Interleukin-21: Basic Biology and Implications for Cancer and Autoimmunity*

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Abstract

Interleukin-21 (IL-21), a potent immunomodulatory four-α-helical-bundle type I cytokine, is produced by NKT and $CD4^+$ T cells and has pleiotropic effects on both innate and adaptive immune responses. These actions include positive effects such as enhanced proliferation of lymphoid cells, increased cytotoxicity of $CD8^+$ T cells and natural killer (NK) cells, and differentiation of B cells into plasma cells. Conversely, IL-21 also has direct inhibitory effects on the antigen-presenting function of dendritic cells and can be proapoptotic for B cells and NK cells. IL-21 is also produced by Th17 cells and is a critical regulator of Th17 development. The regulatory activity of IL-21 is modulated by the differentiation state of its target cells as well as by other cytokines or costimulatory molecules. IL-21 has potent antitumor activity but is also associated with the development of autoimmune disease. IL-21 transcription is dependent on a calcium signal and NFAT sites, and IL-21 requires Stat3 for its signaling. The key to harnessing the power of IL-21 will depend on better understanding its range of biological actions, its mechanism of action, and the molecular basis of regulation of expression of IL-21 and its receptor.

INTRODUCTION

Immune responses to foreign antigens have evolved to comprise the two interacting arms of cellular and humoral responses: the innate and adaptive immune systems. The cellular components of the innate immune system, including dendritic cells (DCs), natural killer (NK) cells, macrophages, and granulocytes, possess pattern recognition receptors capable of detecting conserved structural components of pathogens whose recognition then initiates immediate responses. One of the key responses of the innate immune system is the production of cytokines that then regulate the antigen-driven differentiation of the adaptive immune system, composed of naive lymphoid B and T cells, which then results in the development of antigen-specific effector responses. Cytokines produced by the innate immune system as well as cytokines produced by activated T cells drive the expansion and effector functions of the adaptive immune response as well as the downregulation of responses once the offending agent has been eradicated. Much attention has been paid over the past several decades to the array of cytokines that are produced by these immune cells and play such an important role in the amplification and control of responses to pathogens. Understanding the mechanisms involved in the production and function of these cytokines is key to predicting and employing clinical strategies for controlling these responses.

One of the most important families of cytokines includes the type I four-α-helical-bundle cytokines, which comprise many of the interleukins and colony-stimulating factors as well as a range of other molecules such as erythropoietin, growth hormone, and prolactin. In one subfamily of this set of cytokines, the receptors share the common cytokine receptor γ chain, γ_c, which is mutated in humans with X-linked severe combined immunodeficiency (XSCID) (1), a disease characterized by the absence of T and NK cells but the presence of nonfunctional B cells. This set of cytokines includes IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21, the most recently discovered member (2, 3) (see **Figure 1**).

The IL-21 receptor was discovered in 2000 as an orphan receptor, first denoted as NILR for novel interleukin receptor and now as IL-21R (4, 5). IL-21 was cloned as the ligand for this novel receptor (5) and was first observed to be produced by $CD4^+$ T cells and to modulate the proliferation and effector function of other lymphoid cells. Subsequently, however, IL-21 was observed to act on multiple nonlymphoid lineages as well and to be produced by innate immune natural killer T (NKT) cells and the more recently identified Th17 lineage. This has expanded our understanding of the broad potential roles for this cytokine in the development and control of immune responses. Moreover, IL-21 has been shown to have strong antitumor action via its effects on both NK and $CD8^+$ T cells and also has been identified as a key component in the development of autoimmune disease. The dissection of these beneficial and pathogenic effects of IL-21 has begun to offer a new appreciation of the complexity of the interaction between the innate and adaptive immune responses.

IL-21 Receptor and Ligand Structure

The IL-21 receptor (IL-21R) was first discovered by genomic and cDNA sequencing projects as an open reading frame that putatively encoded a type I cytokine receptor (4, 5). Its predicted amino acid sequence was most related to the IL-2 receptor β chain, and like IL-2Rβ, IL-21R appeared to be lymphohematopoietic restricted. Moreover, IL-21R was located immediately downstream of IL-4Rα on human chromosome 16 (4). Thus, IL-21R was clearly related to the γ_c family of cytokines. Indeed, when identified, the ligand for this novel type I receptor was most similar to IL-2, IL-4, and IL-15 (5). The functional receptor for IL-21 is IL-21R + γ_c (6, 7).

IL-21R was observed initially to be expressed on T, B, and NK cells (4, 8).

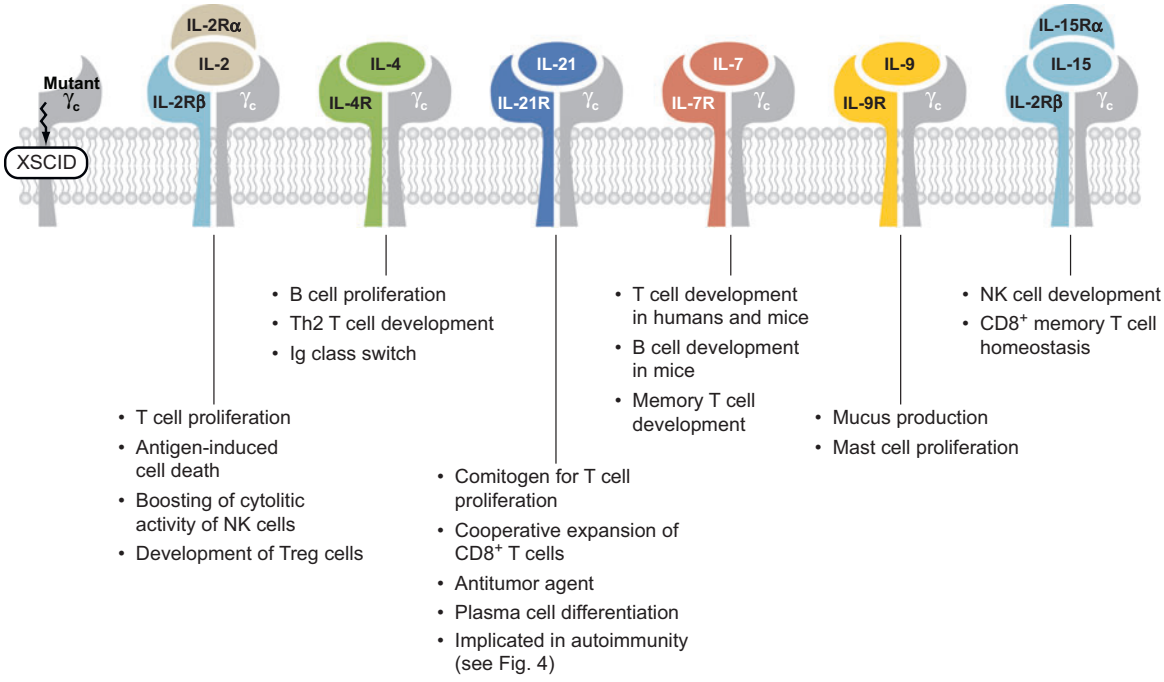

Figure 1

Cytokine receptors containing the common cytokine receptor γ chain (γ_c). The IL-21 receptor is a member of a family of receptors that share γ_c. In addition to γ_c, each of these receptors has one or more distinctive receptor components. Mutations in γ_c result in X-linked severe combined immunodeficiency (XSCID); the severity of this disease results from defective signaling through all these receptors.

Expression on B cells was the highest, even on resting cells, with constitutive expression in a number of cell lines (4, 8). Low-level IL-21R expression on T cells was also observed but was significantly increased following T cell receptor (TCR) stimulation (4, 8). Interestingly, like that of IL-2Rβ, expression of IL-21R was also augmented in cells transformed with HTLV-I (4).

