Interleukin-21 Signaling: Functions in Cancer and Autoimmunity

Ian D. Davis, Kresten Skak, Mark J. Smyth, Paul E. G. Kristjansen, Dennis M. Miller, and Pallavur V. Sivakumar

Abstract

Interleukin-21 (IL-21) is a cytokine with structural and sequence homology to IL-2 and IL-15, yet possesses several biological properties distinct from these cytokines. IL-21 is produced mainly by activated CD4+ T cells and natural killer T cells and mediates its activity by binding to the IL-21 receptor (IL-21R), consisting of an IL-21–specific α chain (IL-21Rα; JAK/STAT) that heterodimerizes with the common γ chain (CD132). Intracellular signaling occurs through the Janus-activated kinase/signal transducer and activator of transcription pathways. Physiologic expression of IL-21R is restricted to lymphoid tissues and peripheral blood mononuclear cells; however, other tissues such as epithelium, synovium, or transformed cells can acquire expression of both components of IL-21R heterodimer. IL-21 has complex activities on a wide variety of cell types, leading to enhancement of adaptive T-cell immunity, antibody production, activation of natural killer cell subtypes, and opposition to suppressive effects mediated by regulatory T cells. Functionally, these activities promote immune responses and point to a physiologic role of IL-21 in autoimmunity and immune enhancement. Therapeutic manipulation of IL-21 activity may allow improved immunotherapy for cancer as well as insights into autoimmune disease. Recently conducted phase 1 trials in metastatic melanoma and renal cell carcinoma have shown that recombinant IL-21 has a favorable safety profile and support its continued investigation as a potential anticancer drug.

IL-21R Signaling Pathways

IL-21R is a heterodimer, consisting of the IL-21–specific IL-21R α-chain (which has no intrinsic signaling ability) plus the common γ-chain (γc; CD132; Fig. 1). IL-21 is thus a member of a family of cytokines defined by the use of γc, which also includes IL-2, IL-4, IL-7, IL-9, and IL-15. Whereas members of the γc family of cytokines share some activities, signaling through IL-21R has several important differences in comparison with other γc family members. Unlike the IL-21R complex, which is a heterodimeric receptor, the receptor for IL-2 is a trimer consisting of γc plus the IL-2R α-chain (CD25) and IL-2R β-chain (CD122); the α and β chains are responsible for high-affinity binding of IL-2 whereas the β and γc chains are responsible for signal transduction (5). IL-15 signaling is unique: expression of the receptor α-chain is widespread; however, only when IL-15 and the IL-15R α-chain are coexpressed can the complex be presented in trans to responding cells bearing the IL-15Rβ and γc complex (6).

IL-21Rα contains six tyrosine residues in the cytoplasmic domain. The specific functions of each residue remain unclear; however, mutation studies have shown that Y510 is sufficient for transduction of a full proliferative signal (7). After binding of IL-21 to the IL-21Rα/γc complex, Janus-activated kinase 1 and Janus-activated kinase 3 undergo autophosphorylation and subsequent phosphorylation of signal transducer and activator of transcription (STAT)-1 and STAT3 and, to a lesser extent, STAT4 and STAT5 (refs. 7–10; Fig. 1). This is different from IL-2 or IL-15, both of which induce strong activation of the STAT5, but not STAT3, pathway. These differences directly influence the differential effects of these cytokines on immune cell subsets. IL-21R signaling leads to up-regulation of SOCS-1.
in CD8+ T cells, which is a likely consequence of STAT1 and STAT3 phosphorylation (11). SOCS-1 is also critical to modulation of the signal because it affects the rate of loss of phosphorylated STAT3 (11). In addition, IL-21R signaling leads to activation of both the mitogen-activated protein kinase and phosphatidylinositol 3-kinase pathways, which are shown to be important for IL-21–mediated cell proliferation (7, 10). IL-21 also reduces expression of Eomesodermin (Eomes), a factor that plays a role in regulating IFN-γ production in CD4+ T cells (12). In B cells and plasma cells, IL-21 signaling induces BIM, BLIMP, and Bcl-6, depending on the costimulatory signal applied, and may be a key factor in survival, differentiation, and apoptosis of these lymphocytes (13, 14). Although signaling pathways in different lymphocyte subsets have been examined, the genes predominantly affected by IL-21 signal transduction remain to be elucidated fully.

