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The modulatory capacity of interleukin-21 in the pathogenesis of autoimmune disease

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1. ABSTRACT

In this review, we discuss recent progress from studies on the biology of IL-21 and the role of this cytokine in the pathogenesis of autoimmunity. Recent studies have demonstrated that IL-21 plays an important and non-redundant role in a number of autoimmune animal models indicating that IL-21 could be a common modulator of the adaptive immune response towards self-tissue constituents in diseases such as systemic lupus erythematosus, models of rheumatoid arthritis, multiple sclerosis and type-1 diabetes. Also, the studies on the production of IL-21 in human autoimmune diseases and its behaviour on human cells *in vitro* are revealing the potential of IL-21 to exacerbate cellular processes that determine the course of autoimmune diseases.

2. INTRODUCTION

Cytokines are regulatory molecules that act on cells of the immune system. They constitute a network that coordinates the development of the immune system and the various stages of the immune response against pathogens. Cytokines govern the initial activation of innate immune cells (dendritic cells, monocytes, granulocytes and natural killer cells) that express pattern recognition receptors for conserved foreign molecules, the induction of adaptive lymphoid cells reactive to the invading pathogen, the capacity to form a circulating memory population of lymphocytes, and the ability to resolve destructive cellular and humoral forces in a timely manner to limit damage to self tissues.

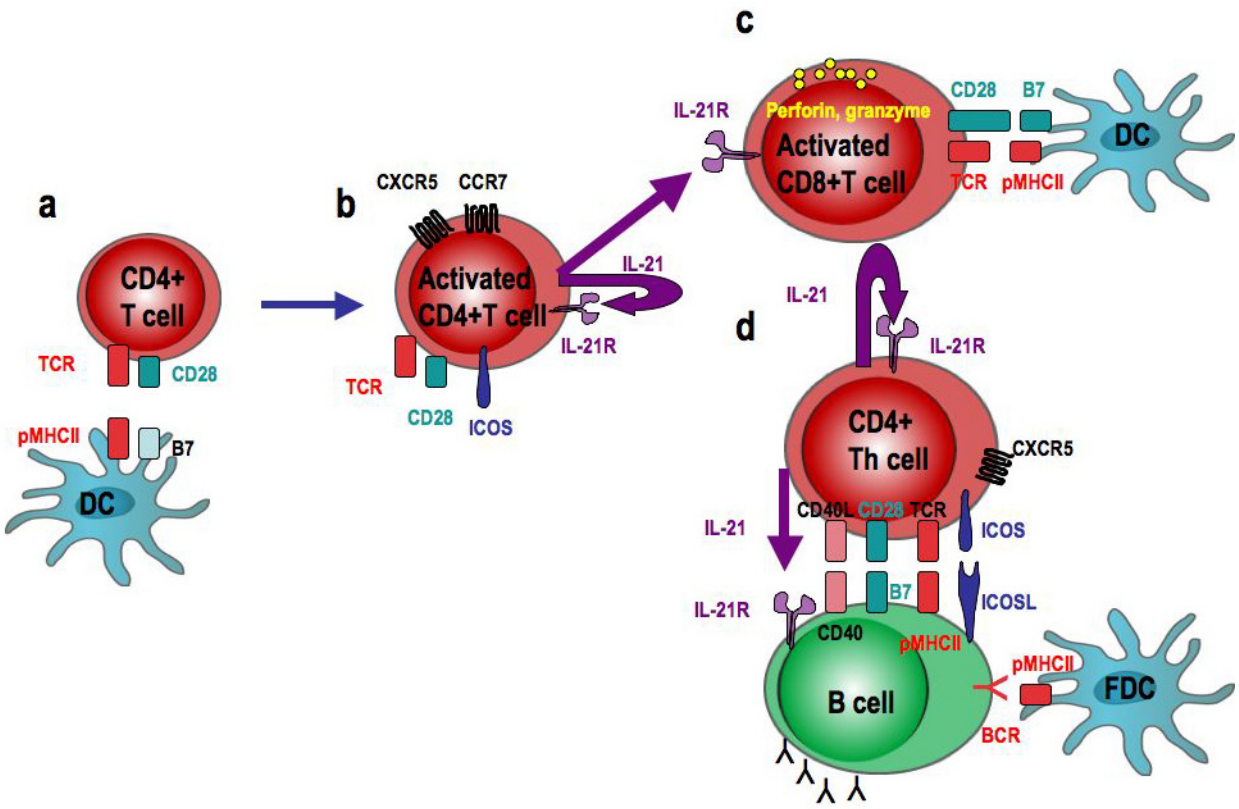

Figure 1. IL-21 acts on T and B lymphocytes to modulate immune responses to antigen. The sustained multi-signal integration necessary for the generation of effector lymphocytes includes IL-21 and expression of costimulatory molecules that can enhance signals through the T cells receptor (TCR) or B cell receptor (BCR). (a) A mature dendritic cell (DC) expressing B7 molecules presents peptide MHC class II ligand (pMHCII) to the TCR of a naïve CD4+ T cell in the T zone expressing CD28. (b) Activated CD4+ T cell produces IL-21 and induces expression of CD28, ICOS and the chemokine receptors CXCR5 and CCR7. (c) For CD8+ T cells, ligation of the TCR with peptide MHC class I ligand expressed on dendritic cell and costimulation through ligation of CD28 by B7 molecule on DC and IL-21 from activated CD4+ T cell, sustains CD28 signalling and induces the expression of effector molecules such as perforin (d) Sustained signalling of activated CD4+ T cell through the TCR and CD28, in the T zone and at the T:B border, leads to modulation of the expression of molecules important for migration, such as CXCR5 and CCR7, and costimulatory receptors including ICOS and CD40 ligand (CD40L). (d) Efficient T cell help from surface molecules, along with the interaction of IL-21 from T helper cell (T follicular B helper cell) with IL-21R expressed on B cell supports the selection of an activated immunoglobulin secreting B cell.

Interleukin (IL)-21 is a recently discovered member of the type 1 cytokine family that signals via the common gamma chain and has pleiotropic effects on the immune system at many levels (Figure 1). The receptor for IL-21 (IL-21R) exists as a heterodimer that comprises the alpha unit (IL-21Rα) and the common gamma chain. The IL-21R is expressed on a variety of immune cells, including T, B, NK and dendritic cells, whereas IL-21 expression is restricted to CD4+ T cells and NKT cells (1-3).

Analyses of immune cell responses to IL-21 *in vitro* and studies on mice deficient in IL-21 or its receptor have indicated a role for IL-21 in lymphocyte activation, proliferation, differentiation and survival (2,4). Other studies resonate with an important role of IL-21 in humoral responses and CD8+ T cell survival and function (Figure 1) (5-13). IL-21 appears to deliver a costimulatory signal to lymphocytes, yet it is not known how such a wide range of consequences can be attributed to a single cytokine (1). Recent molecular studies have revealed signalling pathways downstream from the IL-21R that might account for several effects on lymphocytes, and they include the Janus family tyrosine kinases (JAKs)-STAT, MAPK and PI3K pathways in T cells (14).

In this review we discuss the effects of IL-21 on the adaptive immune response with a focus on the effects of IL-21 on lymphocytes in the systemic autoimmune diseases systemic lupus ertythematosus (SLE) and rheumatoid arthritis (RA), and the organ specific autoimmune diseases, Type 1 diabetes (T1D) and Experimental Allergic Encephalitis (EAE), which is the murine model for multiple sclerosis. Although several studies have implicated the gene for IL-21 as a host genetic factor contributing to the susceptibility to autoimmune diseases (reviewed by N. J. Hill in this volume), even in the absence of a defined

genetic component, IL-21 can facilitate the expansion and differentiation of T and B cells, orchestrating both cellular and humoral inflammation. Thus, we will focus here on the capacity of IL-21 to modulate the immune systems' adaptive response to the environmental and self-antigens in an autoimmune setting.

3. AN OVERVIEW OF THE BIOLOGY OF IL-21

The receptor for IL-21 (IL-21R) is made of a unique cytokine-binding protein (IL-21Rα) and the common γ chain (γc), which is also a component of receptor complexes for IL-2, IL-4, IL-7, IL-9 and IL-15 (4,15-17). The integral role of the common γ chain in the immune response is evident in the severe immune defects observed when its signalling is disrupted. X-linked severe combined immunodeficiency arises from a mutation in the common γ chain and results in a failure to generate T cells, NK cells, and a functional B cell population (17).

