

Molecular mechanisms in allergy and clinical immunology

Series editors: William T. Shearer, MD, PhD, Lanny J. Rosenwasser, MD, and Bruce S. Bochner, MD

IL-21: A novel IL-2–family lymphokine that modulates B, T, and natural killer cell responses

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IL-21 is a recently described type I cytokine produced by activated CD4⁺ T cells that profoundly affects the growth, survival, and functional activation of B, T, and natural killer lymphocytes in concert with other cytokines or activating stimuli. Structurally, IL-21 is predicted to display a 4-helix–bundle–type fold with significant homology to IL-2, IL-4, and IL-15 and mediates its biologic effects through a novel type I cytokine receptor, IL-21R, in conjunction with the common cytokine receptor γ chain (γ c) of the IL-2, IL-4, IL-7, IL-9, and IL-15 receptors. As a new member of the γ c-dependent cytokine family, there is significant interest in IL-21, in part because of its potential to provide new insights into the immunologic phenotype caused by γ c deficiency. IL-21R knockout mice have been generated that have normal lymphoid cell development yet exhibit impaired production of the immunoglobulin IgG₁ and increased IgE responses after immunization. As expected for cytokines that use γ c, recent studies indicate that IL-21 induces Janus kinase 1 (JAK1) and JAK3 activation to initiate signal transduction, but unlike these other γ c-dependent cytokines, which predominantly activate signal transducer and activator of transcription 5 (STAT5), IL-21 preferentially activates STAT1 and STAT3. IL-21 potently enhances primary antigen responses and the effector functions of T and natural killer cells and stimulates IFN- γ production alone or in concert with other cytokines. Thus, on the basis of primary structure, receptor composition, and biologic activities, IL-21 is a new IL-2–family cytokine that participates in both innate and adaptive immunity and might be important for the development of a T_H1 immune response. (*J Allergy Clin Immunol* 2003;112:1033–45.)

Key words: Interleukins, cytotoxicity, (X-linked severe combined) immunodeficiency, inflammation, interferon- γ , lymphoma, apoptosis, proliferation

Abbreviations used

CRM: Cytokine-recognition module
CTL: Cytotoxic T lymphocyte
ERK: Extracellular signal-related kinase
 γ c: Common cytokine receptor γ chain
JAK: Janus kinase
MAPK: Mitogen-activated protein kinase
NK cell: Natural killer cell
SCID: Severe combined immunodeficiency
SH2: Src homology-2
STAT: Signal transducer and activator of transcription
XSCID: X-linked severe combined immunodeficiency

Cytokines are critical regulators of both the development and functional activation of hematopoietic cells, serving as an important communication network within the immune system. Collectively, most of the cytokines that influence the survival, proliferation, differentiation, or homeostasis of lymphoid cells are members of a structurally and functionally related family referred to as the type 1 cytokines.¹ A subset of structurally related soluble proteins within this diverse group, the 4-helix–bundle cytokines, includes most of the ILs that mediate a broad range of biologic functions in a variety of lymphoid and nonlymphoid cell types.² Functionally, these proteins are further related by their utilization of a highly homologous class of cell-surface receptors. This class of single-pass transmembrane molecules is characterized by 1 or 2 extracellular cytokine-recognition modules (CRMs) composed of approximately 200 amino acids, 2 pairs of positionally conserved cysteine residues, and a Trp-Ser-X-Trp-Ser (WSXWS) motif just proximal to the transmembrane domain.¹ Unlike several other cytokine receptors that bear an intracellular tyrosine kinase motif (eg, c-kit receptor, macrophage-colony stimulating factor receptor, c-fms receptor), the type I cytokine receptors instead contain Box 1 and Box 2 motifs to which cytoplasmic tyrosine kinases of the Janus kinase (JAK) family bind and initiate signaling.³ Type II cytokine

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Received for publication July 23, 2003; revised August 27, 2003; accepted for publication August 28, 2003.

Disclosure of potential conflict of interest: T. Habib—none disclosed. A. Nelson—none disclosed. K. Kaushansky—none disclosed.

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0091-6749/2003 \$30.00 + 0
doi:10.1016/j.jaci.2003.08.039

receptors are distinguished by a different pattern of the conserved cysteine residues in the CRM that are thought to form disulfide bonds and bind to the IFNs and IL-10 to modulate primarily antiviral and inflammatory immune response.^{4,5} Distinct paradigms have emerged for ligand-receptor signaling complexes in each receptor class in part on the basis of the solution of their crystal structures.⁶⁻⁸ These findings, together with extensive studies to date on a variety of type I and type II cytokines, have led to the fundamental concept that pleiotropy and redundancy are properties of cytokine function that underlie their critical role as immunomodulators. Thus, a given biologic function is often mediated by multiple cytokines, and each cytokine can elicit multiple functions.¹

Since its discovery as a T-cell growth factor,⁹ IL-2 and its receptor have provided a paradigm for our understanding of type I cytokine signaling. The complete IL-2 receptor (IL-2R) is a heterotrimeric complex comprised of α , β , and γ subunits, although a heterodimeric IL-2R $\beta\gamma$ receptor can also signal in the presence of high concentrations of IL-2 (dissociation constant, ~ 1 nmol/L).¹⁰ Whereas IL-2R β and IL-2R γ are members of the type I cytokine receptor family, IL-2R α displays little homology with the cytokine receptor superfamily but does serve to increase ligand-binding affinity of the IL-2R $\beta\gamma$ dimer by nearly 2 orders of magnitude.¹⁰ IL-2R $\beta\gamma$ is also a signaling subunit of the IL-15 receptor complex, which contains its own distinctive α chain as a high affinity converter.¹¹ Originally cloned as the third component of the IL-2R, IL-2R γ achieved progressive notoriety as subsequent studies demonstrated that this subunit is also a functional component of the receptors for IL-4, IL-7, IL-9, and IL-15.^{12,13} As a result of its involvement in these receptor complexes, IL-2R γ is currently referred to as the common cytokine receptor γ chain (γ_c).¹⁴ Sharing of γ_c by multiple receptors, combined with the discovery by Noguchi et al¹⁵ that mutations in the γ_c gene result in X-linked severe combined immunodeficiency (XSCID) and by Schorle et al¹⁶ that genetic elimination of IL-2 alone fails to result in such immune deficits in mice had profound implications for the role of γ_c in the immune system beyond IL-2R signaling. Targeted disruption of a number of γ_c -dependent cytokines, or their unique receptor subunits, has shown that although γ_c is shared, it mediates distinct developmental and homeostatic functions in lymphocytes when engaged by individual cytokines.¹⁷ Accordingly, IL-7 and IL-7R α/γ_c are essential for thymopoiesis and B-cell development, IL-15-derived signals via IL-15R α , IL-2/15R β , and γ_c are required during natural killer (NK) cell ontogeny, and IL-2/IL-2R $\alpha\beta\gamma_c$, and IL-4/IL-4R α/γ_c regulate the peripheral T- and B-cell compartments.^{11,13,17} Collectively, these findings necessitated further division of the cytokine superfamily into a subgroup on the basis of the shared use of γ_c , referred to as the IL-2 cytokine family, and other subfamilies on the basis of the shared usage of β_c (GM-CSF, IL-3, IL-5) or gp130 (IL-6, IL-11, leukemia inhibitory factor, oncostatin M, and cardiotrophin-1).¹⁴

DISCOVERY AND SEQUENCE CHARACTERIZATION OF THE IL-21 RECEPTOR

As noted above, members of the class I cytokine receptor family share highly conserved structural motifs in their extracellular domain, a finding that facilitates the identification of novel family members on the basis of primary amino acid sequence homologies. With these search criteria, Parrish-Novak et al¹⁸ mined an expressed sequence tag database and identified a sequence with many of the hallmarks of a type I cytokine receptor subunit, leading to the cloning of a novel cDNA encoding a 538 amino acid type I cytokine receptor. Independently, Ozaki et al¹⁹ identified an open reading frame on the genomic sequence of a bacterial artificial chromosome clone from human chromosome 16p11, which predicted a transmembrane protein of identical amino acid sequence to the type I receptor cloned by Parrish-Novak et al. This cDNA, later designated IL-21R, contains 1 copy of the conserved WSXWS motif and 2 paired cysteine residues in the extracellular domain and a large intracellular domain containing the conserved Box 1 and Box 2 elements involved in signal transduction.^{18,19} Overall, the IL-21R protein has highest amino acid sequence homology to IL-2R β (29% identity, 46% similarity) and in domain organization is most similar to IL-4R α , erythropoietin receptor, and growth hormone receptor. Like these receptors, it has a single copy of the CRM. With respect to its genomic organization, the IL-21R gene spans approximately 20 kb of genomic DNA on human chromosome 16p11 and contains 9 coding exons.^{18,20} Two 5' noncoding exons of IL-21R have also been identified in a second overlapping bacterial artificial chromosome clone mapped to 16p11, with the first noncoding exon situated just 39 kb from the IL-4R α gene.^{18,19} Thus, the very close spacing between IL-21R and IL-4R α strongly suggests that these genes arose by a gene duplication event.