Recently, the three-dimensional structure of human IL-21 has been solved by heteronuclear NMR spectroscopy (9). As anticipated, it is a typical up-up-down-down four-α-helical-bundle cytokine. A segment of the molecule involving helix C that is important for receptor binding is relatively unstable, and stabilization of this region in a human IL-21 analog results in a tenfold increase in biological potency (9).

The Molecular Basis for IL-21 Signaling

Like other type I cytokines, IL-21 signals via the Jak-STAT pathway (see **Figure 2**). Analogous to IL-2, IL-4, IL-7, IL-9, and IL-15, Jak1 and Jak3 are the Janus family tyrosine kinases that are activated by IL-21 (4, 6, 7). IL-21 can activate Stat1, Stat3, and both Stat5a and Stat5b (10, 11). However, the activation of Stat5a and Stat5b is relatively weak and transient, whereas the activation of Stat3 is the most sustained. Stat3 appears to be the most important STAT protein for IL-21 signaling. Indeed, there is defective signaling to IL-21 in T cells that lack expression of Stat3 (11). The IL-21R cytoplasmic domain contains six tyrosine residues. One of these, Tyr 510, is phosphorylated and serves as a critical docking site for both Stat1 and Stat3. On the basis

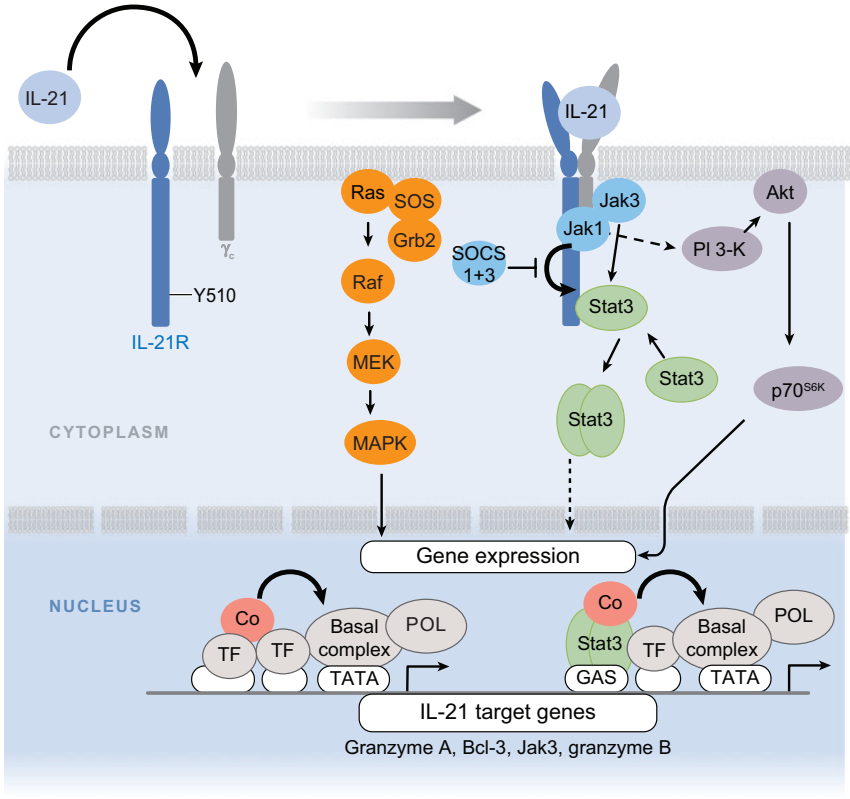

Figure 2

Signaling pathways for the IL-21 receptor (IL-21R). Upon IL-21 binding, Jak1 and Jak3, which interact with IL-21R and γ_c, respectively, are activated and then phosphorylate Stat3 and Stat1 and weakly phosphorylate Stat5 proteins. This leads to STAT dimerization and translocation to the nucleus, with subsequent binding to target gene regulatory elements. A critical tyrosine in the IL-21R cytoplasmic domain (Y510) is primarily responsible for the docking of Stat1 and Stat3. Five other cytoplasmic tyrosines are not shown. Additionally, ligand binding to the IL-21R can lead to activation of the MAP kinase (MAPK) and the PI 3-kinase (PI 3-K) pathways. Target genes activated by IL-21 have been identified, but the involvement of each of these signaling pathways in the regulation of these genes remains to be determined. Co, co-activator; POL, RNA polymerase; TF, transcription factor.

of analysis using the chemical inhibitors wortmannin and PD98059, we can conclude that PI 3-kinase and MAP kinase pathways also contribute to IL-21 signaling. These different signaling pathways may function in distinct phases of lineage development and function.

IL-21 Ligand and Receptor Regulation

IL-21 is produced by $CD4^+$ T cells (5) as well as by NKT cells (13). Although the genes encoding IL-21 and IL-2 are adjacent to each other, the regulation of these genes is significantly distinct. Both cytokines can be induced in $CD4^+$ T cells by signaling via the TCR, but IL-21 mRNA can be induced by a calcium signal alone in preactivated T cells, whereas IL-2 mRNA induction requires both a calcium signal and protein kinase C (14). Nuclear factor of activated T cells (NFAT) binding sites in the IL-21 promoter region contribute to the regulation of IL-21 transcription (14, 15). Interestingly, NFATC2 binds in vivo, but mice lacking NFATC2 still express IL-21, indicating functional redundancy with other NFAT family proteins (14). Mycobacterial antigens

(BCG) upregulate levels of IL-21 in NKT cells in both mouse and human systems (16), revealing that innate immune signals can also induce IL-21 production.

IL-21 receptor expression has been detected on $CD4^+$ T cells, $CD8^+$ T cells, B cells, NK cells, DCs, macrophages, and keratinocytes (4, 5, 8, 17–19), suggesting that IL-21 has a broad range of actions. Although IL-21 is not required for hematopoiesis, as demonstrated by analysis of IL-21R knockout (KO) mice (20), IL-21R mRNA has also been detected on a population of bone marrow progenitors, and IL-21 can expand hematopoietic progenitor cells both in vivo and in vitro (20a). Within the T cell lineage, IL-21R expression is induced as cells differentiate from double negative ($CD4^-CD8^-$) thymocytes to double positive ($CD4^+CD8^+$) thymocytes (8), but this expression may not be absolutely essential for thymocyte differentiation because there is normal thymic development in IL-21R KO mice (20). Low but detectable levels of IL-21R are found on mature $CD4^+$ and $CD8^+$ T cells (8), and these levels are upregulated in response to either TCR or IL-21 (8, 21). TCR-mediated IL-21R expression is regulated in part by the induction and dephosphorylation of the transcription factor Sp1 (21).

Within the B cell lineage, IL-21R is expressed at a low level at the pre–B cell stage of development; this level persists through the first transitional (T1) stage but then increases at the second transitional (T2) stage (22). Mature follicular B cells express higher basal levels of IL-21R than are found on mature T cells (8), and these levels are further increased by signals either through the B cell receptor (BCR) or through CD40 (23). Marginal zone B cells respond to IL-21 but have lower IL-21R expression than do follicular B cells (22). Plasma cells have no detectable surface IL-21R (23), in keeping with their terminally differentiated and nonproliferative state. Interestingly, however, myeloma plasmacytoma cells do express surface IL-21R (24), which may provide a distinct survival advantage for these cells in vivo.