Interactions of IL-21 on Various Cell Types

**B cells.** IL-21 has profound effects on B-cell differentiation and antibody production (Fig. 2). These effects depend on the nature of the costimulatory signal involved. Important mechanisms for stimulation of B cells include ligation of surface CD40 (physiologically mediated by CD40 ligand expressed on activated T cells) or signaling via Toll-like receptor (TLR) subtypes (receptors involved in recognition of pathogens). IL-21 augments proliferation induced by CD40 ligation; however, it augments apoptosis and inhibits proliferation driven by TLR-4 or TLR-9 agonists or by stimulation with anti-IgM cell-surface receptor (1, 15, 16). In resting or naive B cells and in B-cell chronic lymphocytic leukemia, IL-21R signaling is associated with a shift toward proapoptotic signals. The proapoptotic effect is mediated both by down-regulation of antiapoptotic mediators such as Bcl-XL and up-regulation of proapoptotic factors including Bim, JunD/activator protein-1, and Bcl-6; activation of caspase-3 and caspase-8; and increased cleavage of Bid, poly(ADP-ribose) polymerase, and p27Kip-1 (17–19). In some respects, this is the opposite of what might be expected with signaling through STAT3 (20).

In combination with activation via CD40, IL-21 induces isotype switching to production of IgG1 and IgG3 (13, 21, 22) and directly inhibits IL-4–induced Cε transcription and IgE...
production in mice (23, 24). In contrast, IL-21 enhances IgE production in human B cells costimulated with IL-4 and CD40 activation (25). Mice deficient in IL-21R show normal B-cell numbers but have enhanced IgE titers and significant defects in antibody responses to antigenic stimulus (21). In vitro, IL-21 promotes differentiation of plasma cells through increased Blimp-1 expression but does not induce somatic hypermutation (13, 14). Taken together, these data indicate that IL-21 plays a key physiologic role in promoting T-cell–mediated humoral immune responses and antibody production and in the maturation of B cells into primary antibody-producing plasma cells. These properties of IL-21 suggest that it could also be a causative factor in multiple autoimmune diseases involving B cells and autoantibody production.

**T cells.** CD8+ ("effector") T-cell responses are not only required for elimination of intracellular viral infections but also play key roles in antitumor immunity and autoimmunity, whereas CD4+ ("helper") T cells are thought to orchestrate different arms of the immune response, including CD8 and B-cell responses, leading to efficient immune and pathologic responses. Signaling via IL-21R can lead to multiple effects on T cells, including cellular proliferation, differentiation, activation, and changes in chemokine and cytokine production and effector function. T cells are activated through recognition of antigen via the T-cell receptor. CD3 and associated molecules are important in this process, and T-cell activation can be mimicked by cross-linking CD3 using antibodies. IL-21 costimulates proliferation of T cells stimulated with anti-CD3 or antigen (1, 26). IL-21 has effects on both CD8+ T cells and CD4+ T cells. IL-21 synergizes with IL-15 in inducing optimal and sustained antigen-specific CD8+ T-cell responses in vitro and in vivo (27). IL-21 prevents IL-15–induced down-modulation of CD28 (a molecule involved in mediating costimulatory signals to T cells) on CD8+ T cells and leads to higher production of IL-2 and IFN-γ after triggering via the T-cell receptor/CD3 complex in the presence of IL-15 (28), suggesting that IL-21 may be important in promoting T-cell activation and memory. In addition, IL-21 promotes proliferation and expansion of antigen-specific T cells in vitro and in vivo (refs. 26, 29; reviewed in ref. 30).