The discovery of human IL-21R facilitated subsequent cloning of the full-length murine IL-21R cDNA, which encodes a 529 amino acid protein with 62% identity to human IL-21R.^{18,19} Northern blot or RT-PCR analyses showed IL-21R transcripts primarily in lymphoid tissues (spleen, thymus, lymph node, peripheral blood lymphocytes)^{18,19} and in bone marrow.¹⁹ Characterization of specific cell types expressing IL-21R showed surface expression on resting peripheral human B cells, activated CD4⁺ (T_H) and CD8⁺ (cytotoxic) T cells, and in a number of murine and human cell lines of T, B, and NK cell origin.¹⁸⁻²⁰ Flow cytometric and Northern analyses demonstrated IL-21R expression in numerous lymphoma cell lines including IM-9 (B cell), NK-92 (NK cell), and Jurkat (T cell).^{18,20,21} Notably, IL-21R expression levels were found to be regulated in an activation-specific fashion. In human peripheral blood mononuclear cells, IL-21R mRNA levels increased within 1 hour after stimulation of the T-cell antigen receptor with phytohemag-

glutinin and remained high after 24 hours of activation.¹⁹ Moreover, IL-21R message was upregulated during activation of purified peripheral blood CD3⁺ T cells and CD19⁺ tonsillar B cells with phorbol 12-myristate 13-acetate and ionomycin, conditions that closely mimic an antigen encounter.²⁰ Taken together, these findings showed that IL-21R has a lymphoid-restricted expression pattern and suggested an immunomodulatory role for the corresponding ligand.

FUNCTIONAL CLONING AND CHARACTERIZATION OF IL-21

By introducing a chimeric receptor containing the extracellular and transmembrane domains of c-Mpl, the receptor for thrombopoietin, and the intracellular domain of IL-21R into the IL-3-dependent pro-B lymphoid cell line BaF3 (which is not normally responsive to thrombopoietin), the signaling capacity of IL-21R was confirmed; like the full-length c-Mpl receptor,²² the chimeric Mpl-IL-21 receptor transduced a proliferative signal in response to thrombopoietin in engineered BaF3 cells.¹⁸ This finding suggested that a ligand for IL-21R might be able to induce proliferation of BaF3 cells expressing the full-length IL-21R, thus providing a functional assay for ligand cloning. After testing conditioned media from more than 100 primary and immortalized cell lines in this assay system, only human peripheral blood CD3⁺ T cells activated with phorbol 12-myristate 13-acetate and ionomycin were found to stimulate proliferation of BaF3 cells engineered to express IL-21R.¹⁸ Construction of a cDNA library from this source enabled functional screening of progressively smaller pools of cDNA, ultimately yielding cDNA clones encoding a novel polypeptide, designated IL-21. Because the frequency of the IL-21 cDNA in the library was found to be quite low,²⁰ this was indicative of a highly regulated message in activated T cells.

The mature IL-21 polypeptide has a predicted molecular mass of 15 kd and is predicted to fold into a 4-helix-bundle type I cytokine with significant structural homology to IL-2 and IL-15.¹⁸ IL-15 and IL-21 also share a relatively high degree of amino acid homology, including 2 pairs of spatially conserved cysteine residues. Given that 1 pair is conserved in IL-2, IL-4, and GM-CSF, whereas the other pair is unique to IL-15 and IL-21, these similarities indicate that IL-15 is the closest structural relative of IL-21.¹⁸ In this respect it is not surprising that the human IL-21 gene was mapped to chromosome 4 (4q26-q27) approximately 180 kb from the IL-2 gene, with the IL-15 gene positioned distal to IL-2 and IL-21 within this cluster,¹⁸ strongly suggesting that the 3 cytokines arose by gene duplication.

All of the evidence to date indicates that IL-21 is expressed exclusively by activated CD4⁺ T_H cells.^{18,20} Real-time PCR studies have definitively established that conditions that induce high levels of IL-21 message are those that best mimic T-cell activation, namely stimulation of T_H cells with the combination of anti-

CD3 and anti-CD28 antibodies.²⁰ These observations evoke similarities between the expression pattern of IL-21 and that of IL-2, which is also produced primarily by activated CD4⁺ T cells.¹⁰ In contrast, IL-15 is subject to extensive post-transcriptional control and is produced by multiple nonlymphoid cell types under various stimulatory conditions.^{11,23} Thus, IL-21 was initially predicted to play a co-stimulatory role in T-cell-mediated effector functions, both in an autocrine and paracrine fashion.

THE BIOLOGIC ACTIVITIES OF IL-21

T lymphocytes

Although the physiologic role of IL-21 is not yet fully understood, a number of important insights have come from several *in vitro* studies. The findings of Parrish-Novak et al¹⁸ and Kasaian et al²⁴ have borne out the prediction that IL-21 has a co-stimulatory effect on activated murine and human T cells. IL-21 enhances the proliferation of both anti-CD3-activated thymocytes and mature peripheral T cells^{18,24} and acts in concert with IL-2, IL-15, and, to a lesser extent, IL-7 to enhance peripheral T-cell expansion with or without anti-CD3 stimulation.¹⁸ More specifically, the proliferative response of human T cells to IL-21 appears to be limited to the naïve (CD3⁺CD45RA⁺) population, as opposed to the memory (CD3⁺CD45RO⁺) subset.¹⁸ This observation has been corroborated in a recent report that demonstrated that a cytomegalovirus-specific, CD8⁺CD45RA⁺ effector T-cell population can be induced to expand up to at least 6 generations when stimulated by cognate peptide in combination with the T_H-cell-derived cytokines IL-2 or IL-21.²⁵ However, unlike its cousins IL-2 and IL-15, IL-21 alone has no significant proliferative effect on T cells in the absence of anti-CD3 or other co-stimuli.¹⁸

Evidence is also accumulating that demonstrates the involvement of IL-21 in primary effector T-cell differentiation. Kasaian et al²⁴ recently reported that IL-21 dramatically enhances murine T-cell responses to alloantigen in a mixed lymphocyte reaction system. In accord with its effects on anti-CD3 co-stimulation,^{18,24} IL-21 serves as a potent accessory signal to enhance allogeneic-specific proliferation of freshly isolated lymph node T cells.²⁴ Secondary effector responses after allogeneic stimulation, including cytolytic activity and IFN- γ production, were also enhanced by IL-21 acting alone or in concert with IL-2 or IL-15.²⁴ A separate study has demonstrated that IL-21, like IL-15, stimulates IFN- γ gene expression in activated human T cells and enhances the transcription of numerous other genes involved in innate immunity and the T_H1 immune response, including IL-12R β 2, IL-18 receptor components, and the newly described transcription factor T-bet.²⁶ Macrophage-derived IL-12 and IL-18 are well-established regulators of IFN- γ production in NK and T cells.²⁷ Acting alone, these T_H1 cytokines induce low levels of IFN- γ , yet IL-12 together with IL-18 synergistically enhances IFN- γ

production from both NK and T cells.^{27,28} In vitro T-cell culture systems have recently demonstrated a synergistic effect of IL-15 plus IL-18, IL-15 plus IL-21, or IL-18 plus IL-21 on IFN- γ mRNA synthesis and production.²⁸ Collectively, these data implicate IL-21 as a potent inducer of T-cell-mediated effector functions and establish an important role for this lymphokine in the development of a T_H1 response.