The IL-21 receptor was discovered in 2000 as a putative type 1 family receptor bearing close resemblance to the IL-2 receptor β chain (15). High levels of transcript were found in lymphoid tissues from humans and mice (15), and subsequent studies indicated that the IL-21Rα chain was predominantly expressed on B cells, NK cells, and some T cells (1,18,19). This research was closely followed by a description of IL-21 as a four α-helical bundle structure displaying structural homology with IL-2 and IL-15. It was shown to induce *in vitro* growth and proliferation of NK cells, CD4+ T and B lymphocytes in the presence of other γ chain cytokines (1). The structural similarities of IL-21 with IL-2 and IL-15, combined with their proximity on chromosome 3, suggested that these cytokines might have arisen from gene duplication.

IL-21 signalling is mediated via the common γ chain and associated JAKs in concert with the IL-21R. The IL-21R cytoplasmic domain contains six tyrosine residues. One of these, Tyr 510, is phosphorylated upon IL-21 binding and serves as a critical docking site for both STAT1 and STAT3 (14). Phosphorylation of JAK1 and JAK3 upon IL-21 stimulation activates sustained STAT3 and STAT1 signalling (20,21). STAT3 is critically important to the actions of IL-21 since signaling responses to IL-21 are defective in T cells that lack expression of STAT3 (14). IL-21R expression is seen in B cells, T cells, NK cells and dendritic cells, and its effects include both stimulatory and inhibitory impulses among these subsets.

The pluripotency of IL-21 within both arms of both the innate and adaptive immune response indicate an integral role in both the mobilisation of innate immune cells and the transition to antigen specific immune defence. IL-21 is produced by CD4+ T cells and by NKT cells upon mobilisation of intracellular calcium stores following T cell receptor (TCR) cross-linking - controlled by regulatory motifs such as Sp1 and the T cell transcription factor NFATc2 (4,22,23). IL-21 is produced in response to immunization with experimental protein antigens and during infection (24).

4. THE EFFECTS OF IL-21 ON IMMUNE CELLS

4.1. Actions of IL-21 on CD4+ T cells

The functional capabilities of CD4+ T cells during immune responses are thought to vary in a manner dictated by co-stimulation events delivered at the time when the T cell encounters its cognate antigen in the context of MHC class II on antigen presenting cells (APC). This can require co-stimulatory molecules such as the B7 family molecules on APC, and cytokines in the local environment. CD4+ T cells are the main producers of IL-21, and the receptor for this cytokine is upregulated on these cells after activation, suggesting a possible autocrine role. IL-21R is upregulated on T cells in the thymus at the double positive stage, although it may not be involved in thymic development or selection because mice genetically deficient in the IL-21 receptor (IL-21R-/- mice) have normal thymic development (25). Instead, IL-21 begins to play an important role for T cells at a later stage, i.e. during proliferation, differentiation, and acquisition of effector functions in response to antigen. CD4+ T cells express several surface molecules that facilitate interactions with CD8+ T cells and B cells to guide the acquisition of cytotoxic function and antibody production, respectively. IL-21 is produced by CD4+ T cells and acts as a soluble helper molecule for CD8+ T cell and B cell responses. For CD8+ T cells, IL-21 supports proliferation, survival and differentiation into cytotoxic effectors. IL-21 induces proliferation of B cells too, but can lead to either subsequent apoptosis or differentiation into antibody-producing cells.

4.1.1. T helper generation: Th1, Th2 and T_{FH} cells

The role of IL-21 in T helper cell differentiation remains controversial. This is largely due to the fact that IL-21 does not readily fit the established Th1/Th2 cytokine models. Some studies have demonstrated a functional contribution of IL-21 to both the production of Th1 cytokines and to the generation of Th2 responses (26-31). Recent studies have highlighted a controversial new role for IL-21 as a growth factor for newly characterised CD4+ T cell subsets (32). The T helper cell subsets that have been reported to produce IL-21 include Th17 and Th2 cells but the highest levels of IL-21 mRNA transcript has been found in a subset of CD4+ T cells that are thought to provide help to B cells, producing antigen specific immunoglobulin (Ig), termed T follicular B helper cells (T_{FH}) (19,33-37). This non-polarised lineage of T cells expresses chemokine receptors such as CXCR5, which direct T_{FH} cells to the B cell follicles where high levels of IL-21 and co-stimulatory molecules can effectively influence high affinity antigen specific antibody responses by germinal center B cells (32). T_{FH} express significantly greater amounts of IL-21 than other Th subsets (19) and our work on IL-21 and IL-21R deficient mice shows that IL-21 is an autocrine growth factor for T_{FH} cells (A. Vogelzang and C. King, unpublished observations). In addition, studies demonstrate that IL-21 can potently co-stimulate B cell maturation and provide a check-point for regulating the onset of autoimmune antibody production (19,34). Taken together, these findings support an important role for IL-21 in humoral immune responses.

Mice deficient in the IL-21R reveal that IL-21 responsiveness is critical for the differentiation of Th2 cells (31,35). Infection of IL-21R-/- mice with *Toxoplasma Gondii* induces a strong induction of a Th1 response to intracellular pathogens characterised by interferon (IFN)-γ production similar to wildtype mice (38). In contrast, Th2 responses in IL-21R-/- mice are compromised. IL-21R-/- CD4+ T cells migrate poorly to the site of infection and produce fewer Th2 cytokines in response to infection with the extracellular parasite *Nippostrongylus Brasiliensis.* These findings imply that IL-21 is necessary for potent Th2 responses but does not play a role in the polarisation towards Th1 responses (31). A predominant role for IL-21 in Th2, rather than Th1, responses is also supported by studies demonstrating an inhibitory role of IL-21 on the generation of Th1 cells *in vitro*. Exposure of naive Th cell precursors to IL-21 inhibited IFN-γ production from developing Th1 cells through repression of eomesodermin expression (39).

4.1.2. Th17 cells

T helper cells producing IL-17 (Th17) are a pro-inflammatory Th cell subset that attracts neutrophils and other inflammatory cells to the site of the immune response. Differentiation of Th17 cells is controlled at a transcriptional level by the nuclear orphan receptor RORγt (40). IL-17 production can be induced *in vitro* in response to TGF-β and IL-6 alongside TCR signals (36,37). Th17 cells also produce high levels of IL-21, which can, in turn, enhance production of IL-17 *in vitro* and substitute for IL-6 in Th17 differentiation (36,37,40). Furthermore, reduced production of IL-17 during re-stimulation *in vitro* is observed from mice deficient in the IL-21R following peptide immunization (37). However, it remains unclear whether these results reflect what happens *in vivo*

EAE is a murine autoimmune model characterised by an influx of inflammatory cells into the central nervous system. While IL-21 appears necessary for a Th17 response in EAE in mice lacking the key differentiation factor IL-6-/-, it seems unlikely that IL-21 is critical for Th17 generation either during infection or in an autoimmune setting where IL-6 is abundant. Kopf *et al* have shown that IL-21 signalling is redundant for several types of *in vivo* Th17 responses. IL-21R-/- mice are normally susceptible to EAE and to a similar experimental autoimmune response directed against the myosin protein in the heart tissue (31). In summary, since the results in tissue culture experiments imply that IL-21 actions may be more important during T cell activation only in the absence of other factors such as IL-6 or costimulatory molecules such as ICOSL, it is vital to dissect the actions of IL-21 in *in vivo* settings.

4.1.3. Regulatory T cells

Regulatory T cells (Tregs) are characterized by high levels of expression of the α chain of the IL-2 receptor (CD25) and the transcription factor Foxp3. Tregs are known to suppress the proliferation and effector functions of other T cells, however, a definitive mechanism explaining the suppressive abilities of Tregs *in vivo* remains largely elusive. Nevertheless, studies demonstrating that a reduction in Treg number and function precipitate autoimmunity indicate a critical function of these cells in peripheral tolerance to self-antigens. IL-2 signals through STAT5a/b to up-regulate Foxp3 and, as such, is the primary growth factor for Tregs (41). In contrast, IL-21 predominantly induces STAT3 phosphorylation and evidence suggests that IL-21 may have a negative impact on the function of Tregs. Recent studies have shown that IL-21 inhibits TGF-b–driven differentiation of naïve Th cells into $Foxp3^+$ Tregs (42). However, it remains unclear whether IL-21 exerts its effect directly on Tregs or whether IL-21 renders T cells resistant to Treg-mediated suppression (43,44).