Recently, IL-21 was shown to play a key role in Th helper (Th) cell differentiation. Th cells can be divided into three distinct lineages: Th1 T cells are involved in supporting CD8+ effector T-cell function; Th2 T cells tend to promote antibody responses; and the recently described Th17 cells promote inflammatory responses and play a pivotal role in autoimmunity. The hallmark of Th1 responses is production of IFN-γ whereas Th2 responses are characterized by IL-4 and IL-5 production.
and Th17 responses are characterized by production of IL-17A, IL-17F, and IL-22. IL-21 plays a key role in the differentiation of naïve Th cells into Th17 cells, leading to increased IL-17 production and IL-23R expression (31–34). Both IL-21– and IL-6–driven Th17 differentiation are dependent on STAT3 activation. Interestingly, IL-6 and IL-21 also induce IL-21 expression through STAT3 activation; thus, IL-21 induces transcription of the IL-21 gene in an autocrine feedback loop (33, 34). Other studies have shown that IL-21 stimulates genes linked to Th1 responses, such as T-bet and IFN-γ (35). In contrast, IL-21 acts on naïve Th cell precursors to decrease IFN-γ production in a specific fashion without decreasing other Th1 and Th2 cytokines and reduces responsiveness of developing T cells to IL-12 by means of reduction of STAT4 expression (36). In agreement with these findings, studies in IL-21R–deficient mice have indicated that IL-21 is important for development of Th2 responses but is not essential for Th1 responses (37). It is likely that the end result of IL-21 signaling on Th1/Th2/Th17 differentiation is dependent on the context in which it is found.

**Regulatory T cells.** An important distinction between IL-21 and other γc cytokines, such as IL-2 and IL-15, is their effects on regulatory T cells (Treg). Tregs are a population of CD4+CD25+Foxp3+IL-7Rα+ cells that have profound suppressive effects on T-cell proliferation and function (38). Tregs are involved in constitutive suppression of autoreactive T cells; reduction of Treg numbers and function is associated with various autoimmune disorders including inflammatory bowel disease. Both IL-2R and IL-21R complexes are expressed on Tregs. Recent studies have shown that IL-21 inhibits transforming growth factor–β–driven differentiation of naïve Th cells into Foxp3+ Tregs (32, 33). In contrast to IL-2, IL-21 does not enhance the proliferation of Tregs (39, 40). IL-21 allows CD4+ Th cells to become resistant to the suppressive effects of Tregs without directly reversing the function of CD4+CD25+ Tregs (41). In contrast, IL-2, IL-7, and IL-15 have direct effects on Tregs either by enhancing their proliferation or by reversing their anergic effects (41). These contrasting effects of IL-21 versus IL-2 on Tregs could be due to differences in their ability to induce (IL-2) or not induce (IL-21) phosphorylation of STAT5 because it has been shown that STAT5a/b signaling is essential for the up-regulation of Foxp3, the transcription factor that drives generation of Tregs (42). IL-21 may therefore promote a propensity toward autoimmunity and therefore also to antitumor immunity, and these effects may help differentiate IL-21 responses in cancer patients.

**NK and NKT cells.** IL-21 induces maturation and activation and thereby increases the cytolytic potential of NK cells (43), with induction of various genes involved in activation of innate immune responses. These include cytokines such as IFN-γ, IL-10, T-bet, and granulocyte macrophage colony-stimulating factor (GM-CSF; refs. 35, 43, 44) and gene products involved in interactions with cells of the adaptive immune system, including MyD88, IL-2Rα/CD25, IL-12Rβ2, and IL-18R (10, 35). IL-21 also enhances antibody-dependent cellular cytotoxicity by NK cells, leading to secretion of IFN-γ, tumor necrosis factor-α, IL-8, granulocyte macrophage inflammatory protein-1α, and regulated on activation, normal T-cell expressed and secreted (RANTES), and to an enhanced antitumor effect (45). IL-21 both is produced by and acts on NKT cells, enhancing their proliferation in combination with IL-2 or IL-15, particularly in the context of the NKT-cell–activating CD1d ligand α-galactosylceramide (3). In addition, IL-21 also increases granzyme B expression and cytokine production by NKT cells (key mediators of their effector activity) and may also have autocrine positive feedback effects (3). NKT cells can substitute for CD4+ T cells in providing B-cell help, and this process is mediated by CD40/CD40 ligand (46). A role for IL-21 in this process has recently been shown: NKT-cell–induced apoptosis and inhibition of IgE production in B cells was shown to depend on IL-21 produced by NKT cells (2). Such an interaction may be another link between innate and adaptive immunity, a distinction that is becoming increasingly blurred (47).