Interestingly, in the context of T-cell receptor-independent stimulation, IL-21 exerts an antagonistic effect on IL-15-mediated expansion of memory-phenotype CD44^{hi}CD8⁺ T cells.²⁴ Moreover, IL-21 inhibits the IL-15-induced upregulation of receptor components for IL-2, IL-15, and IFN- γ in memory T cells.²⁴ The direct action of IL-15 on the memory CD8⁺ T-cell subset has been proposed by some groups to be an important reactivation mechanism required for bystander (T-cell receptor-independent) proliferation during the early stages of viral infection.²⁹⁻³¹ In this respect, cytokine-driven bystander activation of memory T cells might provide early amplification of the adaptive immune response²⁹; indeed, recent findings indicated that IL-15-induced transcriptional and functional responses of human peripheral blood memory phenotype CD8⁺ cells resemble in many ways the effects of anti-CD3 activation.³¹ Thus, in addition to mobilizing antigen-specific T cells, IL-21 appears to downmodulate TCR-independent memory T-cell functions by limiting IL-15-induced T-cell proliferation once specific immunity has emerged.²⁴ However, it is not yet clear whether this decreased expansion is due to inhibition of IL-15-induced proliferation or to IL-21-induced apoptosis of the dividing cells.

NK cells

The range of responses elicited by IL-21 in cultured NK cells points to a potentially important and complex role for this lymphokine in the innate immune response. It should be noted that the reported effects of IL-21 on NK cells appear to vary depending on the species, activation state, or maturation stage of the cells, as well as the relative concentration of IL-21 (and co-stimulatory factors) used in each study.^{18,20,24} For example, in vitro assays suggest a role for IL-21 in the expansion of mature human CD16⁺CD56⁺ NK cells from bone marrow hematopoietic progenitor cells in synergy with IL-15.¹⁸ The most dramatic effect in this co-culture system is the influence of IL-21 on NK cell maturation; IL-21 promotes a striking increase in the cytotoxic response of this population relative to the CD16⁺CD56⁺ cells that develop after exposure to IL-15 alone. Moreover, IL-21, like IL-2 or IL-15, can stimulate cytolytic activity of mature peripheral human NK cells¹⁸ and, in synergy with IL-15 or IL-18, enhances IFN- γ production in primary human NK cells.²⁸

Contrary to these co-stimulatory roles in human NK cell physiology, Kasaian et al²⁴ have shown that IL-21 inhibits the IL-2- or IL-15-mediated expansion of naïve murine NK cells and enhances effector function only

after preactivation of NK cells in vivo with poly I:C or in vitro with IL-15. Under these conditions, restimulation with IL-15, IL-21, or their combination greatly increased NK cell cytotoxicity and resulted in high levels of IFN- γ production with or without IL-12 co-stimulation. Interestingly, the enhanced effector response to IL-21 or IL-15 plus IL-21 was accompanied by a sharp reduction in cell viability, whereas IL-15 treatment alone prolonged survival. Thus, in this context, IL-21 promotes functional activation yet fails to sustain viability and antagonizes the survival effect of IL-15 on activated murine NK cells. Taken together with the ability of IL-21 to augment antigen-specific T-cell function, Kasaian et al have interpreted the IL-21-induced inhibition of resting NK cell expansion and activated NK cell survival as a possible mechanism to limit their continued participation in the host immune response as the adaptive system becomes engaged. Therefore, as a product of activated CD4⁺ T cells, IL-21 might serve to facilitate the transition between innate and adaptive immunity while greatly enhancing the effector mechanisms of T cells and activated NK cells.²⁴

Given the initial observation that IL-21 potentiates the IL-15-induced expansion of human NK cells from bone marrow hematopoietic progenitor cells or peripheral blood^{18,20} and promotes functional activation of murine and human NK cells,^{18,24} the phenotypically normal NK cell development of IL-21R-deficient mice seemed surprising.²⁴ Although IL-21R^{-/-} mice have normal numbers of mature peripheral NK cells and full cytolytic potential, lymphoid cells from these mice do not exhibit any detectable responses to IL-21 in vitro, including the effects on T-cell proliferation and T and NK cell effector function described above.²⁴ These results confirm the requirement for IL-21R in mediating the effects of IL-21 and indicate that the IL-21/IL-21R system is not essential for NK cell maturation in mice. However, this study does not preclude a role for IL-21 in human NK cell development, and it remains to be determined whether mature, activated T cells are the only source of this cytokine.

B lymphocytes

In accord with the discovery of IL-21R expression on human peripheral blood B lymphocytes and murine splenic B cells, IL-21 was found to regulate a number of aspects of B-cell function. Although IL-21 alone fails to stimulate the proliferation of B cells, it profoundly affects B-cell growth in combination with other co-stimuli. For example, IL-21 synergizes with CD40 co-stimulation to dramatically enhance primary B-cell mitogenesis.¹⁸ In contrast, IL-21 inhibits the proliferation of murine B cells stimulated with LPS or anti-IgM antibodies (to mimic an antigen encounter), as well as human B cells activated with the combination of anti-IgM antibodies and IL-4.^{18,20} The ability of IL-21 to oppose the T cell-independent co-stimulatory effects of LPS on B cells substantiates the argument proposed by Kasaian et al²⁴ that IL-21 down-modulates innate immune responses during the transition to adaptive immunity. Converse-

ly, synergy between IL-21- and CD40-mediated pro-mitogenic pathways might enhance B-cell function after B cell: T_H cell interactions between CD40 and its ligand CD40L. These events are vital for the generation of memory B cells and germinal centers, an integral part of an effective humoral immune response.

A recent study by Ozaki et al³² has confirmed that IL-21 is indeed crucial for the regulation of B-cell function in vivo. Several groups have independently observed that despite outwardly normal lymphocyte development, IL-21R-deficient mice have elevated circulating IgE titers^{20,24,32} and reduced serum levels of IgG₁ and IgG_{2b}.^{24,32} Similarly, elevated total and antigen-specific IgE responses were observed on immunization or infection of IL-21R-/- mice,^{20,32} even under conditions that do not normally elicit an IgE response in mice, such as infection with *Toxoplasma gondii*.³² A priori, there are many possible direct or indirect mechanisms to account for the dysregulated B-cell function in IL-21R-deficient mice, including impaired suppression of IgE synthesis via IFN- γ . Inhibition of Ig synthesis is a well-established property of IFN- γ , and IL-21 requires IL-21R to enhance IFN- γ production from activated NK and T cells.^{24,27} In this respect, it is plausible that decreased production of IFN- γ in the absence of IL-21 signaling during the course of an immune response could lead to excessive IgE production from B cells. Alternatively, these B-cell abnormalities could be secondary to dysregulated T-cell activity. Along these lines, it is possible that IL-21 normally skews the T_H1/T_H2 balance toward T_H1 type cells; thus T_H2 cells, which produce IL-4, might outnumber T_H1 cells in IL-21R-/- mice and thereby influence the extent and specificity of Ig class switching in B cells. This notion is supported by the observation that the shift to IgE overproduction in IL-21R-/- mice is dependent on IL-4³² (see below). Nevertheless, this hypothesis does not readily explain the diminished IgG₁ levels in IL-21R-deficient mice. Moreover, there is recent evidence to discount both of the above indirect mechanisms, because Ozaki et al³² have shown that splenic IL-21R-/- CD4⁺ T cells stimulated with a CD3-specific antibody under T_H1 - or T_H2 -polarizing conditions produce comparable amounts of IFN- γ (T_H1 population) and IL-4 (T_H2 population) relative to that of wild-type mice, suggesting that differentiation of these CD4⁺ T-cell subsets proceeds normally in IL-21R-/- mice. Furthermore, serum IFN- γ levels were similar in wild-type and IL-21R-/- mice after infection with *T gondii*, which is known to induce a potent T_H1 -dependent IFN- γ response during the acute phase of infection and does not elicit an IgE response in mice. Surprisingly, despite similar survival times after infection, serum IgG₁ levels of IL-21R-/- mice were approximately one third that of wild-type mice, whereas IgE levels were increased about 20- to 25-fold.