4.2. CD8+ T cells

Compared to the effects of IL-21 on T cells of the CD4+ T cell lineage, the actions of IL-21 on CD8+ T cells appear relatively straightforward. IL-21 promotes the proliferative response of several lymphocyte subsets. However, transgenic over-expression of murine IL-21 shows that IL-21 predominantly causes the expansion of memory $CD8^+$ T cells (13). The accumulation of memory CD8+ T cells in mice with excessive production of IL-21 is likely to be explained by effects on both their proliferation and survival. Studies demonstrating that IL-21 activates the phosphatidylinositol-3 kinase-signaling cascade and boosts CD8+ T cell numbers *in vivo* support a role for IL-21 in CD8+ T cell survival (12,45). In contrast, there is recent evidence for a pro-apoptotic effect of IL-21 on primate CD8+ T cells through the down-regulation of Bcl-2 (46). Whether these conflicting data reflect species-specific differences in responses to IL-21 or differences in the actions of IL-21 on distinct CD8+ T cell subsets remains unclear. Notably, CD8+ T cell development appears normal in mice made genetically deficient in the IL-21R, indicating that other factors can compensate for IL-21 effects on CD8+ T cell growth (38).

The potent effect of IL-21 on CD8+ T cell growth was initially observed as an accelerated outgrowth of CD8+CD4- cells in cultures of mouse thymocytes (1). IL-21 co-stimulates T cell proliferation and numerous studies demonstrate that IL-21 enhances the proliferation, IFN-γ production, and cytotoxic function of CD8+ effector T cells (47,48). In concert with the γ chain cytokines IL-15 and IL-7, IL-21 can promote proliferation and the acquisition of cytotoxic effector functions of CD8+ T cells both *in vitro* and *in vivo* (11,48). The ability of IL-21 to synergize cytokine-mediated proliferation occurs in the absence of TCR signaling, suggesting that IL-21 may play a cooperative role in antigen-independent expansion. In contrast to other γc signalling cytokines such as IL-2 and IL-15, IL-21 does not augment CD8+ T cell proliferation in the absence of stimulation through the TCR or other γc cytokines and an added complexity to the effect of IL-21 on CD8+ T cells is that the proliferative and functional effects of IL-21 appear to differ for naive and memory CD8+ T cells. As noted above, IL-21 can augment the IL-15 or IL-7 induced proliferation of CD8+ memory T cells but has less effect on antigen-dependent proliferation of memory cells. However, IL-21 effectively co-stimulates antigen-driven proliferation of naïve CD8+ T cells (48). Interestingly, IL-

21 added to CD8+ effector T cells from HIV-infected patients upregulates perforin production in the absence of cell activation or proliferation, whereas IL-15-mediated upregulation of perforin occurs only in the presence of proliferation (49). Although beyond the scope of this review, it is important to note that the effects of IL-21 on cytotoxicity have functional consequences *in vivo* demonstrated by a potent anti-tumor ability against a range of tumor targets (4,50-53).

Taken together, these studies define IL-21 as a co-stimulator of T cell proliferation. The co-stimulation of CD8 T cells *in vitro* by IL-21 invokes a distinct modulation of cell surface molecules including sustained expression of the co-stimulatory molecule CD28 (10) which could increase CD8+ T cell responsiveness to antigen (9). However, whether IL-21 operates via co-stimulatory molecules (such as CD28) or whether IL-21 delivers its own, unique, co-stimulatory signal to T cells remain relevant unanswered questions.

4.3. Opposing effects of IL-21 on B cells

IL-21 applied to purified B cells from humans or mice yields unremarkable effects but can induce interesting paradoxical effects when combined with other stimuli. IL-21 can either deliver co-stimulation to B cells or induce B cell apoptosis - depending on the type of activating signals that accompany it. These implications are important for autoimmunity: costimulation amplifies signals through the BCR to facilitate autoantibody production, whereas apoptosis is necessary for the removal of both the self-reactive clones that could target tissues for destruction and antibody-producing cells generated during infection (which could cause chronic formation of inflammatory immune complexes).

IL-21R is expressed on both immature and mature B cells, and is upregulated further upon antigen binding or stimulation by TLR ligands (25). IL-21 can also increase human B cell proliferation induced by ligation of CD40 *in vitro* (1,6,7,25). However, when paired with BCR stimulation, IL-21 reduces the response and inhibits proliferation of murine B cells induced by TLR ligands - namely LPS and CpG - by inducing apoptosis (25,54,55). These different outcomes on B cells led to the hypothesis that while IL-21 could enhance antigen specific responses, it could also induce apoptosis for B cells that lack T cell help provided by molecules such as CD40L, thus protecting against inappropriate B cell activation (7). However, studies on purified murine splenic B cells demonstrated the induction of apoptosis by IL-21 combined with both mitogens, such as LPS, and T dependent stimulation (54). The addition of IL-21 to these cultures down-regulated the anti-apoptotic molecules BCL-2 and BCL-x_L. Taken together, these divergent survival outcomes may reflect different composition of cell samples in terms of maturity and degree of activation of B cells purified from the spleen of mice compared to those circulating in human blood (54).

The evidence for both an activating and regulatory role of IL-21 for B cells is exemplified by the introduction of a plasmid inducing constitutive high levels of human IL-21 in mice. Increased levels of IL-21 resulted in reduced numbers of mature B cells possibly due to apoptosis of resting cells or, alternatively, a developmental defect. Conversely, there was an increased class-switch to the IgG1 antibody isotype, and upregulation of the germinal center B cell transcription factor Bcl-6 (5). Excessive levels of IL-21 also pushed B cell differentiation toward plasma cells *in vitro* as well as *in vivo* via Blimp-1 upregulation, indicating that some of these effects could be attributed to an intrinsic B cell signaling response to IL-21. While an excess of IL-21 promotes plasma cell differentiation, IL-21R-/- mice have normal circulating mature B cell populations that proliferate normally to mitogens (5). Perhaps surprisingly - considering that IL-21 can induce dramatic B cell outcomes *in vitro* - it seems that IL-21 may not be essential for B cell development and function *in vivo*, and it probably has many overlapping functions with other common gamma-chain cytokines. One exception to the redundant roles of IL-21 on B cells is IgG1 production, which is heavily dependent on the ability of lymphocytes to respond to IL-21 *in vivo*. However, it remains to be seen if this is a B cell intrinsic effect or due to the effect of IL-21 on T cell help. IL-4 and IL-21 together are responsible for the production of high levels of switched antibodies. Mice made genetically deficient in both cytokines display a severe pan-hypogammaglobulinaemia that is almost identical to X-linked SCID phenotype, implying that in combination, but not individually, IL-21 and IL-4 control antibody production (38).

IL-21 also effects IgE production as demonstrated by increased IgE levels in IL-21R-/- mice after immunization with experimental antigens and after infection with the parasite *Toxoplasma Gondii* (31). In support of this observation, injection of exogenous IL-21 during immunization can inhibit germline IgE heavy chain transcription induced by TLR mitogens *in vivo* (56). The mechanism of this inhibition remains unknown, and consideration for the modulation of IL-21 for therapeutic reduction of harmful self-reactive IgG1 production must acknowledge the risk of possible inverse effects from the dominant isotype (IgE) produced in allergy and atopy (56).

In conclusion, the speculated redundancy of IL-21 with other co-stimulation factors makes this cytokine an attractive therapeutic target for modulating chronic B cell activation in autoimmune disease, perhaps without compromising the adaptive immune system and antibody production as much as some current therapies such as B cell depletion.