**Dendritic cells and monocytes/macrophages.** Dendritic cells play a central role in initiating and maintaining immune responses and are involved in cross talk between T cells, B cells, and NK cells (48). Several different types of dendritic cells have been described and IL-21 has effects on each. In monocyte-derived dendritic cells, IL-21 strongly induces expression of SOCS-1 and SOCS-3 genes, which are known to interfere with TLR-4 signaling, although weak up-regulation of TLR-4 expression itself is also induced (49). IL-21 decreases the lipopolysaccharide-induced production of tumor necrosis factor-α, IL-12, CCL5, and CXCL10 (49). Murine bone marrow–derived dendritic cells generated in the presence of IL-21 exhibited an immature phenotype with high antigen-uptake ability and low expression of CD86 and MHC class II and could not induce antigen-specific T-cell–mediated contact hypersensitivity (50). The opposite effect was seen with dendritic cells cultured in the presence of IL-15. This suggests that IL-21 may be involved in the negative regulation of the functions of myeloid dendritic cells, particularly in relation to interactions with T cells, and points to an immunomodulatory role for IL-21 in a broader sense.

Macrophage function is also dependent on IL-21 signaling. In mice lacking IL-21R or treated with an IL-21R-Fc fusion protein as an IL-21 ligand trap, infection with pathogens such as Schistosoma mansoni and Nippostrongylus brasiliensis was associated with reduced granuloma formation and hepatic fibrosis and reduced expression of Th2 cytokines and other relevant mediators (51). IL-21 was associated with up-regulation of IL-4Ra and IL-13Ra expression (51). These findings suggest a role for IL-21 in mediating chronic inflammatory responses.

**Epithelial cells and other cells.** IL-21R is expressed and IL-21 has biological activity on many other nonimmune cell types, including intestinal epithelium (52), intestinal fibroblasts in the setting of inflammatory bowel disease (53), gastric epithelium in Helicobacter pylori infection (54), rheumatoid synovium (55), and hematopoietic progenitors (56). It is likely that such effects of IL-21 in physiologic immune responses or pathologic autoimmune responses in these tissues are mediated both through immune effector cells as well as directly on the cellular components of the target tissues. However, analysis of IL-21R– or IL-21–deficient mice has shown that basal homeostasis and development of various cell types and tissues are not affected in these mice, suggesting that the IL-21 pathway is not crucial for these aspects (21).
Functional Interactions

The properties of IL-21 as described above suggest several important functional interactions. IL-21 is involved with interactions between B cells, T cells, NK cells, and dendritic cells and may act as a key cytokine linking innate and adaptive immune responses (Fig. 2). IL-21 may be regarded as a CD4+ T-cell–derived helper cytokine that enhances antigen-specific immune responses by driving generation and survival of long-term CD8+ T cells and by differentiating B cells into potent antibody-producing plasma cells. IL-21 also mediates effects on nonimmune cells and plays a role in various inflammatory conditions probably through its ability to drive lineage specification of Th17 cells. IL-21R is expressed on intestinal epithelial cells and is functionally active (52). Expression of IL-21R on intestinal epithelial cells is higher in patients with inflammatory bowel disease than in controls. Increased IL-21 production was also shown in gut T cells isolated from inflammatory bowel disease patients in these studies. Signaling through IL-21R on these cells leads to phosphorylation of downstream intermediaries and increased production of macrophage inflammatory protein-3α (MIP-3α), a chemokine involved in attraction of T cells to sites of inflammation. A neutralizing anti–IL-21 antibody reduced MIP-3α production (52), suggesting that IL-21 may have direct effects on gut epithelial cells in the promotion of inflammatory bowel disease. Other studies showing effects of IL-21 on expression of metalloproteases by gut fibroblasts (53) support the hypothesis that IL-21 mediates these effects, at least in part, by acting on nonimmune cells.