Given that T_H -cell-derived cytokine expression appeared normal in IL-21R-/- mice, Ozaki et al³² evaluated whether the impaired production of IgG₁ and augmented IgE response of IL-21R-/- mice could be attributed to an intrinsic B-cell defect. They found that the in

vitro production of both IgG₁ and IgE was similar in IL-21R-/- and wild-type B cells after culture with anti-CD40 plus IL-4 or LPS plus IL-4, indicating that ablation of IL-21R does not impact the response to these stimuli. However, production of IgG₁ from IL-21R-deficient B cells stimulated with anti-CD40 antibodies plus IL-4 and IL-21 was significantly lower than that of wild-type B cells, indicating that intrinsic B-cell function is abnormal in IL-21R-/- mice. Moreover, although the ability of wild-type and IL-21R-/- mice to develop splenic memory B cells appeared similar after immunization with a T-cell-dependent antigen, the numbers of antigen-specific IgG₁-producing splenic B cells from IL-21R-/- mice were substantially lower than those of wild-type mice.³² Collectively, these findings indicate that IL-21 influences IgG₁ production in normal murine B cells and participates in the development of antigen-specific IgG₁-producing B cells in mice.³² To help explain the augmented IgE production in IL-21R-/- mice, investigators have explored the effect of IL-4 ablation in the context of IL-21R deficiency (see below).³²

IL-4 is well known for its ability to direct isotype switching in activated murine B cells from IgM to IgG₁ and IgE.³³ To determine whether IL-21 regulates IL-4-mediated immune responses, Suto et al³⁴ also examined the effect of IL-21 on antigen-specific IgE production in mice. They found that IL-21 inhibits the in vitro production of IgE from LPS/IL-4-stimulated murine B cells, yet it increases IgG₁ production from LPS-stimulated B cells.³⁴ Although it is not yet clear how IL-21 regulates Ig production in B cells, this study also demonstrates that IL-21 downregulates IL-4-dependent isotype switching to IgE at the level of germ line C ϵ transcription. Interestingly, IL-21-induced inhibition of germ line C ϵ transcription in B cells is not mediated by direct effects on IL-4-induced activation of signal transducer and activator of transcription 6 (STAT6) and nuclear translocation or by inhibition of STAT6-mediated transcription from the core germ line C ϵ promoter. Negative effects on transcript stability, competition for γ c, and the induction of negative regulators that inhibit IL-4 proximal signaling events upstream of STAT6 activation have also been excluded as IL-21-mediated inhibitory mechanisms. Rather, it is suggested that IL-21 modulates germ line C ϵ transcript levels through regulation of an undefined response element located outside the core promoter region of the germ line C ϵ gene or possibly by recruiting chromatin remodeling factors directly to the germ line C ϵ gene.³⁴ In this regard, transcriptional crosstalk might emerge as a general immunomodulatory mechanism by which IL-21 exerts its antagonistic effects on lymphocyte function.

In addition to regulating normal B-cell proliferation, IL-21 has been shown to be a growth and survival factor in multiple myeloma, a cancer of terminally differentiated B lymphocytes.³⁵ IL-21 alone can augment the proliferation of several IL-6-dependent human myeloma cell lines that endogenously express IL-21R and, together with tumor necrosis factor, synergistically enhances

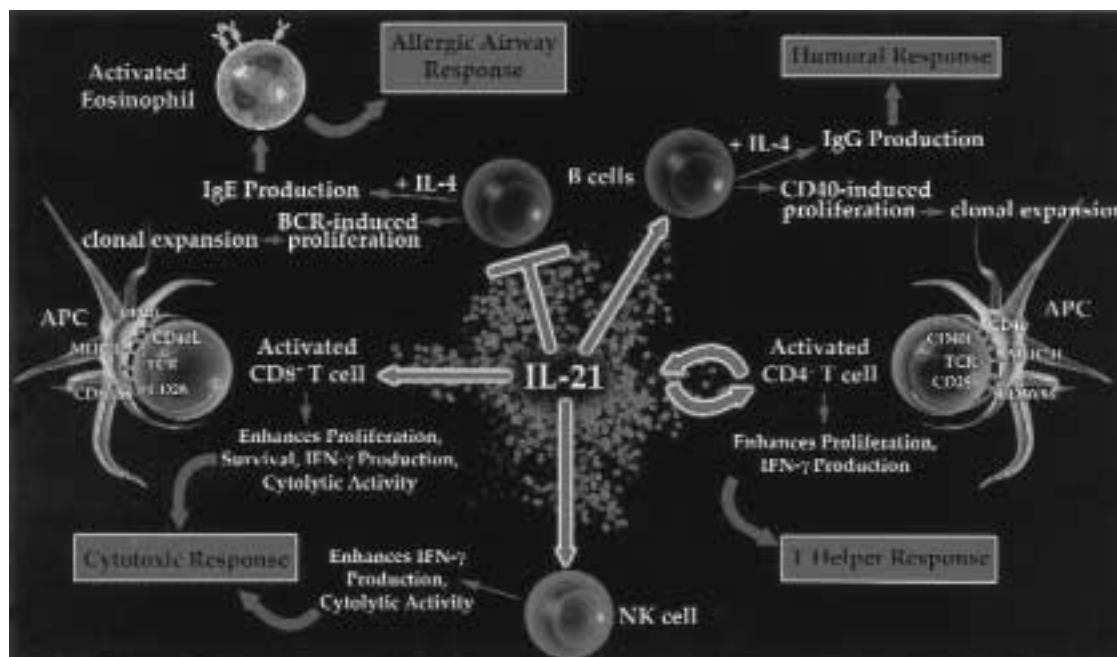


FIG 1. The biologic actions of IL-21: potential role in innate and adaptive immunity. IL-21, a T_H1 -like lymphokine, is produced by activated $CD4^+$ T cells and has pleiotropic effects on T, B, and NK cells. The effect of IL-21 (green arrows) leads to various immunologic processes (red arrows), including enhanced alloantigen stimulation and effector responses of T cells, functional activation of NK cells, and regulation of Ig production by B cells. IL-21 increases cytolytic activity and $IFN-\gamma$ production from activated NK and T cells and potentially enhances antitumor responses in syngeneic murine models of cancer. These activities suggest that IL-21 has potential as an immunotherapeutic for oncology. As a mediator of an effective humoral immune response, IL-21 positively influences IgG production in concert with IL-4 and synergizes with CD40 co-stimulation to enhance B-cell proliferation. In contrast, IL-21 inhibits the proliferative response of B cells to antigen receptor (BCR) activation. The ability of IL-21 to suppress IL-4-dependent IgE production provides a potential mechanism to explain the reduced pulmonary inflammation that occurs in a murine ovalbumin-challenge asthma model.

myeloma cell mitogenesis.³⁵ Interestingly, IL-21R is also expressed in a variety of lymphoma tumor cell lines derived from B, T, and NK cell origin, and IL-21 has been shown to directly inhibit the proliferation of a subset of B-cell lymphoma cell lines, such as human IM-9 cells, both in vitro and in a severe combined immunodeficiency (SCID) mouse model of lymphoma.^{20,36} Therefore, IL-21 appears to play dramatically different roles in B-cell tumorigenesis, perhaps depending on the stage of B-cell maturation during this process, which underscores the importance of characterizing the signaling events downstream of the IL-21 receptor in both normal and transformed primary B cells and B-cell lines. Fig 1 depicts the major biologic effects of IL-21 as discussed in this section and the potential role of this cytokine in innate and adaptive immunity.