IL-21 AND B CELL FUNCTION

IL-21 Plays a Critical Role in Immunoglobulin Production

The role of IL-21 in B cell function has been investigated in both in vitro studies and in vivo systems employing IL-21R KO and IL-21 transgenic mice. IL-21 is not essential for B cell development; no defects in B cell subsets within bone marrow or periphery have been observed in IL-21R KO mice (20). B cells from IL-21R KO mice normally proliferate in response to lipopolysaccharide (LPS), anti-CD40, or the combination of IL-4 plus anti-IgM (20). The most striking defect in naive IL-21R KO mice is a reduced level of serum IgG1, yet an increased level of IgE (20). Upon immunization with T cell–dependent antigens, IL-21R KO mice have strikingly impaired production of antigen-specific IgG1 and significantly higher levels of antigen-specific IgE (20), an unexpected result given that IgG1 and IgE are usually coordinately regulated. These elevated levels of IgE in IL-21R KO mice were consistent with experiments demonstrating that IL-21 administered to wild-type (WT) mice at the time of immunization can lead to reduced IgE responses, as well as with in vitro experiments showing that IL-21 can reduce levels of germline Cε transcripts, leading to reduced IgE-specific switching (25). Interestingly, in vitro experiments using human peripheral blood B cells revealed that IL-21 can both positively and negatively regulate IgE production, depending on the context. For example, IL-21 in combination with PHA and IL-4 inhibited IgE, whereas IL-21 in combination with anti-CD40 plus IL-4 led to increased IgE levels (26). The precise molecular mechanism(s) by which IL-21 regulates IgE production remains to be fully delineated, particularly because IL-21 can also be proapoptotic for B cells (see below).

IL-4 is required for IgE production, and as expected, IL-4/IL-21R double-knockout (DKO) mice could not produce IgE,

confirming that IL-4 is indeed necessary for the enhanced levels of IgE seen in the IL-21R KO mice. But surprisingly, the DKO mice exhibited a pan-hypogammaglobulinemia, with essentially absent levels of IgG1, IgG2a, IgG2b, and IgG3 and greatly reduced levels of IgM (20). Thus, IL-21 and IL-4 cooperatively regulate immunoglobulin (Ig) production. These observations may also explain the B cell phenotype in humans with XSCID (2, 20). In this severe immunodeficiency, B cells develop normally but are nonfunctional; patients exhibit a severe pan-hypogammaglobulinemia. In the mouse, elimination of γ_c results in a loss not only of T cells but also of B cells, given a critical role in the mouse, but not human, for IL-7 signaling in B cell development (27). By keeping IL-7 signaling intact and thus allowing B cells to develop but by eliminating signaling by IL-4 and IL-21, we apparently have mimicked in mice the human XSCID B cell phenotype (20).

IL-21 Induces B Cell Apoptosis in a Context-Dependent Manner

One of the most puzzling aspects of IL-21 biology is that, in contrast to other members of the γ_c-dependent cytokines, IL-21 can be potently proapoptotic for B cells. Initially, IL-21 was found to augment anti-CD40-induced human B cell proliferation but inhibit proliferation to anti-IgM and IL-4 (5). The inhibition of LPS-induced proliferation by IL-21 results at least in part from a strong proapoptotic signal from IL-21 (8, 28, 29). The degree of IL-21-induced apoptosis is dependent on the context of B cell activation: Apoptosis dominates when B cells are activated with Toll-like receptor (TLR) signals such as LPS or CpG but augments proliferation when B cells are activated with BCR signals (anti-IgM) plus T cell–derived costimulatory signals such as those provided by anti-CD40 (8, 28, 29). The apoptotic signal is caspase dependent because it can be inhibited with caspase inhibitors (28). Although apoptosis can be prevented by the overexpression of the antiapoptotic Bcl-2 protein (28, 29), IL-21 has no effect on Bcl-2 protein levels in B cells (29). Analysis of genes involved in apoptosis revealed that IL-21 increases mRNA and protein levels of the proapoptotic mitochondrial protein Bim and decreases levels of Bcl-xL (8). IL-21-induced apoptosis was eliminated in B cells from Bim KO mice, confirming that Bim-1 plays a role in the IL-21-mediated death of B cells. Other antiapoptotic proteins may be involved in the rescue from IL-21-mediated apoptosis. For example, IL-4 can rescue B cells from LPS + IL-21-induced cell death through upregulation of Bcl-xL, and this rescue is dependent on the presence of Bcl-6 (30). IL-21-mediated induction of apoptosis may in part also account for the inhibitory effects of IL-21 on IgE production. Vaccination with *Mycobacterium bovis* bacillus (BCG) activated Vα14 NKT cells to express high levels of IL-21, which in turn preferentially induced apoptosis of IgE-expressing B cells but not apoptosis of other Ig-isotype-expressing B cells (16). The mechanism for this specific apoptosis seems to involve IL-21-induced formation of a complex between Bcl-2 and the proapoptotic molecule Bcl-2-modifying factor (Bmf), which is specifically expressed in the IgE-expressing population of B cells. Bmf thereby inhibits the usual antiapoptotic activity of Bcl-2, leading to specific apoptosis of IgE-producing B cells and the subsequent loss of IgE production.

The above results indicate that IL-21 can differentially influence the outcome of an antibody response, depending on the costimulatory signals present at the time that B cells encounter antigen (see **Figure 3**). B cells receiving a polyclonal, nonspecific signal such as those mediated by the TLRs would thus potentially produce nonantigen-specific Igs, including autoreactive Igs. Expansion of this potentially deleterious population would be prevented by the presence of IL-21 at the time of encounter. In contrast, a B cell that interacts specifically via its BCR and receives specific

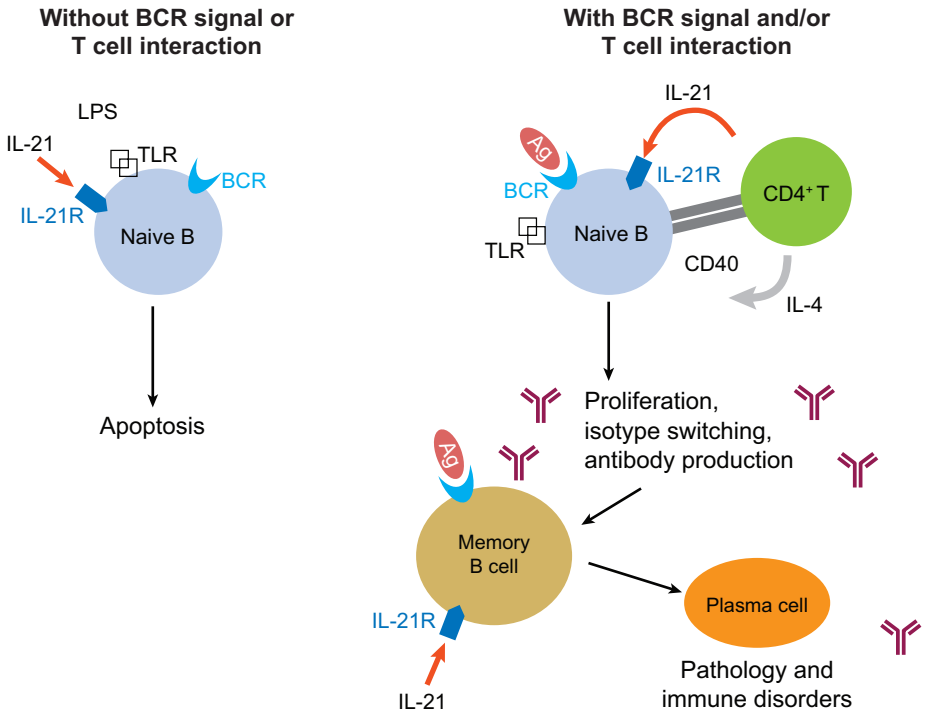

Figure 3

Effects of IL-21 on B cell differentiation and survival. Signaling through the IL-21 receptor (IL-21R) has two potential outcomes for naive B cells. (*Left*) In the absence of a B cell receptor (BCR) or T cell interaction signal or in the presence of a Toll-like receptor (TLR)-dependent signal, IL-21 induces apoptosis of a naive B cell. (*Right*) In the presence of a BCR signal and/or costimulatory interactions with T cells, IL-21 induces proliferation, isotype class switching, and differentiation to either memory B cells or terminally differentiated plasma cells. Memory B cells maintain IL-21R and respond to IL-21 plus antigen signals to differentiate into plasma cells, with subsequent downregulation of IL-21R expression.

