknown mechanisms. Interestingly, when IL-21 is added during naive CD4⁺ T cell priming, it downregulates IFN- γ production without affecting other Th1 cytokines [25] (Figure 2).

Role of IL-21 in Th17 differentiation

Th17 cells play a major role in promoting inflammatory responses in T cell-mediated autoimmune diseases [26]. Although there are some unresolved issues regarding factors influencing human vs murine Th17 differentiation, TGF- β and IL-6 are critical in murine Th17 differentiation, with IL-6 shifting the balance from TGF- β -mediated induction of regulatory T cells (Treg cells) to Th17 cells [27]. IL-21 is induced by IL-6 or by transduction of ROR γ t [20**], a nuclear hormone receptor essential for Th17 differentiation [28]. Interestingly, IL-21 is more highly expressed in Th17 cells than in Th1 or Th2 cells [21**,22**] (Figure 2). Induction of IL-21 by IL-6 and ROR γ t leads to augmented induction of IL-21 itself and amplification of this pathway. Th17 commitment is stabilized by IL-21-induced upregulation of IL-23R [20**]. IL-23 plays a key role in inflammatory disease [29] but its receptor is not present on naive T cells. The up-regulation of IL-23R by IL-21 promotes responsiveness to IL-23, Th17 commitment and expansion in vivo of these cells.

Role of IL-21 in Treg differentiation and function

Because IL-21 in combination with TGF- β can initiate an IL-6-independent pathway to Th17 development, it is not surprising that IL-21 also down-regulates TGF- β -regulated induction of FoxP3⁺ Treg cells [21**,22**]. Enhanced FoxP3 expression was observed in CD4⁺ T cells lacking IL-21, IL-6, or Stat3 following in vitro Th17 differentiation [21**,22**], revealing the negative regulation of Treg development by IL-21 and IL-6. Naive Treg cells express low levels of IL-21R, but TCR stimulation upregulates these levels. In contrast to the ability of IL-2, IL-7, and IL-15 to induce proliferation of Treg cells, IL-21 cannot and inhibits the suppressive function of Treg cells on either CD4⁺ [30] or CD8⁺ [31] T cells, but it is unclear whether this results from actions of IL-21 on Treg or responder cells. These studies suggest that IL-21 can overcome Treg-mediated immunosuppression, either by inhibiting their

development or their function. These findings have implications for the clinical manipulation of IL-21 levels in autoimmune and inflammatory diseases.

IL-21 regulates proliferation and function of CD8⁺ T cells

Although IL-21 is produced by CD4⁺ T cells, most of its proliferative effects are on CD8⁺ T cells. Naïve CD8⁺ T cells express low IL-21R levels [8], and IL-21 does not by itself induce proliferation of this subset [32]. However, IL-21 acts synergistically with IL-15 or IL-7 to induce proliferation of both naïve and memory phenotype CD8⁺ T cells [32]. The proliferative response of these cells to IL-21 in the absence of TCR signals implies a role for IL-21 in innate immune responses. In the presence of self-antigen signals, IL-21 can induce CD8⁺ T-cell expansion, with a cytolytic phenotype characterized by higher avidity TCR interactions and increased surface CD28 [33]. Interestingly, IL-21 can increase perforin expression in CD8⁺ T cells from HIV patients in the absence of either activation or proliferation, suggesting that IL-21 can upregulate CD8⁺ cytolytic activity without affecting endogenous HIV gene expression or virus production [34]. The ability of IL-21 to expand antigen-specific cytotoxic CD8⁺ T cells is limited by Treg cells, as depleting Treg cells augmented IL-21-mediated expansion of tumor-specific CTL [31].

Anti-tumor strategies using IL-21-induced CTL activity

The effects of IL-21 on CD8⁺ T and NK cell differentiation and cytolytic activity suggested its potential utility in the treatment of tumors and indeed IL-21 appears to possess greater anti-tumor activity than other γ_c family cytokines. In a study using syngeneic thymomas, IL-21 treatment resulted in longer survival (> 4 months) than seen with IL-2 or IL-15, accompanied by persistent CD8⁺ memory T cells that responded to secondary tumor challenge [35]. In a study of melanoma and renal tumors, when treatment was administered early after tumor initiation, subcutaneous administration of IL-21 augmented tumor regression and tumor infiltration by CD8⁺ T cells [36], suggesting that IL-21 might influence the homing of CD8⁺ T cells to the tumor.

Importantly, IL-21 can synergize with other agents that alone have suboptimal anti-tumor efficacy. For example, when mice with large pre-established poorly antigenic melanomas received in vitro activated tumor-specific Pmel-1 TCR transgenic CD8⁺ T cells plus peptide vaccine, better tumor regression was seen with the combination of IL-15 plus IL-21 than with individual cytokines [32], consistent with the synergistic effect that IL-15 plus IL-21 have on CD8⁺ T cell proliferation.

In another study, following adoptive transfer of naïve tumor-specific CD8⁺ T cells, IL-21 and suboptimal IL-2 synergized in treating pre-established melanomas [37], yielding long-term survival of approximately half of mice and persistence of memory phenotype tumor-specific CD8⁺ T cells. Although IL-2 and IL-21 had synergistic anti-tumor activity when administered in vivo after the delivery of naïve CD8⁺ T cells, in vitro activation with IL-21 enhanced the ability of cells to mediate tumor regression upon adoptive transfer, whereas IL-2 impaired the anti-tumor efficacy of transferred cells [38*].