THE MOLECULAR MECHANISMS OF IL-21 ACTION

Nearly all of the cytokines that regulate immune function and hematopoiesis do so by inducing the phosphorylation of a number of vital intracellular substrates, thereby triggering protein-protein interactions that lead to transcriptional and other post-transcriptional signal-

ing events. Several cytokines important for hematopoietic cell homeostasis, stem cell factor (also termed steel factor or c-kit ligand), Flt-3 ligand, and M-CSF, act by engaging receptors that contain intrinsic protein tyrosine kinase domains.³⁷ However, most lymphokine receptors are devoid of such enzymatic activities and instead recruit cytoplasmic protein tyrosine kinases of the JAK family.³ Of the 4 members of the mammalian JAK family, JAK1, JAK2, and Tyk2 are expressed ubiquitously, whereas JAK3 is restricted in its expression pattern to cells of the myeloid and lymphoid lineages.³⁸ Consistent with this finding, biochemical studies have implicated JAK3 in signaling by all γ_c -dependent receptors, because JAK3 associates exclusively with the γ_c chain.³⁹ The tyrosine phosphorylation (activation) of JAK1 and JAK3 is among the most proximal biochemical signals triggered by ligand binding to γ_c -containing receptors.⁴⁰ JAKs are brought into proximity as a result of ligand-induced conformational changes in the receptor subunits (best illustrated for the erythropoietin receptor⁶), which in turn promotes their catalytic activation. Once activated, the JAKs phosphorylate their primary substrates, target tyrosine residues on the receptor subunits, which then serve as docking sites for additional JAK substrates, including the STATs.⁴¹ Cytokines regulate

numerous aspects of immune function and hematopoiesis through activation of the JAK-STAT signaling pathway. This pathway couples cytokine action directly to the transcriptional apparatus of specific target genes, providing a rapid mechanism for reprogramming patterns of gene expression.

Indispensable roles for JAK1 and JAK3 in lymphocyte development have been shown in recent years through knockout mouse studies and naturally occurring human immunodeficiency states.⁴²⁻⁴⁵ JAK1 deficiency in mice severely impairs biologic responses induced by receptors that use γ_c . Consequently, lymphopoiesis, but not myelopoiesis, is blocked.⁴² Similarly, mutations affecting the expression of JAK3 in mice and humans have been shown to result in autosomal recessive SCID.⁴³⁻⁴⁷ Mice deficient in JAK3 develop a form of SCID that mimics the phenotype in γ_c -deficient mice, characterized by impaired B-cell maturation and aberrant T-cell activation.⁴⁵ In addition to the severe B-cell and T-cell lymphopenia observed in JAK3^{-/-} mice,⁴³ NK cells are completely absent.⁴⁴ Thus, the significance of characterizing the JAK-STAT pathways downstream of the IL-21 receptor is underscored by the involvement of IL-21 in crucial aspects of lymphocyte function.

IL-21 IS A γ_c -DEPENDENT CYTOKINE

On the basis of the homology of IL-21R to IL-2R β and IL-4R α and the structural homology between IL-2, IL-15, and IL-21, it was proposed that IL-21 would engage γ_c and stimulate the activation of JAK1 and JAK3. By using 3 different cell lines that endogenously or exogenously express IL-21R, we and others have demonstrated that IL-21 induces tyrosine phosphorylation of JAK1 and JAK3 within minutes of its addition to cultures of cytokine-starved cells.^{48,49} These results have been extended to primary murine splenic B cells.⁴⁹ The functional role of γ_c in IL-21 signaling has also been explored. Two studies from separate laboratories have now established that γ_c is a required signaling component of the IL-21R complex^{48,49} and that JAK3 is an essential transducer of γ_c -dependent mitogenic signals induced by this cytokine.⁴⁹

Early reports suggested that IL-21R is capable of signal transduction through homodimerization, as is IL-4R α , in part on the basis of the ability of the chimeric receptors Mpl-IL-21R¹⁸ and EPOR-IL-21R¹⁹ to support proliferation of the pro-B lymphoid cell line BaF3. Moreover, the initial studies of Parrish-Novak et al¹⁸ indicated that a soluble, homodimeric form of IL-21R was capable of blocking IL-21 activity in vitro. However, the experiments with the above chimeric receptors^{18,19} were performed by using cells bearing the γ_c chain and did not exclude the possibility that IL-21R might physiologically associate in a heteromeric receptor complex with γ_c on ligand binding. Other evidence suggested that IL-21R interacts with γ_c , including the results of an electrochemiluminescence dimerization assay that showed heterodimerization of IL-21R and γ_c , but not IL-21R homodimerization, in the pres-

ence of IL-21.²⁰ By using a panel of monoclonal antibodies that block, to different extents, the function of γ_c , we were able to partially inhibit IL-21-induced proliferation of BaF3 cells expressing full-length IL-21R (BaF3/IL-21R).⁴⁹ The addition of anti- γ_c antibodies to cultures of primary murine splenic B cells also partially blocked proliferation induced by the combination of IL-21 and anti-CD40,²⁰ suggesting that γ_c is involved in IL-21 signaling in B cells. However, because the affinity of these antibodies is modest, additional strategies were used to implicate γ_c and γ_c -associated JAK3 in IL-21R signaling. The addition of the JAK3-specific inhibitor WHI-P131 to BaF3/IL-21R cell cultures substantially reduced IL-21-induced proliferation of this cell line, providing further support for the involvement of γ_c in IL-21 function.⁴⁹

Definitive evidence of the role played by γ_c in IL-21 signaling came from studies with cell lines deficient in γ_c . By using an Epstein-Barr virus-transformed B lymphoid cell line derived from a patient with γ_c -deficient X-linked SCID (JT cells), we showed that despite the presence of IL-21R, IL-21 failed to induce JAK1 or JAK3 tyrosine phosphorylation or proliferation of JT cells. However, retroviral-mediated transduction of wild-type γ_c into these cells restored function to the IL-21R, as shown by IL-21-induced tyrosine phosphorylation of JAK1 and JAK3, downstream activation of STAT proteins, and a modest increase in cell proliferation.⁴⁹ In a similar study, Asao et al⁴⁸ demonstrated that IL-21 is unable to transduce intracellular signals in the γ_c -deficient human T-cell leukemia virus-I (HTLV-I)-transformed cell line ED40515⁻, whereas exogenous expression of wild-type γ_c conferred IL-21 responsiveness, as shown by activation of JAK1, JAK3, STAT1, STAT3, and STAT5. Chemical crosslinking studies with the γ_c -reconstituted HTLV-I cell line further indicated that the IL-21R complex contains γ_c as an essential component.⁴⁸ Thus, several lines of evidence have established that IL-21 is a γ_c -dependent cytokine and a member of the IL-2 cytokine family. More recently, it has been determined that human IL-21 and IL-4 bind to partially overlapping epitopes of γ_c , as defined by alanine-scanning mutational analysis.⁵⁰ Although the binding affinity of IL-21 to the IL-21 receptor complex on whole cells has not yet been reported, the ligand-binding properties of IL-21 and IL-21R/ γ_c have been analyzed by using a biosensor technique (Biacore, Piscataway, NJ) in conjunction with recombinant ectodomains of IL-21R and γ_c .⁵⁰ The formation of a ternary complex between γ_c and immobilized IL-21/IL-21R resulted in an apparent dissociation constant of 160 nmol/L, a 25-fold higher binding affinity than that observed between IL-4 and the IL-4R α ectodomain under similar conditions.⁵⁰

As might be expected, impaired IL-21R signaling has recently been linked to the developmental and functional deficits observed in the lymphoid compartment of γ_c -deficient XSCID patients.³² As discussed previously, B lymphocytes from patients with XSCID exhibit profound defects in B-cell function, in part because of their inability to undergo isotype class switching from IgM to IgG.⁵¹