T cell help would receive a positive costimulatory signal from IL-21.

IL-21 Drives Terminal Differentiation of B Cells to Plasma Cells

The analysis of IL-21R KO mice had indicated a critical role for IL-21 in Ig production. The role of IL-21 in antibody responses was further investigated through the use of IL-21 transgenic mice as well as through hydrodynamic transfection of mice with an IL-21 expression plasmid (29). In both systems, IL-21-induced apoptosis could be detected by annexin V staining of naive B cells ex vivo. Surprisingly, in both mouse models, there were increased numbers of total splenic B cells rather than the expected decrease resulting from IL-21-induced apoptosis. Most of the increase in B cell numbers resulted from increases in the number of immature B cells and in the number of postswitch B cells and plasma cells. However, there was no change in the number of mature B cells. This was consistent with the increased concentrations of serum IgM and IgG1. In vitro experiments using murine splenic B cells showed that IL-21 in combination with anti-IgM could directly induce the differentiation and accumulation of Syndecan-1$^+$ plasma cells in these cultures (29). The ability of IL-21 to promote differentiation to plasma cells was explained by its potent induction of B lymphocyte–induced

maturation protein-1 (Blimp-1) (29), a transcription factor that acts as a master switch for the program of transcriptional changes involved in terminal differentiation of B cells to plasma cells (31). Surprisingly, in both primary splenic B cells as well as in a B cell line, IL-21 also induced the production of the transcription factor Bcl-6 (29). Blimp-1 and Bcl-6 function as mutually exclusive transcription factors that negatively regulate the expression of each other and correlate with the plasma cell and the memory cell phenotype, respectively (31, 32). It is not yet clear whether both transcription factors are induced by IL-21 within the same individual cells; if so, their coinduction by IL-21 may be limited to a specific stage of B cell development prior to the commitment to either memory cell or plasma cell lineages.

Corresponding to its effects on mouse B cells, IL-21 also plays a major role in stimulating the differentiation of human B cells. Naive cord blood B cells as well as postswitch memory B cells can be driven to differentiate to plasma cells by IL-21 in combination with either BCR and/or CD40 signals (33). IL-21 costimulation of human B cells also induced high levels of Blimp-1 as well as activation-induced cytidine deaminase (AID), but surprisingly this did not induce somatic hypermutation (33). Although IL-21 acted as a switch factor for production of both IgG1 and IgG3 by human peripheral B cells (34), costimulation of naive cord blood B cells with IL-21 plus anti-CD40 induced predominantly the IgG3 isotype (33), suggesting that molecular differences in the responding populations can account for switch preferences.

Interestingly, although IL-21 and IL-4 cooperate in the production of Ig, as seen in the absence of Ig responses in IL-4/IL-21R DKO mice (20), these two cytokines appear antagonistic in their effects in both murine and human B cell differentiation into plasma cells: IL-4 inhibited IL-21-mediated plasma cell differentiation by B cells stimulated with either anti-IgM or anti-IgM plus anti-CD40 but did not inhibit B cells stimulated with anti-CD40 alone (29, 33). The functional interaction between IL-4 and IL-21 is thus complex and dependent on the nature of costimulatory signals as well as the developmental stage of the target B cell.

Although IL-21R levels are higher on naive than on memory B cells (23), IL-21 can also induce rapid plasma cell differentiation in a population of human marginal zone memory B cells (35). This differentiation occurs in response to the combination of IL-21 plus BAFF/BLyS, a TNF family cytokine produced by the local DC population (36). The combination of IL-21 with BAFF leads to the synergistic induction of both Blimp-1 and AID, providing a possible mechanism for rapid upregulation of IgG-secreting plasma cells in an antigen-independent manner (35).

IL-21 EFFECTS ON CD4$^+$ T CELL FUNCTION

IL-21 Is Produced by Multiple T Helper Populations

The functional capacity of CD4$^+$ T cell populations is highly dependent on the cytokines that are available in the environment at the time of TCR priming. Th1 cells arise in response to DC-derived IL-12, produce IFN-γ and TNF-α, and are involved in mediating strong inflammatory responses to intracellular pathogens. IL-4-mediated Th2 cell differentiation results in cells that produce cytokines, including IL-4, IL-5, and IL-13, which mediate antibody responses to extracellular pathogens. Th17 cells, the most recently identified CD4$^+$ T cell subset, differentiate in response to TGF-β and IL-6 signals and produce IL-17, which mediates neutrophil differentiation and infiltration during various infections (37). Each of these CD4$^+$ T cell populations can produce IL-21, although to different extents, and can respond to IL-21 with distinct differentiative responses. Nevertheless, the subset(s) of cells responsible for IL-21 production at specific phases of various in vivo immune responses is not yet fully clear.

Several laboratories originally examined the Th subset expression of IL-21 and obtained ostensibly inconsistent results. One study reported that IL-21 mRNA and protein are produced by Th2-polarized cells but not by Th1-polarized cells (38), but another used a DNA array analysis of expression in human Th1- and Th2-polarized CD4 T cells and showed that IL-21 mRNA was expressed predominantly in Th1 cells (39). Furthermore, IL-21 mRNA expression was highest in a population of follicular Th cells that could augment B cell antibody responses to antigen (39). Recent work has identified Th17 cells as producing significantly higher levels of IL-21 mRNA and protein than either Th1 or Th2 cells do (40, 41). IL-21 itself and IL-6 were identified as essential for the upregulation of IL-21 in this lineage (40–42); Stat3 signaling was essential (41, 42). In the committed Th17 cell, TCR signaling can further upregulate IL-21 production. The functional significance of Th17 expression of IL-21 is further discussed below.

Regulation of Th1 versus Th2 Differentiation In Vitro by IL-21

Although both Th1 and Th2 cells produce IL-21, the effects of IL-21 on differentiation within these subsets are only beginning to be delineated. Analogous to effects of IL-21 on B cell proliferation and function, the end result may depend on other cytokines or immune populations as well as on the developmental stage of the target cell. The initial observation that IL-21 can function as a Th2 cytokine was based in part on in vitro experiments showing that IL-21 could inhibit IFN-γ expression only when IL-21 was present at the time of naive $CD4^+$ T cell priming under Th1 conditions (38). There was not, however, a general downregulation of the Th1 program in that IL-21 had no effect on T-bet or IL-12Rβ2 expression, both of which are induced in Th1 cells even when Th1 cells are primed in the presence of IL-21. The specific decrease of IFN-γ by IL-21 was mediated by the direct repression of Eomesodermin, a T-box transcription factor important for IFN-γ induction (43). Moreover, in populations of human peripheral blood T cells that were preactivated with TCR, IL-21 could induce the expression of a panel of Th1 genes, including those encoding IFN-γ, T-bet, and IL-12Rβ2, suggesting that the effects of IL-21 on already activated cells may be distinct from those on naive T cells (44).