4.4. NKT cells and NK cells

NKT cells are a distinct subset of T cells that recognize lipid antigens presented by CD1d. Following activation by CD1d-restricted glycosphingolipid antigens, NKT cells are prolific producer of cytokines which can in turn influence the generation of immune responses. Early studies document an association between decreased numbers and function of NKT cells and the progression of autoimmune disease (57-62). In addition, treatment of type-1 diabetes-prone non-obese diabetic (NOD) mice with α-

galactosylceramide, known to activate NKT cells, reduced the severity of autoimmune diabetes in a CD1-dependent manner (63). More recently, IL-21 was shown to enhance NKT cell survival and increase the proliferation of NKT cells in the response to α-galactosylceramide, and in combination with IL-2 or IL-15 *in vitro*. In addition, NKT cells produced IL-21 and IL-21 was shown to increase granzyme B expression, suggesting autocrine stimulation of NKT cells by IL-21 (3). However, whether IL-21 production by NKT cells negatively or positively impacts on autoimmune disease awaits future studies.

This review focuses on the role of IL-21 in the adaptive arm of the immune response, but IL-21 has well-established effects on innate immune cells. The ability of IL-21 to modulate NK cell function is of interest to autoimmune disease pathogenesis since the interaction between IL-21 and NK cells has been reported to influence the course of EAE (64). IL-21 has been shown to stimulate the production of IFN-inducible genes important in innate immunity and to enhance the cytotoxic activity and IFNγ production in NK cells, but did not support their viability (26, 47). IL-21 also has potent antitumor activity, which has been attributed, in part, to its effects on NK cells (4). Expression of the effector molecules perforin and granzyme A and B was up-regulated in human NK cells by IL-21 at both mRNA and protein levels (65). In contrast, IL-21 blocked IL-15-induced expansion of both NK cells and memory-phenotype CD8+ T cells (47).

5. THE ROLE OF IL-21 IN HUMAN AUTOIMMUNE DISEASES AND LESSONS FROM ANIMAL MODELS

The immune system operates to protect tissues from foreign pathogens. This reaction is inherently self-limiting to prevent chronic tissue damage. Autoimmune disease occurs when the immune system damages host tissues following mechanical injury or infection or by unknown triggering factors.

IL-21 can contribute to the development of autoimmune diseases in animal models of SLE, EAE and RA (2,4), although the mechanisms underlying its role remain obscure.

5.1. Systemic Lupus Erythematosus (SLE)

A myriad of different genetic and environmental factors contribute to SLE. A genetic association between two common single nucleotide polymorphisms in the *IL-21* gene has been recently established in SLE, and its known effects on plasma cell differentiation and T cell activation make IL-21 an interesting focus in SLE studies (66). Serum levels of IL-21 are high in patients with SLE and correlate with severity of the disease, although the clinical significance of these findings remains unknown (67). The identification of an autoimmune regulator gene, named *Roquin*, and studies on the knockout mouse *sanroque* also implicated IL-21 in a lupus like disease (68). Without the action of the ubiquitin ligase *roquin,* these mice displayed dysregulated expression of the co-stimulator molecule ICOS, excessive generation of CD4+ T_{FH} cells and increased IL-21 production, leading to spontaneous formation of germinal centers producing auto-reactive antibodies (68). It has not been established whether excessive IL-21 production is necessary for pathogenic germinal center formation in SLE and similar diseases, or whether it is the result of other factors causing activation of diverse CD4+ T helper cell clones that produce IL-21. In a human study with implications for antibody mediated autoimmune diseases such as SLE, the application of CD40L and IL-21 to mimic co-stimulation by activated CD4+ T cells was found to increase the viability of purified tonsillar germinal center B cells. These cells are responsible for the generation of the high affinity, class switched antibody that can be harmful in chronic activation of B cells by self-antigen (7).

Elevated IL-21 production has been reported in a genetic model of SLE, the BXSB-Yaa mouse strain (5). Promising therapeutic studies indicate that neutralisation of circulating IL-21 can delay the progression of disease in BXSB-Yaa mice, with increased survival in treatment groups and lower levels of serum anti-DNA antibodies. Intriguingly, it appears that sequestration of excessive IL-21 may have a dual effect in that it reduces inappropriate activation of self-reactive B cell clones as well as inhibits protective processes mediated by CD8+ suppressor cells that require IL-21 (69).

The combination of pro- and anti-inflammatory effects of IL-21 on various immune cells will likely challenge researchers aiming to develop studies with clinical benefits from manipulation of IL-21 signalling in SLE.

5.2. Rheumatoid Arthritis (RA)

Rheumatoid arthritis is a humoral autoimmune disease in which tissue damage of the joints is excited by the buildup of large, pro-inflammatory immune complexes. Antibody complexes recruit a cellular infiltrate to the synovial lining, consisting of T and B cells as well as macrophages, neutrophils and fibroblasts. The initiation of this inappropriate response against unknown self-antigens in the joint are not as well understood as the downstream pathological cycle of cellular inflammation. IL-21 plays a critical role supporting antibody production and there have been several recent studies investigating its role in RA. Just as the Idd3 genetic locus, containing the genes for *IL-21* and *IL-2*, has been linked to higher incidence of T1D (discussed below), SNPs in the region have also been confirmed in linkage studies with RA (70), leading to the hypothesis that this region may confer a general genetic susceptibility to autoimmune diseases that share some common mechanisms in aetiology.

The analyses of inflamed synovial membranes by both histological and molecular biology methods reveals that IL-21 and its receptor are overexpressed in RA patients and, despite an ambiguous role in the disease process, studies of therapeutic modulation of IL-21 in RA have been initiated (71,72). Treatment with IL-21R conjugated to an Fc fragment reduces clinical outcomes in models of RA in both rats and mice, perhaps by reducing IgG1 titers in these animals and the subsequent release of harmful cytokines

from the cells encountering immune complexes in the joints (73).

5.3. Multiple Sclerosis (MS) and Experimental Allergic Encephalitis (EAE)

MS is a disease of the central nervous system (CNS) that occurs when the immune system attacks the protective myelin coating around nerve cells with resulting inflammation disrupting nerve signals. A genetic association of IL-21 with MS has been proposed but remains to be established (74). Animal models of CNS inflammation, such as EAE are used to study MS and offer direct support for IL-21 contributing to the disease process. EAE is induced by immunization of mice with myelin antigen in the presence of adjuvants. A recent study suggests that IL-21 acts predominantly on the priming of T cell responses to myelin antigen (64). The administration of IL-21 to mice before the induction of disease increases disease severity - characterized by increased numbers of inflammatory cells in the central nervous system (64). In contrast, IL-21 did not affect disease severity when administered after the disease has been initiated. A role for NK cells is suggested, since depletion of NK cells before disease induction abrogates the effect of IL-21 (64).

Th17 cells have a critical role in EAE (75) and recent studies have linked IL-21 to the induction and expansion of the Th17 population and disease severity in EAE (36,37,75). As noted above, Th17 cells produce IL-21 and since IL-21 may support Th17 differentiation but have a negative effect on FoxP3+ Tregs, a potential interplay between Th17 cells and Tregs has been suggested (36,37,42,76-78). In this context, the enhanced autoimmune symptoms in mice injected with IL-21 before the initiation of EAE may be the result of increased numbers of Th17 cells and reduced numbers of Tregs. Conversely, the blockade of IL-21 before and after the induction of EAE has been shown to enhance the influx of inflammatory cells into the CNS (79). Intriguingly, when EAE was induced following adoptive transfer of proteolipid peptide (PLP(139-151))-reactive T cells, the blockade of IL-21 induced the proliferation of these self-reactive T cells and decreased the number and function of Tregs (79). These divergent results may reflect the suppressive effect of IL-21 on dendritic cell function or, alternatively, these models might exploit the agonistic versus antagonistic effects of IL-21 on T cell and NK cell expansion to IL-15, respectively (80,81,47). However, whether these conflicting data for IL-21 in EAE reflect differences in the models, strains of mice used or fundamental distinctions in the role of IL-21 during the course of CNS inflammation await further studies.