IL-4 signaling via STAT6 is known to play a crucial role in Ig class switching to IgG₄ and IgE in humans (IgG₁ and IgE in mice) and acts as a co-mitogen for B-cell growth.³³ In fact, in IL-4 and IL-4 receptor knockout mice, as well as in STAT6-deficient mice, IgE production is diminished by a factor of at least 100-fold³³; however, residual IgG₁ production has been observed, suggesting the existence of additional regulators of IgG₁ production, such as IL-21, in these animals.^{32,34} Of note, although IL-21R^{-/-} murine B cells exhibit an impaired IgG₁ response, they are not as defective as XSCID B cells.³² Thus, it is likely that the XSCID B-cell phenotype can be explained by impaired signaling via IL-4R, IL-21R, and potentially additional γ c-dependent cytokine receptor systems. Indeed, ablation of both IL-4 and IL-21R in mice has been shown to generate a phenotype similar to that found in human XSCID.³² Interestingly, on immunization, IL-4^{-/-}IL-21R^{-/-} mice exhibit a significantly more pronounced deficiency in production of IgG subclasses than IL-21R^{-/-} mice; however, the striking IgE upregulation observed in IL-21R^{-/-} mice is absent.³² Moreover, although germinal center formation appears normal in IL-4^{-/-} or IL-21R^{-/-} mice, the double knockouts have disorganized germinal centers, characterized by abnormal histologic features indicative of reduced B-cell somatic hypermutation. Thus, taken together with the apparent defects in IL-21R-deficient mice, these findings indicate that the dramatic increase in IgE production observed on immunization of IL-21R^{-/-} mice is dependent on IL-4 activity and show a cooperative role for IL-4 and IL-21 in normal germinal center formation and IgG₁ production.³² Importantly, both γ c-dependent cytokines have now been linked to the B-cell defect in humans with XSCID. Future investigation of the signaling events initiated by IL-21 in activated B cells will likely help to explain the role of this cytokine in Ig production.

IL-21 RECEPTOR SIGNALING PATHWAYS

The molecular mechanisms governing the complex biologic activities of IL-21 are just beginning to be unraveled. Most of the available data in the literature have focused on the IL-21-induced STAT activation profile and potential STAT target genes in T, B, and NK cells. The role of STATs in cytokine signaling and their impact on cellular function have been recently reviewed.^{38,52-59} Seven distinct STATs have been identified (STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6) that play a crucial role in regulating the expression of target genes that control cell proliferation, differentiation, survival, and apoptosis.⁴¹ On their recruitment to cytokine receptors, monomeric STATs are phosphorylated on a single conserved tyrosine residue by JAK kinases (or in some cases, src family kinases), resulting in their dissociation from receptors and immediate dimerization through bivalent, reciprocal phosphotyrosine–src homology-2 (SH2) domain interactions.⁵² The activated STAT dimers are then competent to translocate to the nucleus, in which they bind to specific response elements and transactivate

a myriad of target genes. Positive regulation can occur through additional post-translational modifications such as serine phosphorylation, which has been shown to be required for full transcriptional activity of STAT1 and STAT3 in numerous systems.⁶⁰ STAT2, STAT4, and STAT6 are largely restricted to IFN- α , IL-12, and IL-4/13 signaling, respectively⁵²; in contrast, STAT1, STAT3, and STAT5 are activated by many different cytokines and mediate distinct *in vivo* responses depending on their pattern of activation.^{56,61} The JAK kinases alone are clearly not an absolute determinant of specificity in STAT-dependent signaling by IL-2 family cytokines, because all γ c-dependent cytokines activate JAK1 and JAK3.² Thus, functional specificity is determined at multiple levels within the JAK-STAT pathway, eg, different STAT SH2 domain-receptor phosphotyrosine interactions dictate the pattern of STAT dimerization, and activated STAT dimers recognize sequence-specific target gene response elements in the nucleus.⁶²

In accord with the findings of Asao et al,⁴⁸ Strengell et al,^{26,28} and Bennett et al,⁶³ we have found that IL-21 signaling via IL-21R/ γ c leads predominantly to co-activation of STAT1 and STAT3 and consistently weak activation of STAT5 in a variety of lymphoid cell lines as well as primary splenic murine B cells⁴⁹ (unpublished observations). A consistent theme in the literature is that STAT1 generally serves as a negative regulator of cell growth and survival, in contrast to STAT3 and STAT5, which function primarily to promote cell survival, cell cycle progression and differentiation.⁵⁴⁻⁵⁷ Given these fundamental functional differences between the STATs, the above results appear to be consistent with the observation that IL-21 alone is an inadequate stimulus of lymphoid cell growth and acts primarily to modulate immune responses in concert with other cytokines or co-stimuli. Parrish-Novak et al¹⁸ have reported that IL-21 inhibits the expansion of human and murine B cells stimulated through the B-cell receptor with anti-IgM, yet it dramatically enhances B-cell proliferation in synergy with CD40 engagement. Although a number of models could be proposed to explain these divergent effects of IL-21 on B cells, it is conceivable that specific co-stimulatory signals might impart differential sensitivity to the growth inhibitory effects of STAT1. In this regard, B-cell receptor activation might sensitize B cells to the inhibitory effects of STAT1, perhaps by influencing levels of STAT1 serine phosphorylation, or the kinetics of STAT1 activation and nuclear import/export. Conversely, CD40-derived positive signals might downregulate the antagonistic effects of STAT1 on pro-mitogenic or survival gene expression by inducing STAT tyrosine phosphatase activity or protein inhibitor of activated STAT1 binding to STAT1.⁵⁴ Competition between CD40-activated transcription factors and STAT1 for transcriptional co-activators could similarly attenuate STAT1-mediated growth inhibitory signals. In support of this idea, Mehta et al⁶⁴ have recently reported that IL-21 induces the apoptosis of resting primary murine B cells, an effect that correlates with downregulation of the antiapoptotic family

members Bcl-2 and Bcl-x_L. In addition, short-term pre-activation of resting B cells with anti-CD40, through Bcl-x_L upregulation, confers protection from IL-21-mediated apoptosis. Because STAT1 has been shown to regulate the expression of genes involved in cell survival,^{65,66} these findings implicate STAT1 as a potentially important mediator of the proapoptotic/antiproliferative effects of IL-21 in primary B cells.

As previously noted, IL-21 inhibits the *in vitro* proliferation of a subset of B-cell lymphoma cell lines that endogenously express IL-21R, such as the human cell line IM-9.^{20,36} These findings have led us to speculate that STAT1 plays a significant role in IL-21-mediated growth arrest of IM-9 cells. Consistent with this idea, a recent report has demonstrated that IL-4 inhibits the growth of colon carcinoma cell lines in a STAT1-, but not STAT6-, dependent manner.⁶⁷ Expression of a dominant-negative version of STAT1 in these colon cancer cells, which acts to block activation of endogenous STAT1 protein, reversed the IL-4-induced growth inhibition even at high concentrations of cytokine. Moreover, IL-4 induced the co-activation of STAT1 and STAT3 in colon carcinoma cells, as well as normal mouse splenocytes, although the significance of STAT3 activation in IL-4-mediated growth arrest is not yet clear. Because IL-4 is also known to promote cell growth via activation of insulin receptor substrate-1,³³ these recent findings provide an example of how a negative signal (STAT1) can override a pro-mitogenic signaling pathway in a particular cellular context and provide a basis for future investigation of this concept in IL-21-treated lymphomas.