Monoclonal antibody therapy directed against the TRAIL/DR5 ligand/receptor that controls apoptosis of some tumors was much more effective in suppressing metastases when subsequent IL-21 treatment was added. This suggests that tumor cell apoptosis can prime CTLs to tumor-specific antigens and that IL-21 enhances this priming and CTL effector function [39].

The successful treatment of tumors by IL-21 in animal models led to human clinical trials (reviewed in [1**]). In Phase I clinical trials in patients with advanced metastatic melanoma [40], IL-21 was well-tolerated, with minimal adverse side effects and efficacy in tumor control

or regression. Future clinical studies with IL-21 will likely incorporate combination therapies that have been effective in animal models.

IL-21 in autoimmune diseases

Early studies suggested that IL-21 might play a role in autoimmunity, with elevated IL-21 being found in two mouse models of systemic lupus erythematosus. In BXSB.B6-*Yaa*⁺ mice, levels of IL-21 correlated with increased serum Ig and development of disease, consistent with the role of IL-21 in plasma cell differentiation [7]. In the Sanroque mutant mouse, there is a defect in Roquin, a protein that negatively regulates follicular T helper cells, a population that produces high levels of IL-21 and regulates the differentiation of follicular B cells [41]. These mice also have high serum levels of IL-21 and develop lupus-like symptoms. The mechanisms leading to elevated IL-21 are unknown but this is believed to lead to increased production of auto-antibodies. One of the genetic loci associated with autoimmune diabetes is the *Idd3* locus that contains the genes encoding both IL-21 and IL-2. Although a study of the NOD diabetic mouse model found elevated levels of IL-21 mRNA in T cells [42], a recent study concluded that the critical *Idd3*-encoded gene that leads to the development of diabetes corresponds to the *Il2* rather than the *Il21* gene [43].

Therapeutic efforts for the treatment of autoimmune responses with agents that block IL-21 have shown partial success. In the lupus-prone MRL-lpr mouse strain, treatment with soluble IL-21R-Fc fusion protein partially reduced disease [44]. Rheumatoid arthritis is an autoimmune disease in which synovial fluid and tissue have enhanced inflammatory responses to IL-21 and elevated IL-21R expression [45]. In two animal models, collagen-induced arthritis and adjuvant-induced arthritis, treatment with an IL-21-blocking agent ameliorated disease and/or reversed established disease and also lowered levels of IL-6 and IL-17 [46].

In experimental allergic encephalitis (EAE), a model for multiple sclerosis, it was shown that IL-21 is integral in the development of Th17 cells. IL-21 and IL-21R KO mice have dramatically reduced Th17 cells [20**,21**,22**] and greatly reduced progression of the EAE disease [21**,22**]. Although IL-21 treatment at the time of disease initiation can exacerbate EAE symptoms [47], in one study, IL-21-blocking agents have surprisingly exacerbated EAE symptoms, augmenting IL-17 and reducing Tregs numbers and activity [48]. The mechanisms underlying these conflicting results are unknown. A future focus is to understand how/when IL-21 is involved in the development of these autoimmune diseases.

Conclusions

The existence of IL-21 and its receptor were first reported in 2000, with the receptor having been identified as an orphan receptor for which the ligand was then cloned [49,50]. In just over seven years, an enormous amount has been learned about the biology of this sixth γ_c family cytokine. Not only does its inactivation explain part of the XSCID phenotype, but a range of studies reveal its critical actions for plasma cell differentiation, Th17 differentiation, and other effects on B, T, NK, and dendritic cells. These studies also underscore that there may be a dangerously fine line between levels of IL-21 that function as an effective anti-tumor agent and those levels that lead to pathogenic effects in autoimmunity. The challenge in the field is to understand how this balance is maintained within the normal immune system as well as properly manipulating it in the treatment of pathogenic states.

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Figure 1. Effects of IL-21 on B cell survival and function

The outcome of IL-21 signaling to the naïve B cell is context dependent. (A) In the absence of a signal to the B cell antigen receptor but in the presence of a pathogen-derived signal to toll-like receptors (TLR), IL-21 induces apoptosis of naïve B cells. (B) In the presence of a B cell antigen receptor signal as well as a T cell-derived signal such as IL-4, IL-21 enhances naïve B cell proliferation, leading to the cooperative regulation of immunoglobulin production mediated by the IL-21 induction of the master switch transcription factor Blimp-1 that leads to plasma cell differentiation. Although IL-21 signals to IgG-expressing B cells are positive, IL-21 induces selective apoptosis of IgE-committed B cells, leading to reduced production of IgE in the presence of IL-21.

Figure 2. Pleiotropic effects of IL-21 on CD4⁺ T cell subsets

When IL-21 is present during priming of naïve CD4⁺ T cells under conditions leading to Th1 commitment (IL-12 plus anti-IL-4), levels of the transcription factor Eomes are down-regulated, leading to reduced production of IFN-γ. In contrast, the presence of IL-21 during priming under conditions leading to Th2 differentiation (IL-4 plus anti-IFN-γ) has no apparent effect on the production of Th2 cytokines such as IL-4. Priming of naïve CD4⁺ T cells with TGF-β plus IL-6 leads to expression of ROR_γt, which initiates Th17 lineage commitment. This leads to the production of high levels of IL-21 which has the further effect of inducing the IL-23R on T cells, thus allowing Th17 cells to respond to IL-23 with further expansion and production of IL-17, IL-21, and IL-22, all of which are involved in the propagation of inflammatory responses.