IL-21 seems to have a defined role in the maintenance and possibly initiation of autoimmune and mediates these effects through activation of T and B cells. In animal models of disease, such as mouse models of systemic lupus erythematosus, a disease largely dependent on autoantibody production, increased IL-21 has been shown in serum (13) and depletion of IL-21 improves outcomes (57). IL-21–deficient mice are less prone to the development of experiment autoimmune encephalomyelitis, a murine model of multiple sclerosis (33). In a murine model of rheumatoid arthritis, which is dependent on both T-cell and B-cell functions, IL-21 depletion improved physical and histologic signs and was associated with a reduction in IL-6 and an increase in IFN-γ (58). This is consistent with observations that IL-21 is increased in animals recovering from induced lymphopenia or with constitutive lymphopenia (59), both conditions in which autoimmunity occurs. There is increasing evidence that IL-21 is involved in human autoimmune conditions. T cells derived from peripheral blood or synovial fluid of humans with rheumatoid arthritis secrete higher levels of tumor necrosis factor-α and IFN-γ in response to IL-21, compared with controls (55). In patients with Crohn’s disease, IL-21 enhances Th1 responses and IFN-γ expression (60). However, considering the recent discovery of the function of IL-21 in induction of Th17 cells and the possible inhibition of Tregs, IL-21 may also promote autoimmune disease through its effects on Th17 cells. Therefore, targeting IL-21 may benefit autoimmune conditions driven by both T-cell and B-cell activation and enhanced autoreactive responses.

Considering its potential for immune activation, it is not surprising that IL-21 has antitumor effects. These have been observed in animal models including melanoma, sarcoma, and bladder and renal cell carcinoma (61–64). Through depletion studies, the antitumor activity of IL-21 was shown to depend on NK cells or CD8+ T cells, or both, depending on the tumor model. Tumor rejection requires perforin but not IFN-γ, IL-12, IL-4, or IL-10 (62), and in some models, rejection occurs via an NKG2D-dependent mechanism (65). Other models have shown that antitumor responses are associated with tumor infiltration by CD8+ T cells and with induction of central and effector memory cells (63, 64, 66) that can be sustained over long periods in vivo (29) or, in immunodeficient mice, by activation of NK cells. This disparity may be due to unanticipated variables such as the route of injection or the nature of the vaccine (e.g., in the Renca renal cell carcinoma and B16 melanoma model, s.c. injection of IL-21 was superior to i.p. treatment; ref. 64). Tumor immunity induced by local high concentrations of IL-21 due to gene transfer techniques can lead to regression of distant tumor deposits (63), raising the possibility that intratumoral administration of IL-21 may be an alternative to systemic therapy. The ability of IL-21 to enhance endogenous tumor-specific antibody responses may also be important in models in which the relevant tumor antigen is on the cell surface (67). IL-21 can therefore induce antitumor responses not only by activating T and NK cells but also by facilitating tumor-specific antibody production and enhancing antibody-dependent cellular cytotoxicity.

Clinical/Translational Advances

The unique biology of IL-21 provides many opportunities for its manipulation in different clinical conditions. In the setting of autoimmune disease, manipulations to decrease the effect of IL-21 may lead to improved clinical outcomes, especially in diseases that are driven by enhanced autoantibody responses, like systemic lupus erythematosus, scleroderma, rheumatoid arthritis, or multiple sclerosis. Because Treg numbers and function are integral to the pathogenesis of some, if not all, autoimmune conditions, it will be important to achieve a greater understanding of the interactions of IL-21 with Tregs. Nevertheless, it is possible that neutralization of IL-21 by means of specific antibodies or soluble IL-21R ligand trap constructs may lead to meaningful clinical remissions in some autoimmune diseases, particularly those in which inflammatory cytokines are known to have a key role.