Regulation of Th1 versus Th2 Responses In Vivo by IL-21

The role that IL-21 plays in the in vivo regulation of Th1 versus Th2 polarization has been studied via the use of IL-21R KO mice in a number of immunization and infection models. IL-21R KO mice exhibit normal development of $CD4^+$ T cells both in the thymus and in the periphery (20). Additionally, in vitro stimulation of naive $CD4^+$ T cells under Th1- or Th2-polarized conditions showed no differences in WT versus IL-21R KO levels of IFN-γ or IL-4, suggesting that IL-21 is not essential for the normal differentiation of these two subsets (20). When IL-21R KO mice were examined in a delayed-type hypersensitivity model in which footpad swelling was measured after an antigenic challenge, these KO mice had higher inflammatory responses than did WT mice (38). Ex vivo antigen-specific challenge of $CD4^+$ T cells in these challenged mice revealed higher production of IFN-γ by KO than by WT cells. Another group examined the expression of IL-21 during the time course of infection with *Schistosoma mansoni*, a parasite that induces a Th2-dependent granuloma formation (45). IL-21 levels were measured in strains of mice that developed massive Th1 (IL-4/IL-10 DKO) or Th2 (IL-12/IL-10 DKO) responses to the parasite. Although infection of these mice with schistosome eggs induced highly polarized responses in the lungs, as evaluated by the production of IL-13 and IFN-γ, IL-21 was produced during the infection in both strains of mice, indicating that IL-21 does not

behave as a classical Th1 or Th2 cytokine (45). When IL-21R KO mice were infected with *S. mansoni*, there was not a corresponding increase in Th1 cytokine production, despite a reduced Th2 response (i.e., decreased production of IL-4, IL-10, and IL-13) to the parasite in the granulomatous tissues of the lungs. Consistent with the decreased Th2 response in the IL-21R KO mice, there was more rapid resolution of the lung granulomas in these mice, indicating that IL-21 plays a role in the initiation and maintenance of a granulomatous inflammatory response. In spite of the reduced Th2 responses in the *S. mansoni*–infected mice, there was no difference in the cytokine profile of $CD4^+$ T cells stimulated ex vivo with antigen, suggesting that in vivo IL-21 deficiency may not alter the Th priming of $CD4^+$ T cells per se but rather may lead to a depressed Th2 response through mechanisms that are not yet clear. In a separate study, IL-21R KO mice that were infected with *Heligmosomoides polygyrus* intestinal parasites developed fewer and smaller granulomas and had a reduced eosinophilia in the blood, suggesting a defective Th2 type response (46). However, analysis of ex vivo cytokine production from infected mice revealed no differences in levels of IFN-γ or IL-4 (46).

IL-21 Critically Regulates Th17 Development

IL-17-producing Th17 cells have distinctive developmental and functional properties that differ from those ascribed to Th1 and Th2 $CD4^+$ effector T cells (47, 48). TGF-β, an immunosuppressive cytokine with a role in the generation of T regulatory (Treg) cells (49), also plays a key role in the induction of the Th17 differentiation pathway (50). However, Treg and Th17 cells are induced by TGF-β in a mutually exclusive manner; IL-6 shifts the balance in favor of Th17 cells and decreases the development of Treg cells (51).

An analysis of TCR-stimulated $CD4^+$ T cells revealed that the IL-21 gene was one of the genes most highly induced by IL-6 (42). Indeed, IL-21 mRNA and protein are very highly expressed in Th17 cells, at levels approximately fivefold higher than in Th1- and Th2-polarized cells (40, 41). The induction of IL-21 leads to a further autocrine upregulation of IL-21 (42). Interestingly, IL-21 induced IL-23R expression on these $CD4^+$ T cells (41, 42). IL-23R forms dimers with the IL-12Rβ1 subunit, which is shared by both IL-12R and IL-23R (52). Although IL-23 is an important factor in inflammatory disease in both mice and humans, its receptor is not present on naive T cells, and IL-23 may play a role in the expansion of already differentiated Th17 cells (53). The induction of IL-23R by IL-21 in naive $CD4^+$ T cells is therefore a critical step in the differentiation and possibly in the expansion of Th17 cells in vivo. IL-21 and IL-23 both upregulated expression of the orphan nuclear receptor RORγt, which is essential for Th17 differentiation (54) and which leads to further upregulation of IL-21 (42). IL-17 production was significantly lower in $CD4^+$ T cells from IL-21R KO mice that were induced in vitro with TGF-β and IL-6, demonstrating that the induction of IL-21 by IL-6 leads to an amplification of this differentiation pathway (41, 42). In fact, no IL-23R was induced by TGF-β + IL-6 in $CD4^+$ T cells from IL-21 KO or IL-21R KO mice, indicating a critical role for IL-21 in controlling IL-23R expression (41, 42). The initial induction of IL-21 in Th17 cells therefore is critical for the establishment of an autocrine amplification pathway for maximal IL-17 production.

IL-21 and Treg Induction

IL-21 also plays an indirect role in the regulation of Treg differentiation. Interestingly, IL-6 is critical in the inhibition of Treg differentiation by TGF-β and in the induction of Th17 differentiation (51). Indeed, IL-6 KO mice do not produce Th17 cells but produce a dominant $FoxP3^+$ Treg population (40). However, in mice that are deficient in both IL-6 and $FoxP3^+$ Treg cells, Th17 cells are once again

present, and these cells are produced in response to IL-21 in combination with TGF-β, demonstrating the presence of an IL-6-independent pathway for Th17 production. The negative effects of IL-21 on Treg production are further demonstrated by the presence of a three- to fourfold increased FoxP3$^+$ CD4$^+$ T cell population in IL-21 KO mice (41).

IL-21 Regulates Proliferation and Effector Function of CD8$^+$ T Cells

IL-21 is produced by CD4$^+$ T cells and, as indicated above, influences the development of specific functional subsets within this lineage; however, the CD8$^+$ T cell lineage is perhaps the primary target of the proliferative effects of IL-21. Nevertheless, CD8$^+$ T cell development appears normal in the IL-21R KO mice (20), indicating compensatory redundancy of these proliferative effects. In vitro experiments demonstrate that IL-21 by itself has very little if any effect on naive or memory phenotype CD8$^+$ T cell proliferation and expansion but that it has a profound synergistic effect on proliferation in combination with either IL-7 or IL-15 (55), cytokines previously identified as playing major roles in homeostatic expansion of naive or memory CD8$^+$ T cells (56, 57). This synergistic effect is especially evident when CD8$^+$ T cells are stimulated in the absence of TCR signals (55), suggesting that IL-21 may play a role in antigen-independent expansion of this lineage in vivo. Gene expression analysis by DNA microarrays reveals that subsets of mRNAs are regulated individually by IL-21 or IL-15, but an additional set of mRNAs are cooperatively or distinctively regulated by the combination of IL-21 and IL-15, including granzyme B, which is important in cytolytic function of CD8$^+$ T cells, as well as c-jun, which plays a role in the control of proliferative responses (55). The molecular mechanism of this synergistic transcriptional activity by these two cytokines remains to be determined. It is interesting that IL-21 activates Stat1 and Stat3 whereas IL-15 activates Stat5; however, differential STAT protein activation alone cannot be the full explanation given that the cooperative effect of IL-15 with IL-21 cannot be mimicked by IL-2, even though, like IL-15, IL-2 is an activator of Stat5 proteins.