5.3. Type-1 diabetes (T1D)

Diabetes is the name given to disorders in which the body has trouble regulating its blood glucose levels. Type 1 or insulin-dependent diabetes mellitus (T1D) in humans is a chronic autoimmune disease involving multiple genes that are under strong environmental influence and promote the destruction of the insulin-producing β cells in the islets of Langerhans of the pancreas. As in humans, the target insulin-producing β cells in the pancreas of the non-obese diabetic (NOD) mouse are attacked and destroyed by activated T cells. The chronic inflammation of the islets of Langerhans in the T1D-prone NOD mouse begins at 4–6 weeks of age. The infiltrate consists of T cells, B cells, dendritic cells and macrophages, and begins as an apparently inconsequential inflammation around the islets (peri-insulitis) progressing to insulitis over the next 2-3 months and culminating in clinical T1D (82-84). Experiments utilizing knockout mice and antibody depletion indicate that all of these cell types have a part in the T1D autoimmune disease process. The transition from mild peri-insulitis to the destructive form of insulitis is a critical checkpoint in the progression of autoimmune diabetes (85,86). This transition is necessary for the expression of clinical diabetes and it is defined by the movement of immune cells into the islet, a subsequent loss of insulin production from β cells and a reduction in the β cell mass (85,86). Determining the factors and conditions that fuel the destructive transformation of pancreatic islet inflammation are vital questions for a better understanding of the pathogenesis of T1D and possible identification of better therapeutic targets.

There are a number of genetic loci that are associated with susceptibility to autoimmune diabetes in humans and in the NOD mouse model. One locus that has garnered much interest lies on chromosome 4 in humans and chromosome 3 (Idd3) in mice has a major impact on insulitis and encodes the cytokines IL-2 and IL-21 (87,88). Reduced expression of the NOD IL-2 allele has been demonstrated and is thought to explain much of the effect of the Idd3 susceptibility locus (89). In contrast, the relative importance of IL-21 in the Idd3 locus effect remains controversial and studies are confounded by the fact that IL-2 and IL-21 are in strong linkage disequilibrium, such that a haplotype of IL-2 is inherited with the same haplotype of IL-21 (90). The NOD mouse has elevated levels of IL-21 but whether the effect of IL-21 on T1D pathogenesis is due to the IL-21 gene itself or is secondary to a defect in IL-2 production remains unclear (91). Genetic linkage studies support an association of the IL2/IL21 region on human chromosome 4q27 with T1D and this finding has been supported by a recent study in both T1D and RA patients as well as in a recent genome wide association study of celiac disease (70,92,93). In addition, polymorphic variants of IL-21 and its receptor have been associated with genetic susceptibility to T1D in an additive manner (94).

Functional analyses are lacking in regard to the function of IL-21 in T1D. One likely target of IL-21-necessary for the development of diabetes in the NOD model - is the CD8+ T cell. CD8+ T cells proliferate to an increased extent in association with elevated IL-21 levels in the lymphoid organs of NOD mice (91). As discussed above, IL-21 also has a profound effect on B cell proliferation and antibody production and studies showing that a genetic defect in B cells prevents diabetes indicate that B cells play a part in T1D in the NOD mouse. The

exact role of B cells in T1D pathogenesis remains unclear, however, studies point to their role as antigen-presenting cells (95).

6. CONCLUDING REMARKS

Identifying the cytokines that participate in the development of autoaggressive T cell responses and the mechanisms underlying their involvement in the disease processes will enable the design of more specific immunotherapies for the treatment of autoimmune disease. IL-21 is an interesting candidate for immunosuppressive therapy because of its involvement in the generation of both cytotoxic and antibody responses against self-antigen. Blockade of the IL-21/IL-21R interactions prevents or limits the development of a range of autoimmune diseases. These studies demonstrate that therapeutic inhibition of IL-21 actions have beneficial anti-inflammatory effects in autoimmunity including the reduction of autoantibody production and the prevention of the differentiation of self antigen destructive T cells.

The effects of IL-21 on T and B cell mediated autoimmune diseases are likely to reflect its ability to co-stimulate the activation of antigen-driven responses. However, specific mechanisms explaining a qualitative or quantitative effect of IL-21 on immune responses remain unclear, and the several attempts to define a role for IL-21 in the immune system are confounded by a considerable overlap of the actions of the γc family of cytokines. For instance, it remains to be established whether IL-21 acts primarily on naïve or memory CD8+ T cells. Current data indicate that IL-21 has a critical role in CD8+ T cell responses as well as on the GC reaction to promote antibody responses through its effects on both B cells and CD4+ T cells. However, a number of questions remain that are highly relevant to the pathogenesis of certain autoimmune diseases, including the manner in which IL-21 influences T and B cell differentiation during immunization or infection, and whether IL-21 affects the migration of T cell subsets to lymphoid or tissue sites. The role of IL-21 in Th17 differentiation remains controversial and further analyses of the contribution of IL-21 to T helper cell differentiation will be beneficial to further our understanding of the role of IL-21 in autoimmune disease. Finally, determining whether there is there a lymphoid microenvironment where IL-21 is predominantly produced would better define the context in which IL-21 exerts its effects on the immune system.

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8. REFERENCES

1. Parrish-Novak, J., S. R. Dillon, A. Nelson, A. Hammond, C. Sprecher, J. A. Gross, J. Johnston, K. Madden, W. Xu, J. West, S. Schrader, S. Burkhead, M. Heipel, C. Brandt, J. L. Kuijper, J. Kramer, D. Conklin, S. R. Presnell, J. Berry, F. Shiota, S. Bort, K. Hambly, S. Mudri, C. Clegg, M. Moore, F. J. Grant, C. Lofton-Day, T. Gilbert, F. Rayond, A. Ching, L. Yao, D. Smith, P. Webster, T. Whitmore, M. Maurer, K. Kaushansky, R. D. Holly & D. Foster: Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function. *Nature*, 408, 57-63(2000)

2. Leonard, W. J. & R. Spolski: Interleukin-21: a modulator of lymphoid proliferation, apoptosis and differentiation. *Nat Rev Immunol*, 5, 688-98(2005)

3. Coquet, J. M., K. Kyparissoudis, D. G. Pellicci, G. Besra, S. P. Berzins, M. J. Smyth & D. I. Godfrey: IL-21 is produced by NKT cells and modulates NKT cell activation and cytokine production. *J Immunol*, 178, 2827-34(2007)

4. Spolski, R. & W. J. Leonard: Interleukin-21: Basic Biology and Implications for Cancer and Autoimmunity. *Annu Rev Immunol*(2007)

5. Ozaki, K., R. Spolski, R. Ettinger, H. P. Kim, G. Wang, C. F. Qi, P. Hwu, D. J. Shaffer, S. Akilesh, D. C. Roopenian, H. C. Morse, 3rd, P. E. Lipsky & W. J. Leonard: Regulation of B cell differentiation and plasma cell generation by IL-21, a novel inducer of Blimp-1 and Bcl-6. *J Immunol*, 173, 5361-71(2004)

6. Ettinger, R., G. P. Sims, A. M. Fairhurst, R. Robbins, Y. S. da Silva, R. Spolski, W. J. Leonard & P. E. Lipsky: IL-21 induces differentiation of human naive and memory B cells into antibody-secreting plasma cells. *J Immunol*, 175, 7867-79(2005)

7. Good, K. L., V. L. Bryant & S. G. Tangye: Kinetics of human B cell behavior and amplification of proliferative responses following stimulation with IL-21. *J Immunol*, 177, 5236-47(2006)

8. Bryant, V. L., C. S. Ma, D. T. Avery, Y. Li, K. L. Good, L. M. Corcoran, R. de Waal Malefyt & S. G. Tangye: Cytokine-Mediated Regulation of Human B Cell Differentiation into Ig-Secreting Cells: Predominant Role of IL-21 Produced by CXCR5+ T Follicular Helper Cells. *J Immunol*, 179, 8180-90(2007)

9. Li, Y., M. Bleakley & C. Yee: IL-21 influences the frequency, phenotype, and affinity of the antigen-specific CD8 T cell response. *J Immunol*, 175, 2261-9(2005)

10. Alves, N. L., F. A. Arosa & R. A. van Lier: IL-21 sustains CD28 expression on IL-15-activated human naive CD8+ T cells. *J Immunol*, 175, 755-62(2005)

11. Zeng, R., R. Spolski, S. E. Finkelstein, S. Oh, P. E. Kovanen, C. S. Hinrichs, C. A. Pise-Masison, M. F. Radonovich, J. N. Brady, N. P. Restifo, J. A. Berzofsky & W. J. Leonard: Synergy of IL-21 and IL-15 in regulating CD8+ T cell expansion and function. *J Exp Med*, 201, 139-48(2005)