In contrast to IM-9 cells, IL-21 has potent growth-promoting and antiapoptotic effects on the IL-6-dependent myeloma cell lines ANBL-6, OH-2, and IH-1.³⁵ Like IL-6, IL-21 was shown to activate both the JAK-STAT pathway and the mitogen-activated protein kinase (MAPK) pathway in myeloma cells. Interestingly, STAT3, but not STAT1 and STAT5, was activated by IL-21 in this context, in addition to the MAPK family members extracellular signal-related kinase (ERK) 1/2. Normally, the IL-6/gp130/STAT3 signaling pathway is involved in B-cell growth and differentiation into plasma cells.⁶⁸ STAT3 is essential for the proliferation and survival of myeloma cells and likely contributes to the generation of plasma cell oncogenesis because it has been found to be constitutively activated in bone marrow mononuclear cells from patients with multiple myeloma.⁶⁸ Thus, IL-21 might be among a growing list of factors that contribute to the pathology of multiple myeloma. Because this list includes IL-15, the question again arises as to whether IL-21 can also be detected in the bone marrow microenvironment of normal individuals, as well as patients with myeloma.³⁵

A notable feature of IL-21 is its ability to potently enhance the effector functions of primary activated T cells and NK cells, namely IFN- γ secretion and cytotoxic activity, alone or in concert with other cytokines such as IL-2, IL-12, IL-15, or IL-18.^{24,28} Thus, a differentiative role for STAT1 or STAT3 in these cell types is certainly likely. In primary human T and NK cells, IL-21

has very recently been shown to stimulate STAT1-, STAT3-, and, to a lesser extent, STAT4-binding to the IFN- γ promoter,²⁸ which strongly implicates these molecules in IL-21-induced IFN- γ gene expression. STAT4 has previously been associated with T_H1 development in the context of IL-12 signaling²⁷; therefore, it is somewhat surprising that STAT4 DNA binding occurred in response to both IL-15 and IL-21 in this study (although more weakly than that of IL-12). Nevertheless, another γ c-dependent cytokine, IL-2, has been shown to activate STAT4, albeit in NK cells.⁶⁹ This might reflect a specialized role for STAT4 in potentiation of NK cell cytotoxicity by IL-2⁶⁹; similarly, the ability of IL-15 or IL-21 to enhance NK cell cytolytic activity overlaps with the effects of IL-12,⁶⁹ which might help to explain the shared use of a downstream signaling pathway. It has also been suggested that IL-15- or IL-21-induced STAT4 activation in NK cells contributes to IFN- γ production during the early stages of viral infection, when macrophages secrete IL-15 and trigger T-cell-derived IL-21 release via presentation of viral antigens.²⁸ Thus, taken together, these studies further support the contention that IL-21 is a T_H1-like cytokine that plays a significant role in the activation of innate immune responses.

In vivo, IL-21 has been shown to stimulate the elimination of solid tumors through indirect effector cell mechanisms mediated by NK and cytotoxic T cells.^{36,70} Interestingly, despite the ability of IL-21 to enhance IFN- γ production by both NK and CD8⁺ T cells, recent *in vivo* tumor studies with cytokine-deficient mice have indicated that IFN- γ is not required for the antitumor responses mediated by IL-21 in the B16F1 melanoma tumor model.⁷¹ However, in this system, IL-21-induced tumor rejection is mediated via a perforin-dependent cytotoxic pathway, because IL-21 failed to eliminate B16F1 tumor growth in perforin-deficient mice.⁷¹ Defective tumor rejection has previously been observed in mice lacking STAT1, which correlates with a failure to develop cytolytic T cells.⁷² Moreover, the identification of a functional STAT-binding element in the human perforin promoter has previously been described, in which binding of STAT1 α and STAT5 occurred in response to IL-6 and IL-2, respectively.⁷³ Thus, there might be a direct role for STAT1 in the upregulation of genes encoding cytotoxic granule proteins (perforin and granzymes) in IL-21-stimulated T and NK cells. In other systems in which IL-21 has exhibited direct killing activity against a variety of B-cell tumors both *in vivo* and *in vitro*,³⁶ STAT1 might play a crucial proapoptotic or antiproliferative role in this process. Future studies will further clarify the STAT-dependent pathways involved in IL-21-mediated T and NK cell responses, which are likely to have therapeutic utility in the treatment of cancer (see below). Fig 2 summarizes the known intracellular signaling pathways activated by IL-21 in lymphocytes and depicts the potential roles of the STAT proteins in mediating cell growth and survival signals, as well as T_H1 and cytotoxic immune responses, transduced through the IL-21 receptor.

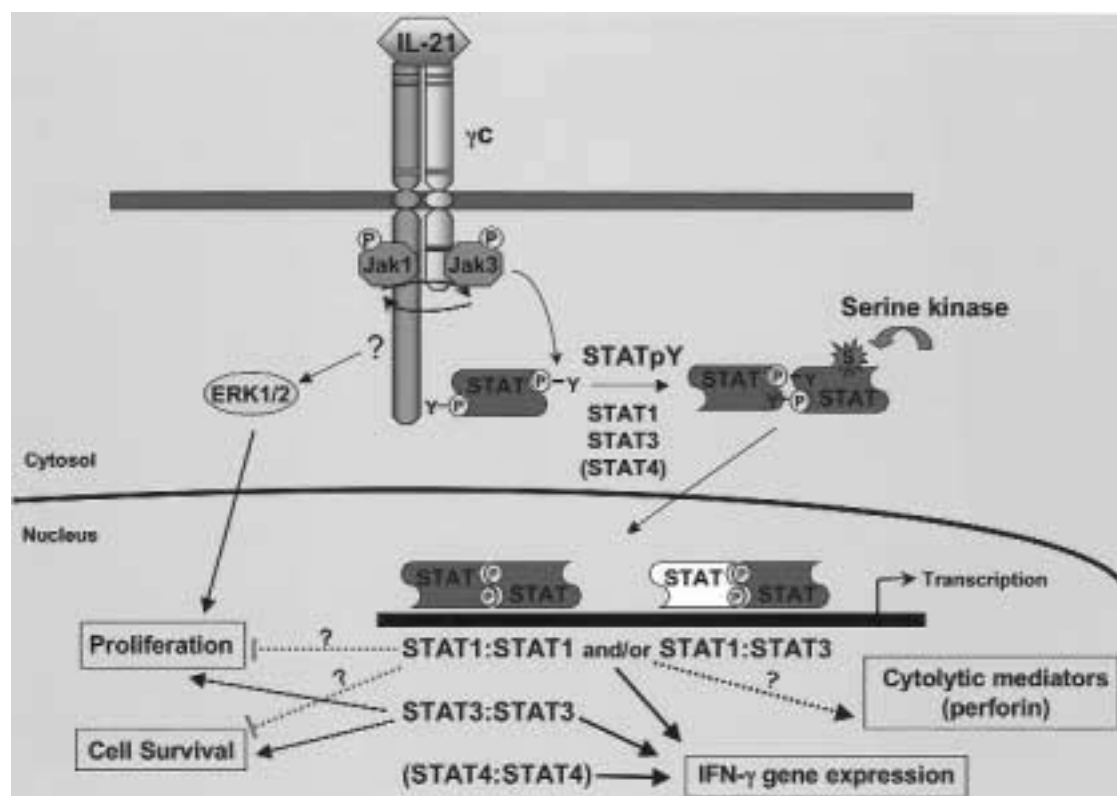


FIG 2. Current model for IL-21 signal transduction. IL-21 binds to a heterodimeric complex of IL-21R and the γ_c chain, thereby triggering catalytic activation of preassociated JAK1 and JAK3, respectively. Activation of the JAK kinases in turn results in the phosphorylation of tyrosine residues on IL-21R, creating docking sites for STAT proteins and other as yet uncharacterized signaling modules (depicted as ? upstream of ERK activation). On recruitment, STATs undergo tyrosine phosphorylation (activation) and are favored to form dimers (either homodimers or, in some cases, heterodimers), which translocate to the nucleus and modulate expression of target genes. Maximal transcriptional activity of STAT1 and STAT3 is thought to require additional phosphorylation on a conserved C-terminal serine residue. Unlike the other γ_c -dependent cytokines, IL-21 preferentially activates STAT1 and STAT3 in lymphocytes, and more recently STAT4 activation has been reported in human T and NK cells. In human myeloma cell lines and primary myeloma cells, IL-21 mediates its proliferative and survival effects through ERK1/2 and STAT3 activation. Recent data strongly suggest that IL-21-activated STATs are also involved in the upregulation of IFN- γ gene expression in NK and T cells, because STAT1, STAT3, and STAT4 can be induced to bind to putative regulatory elements in the IFN- γ gene promoter. STAT1 homodimers or STAT1:STAT3 heterodimers are potential candidates to mediate the proapoptotic or antiproliferative effects of IL-21 on primary T, B, and NK cells, as well as on B-cell tumors expressing IL-21R (represented by the *dashed red lines with a ?*). Similarly, there might be a direct role for STAT1 in functional activation of T and NK cells via induction of cytolytic mediators such as perforin (represented by the *dashed black arrow with a ?*), which is indispensable for IL-21-induced tumor rejection in the B16F1 melanoma tumor model.