Despite the ability of IL-21 to synergistically upregulate proliferation in combination with either IL-7 or IL-15, IL-21 has distinct effects on the differentiation of CD8$^+$ T cells. IL-15 treatment of naive CD8$^+$ T cells induces an effector phenotype in CD8$^+$ T cells characterized by reduced CD28 and CD62L surface proteins, but IL-21 acts to prevent the downregulation of these proteins and potentially serves to maintain the important costimulatory function mediated by them (58). Although IL-21 alone can lead to the downregulation of CD44 expression on CD8$^+$ T cells, the combination of IL-21 with IL-15 enhanced the accumulation of CD44^{high} CD8$^+$ T cells (55). With regard to cytokine production by naive CD8$^+$ T cells, IL-21 alone induced no accumulation of IFN-γ-producing cells, and IL-15 alone could induce these cells, but the combination led to a further increase in the number of IFN-γ-producing cells (55).

The effects of IL-21 on antigen-specific CD8$^+$ T cell proliferation and effector function have been examined in several experimental systems. Primary immunization of mice with vaccinia virus expressing HIV gp160 antigen induced significantly lower expansion and cytolytic activity in IL-21R KO mice than in WT mice (55), indicating a role for IL-21 in antigen-specific expansion and functional differentiation of naive CD8$^+$ T cells in vivo. When naive human CD8$^+$ T cells were stimulated in vitro with mature DCs presenting a tumor-associated peptide, there was a greatly augmented proliferation when IL-21 was added, leading to the accumulation of a population of cytotoxic cells characterized by a CD28^{high} surface phenotype and a tenfold higher affinity for antigen and a significantly increased production of IL-2, as compared with cells stimulated in the absence of IL-21 (59). In contrast to the ability of IL-21 to

augment the antigen-independent proliferation of both naive and memory $CD8^+$ T cells, IL-21 augmented proliferative and differentiative effects of antigen-dependent stimulation with naive but not with memory $CD8^+$ T cells. The basis for this difference is not yet known. A study of the effects of IL-21 on $CD8^+$ effector T cells in HIV-infected patients revealed that IL-21 could upregulate perforin production in the absence of cell activation or proliferation, whereas IL-15-mediated upregulation of perforin was less substantial and occurred only in the presence of proliferation (60). This induction of perforin by IL-21 in memory T cells was greater in cells from HIV patients than from normal controls. Thus, the proliferative and functional effects of IL-21 differ for naive and memory $CD8^+$ T cells.

Actions of IL-21 on Natural Killer Cells

NK cell development depends on the function of γ_c cytokines; γ_c KO mice are devoid of mature NK cells (61, 62). IL-21R KO mice have normal numbers of fully functional NK cells (20, 63), indicating that IL-21 is not required for NK cell development, but it has become clear that IL-21 plays a role in NK cell maturation and functional development and that the actions of IL-21 on this lineage are stage specific. The original observation of an effect of IL-21 on NK cells was that IL-21 enhanced in vitro generation of NK cells from bone marrow precursors (5). Although γ_c KO mice lack mature NK cells, bone marrow NK cell precursors ($CD122^+NK1.1^-CD49b^-$) develop even in the absence of γ_c-mediated signals, and a small subset of these precursors expresses IL-21R (64). Increases in IL-21R levels on these precursors depended on IL-15, suggesting that IL-15 may regulate the ability of NK precursors to respond to IL-21 (64). Although IL-21 has not been found to affect NK cell generation, experiments using human cord blood NK cell precursors showed that these cells progressed further along the maturation pathway if IL-21 was added to the combination of IL-15, Flt3, stem cell factor, and IL-7, a set of cytokines required for in vitro generation of NK cells (65). Once committed immature NK cells are generated, IL-21 can also enhance their proliferative response to suboptimal IL-2 or IL-15 concentrations while having no effect on proliferation by itself (66). Interestingly, IL-21 had a biphasic effect on the growth of immature NK cells: Low doses of IL-21 enhance proliferation and high doses inhibit proliferation, even in the presence of IL-2 or IL-15 (66).

IL-21 also has effects on mature NK cells, including effects on both proliferation and survival as well as on NK cell–specific cell surface receptors. IL-21 had a negative effect on the proliferation of NK cells that had been activated by IL-15, either inhibiting proliferation or increasing their apoptosis (67). In spite of the reduced proliferative and survival effects of IL-21 on NK cells, these cells exhibited enhanced cytolytic function, increased IFN-γ production, and conversion to a large granular phenotype, all indicative of enhanced effector function of these cells (63, 67). In addition, IL-21 inclusion in the in vitro–generated NK cell cultures resulted in changes in the expression of several NK cell inhibitory and activating receptors. For example, IL-2 and IL-15 could induce inhibitory Ly49 receptors on mature NK cells, but the inclusion of IL-21 downregulated these receptors (68). The NKG2D receptor was similarly downregulated by IL-21 in cultures of human NK cells (69). This decreased expression was mediated by transcriptional repression of the DAP10 adaptor through which NKG2D signals. Consistent with these changes in NK receptor expression, IL-21 modestly inhibited NK cell lysis of NKG2D-sensitive targets (69). Although IL-21 repressed NKG2D expression on mature peripheral blood NK cells, IL-21 enhanced NKG2D expression on murine bone marrow–derived NK cells, which represent a less mature population of NK cells. This underscores

the stage-specific effects of IL-21 on NK cells (67). Thus, although IL-21 is not required for NK cell development, it influences the proliferation and functional activity of this lineage.

IL-21 Effects on Natural Killer T Cells

NKT cells are a population of T cells expressing a restricted TCR repertoire; they recognize glycolipids presented by CD1d as well as inhibitory and activating NK receptors (70). NKT cells have immunoregulatory activity on other subsets that is related to their secretion of cytokines and their potent cytotoxic activity. Similar to its effect on NK cells, IL-21 can increase the proliferation of NKT cells in response to in vitro stimulation with anti-CD3 but only when combined with either IL-2 or IL-15 (13). IL-21 can also stimulate the in vitro release of increased levels of IL-4 and IL-13 by NKT cells. In addition, IL-21 upregulated effector function in NKT cells through the induction of granzyme B and conversion to a large granular cell morphology similar to what was found for NK cells (13). Interestingly, NKT cells are also potent producers of IL-21 when stimulated in vitro with anti-CD3 or in vivo with α-GalCer, a stimulatory glycolipid specific for NKT cells (13). Levels of IL-21 protein secreted by NKT were significantly higher than those produced by splenic $CD4^+$ T cells in response to anti-CD3 stimulation. The ability of NKT cells to produce large amounts of IL-21 in response to microbial stimuli opens the possibility that these innate immune cells can regulate the initial steps in the formation of an adaptive immune response by B and T cells.

IL-21 Inhibits Dendritic Cell Maturation and Function

DCs are peripheral myeloid cells that have the capacity to recognize microbial components via surface receptors, endocytose these microbes, and then undergo maturation in response to some of these microbial components. Subsequent to this maturation, DCs migrate to lymphoid organs, where they function as antigen-presenting cells for T cells. The initial evidence that IL-21 can affect the proliferation or differentiation of myeloid cells came from the observation that injection of an IL-21-encoding plasmid into WT mice led to increases in the numbers of both $CD11b^+$ and $Gr1^+$ cells in the periphery (71). Although most of the effects of IL-21 on lymphoid cells are stimulatory, involving enhanced proliferation or effector function, effects of IL-21 on DCs are largely inhibitory. DCs can be generated and expanded in vitro by culturing bone marrow precursor cells with GM-CSF. When DC cultures were expanded in this manner in the presence of either IL-21 or IL-15, differences in their phenotype and function were evident (17). Although IL-15-treated DCs behaved as mature DCs and could present antigen in both in vivo and in vitro assays, IL-21-treated DCs maintained an immature phenotype that was characterized by low MHC class II expression accompanied by increased uptake of antigen and low-level expression of CC-chemokine receptor 7 (CCR7) (17). When IL-21-primed DCs were stimulated with LPS, there was no upregulation of MHC class II, CD86, or CD80 costimulatory proteins, in contrast to the upregulation that is seen with IL-15-primed DCs, and the IL-21-primed DCs had inhibitory effects on T cell responses. Even when DCs were treated for only 2 h in vitro with IL-21 plus ovalbumin antigen and then adoptively transferred in vivo, they could inhibit T cell–mediated contact hypersensitivity responses (17).