12. Moroz, A., C. Eppolito, Q. Li, J. Tao, C. H. Clegg & P. A. Shrikant: IL-21 enhances and sustains CD8+ T cell responses to achieve durable tumor immunity: comparative evaluation of IL-2, IL-15, and IL-21. *J Immunol*, 173, 900-9(2004)

13. Allard, E. L., M. P. Hardy, J. Leignadier, M. Marquis, J. Rooney, D. Lehoux & N. Labrecque: Overexpression of IL-21 promotes massive CD8(+) memory T cell accumulation. *Eur J Immunol*, 37, 3069-77(2007)

14. Zeng, R., R. Spolski, E. Casas, W. Zhu, D. E. Levy & W. J. Leonard: The molecular basis of IL-21-mediated proliferation. *Blood*(2007)

15. Ozaki, K., K. Kikly, D. Michalovich, P. R. Young & W. J. Leonard: Cloning of a type I cytokine receptor most related to the IL-2 receptor beta chain. *Proc Natl Acad Sci U S A*, 97, 11439-44.(2000)
16. Asao, H., C. Okuyama, S. Kumaki, N. Ishii, S. Tsuchiya, D. Foster & K. Sugamura: Cutting edge: the common gamma-chain is an indispensable subunit of the IL-21 receptor complex. *J Immunol*, 167, 1-5(2001)
17. Leonard, W. J.: X-linked severe combined immunodeficiency: from molecular cause to gene therapy within seven years. *Mol Med Today*, 6, 403-7(2000)
18. Ueda, M., K. Imada, A. Imura, H. Koga, M. Hishizawa & T. Uchiyama: Expression of functional interleukin-21 receptor on adult T-cell leukaemia cells. *Br J Haematol*, 128, 169-76(2005)
19. Chtanova, T., S. G. Tangye, R. Newton, N. Frank, M. R. Hodge, M. S. Rolph & C. R. Mackay: T follicular helper cells express a distinctive transcriptional profile, reflecting their role as non-Th1/Th2 effector cells that provide help for B cells. *J Immunol*, 173, 68-78(2004)
20. Habib, T., S. Senadheera, K. Weinberg & K. Kaushansky: The common gamma chain (gamma c) is a required signaling component of the IL-21 receptor and supports IL-21-induced cell proliferation via JAK3. *Biochemistry*, 41, 8725-31(2002)
21. Parrish-Novak, J., D. C. Foster, R. D. Holly & C. H. Clegg: Interleukin-21 and the IL-21 receptor: novel effectors of NK and T cell responses. *J Leukoc Biol*, 72, 856-63(2002)
22. Kim, H. P., L. L. Korn, A. M. Gamero & W. J. Leonard: Calcium-dependent activation of interleukin-21 gene expression in T cells. *J Biol Chem*, 280, 25291-7(2005)
23. Wu, Z., H. P. Kim, H. H. Xue, H. Liu, K. Zhao & W. J. Leonard: Interleukin-21 receptor gene induction in human T cells is mediated by T-cell receptor-induced Sp1 activity. *Mol Cell Biol*, 25, 9741-52(2005)
24. Holm, C., C. G. Nyvold, S. R. Paludan, A. R. Thomsen & M. Hokland: Interleukin-21 mRNA expression during virus infections. *Cytokine*, 33, 41-5(2006)
25. Jin, H., R. Carrio, A. Yu & T. R. Malek: Distinct activation signals determine whether IL-21 induces B cell costimulation, growth arrest, or Bim-dependent apoptosis. *J Immunol*, 173, 657-65(2004)
26. Strengell, M., T. Sareneva, D. Foster, I. Julkunen & S. Matikainen: IL-21 up-regulates the expression of genes associated with innate immunity and Th1 response. *J Immunol*, 169, 3600-5(2002)
27. Strengell, M., S. Matikainen, J. Siren, A. Lehtonen, D. Foster, I. Julkunen & T. Sareneva: IL-21 in synergy with IL-15 or IL-18 enhances IFN-gamma production in human NK and T cells. *J Immunol*, 170, 5464-9(2003)
28. Fina, D., M. Sarra, R. Caruso, G. Del Vecchio Blanco, F. Pallone, T. T. Macdonald & G. Monteleone: Interleukin-21 Contributes To The Mucosal T Helper Cell Type 1 Response In Celiac Disease. *Gut*(2007)
29. Wurster, A. L., V. L. Rodgers, A. R. Satoskar, M. J. Whitters, D. A. Young, M. Collins & M. J. Grusby: Interleukin 21 is a T helper (Th) cell 2 cytokine that specifically inhibits the differentiation of naive Th cells into interferon gamma-producing Th1 cells. *J Exp Med*, 196, 969-77(2002)
30. Mehta, D. S., A. L. Wurster, A. S. Weinmann & M. J. Grusby: NFATc2 and T-bet contribute to T-helper-cell-subset-specific regulation of IL-21 expression. *Proc Natl Acad Sci U S A*, 102, 2016-21(2005)
31. Frohlich, A., B. J. Marsland, I. Sonderegger, M. Kurrer, M. R. Hodge, N. L. Harris & M. Kopf: IL-21 receptor signaling is integral to the development of Th2 effector responses in vivo. *Blood*, 109, 2023-31(2007)
32. King, C., S. G. Tangye & C. R. Mackay: T Follicular Helper (T(FH)) Cells in Normal and Dysregulated Immune Responses. *Annu Rev Immunol*(2008)
33. Rasheed, A. U., H. P. Rahn, F. Sallusto, M. Lipp & G. Muller: Follicular B helper T cell activity is confined to CXCR5(hi)ICOS(hi) CD4 T cells and is independent of CD57 expression. *Eur J Immunol.*, 36, 1892-903.(2006)
34. Schaerli, P., K. Willimann, A. B. Lang, M. Lipp, P. Loetscher & B. Moser: CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. *J Exp Med*, 192, 1553-62(2000)
35. Pesce, J., M. Kaviratne, T. R. Ramalingam, R. W. Thompson, J. F. Urban, Jr., A. W. Cheever, D. A. Young, M. Collins, M. J. Grusby & T. A. Wynn: The IL-21 receptor augments Th2 effector function and alternative macrophage activation. *J Clin Invest*, 116, 2044-55(2006)
36. Nurieva, R., X. O. Yang, G. Martinez, Y. Zhang, A. D. Panopoulos, L. Ma, K. Schluns, Q. Tian, S. S. Watowich, A. M. Jetten & C. Dong: Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. *Nature*(2007)
37. Korn, T., E. Bettelli, W. Gao, A. Awasthi, A. Jager, T. B. Strom, M. Oukka & V. K. Kuchroo: IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. *Nature*(2007)
38. Ozaki, K., R. Spolski, C. G. Feng, C. F. Qi, J. Cheng, A. Sher, H. C. Morse, 3rd, C. Liu, P. L. Schwartzberg & W. J. Leonard: A critical role for IL-21 in regulating immunoglobulin production. *Science*, 298, 1630-4(2002)
39. Suto, A., A. L. Wurster, S. L. Reiner & M. J. Grusby: IL-21 inhibits IFN-gamma production in developing Th1 cells through the repression of Eomesodermin expression. *J Immunol*, 177, 3721-7(2006)
40. Zhou, L., Ivanov, II, R. Spolski, R. Min, K. Shenderov, T. Egawa, D. E. Levy, W. J. Leonard & D. R. Littman: IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat Immunol*, 8, 967-74(2007)
41. Yao, Z., Y. Kanno, M. Kerenyi, G. Stephens, L. Durant, W. T. Watford, A. Laurence, G. W. Robinson, E. M. Shevach, R. Moriggl, L. Hennighausen, C. Wu & J. J. O'Shea: Nonredundant roles for Stat5a/b in directly regulating Foxp3. *Blood*, 109, 4368-75(2007)
42. Fantini, M. C., A. Rizzo, D. Fina, R. Caruso, C. Becker, M. F. Neurath, T. T. Macdonald, F. Pallone & G. Monteleone: IL-21 regulates experimental colitis by modulating the balance between T(reg) and Th17 cells. *Eur J Immunol*, 37, 3155-63(2007)
43. Li, Y. & C. Yee: IL-21 mediated Foxp3 suppression leads to enhanced generation of antigen-specific CD8+ cytotoxic T lymphocytes. *Blood*, 111, 229-35(2008)
44. Peluso, I., M. C. Fantini, D. Fina, R. Caruso, M. Boirivant, T. T. MacDonald, F. Pallone & G. Monteleone: IL-21 counteracts the regulatory T cell-mediated