POTENTIAL THERAPEUTIC APPLICATIONS OF IL-21

IL-21 affects the response of multiple types of immune cells. As such, there are several clinical situations that reflect dysregulation of the immune system and hence are potentially amendable to IL-21-induced immune modulation for therapeutic benefit. Although IL-21 is among the most recent immunomodulatory cytokines to be identified and characterized, several clinical situations have already been considered, including asthma and cancer.

In both animal models and asthmatic patients, IL-4 has been implicated as an important inflammatory mediator in

allergic inflammation.⁷⁴ Among the numerous biologic effects of IL-4 relevant to this process are the proliferation of mast cells and activated T_H2 and B cells, Ig class switching, and IgG/IgE production by B cells.⁷⁴ The inflammatory response after inhaled allergen challenge occurs as an immediate reaction, triggered by mast cell activation, and a late reaction several hours later, characterized by an influx of eosinophils, lymphocytes, and macrophages throughout the airway wall and into the bronchial fluid.⁷⁴ Secretion of IL-4, IL-5, IL-13, and eotaxin by the CD4⁺ and CD8⁺ T-cell infiltrates in turn stimulates increased production and trafficking of eosinophils, which amplify the inflammatory response. It has recently been shown that IL-21 administered into

BALB/C mice during the course of immunization with ovalbumin effectively decreased antigen-induced eosinophil recruitment into the airway of mice after subsequent challenge with inhaled ovalbumin.³⁴ In contrast, IL-21 did not affect antigen-induced lymphocyte or macrophage recruitment in this preclinical model system. An assessment of the amounts of antiovalbumin IgE, IgG₁, and IgG_{2b} antibodies in the sera of immunized mice showed that IL-21 significantly decreased antiovalbumin IgE and IgG₁ production, but not IgG_{2b} production. The reduced levels of IgE were not a consequence of IL-21-mediated inhibition of T_H2 cell differentiation or IL-4 production; rather, IL-21 directly antagonized IL-4-induced IgE production from B cells at the level of IL-4-induced germ line C ϵ transcription.³⁴ These results suggest that IL-21 or IL-21 mimetics might have beneficial anti-inflammatory effects in asthmatic patients and thus might be useful therapeutically for the treatment of this IgE-dependent allergic disease.³⁴ A soluble IL-4 receptor, developed by Amgen-Immunex (Thousand Oaks, Calif), has previously been shown to be effective in blocking the pulmonary inflammation that occurs in a murine ovalbumin-challenge asthma model.⁷⁴ The ability of IL-21 to block eosinophil infiltration and downregulate IL-4-dependent isotype switching to IgE in B cells (Fig 1) suggests that IL-21 might also have usefulness, alone or in combination with the anti-IL-4 reagent, in the immediate and late phase inflammatory responses in allergic asthma.

Given the capacity of IL-21 to stimulate the innate immune system in humans and mice and to directly inhibit the proliferation of a number of tumor cell lines, IL-21 was tested for antitumor activity in murine models of cancer. IL-21 has been shown to have direct killing activity against a variety of B- and T-cell tumor lines expressing IL-21R (ie, IM-9, HS SULTAN, Hut-70), with both in vitro and in vivo model systems.³⁶ In vivo, IL-21 treatment provides a survival benefit for IM-9-injected SCID mice, and consistent with its direct action on tumor cells, NK cell depletion does not reduce IL-21 efficacy.³⁶ In addition, IL-21 can inhibit the growth of a variety of solid tumors that lack IL-21R and thus are likely to be inhibited indirectly through T- and NK-cell effector mechanisms. For example, Ugai et al⁷⁰ modified the colon carcinoma cell line, Colon 26, to express IL-21 in an attempt to enhance local T_H1- and NK cell-mediated antitumor immunity. They found that after the inoculation of transduced cells into syngeneic and T-cell-deficient nude mice, protective immunity developed, such that the growth of inoculated tumor cells was substantially diminished, and the mice were resistant to subsequent inoculation of nontransduced Colon 26 cells. In contrast, tumors derived from parental Colon 26 cells continued to grow. Because depletion of NK cells resulted in similar tumor growth rates of parent and IL-21-producing cells in nude mice, this suggested that NK cells are involved in the antitumor response. The antitumor effects of IL-21 were also likely mediated through enhanced T-cell effector functions, because spleen cells from mice that had rejected Colon 26/IL-21 cells pro-

duced more IFN- γ and exhibited more potent cytolytic activity than spleen cells from naïve mice when cultured with irradiated Colon 26 cells. However, irrelevant tumor cells did not stimulate production of significant amounts of IFN- γ from either splenic population.

Similarly, Ma et al⁷¹ have demonstrated that IL-21 mediates antitumor responses in the poorly immunogenic B16F1 melanoma and potentially immunogenic MethA fibrosarcoma murine tumor models. Syngeneic tumor cells retrovirally transduced to secrete IL-21 in a paracrine fashion (B16F1-IL-21 or MethA-IL-21) were eradicated in vivo by immunocompetent mice in both model systems. In vivo depletion of various lymphocyte lineages by direct administration of anti-CD4, anti-CD8, or anti-NK cell antibodies demonstrated that the protective effects of IL-21 required NK and CD8⁺ T cells, but not CD4⁺ T cells. To determine whether other major T_H1/T_H2 cytokines are involved in IL-21-induced antitumor responses, B16F1-IL-21 cells were injected into mice deficient in IFN- γ , IL-4, IL-10, or IL-12. These studies showed that IL-21-mediated tumor rejection is independent of all of these cytokines, because none of the cytokine-deficient mice formed tumors after B16F1-IL-21 cell injection; however, B16F1-IL-21 tumors grew in perforin-deficient mice (albeit more slowly than control B16F1 tumors), suggesting that a perforin-dependent pathway, as well as other as yet unidentified IL-21-induced mechanisms, are involved in the enhancement of NK and tumor-specific T-cell cytotoxicity.⁷¹ The finding that IL-21-induced tumor rejection is IFN- γ -independent might have pharmacologic relevance, because IFN- γ is among the inflammatory mediators associated with the severe side effects that accompany a vigorous antitumor response.⁷¹ Collectively, these findings indicate that introduction of the IL-21 gene into malignant cells can generate systemic immunity against tumors and might provide a therapeutic strategy for treatment of a wide range of tumor cell types.

More recently, Nelson et al⁷⁵ tested exogenously administered IL-21 against 2 murine tumor cell models, the moderately antigenic EG7 thymoma cell line, and the poorly antigenic B6F10 melanoma line. With the thymoma line, it was found that as few as 4 injections of purified IL-21, administered every other day, cured syngeneic mice of inoculated tumor cells, and the mice were resistant to subsequent challenge with fresh EG7 cells (without the need for further therapy with IL-21). Tumor-specific cytotoxic T lymphocytes (CTLs) generated from these mice exhibited increased cytolytic activity against EG7 target cells in vitro when cultured in the presence of IL-21. Notably, IL-21 was also shown to inhibit the apoptosis of EG7-specific CTLs in vitro and enhance the survival of adoptively transferred tumor-specific CTLs in vivo to promote sustained CD8⁺ T-cell responses against lymphoma. Although the injection of IL-21 into B6F10 mice did not cure the tumor-bearing animals, the number of metastatic pulmonary lesions was reduced, and CTLs from these mice exhibited enhanced specific cytolytic activity against B16 melanoma cells in vitro.⁷⁵ This lat-

ter finding is significant, in that a strong CTL response is not usually seen with this type of poorly immunogenic tumor. In addition, the pharmacologic effects of IL-21, as compared with IL-2, in the B16F10 tumor model system demonstrate that IL-21 has significant antitumor activity without the systemic side effects (cytokine release) that currently limit IL-2 antitumor therapy.⁷⁶

Although clearly in its infancy, the use of IL-21 to bolster antitumor immunity or to shift an overactive T_H2 response in asthma to a more balanced response could provide an important new tool in a rationally based arsenal designed to fine-tune the immune system toward better health. It is hoped that application of this novel cytokine to these initial models of disease will soon be followed by clinical trials to test the efficacy of IL-21 in patients with important maladies.

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