IL-21 can also exert proinflammatory effects on immune responses through the induction of the neutrophil chemoattractant CXC-chemokine ligand 8 (CXCL8) in macrophages (72). Neutrophils apparently lack IL-21R but can be recruited to sites of inflammation indirectly through IL-21-mediated CXCL8 induction on macrophages. Additionally, the critical role played by IL-21 in the differentiation and expansion of Th17 cells leads to the

production of IL-17 family cytokines that affect neutrophil recruitment and function (40–42). Thus, IL-21 can either dampen immune responses or exacerbate them, depending on the myeloid population that is targeted and the timing of exposure to IL-21 during the course of an immune response.

IL-21 Mediates Potent Antitumor Responses

The ability of IL-21 to regulate both T cell–mediated and NK cell–mediated immune responses suggested possible antitumor effects. Indeed, IL-21 has been examined in a number of in vivo tumor models, and these studies have confirmed the potent effects of IL-21 as an antitumor agent in animal models. The success in these models has led to the use of IL-21 in several phase I clinical trials in advanced-stage melanoma patients. The mechanisms of the antitumor action of IL-21 involve augmented NK cell and $CD8^+$ T cell cytotoxicity.

Systemic expression of IL-21 by plasmid-mediated in vivo delivery led to an inhibition of the growth of large preestablished melanomas and fibrosarcomas (71). These effects were mediated predominantly by NK cells; ablation of this population reduced the antitumor effect, with only minimal effects seen by ablation of the $CD8^+$ T cell population. Significantly, there were no major in vivo toxic effects, even at high doses of IL-21 (71), unlike the severe toxicity observed with similar doses of IL-2 or IFN-α. In another study that used a different approach to achieve high systemic levels of cytokine, IL-21 and IL-23 were constitutively expressed in pancreatic carcinomas, leading to retarded tumor growth in nude mice, an effect that was again predominantly mediated by NK cells (73). IL-21 overexpression in mammary adenocarcinoma cells also led to the prevention of tumor initiation, although in this system there was no role for NK cells, and the tumor prevention was completely dependent on $CD8^+$ T cells (74).

NK cell–mediated killing of tumors after treatment with IL-21 appears to depend on the presence of NKG2D ligands on the tumor target because there was no IL-21-mediated enhancement of rejection of tumors that did not express these ligands and killing could be blocked by antibodies to NKG2D (75). IL-21 could enhance killing of ligand-positive tumors even in Rag2 KO mice, demonstrating that this is an NK cell–mediated event not requiring an adaptive immune response. These experiments suggest that the action of IL-21 on NK cells may be limited to tumors involving the NKG2D recognition system. Although this study found no effects of IL-21 on NK cell expression of this receptor, other studies did find either positive or negative effects of IL-21 on NKG2D expression (67, 69).

Other tumor systems have allowed the delineation of IL-21-induced $CD8^+$ T cell–mediated killing mechanisms. One study compared the antitumor activity of intraperitoneally delivered IL-2, IL-15, and IL-21 with syngeneic E.G7 thymomas. This study found that, although all three cytokines could induce greater survival than PBS, IL-21 was the most potent, and the administration of IL-21 resulted in a doubling of the 50% survival time, with 20–30% of the mice surviving for more than four months after IL-21 was administered (76). When these long-term survivors were rechallenged with thymoma, all the mice survived for more than 100 days, and this survival was dependent on the presence of a persistent $CD8^+$ T cell memory population that was less susceptible to apoptosis than were $CD8^+$ T cells induced by treatment with IL-2 (76).

In another study, mice with large, established melanomas were treated by adoptive transfer of in vitro expanded tumor-specific $CD8^+$ T cells plus peptide vaccine, followed by intraperitoneal administration of either IL-2, IL-15, or IL-21 or the combination of IL-15 plus IL-21 (55). Treatment with either IL-15 or IL-21 led to partial tumor regression, but consistent with the synergistic effects of these two cytokines on $CD8^+$ T cell

proliferation, the combination of IL-15 and IL-21 led to complete regression of a subset of these melanomas and long-term survival of the majority of the treated mice (55).

Although treatment with IL-2 alone was less effective therapy than treatment with either IL-15 or IL-21, IL-21 acted synergistically with low-dose IL-2 after the adoptive transfer of naive tumor-specific $CD8^+$ T cells into mice with preestablished melanomas (77). Nearly half of these treated mice survived for more than 150 days. When these long-term survivors were rechallenged with melanoma, all were protected, indicating the induction of long-term immunity as a result of the combination of IL-2 with IL-21.

Although IL-21 has shown great potential as a cancer chemotherapeutic agent either alone or in combination with other cytokines, it also has potential for being used with other forms of therapy. The TRAIL/DR5 ligand/receptor pair controls apoptosis and has been the target for monoclonal antibody therapy in some tumors (78). Anti-DR5 mAb can inhibit tumor growth in an FcR-dependent fashion mediated by NK cell antibody-dependent cellular cytotoxicity (79). IL-21 can enhance the ability of NK cells to lyse antibody-coated cancer cells (80). After anti-DR5 treatment of tumor-bearing mice, some tumor cells die, and these apoptotic tumor cells prime CTL to respond to tumor-specific antigens. When tumor-bearing mice were treated with anti-DR5, followed by IL-21, there was enhanced suppression of metastasis of small, preestablished tumors as well as an enhanced $CD8^+$ memory T cell response to secondary tumor challenge. In contrast, similar treatment did not eradicate large, established tumors (81).

Another method for enhancing the innate immune response involves the use of the CD1d-reactive glycolipid α-GalCer. In vivo treatment of mice with α-GalCer potently activates NKT cells that then stimulate the activation and proliferation of other lymphoid populations (82). The combination of α-GalCer treatment and IL-21 administration resulted in synergistically enhanced prevention of tumor metastasis (83). Transfer of DCs pulsed with α-GalCer, followed by IL-21, reduced already established metastatic tumors (83). Overall, these results suggest that the ability of IL-21 to enhance NK, NKT, and $CD8^+$ T cell function can potentially be used in combination with numerous chemotherapeutic protocols to lead to further advances in the eradication of tumors.

These preclinical studies have shown that IL-21 has significant antitumor activity against a variety of tumors that is mediated by multiple mechanisms involving both the innate and adaptive immune systems. IL-21 has entered human clinical trials, and phase I results in patients with metastatic melanoma have been reported (84). Consistent with the animal models, IL-21 was well tolerated, and there were few adverse effects, unlike the capillary leak syndrome or neurotoxicity resulting from IL-2 and IFN-α therapy. IL-21 potently upregulated perforin and granzyme B mRNA in patients, at all except the lowest dose tested. One patient in the phase I trial achieved complete remission, and 9 of 29 had stable disease at the end of the study.

Role of IL-21 in Autoimmune Disease

In light of the pleiotropic effects of IL-21 on the function of different components of the innate and adaptive immune systems, it was difficult to predict the role that IL-21 would play in the various autoimmune diseases. The initial observation that suggested that IL-21 might play a role in the progression of B cell–mediated autoimmune disease was that, in the BXSB.B6-*Yaa*$^+$ mouse model of systemic lupus erythematosus (SLE), the development of disease correlated with an increased serum expression of IL-21 (29). This was consistent with the increased serum Igs in these mice and the role for IL-21 in plasma cell differentiation.