suppression of human CD4+ T lymphocytes. *J Immunol*, 178, 732-9(2007)
45. Ostiguy, V., E. L. Allard, M. Marquis, J. Leignadier & N. Labrecque: IL-21 promotes T lymphocyte survival by activating the phosphatidylinositol-3 kinase signaling cascade. *J Leukoc Biol*, 82, 645-56(2007)
46. Barker, B. R., J. G. Parvani, D. Meyer, A. S. Hey, K. Skak & N. L. Letvin: IL-21 induces apoptosis of antigen-specific CD8+ T lymphocytes. *J Immunol*, 179, 3596-603(2007)
47. Kasaian, M. T., M. J. Whitters, L. L. Carter, L. D. Lowe, J. M. Jussif, B. Deng, K. A. Johnson, J. S. Witek, M. Senices, R. F. Konz, A. L. Wurster, D. D. Donaldson, M. Collins, D. A. Young & M. J. Grusby: IL-21 limits NK cell responses and promotes antigen-specific T cell activation: a mediator of the transition from innate to adaptive immunity. *Immunity*, 16, 559-69(2002)
48. Liu, S., G. Lizee, Y. Lou, C. Liu, W. W. Overwijk, G. Wang & P. Hwu: IL-21 synergizes with IL-7 to augment expansion and anti-tumor function of cytotoxic T cells. *Int Immunol*, 19, 1213-21(2007)
49. White, L., S. Krishnan, N. Strbo, H. Liu, M. A. Kolber, M. G. Lichtenheld, R. N. Pahwa & S. Pahwa: Differential effects of IL-21 and IL-15 on perforin expression, lysosomal degranulation, and proliferation in CD8 T cells of patients with human immunodeficiency virus-1 (HIV). *Blood*, 109, 3873-80(2007)
50. Ma, H. L., M. J. Whitters, R. F. Konz, M. Senices, D. A. Young, M. J. Grusby, M. Collins & K. Dunussi-Joannopoulos: IL-21 activates both innate and adaptive immunity to generate potent antitumor responses that require perforin but are independent of IFN-gamma. *J Immunol*, 171, 608-15(2003)
51. Cretney, E., K. Takeda & M. J. Smyth: Cancer: novel therapeutic strategies that exploit the TNF-related apoptosis-inducing ligand (TRAIL)/TRAIL receptor pathway. *Int J Biochem Cell Biol*, 39, 280-6(2007)
52. Yoon, J. S., S. M. Newton, M. Wysocka, A. B. Troxel, S. D. Hess, S. K. Richardson, J. H. Lin, B. M. Benoit, M. Kasprzycka, M. A. Wasik & A. H. Rook: IL-21 Enhances Antitumor Responses without Stimulating Proliferation of Malignant T Cells of Patients with Sezary Syndrome. *J Invest Dermatol*(2007)
53. di Carlo, E., D. de Totero, T. Piazza, M. Fabbi & S. Ferrini: Role of IL-21 in immune-regulation and tumor immunotherapy. *Cancer Immunol Immunother*, 56, 1323-34(2007)
54. Mehta, D. S., A. L. Wurster, M. J. Whitters, D. A. Young, M. Collins & M. J. Grusby: IL-21 induces the apoptosis of resting and activated primary B cells. *J Immunol*, 170, 4111-8(2003)
55. Jin, H. & T. R. Malek: Redundant and unique regulation of activated mouse B lymphocytes by IL-4 and IL-21. *J Leukoc Biol*, 80, 1416-23(2006)
56. Suto, A., H. Nakajima, K. Hirose, K. Suzuki, S. Kagami, Y. Seto, A. Hoshimoto, Y. Saito, D. C. Foster & I. Iwamoto: Interleukin 21 prevents antigen-induced IgE production by inhibiting germ line C(epsilon) transcription of IL-4-stimulated B cells. *Blood*, 100, 4565-73(2002)
57. Godfrey, D. I., S. J. Kinder, P. Silvera & A. G. Baxter: Flow cytometric study of T cell development in NOD mice reveals a deficiency in alphabetaTCR+CDR-CD8- thymocytes. *J Autoimmun*, 10, 279-85.(1997)
58. Baxter, A. G., S. J. Kinder, K. J. Hammond, R. Scollay & D. I. Godfrey: Association between alphabetaTCR+CD4-CD8- T-cell deficiency and IDDM in NOD/Lt mice. *Diabetes*, 46, 572-82.(1997)
59. Falcone, M., B. Yeung, L. Tucker, E. Rodriguez & N. Sarvetnick: A defect in interleukin 12-induced activation and interferon gamma secretion of peripheral natural killer T cells in nonobese diabetic mice suggests new pathogenic mechanisms for insulin-dependent diabetes mellitus. *J Exp Med*, 190, 963-72.(1999)
60. Lehuen, A., O. Lantz, L. Beaudoin, V. Laloux, C. Carnaud, A. Bendelac, J. F. Bach & R. C. Monteiro: Overexpression of natural killer T cells protects Valpha14-Jalpha281 transgenic nonobese diabetic mice against diabetes. *J Exp Med*, 188, 1831-9.(1998)
61. Laloux, V., L. Beaudoin, D. Jeske, C. Carnaud & A. Lehuen: NK T cell-induced protection against diabetes in V alpha 14-J alpha 281 transgenic nonobese diabetic mice is associated with a Th2 shift circumscribed regionally to the islets and functionally to islet autoantigen. *J Immunol*, 166, 3749-56.(2001)
62. Carnaud, C., J. Gombert, O. Donnars, H. Garchon & A. Herbelin: Protection against diabetes and improved NK/NKT cell performance in NOD.NK1.1 mice congenic at the NK complex. *J Immunol*, 166, 2404-11.(2001)
63. Wang, B., Y. B. Geng & C. R. Wang: CD1-restricted NK T cells protect nonobese diabetic mice from developing diabetes. *J Exp Med*, 194, 313-20.(2001)
64. Vollmer, T. L., R. Liu, M. Price, S. Rhodes, A. La Cava & F. D. Shi: Differential effects of IL-21 during initiation and progression of autoimmunity against neuroantigen. *J Immunol*, 174, 2696-701(2005)
65. Skak, K., K. S. Frederiksen & D. Lundsgaard: Interleukin-21 activates human natural killer cells and modulates their surface receptor expression. *Immunology*(2007)
66. Sawalha, A. H., K. M. Kaufman, J. A. Kelly, A. J. Adler, T. Aberle, J. Kilpatrick, E. K. Wakeland, Q. Z. Li, A. E. Wandstrat, D. S. Karp, J. A. James, J. T. Merrill, P. Lipsky & J. B. Harley: Genetic association of IL-21 polymorphisms with systemic lupus erythematosus. *Ann Rheum Dis*(2007)
67. Wang, X. F., S. L. Yuan, L. Jiang, X. L. Zhang, S. F. Li, Y. Guo, C. L. Wu & J. J. Chen: [Changes of serum BAFF and IL-21 levels in patients with systemic lupus erythematosus and their clinical significance.]. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi*, 23, 1041-2(2007)
68. Vinuesa, C. G., M. C. Cook, C. Angelucci, V. Athanasopoulos, L. Rui, K. M. Hill, D. Yu, H. Domaschenz, B. Whittle, T. Lambe, I. S. Roberts, R. R. Copley, J. I. Bell, R. J. Cornall & C. C. Goodnow: A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. *Nature*, 435, 452-8.(2005)
69. Bubier, J. A., S. M. Bennett, T. J. Sproule, B. L. Lyons, S. Olland, D. A. Young & D. C. Roopenian: Treatment of BXSB-Yaa mice with IL-21R-Fc fusion protein minimally attenuates systemic lupus erythematosus. *Ann N Y Acad Sci*, 1110, 590-601(2007)