In the setting of vaccination, it may be predicted that concurrent administration of IL-21 might increase the magnitude, affinity, and duration of T-cell responses. Other strategies aimed at improving T-cell priming or recall responses, or decreasing Treg numbers or function, may also be improved in combination with IL-21. The differential effects of the γc cytokines might be used to advantage in other conditions such as HIV infection. Culture of HIV-infected CD8+ T cells with IL-21 leads to an increase in perforin expression of both memory and effector subsets, including HIV-specific T cells, without causing T-cell activation or proliferation or increasing degranulation induced by ligation of the T-cell receptor (68). In contrast, IL-15 increases TCR-mediated degranulation (68). This suggests that IL-21 treatment may improve T-cell function in HIV-infected patients without inducing nonspecific T-cell activation (69).
The preclinical activity of IL-21 in various tumor models points to its obvious application as an anticancer agent. Clinical development for this indication has already commenced and results from two phase 1 trials have been reported using two treatment schedules: daily intravenous dosing for 5 days followed by a 9-day break ("5 + 9") or continuous thrice-weekly dosing ("3/wk"). These two trials involved patients with advanced melanoma or renal cell carcinoma. The objectives of the trials were to determine the safety of IL-21, to define a maximum tolerated dose and optimal treatment regimen, to document the effects of IL-21 on various biologic markers, and to describe any antigenic effects. These trials have shown that IL-21 has mild toxicity and is generally well tolerated. In particular, capillary leak syndrome has not been observed even at the lowest doses tested (1-3 μg/kg), and some patients with melanoma or renal cell carcinoma have had clinical responses (4, 70). A phase 2 trial of IL-21 monotherapy in melanoma has now been completed (clinicaltrials.gov identifier NCT00336986). Other treatment regimens are being tested, including s.c. dosing (Eudract no. 2006-000376-32). The ability of IL-21 to enhance antibody-dependent cellular cytotoxicity preclinically provides the justification for current studies in combination with rituximab in CD20+ B-cell non-Hodgkin's lymphoma (clinicaltrials.gov identifier NCT00347971) or with cetuximab in colorectal cancer (Eudract no. 2006-004231-30). It is likely that IL-21 will enhance responses to active vaccination against cancer antigens; however, no such treatments or studies are currently under investigation. Possible initial candidates for such vaccine combination studies might be in the setting of virus-related cancers, such as human papillomavirus vaccine or hepatitis B surface antigen vaccine. Combinations of IL-21 with cytotoxic chemotherapy or small-molecule targeted therapies could be justified on the basis that chemotherapy-induced cell death may enhance access of tumor antigens to antigen-presenting cells, with subsequent improvement of T-cell responses due to concurrent IL-21 administration. A phase 1/2 trial combining IL-21 with sorafenib in renal cell carcinoma is now recruiting (clinicaltrials.gov identifier NCT00389285). More novel combinations, such as concurrent treatment with IL-21 and agonistic antibodies against tumor necrosis factor–related apoptosis-inducing ligand receptors (71, 72), may also provide further insights into the anticancer mechanisms of IL-21, but presently no such clinical studies are planned.

IL-21 is a complex cytokine with pleiotropic activities. A greater understanding of the network of interactions mediated by IL-21 signaling has revealed a wealth of potential clinical conditions in which manipulation of IL-21–related responses may lead to benefit for patients. It is remarkable that a cytokine with such a plethora of activities has such little toxicity. The results of further clinical trials are awaited with interest.

References

www.aacrjournals.org Clin Cancer Res 2007;13(23) December 1, 2007 6931 

Molecular Pathways


67. Gu XK, Yue FY, Kovacs CM, Ostrowski MA. The role of cytokines which signal through the common γ chain cytokine receptor in the reversal of HIV-specific CD4 and CD8 T cell anergy. PLoS ONE 2007;2:e300.


Interleukin-21 Signaling: Functions in Cancer and Autoimmunity

Ian D. Davis, Kresten Skak, Mark J. Smyth, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/13/23/6926

Cited articles
This article cites 71 articles, 44 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/13/23/6926.full#ref-list-1

Citing articles
This article has been cited by 10 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/13/23/6926.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.