Another autoimmune mouse strain, the *sanroque* mutant, has a defect in the function

of a protein, Roquin, which is a negative regulator of the production of a unique population of follicular T helper (TFH) cells (85). These TFH cells produce high levels of IL-21, and *sanroque* mutant mice have increased levels of these TFH cells as well as increased production of IL-21 accompanied by augmented levels of antinuclear antibodies, glomerulonephritis, and peripheral lymphadenopathy (85). The hypothesis is that the increased levels of IL-21 lead to increased formation of high-affinity autoreactive antibodies by follicular B cells.

That these autoimmune phenotypes were accompanied by high levels of IL-21 suggested that blocking the IL-21 signal might ameliorate autoimmune symptoms. Such a study was performed in the lupus-prone MRL-Fas/*lpr* mouse model through the use of IL-21R-Fc fusion proteins as blocking agents (86). Analogous to the *Yaa* mice results, *lpr* $CD4^+$ T cells produced higher levels of IL-21.

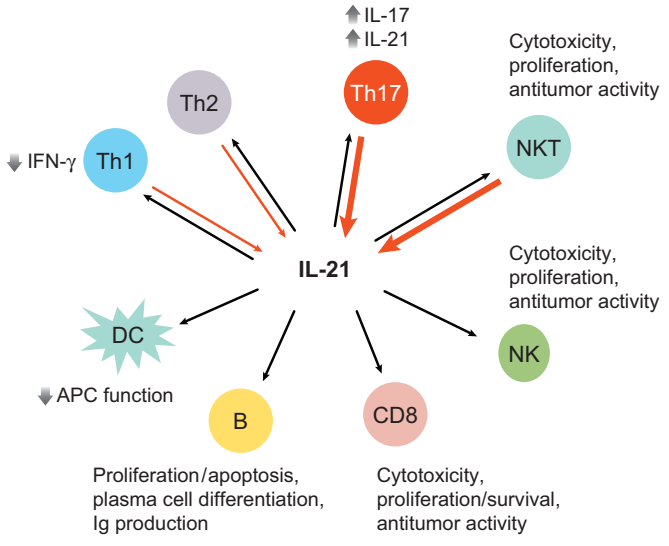

Figure 4

IL-21 has pleiotropic effects on multiple target cells. IL-21 is produced by multiple subpopulations of $CD4^+$ T cells and by natural killer T (NKT) cells (indicated by the *red arrows*), although the amounts secreted by T helper (Th) 17 and NKT cells are significantly higher than those secreted by Th1 and Th2 cells. IL-21 can then function as an autocrine factor for these populations, with the indicated effects, or can then exert varied positive or negative effects on lineages that do not themselves produce IL-21.

Treatment of these mice with the IL-21R-blocking agent led to a partial reduction of lymphadenopathy, morphological changes in kidney glomeruli, and slightly reduced levels of IgG1 and IgG2a. In a mouse model of collagen-induced arthritis, the IL-21R-blocking agent also slightly reduced inflammation (87), suggesting that interruption of the IL-21 signaling pathway may be beneficial in several autoimmune diseases.

One of the genetic loci that are associated with the autoimmune diabetic phenotype in the nonobese diabetic mouse (NOD) is the insulin-dependent diabetes susceptibility 3 locus (Idd3). This locus contains the genes encoding both IL-21 and IL-2 (88). Because these cytokines are known to play roles in the proliferation and function of $CD8^+$ T cells and Treg cells, attempts have been made to identify mutations within this region that associate with diabetes prevalence in the population. One study in the NOD mouse found increased levels of IL-21 mRNA in T cells and suggested that high levels of IL-21 protein may promote homeostatic proliferation of the autoreactive $CD8^+$ T cells that mediate destruction of the pancreatic islet β cells (89). However, a recent study has ruled out the possibility that IL-21 is the genetic determinant of *Idd3* that predisposes one to the development of diabetes (90).

IL-21 also has disease-promoting effects in experimental allergic encephalitis (EAE) (91), an experimental model of human multiple sclerosis, which is induced by immunization of mice with myelin antigen in the presence of adjuvants. When IL-21 is administered to mice before induction of disease, there is increased severity of disease characterized by increased numbers of inflammatory cells in the central nervous system. However, if IL-21 is administered after disease has been initiated, there is no effect on the disease severity. The ability of IL-21 to exacerbate disease is totally dependent on the presence of NK cells because depletion of these cells before disease induction abrogates the effect of IL-21 (91). Although these effects were

attributed to the ability of IL-21 to activate NK cell–mediated inflammatory responses, recent studies have pointed to the role that IL-21 plays in the induction and expansion of the Th17 population in this EAE model (40, 41). IL-21 or IL-21R KO mice have a tenfold reduction in the number of IL-17-producing cells and greatly reduced EAE disease progression (40, 41), as do mice deficient in IL-17 (92). Interestingly, IL-21 KO mice have increased numbers of Treg cells (41). The enhanced autoimmune symptoms in the mice injected with IL-21 before the initiation of EAE may be the result of increased numbers of Th17 cells and reduced numbers of Treg cells (40).

CONCLUDING REMARKS

Since the discovery of IL-21 and IL-21R in 2000, this γ_c family cytokine system has been demonstrated to have effects on an extremely broad set of target cells, including T cells, B cells, NK cells, NKT cells, and DCs (see **Figure 4**). The actions of IL-21 on each of these target cells can be either stimulatory or suppressive, and the ultimate outcome depends on the manner by which the IL-21 signal is integrated with other signals received by a target cell. Although IL-21 was initially thought to be produced solely by antigen-stimulated $CD4^+$ T cells, the discovery that IL-21 is produced by NKT cells implies that it is a key player in early innate immune responses as well. The recent discovery that IL-21 is produced by and plays a major role in the differentiation of the Th17 lineage has expanded our understanding of the ways that IL-21 may contribute to inflammatory responses. Moreover, studies of cancer and autoimmune models suggest that administering IL-21 or blocking the action of IL-21 holds promise in a number of disease settings. IL-21 is thus an exciting cytokine with pleiotropic actions on multiple lineages whose modulation has clear therapeutic benefits in animal models. Understanding how and when it exerts various effects in vivo are some of the major basic science challenges, with the goal that future studies will both advance our scientific knowledge and contribute to moving IL-21 into the therapeutic setting.

FUTURE ISSUES

1. To be able to specifically amplify or neutralize the effects of IL-21 in pathological situations, investigators will require an understanding of the stage-specific and context-specific signaling events involved in the response to IL-21.
2. The in vivo sites where IL-21-producing cells are found are not yet defined. This awaits the construction of reporter mice, with the hope that mRNA expression will correlate with protein expression. The availability of these mice will allow an understanding of the physiological expression of IL-21 as well as mechanisms for controlling levels of IL-21.
3. An in-depth understanding of the molecular basis of IL-21 and IL-21R gene expression will be important to better understand and develop ways of controlling the expression of this cytokine and its receptor. Analogously, a more detailed understanding of IL-21-induced signaling pathways is also important.
4. A major clinical issue will be balancing the immunostimulatory effects of IL-21 on lymphoid lineages with the largely immunosuppressive actions on DCs and the apoptotic effects on inappropriately stimulated B cells.

5. The effects of IL-21 on $CD8^+$ T cell phenotype and function suggest that IL-21 will have an impact on immunological memory, with potential ramifications for vaccination strategies.

DISCLOSURE STATEMENT

The authors have issued patents and/or patent applications related to IL-21.

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