70. Zhernakova, A., B. Z. Alizadeh, M. Bevova, M. A. van Leeuwen, M. J. Coenen, B. Franke, L. Franke, M. D. Posthumus, D. A. van Heel, G. van der Steege, T. R. Radstake, P. Barrera, B. O. Roep, B. P. Koeleman & C. Wijmenga: Novel association in chromosome 4q27 region with rheumatoid arthritis and confirmation of type 1 diabetes point to a general risk locus for autoimmune diseases. *Am J Hum Genet*, 81, 1284-8(2007)
71. Li, J., W. Shen, K. Kong & Z. Liu: Interleukin-21 induces T-cell activation and proinflammatory cytokine secretion in rheumatoid arthritis. *Scand J Immunol*, 64, 515-22(2006)
72. Jungel, A., J. H. Distler, M. Kurowska-Stolarska, C. A. Seemayer, R. Seibl, A. Forster, B. A. Michel, R. E. Gay, F. Emmrich, S. Gay & O. Distler: Expression of interleukin-21 receptor, but not interleukin-21, in synovial fibroblasts and synovial macrophages of patients with rheumatoid arthritis. *Arthritis Rheum*, 50, 1468-76(2004)
73. Young, D. A., M. Hegen, H. L. Ma, M. J. Whitters, L. M. Albert, L. Lowe, M. Senices, P. W. Wu, B. Sibley, Y. Leathurby, T. P. Brown, C. Nickerson-Nutter, J. C. Keith, Jr. & M. Collins: Blockade of the interleukin-21/interleukin-21 receptor pathway ameliorates disease in animal models of rheumatoid arthritis. *Arthritis Rheum*, 56, 1152-63(2007)
74. Forte, G. I., P. Ragonese, G. Salemi, L. Scola, G. Candore, M. D'Amelio, A. Crivello, N. Di Benedetto, D. Nuzzo, A. Giacalone, D. Lio & C. Caruso: Search for genetic factors associated with susceptibility to multiple sclerosis. *Ann N Y Acad Sci*, 1067, 264-9(2006)
75. Chen, Z. & J. J. O'Shea: Th17 cells: a new fate for differentiating helper T cells. *Immunol Res*(2008)
76. Ivanov, II, L. Zhou & D. R. Littman: Transcriptional regulation of Th17 cell differentiation. *Semin Immunol*(2007)
77. Wei, L., A. Laurence, K. M. Elias & J. O'Shea J: IL-21 is produced by TH17 cells and drives IL-17 production in a STAT3-dependent manner. *J Biol Chem*(2007)
78. Bettelli, E., M. Oukka & V. K. Kuchroo: T(H)-17 cells in the circle of immunity and autoimmunity. *Nat Immunol*, 8, 345-50(2007)
79. Piao, W. H., Y. H. Jee, R. L. Liu, S. W. Coons, M. Kala, M. Collins, D. A. Young, D. I. Campagnolo, T. L. Vollmer, X. F. Bai, A. La Cava & F. D. Shi: IL-21 modulates CD4+ CD25+ regulatory T-cell homeostasis in experimental autoimmune encephalomyelitis. *Scand J Immunol*, 67, 37-46(2008)
80. Strengell, M., A. Lehtonen, S. Matikainen & I. Julkunen: IL-21 enhances SOCS gene expression and inhibits LPS-induced cytokine production in human monocyte-derived dendritic cells. *J Leukoc Biol*, 79, 1279-85(2006)
81. Brandt, K., S. Bulfone-Paus, A. Jenckel, D. C. Foster, R. Paus & R. Ruckert: Interleukin-21 inhibits dendritic cell-mediated T cell activation and induction of contact hypersensitivity in vivo. *J Invest Dermatol*, 121, 1379-82(2003)
82. Bach, J. F. & D. Mathis: The NOD mouse. *Res Immunol*, 148, 285-6(1997)
83. Green, E. A. & R. A. Flavell: The initiation of autoimmune diabetes. *Curr Opin Immunol*, 11, 663-9(1999)
84. Ludewig, B., B. Odermatt, S. Landmann, H. Hengartner & R. M. Zinkernagel: Dendritic cells induce autoimmune diabetes and maintain disease via de novo formation of local lymphoid tissue. *J Exp Med*, 188, 1493-501(1998)
85. Andre-Schmutz, I., C. Hindelang, C. Benoist & D. Mathis: Cellular and molecular changes accompanying the progression from insulitis to diabetes. *Eur J Immunol*, 29, 245-55(1999)
86. Gazda, L. S., B. Charlton & K. J. Lafferty: Diabetes results from a late change in the autoimmune response of NOD mice. *J Autoimmun*, 10, 261-70(1997)
87. Denny, P., C. J. Lord, N. J. Hill, J. V. Goy, E. R. Levy, P. L. Podolin, L. B. Peterson, L. S. Wicker, J. A. Todd & P. A. Lyons: Mapping of the IDDM locus Idd3 to a 0.35-cM interval containing the interleukin-2 gene. *Diabetes*, 46, 695-700.(1997)
88. Todd, J. A. & L. S. Wicker: Genetic protection from the inflammatory disease type 1 diabetes in humans and animal models. *Immunity*, 15, 387-95.(2001)
89. Yamanouchi, J., D. Rainbow, P. Serra, S. Howlett, K. Hunter, V. E. Garner, A. Gonzalez-Munoz, J. Clark, R. Veijola, R. Cubbon, S. L. Chen, R. Rosa, A. M. Cumiskey, D. V. Serreze, S. Gregory, J. Rogers, P. A. Lyons, B. Healy, L. J. Smink, J. A. Todd, L. B. Peterson, L. S. Wicker & P. Santamaria: Interleukin-2 gene variation impairs regulatory T cell function and causes autoimmunity. *Nat Genet*, 39, 329-37(2007)
90. Ikegami, H., T. Fujisawa, S. Makino & T. Ogihara: Congenic mapping and candidate sequencing of susceptibility genes for Type 1 diabetes in the NOD mouse. *Ann N Y Acad Sci*, 1005, 196-204(2003)
91. King, C., A. Ilic, K. Koelsch & N. Sarvetnick: Homeostatic expansion of T cells during immune insufficiency generates autoimmunity. *Cell*, 117, 265-77(2004)
92. Todd, J. A., N. M. Walker, J. D. Cooper, D. J. Smyth, K. Downes, V. Plagnol, R. Bailey, S. Nejentsev, S. F. Field, F. Payne, C. E. Lowe, J. S. Szeszko, J. P. Hafler, L. Zeitels, J. H. Yang, A. Vella, S. Nutland, H. E. Stevens, H. Schuilenburg, G. Coleman, M. Maisuria, W. Meadows, L. J. Smink, B. Healy, O. S. Burren, A. A. Lam, N. R. Ovington, J. Allen, E. Adlem, H. T. Leung, C. Wallace, J. M. Howson, C. Guja, C. Ionescu-Tirgoviste, M. J. Simmonds, J. M. Heward, S. C. Gough, D. B. Dunger, L. S. Wicker & D. G. Clayton: Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. *Nat Genet*, 39, 857-64(2007)
93. Van Heel, D. A., L. Franke, K. A. Hunt, R. Gwilliam, A. Zhernakova, M. Inouye, M. C. Wapenaar, M. C. Barnardo, G. Bethel, G. K. Holmes, C. Feighery, D. Jewell, D. Kelleher, P. Kumar, S. Travis, J. R. Walters, D. S. Sanders, P. Howdle, J. Swift, R. J. Playford, W. M. McLaren, M. L. Mearin, C. J. Mulder, R. McManus, R. McGinnis, L. R. Cardon, P. Deloukas & C. Wijmenga: A genome-wide association study for celiac disease identifies risk variants in the region harboring IL2 and IL21. *Nat Genet*, 39, 827-9(2007)
94. Asano, K., H. Ikegami, T. Fujisawa, M. Nishino, K. Nojima, Y. Kawabata, S. Noso, Y. Hiromine, A. Fukai & T. Ogihara: Molecular scanning of interleukin-21 gene and

genetic susceptibility to type 1 diabetes. *Hum Immunol*, 68, 384-91(2007)
95. Silveira, P. A., J. Dombrowsky, E. Johnson, H. D. Chapman, D. Nemazee & D. V. Serreze: B cell selection defects underlie the development of diabetogenic APCs in nonobese diabetic mice. *J Immunol.*, 172, 5086-94.(2004)

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