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# Clinical significance and immunobiology of IL-21 in autoimmunity

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ABSTRACT

Interleukin-21 (IL-21), an autocrine cytokine predominantly produced by follicular helper T (Tfh) and T helper 17 (Th17) cells, has been proven to play an important role in the immune system, for example, by promoting proliferation and the development of Tfh and Th17 cells, balancing helper T cell subsets, inducing B cell generation and differentiation into plasma cells, and enhancing the production of immunoglobulin. These effects are mainly mediated by activation of the JAK/STAT, MAPK and PI3K pathways. Some IL-21 target genes, such as B lymphocyte induced maturation protein-1 (Blimp-1), suppressor of cytokine signaling (SOCS), CXCR5 and Bcl-6, play important roles in the immune response. Therefore, IL-21 has been linked to autoimmune diseases. Indeed, IL-21 levels are increased in the peripheral blood and tissues of patients with systematic lupus erythematosus (SLE), rheumatoid arthritis (RA), type 1 diabetes (T1D), immune thrombocytopenia (ITP), primary Sjogren's syndrome (pSS), autoimmune thyroid disease (AITD) and psoriasis. This increased IL-21 even positively associates with Tfh cells, plasma cells, autoantibodies and disease activity in SLE and RA. Additionally, IL-21 has been utilized as a therapeutic target in SLE, RA, T1D and psoriatic mouse models. Profoundly, clinical trials have shown safety and improvement in RA patients. However, tolerance and long-term pharmacodynamics effects with low bioavailability have been found in SLE patients. Therefore, this review aims to summarize the latest progress on IL-21 function and its signaling pathway and discuss the role of IL-21 in the pathogenesis of and therapy for autoimmune diseases, with the hope of providing potential therapeutic and diagnostic strategies for clinical use.

## 1. The history of the discovery of IL-21

IL-21, discovered in 2000, has been identified as a multifunctional cytokine and is principally produced by follicular helper T (Tfh), T helper 17 (Th17), and natural killer (NK) cells [1–3]. As a receptor for IL-21, IL-21R, a class I cytokine heterodimeric receptor, shares a common cytokine receptor γ chain with other cytokine families, including IL-2, IL-4, IL-7, IL-9, and IL-15 [1,4]. IL-21R is mainly expressed on lymphoid cells such as thymocytes, splenocytes, and cells from lymph nodes [1,4–6]. In the past decade, IL-21R has been detected on other nonlymphocytic cells and tissues, including thyroid cells, synovial fibroblasts, and keratinocytes [7–10], suggesting that these nonlymphocytic cells are regulated by IL-21. By ligating with IL-21R, IL-21 can regulate the generation and polarization of T and B cells and profoundly affect the function of Th cells, plasmablasts, plasma cells and cells from germinal centers [8,11–16] via the JAK/STAT, MAPK, and PI3K pathways [4,17,18], which will be elaborated upon in the following sections. Based on the evidence that IL-21 regulates numerous immune cells, it is not surprising that IL-21 plays an important role in autoimmune disorders. Indeed, accumulating evidence has shown that IL-21 plays multifarious roles in autoimmune diseases, allergies, inflammatory diseases and cancers [19–23]. More recently, IL-21 has been suggested to be an especially attractive target in systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), type 1 diabetes (TID) and cancer therapy. In this review, we will focus on the latest progress in studying the role of IL-21 in the pathogenesis of autoimmune disorders and therapies using *anti*-IL-21 or IL-21R blockade and shed a light on IL-21-targeting therapies in the future.

## 2. The source and effects of IL-21

As a type I four-α-helical bundle cytokine, IL-21 was first found to be produced by $CD4^+$ T and NK cells and regulate the proliferation and function of other immune cells [1,2,7]. Subsequently, Tfh cells, Th17, and regulatory T (Treg) cells have been discovered to be the main sources of IL-21 [1,6,24–27]. In recent years, a newly discovered $CD4^+$ T cell subset was also found to express IL-21; this subset, characterized as $PD\text{-}1^+$ $CXCR5^-$ and expressing ICOS, CXCL13 and MAF, was named peripheral helper T (Tph) cells [28,29] and is mainly located in inflammatory sites.

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Derived from these cells, IL-21 has multiple effects on both humoral and cellular immune responses, including beneficial effects, such as enhancing the proliferation of lymphoid cells, the cytotoxicity of $CD8^+$ T cells, the function of NK cells, immunoglobulin class switching, and differentiation of B cells into plasma cells [2,3,30,31]. Although Tfh cells secrete IL-21, the initial development of Tfh cells still relies on IL-21 stimulation [2,25]. IL-21 likely is produced by Th17 cells and is also a critical regulator of Th17 development [14,16]. Therefore, IL-21 is an autocrine cytokine for Tfh and Th17 cells. Via co-stimulation with anti-CD3, IL-21 promotes the expansion and differentiation of T cells [1,32]. In addition, IL-21 promotes mature B cell expansion, the function of globulin production and co-stimulation in response to CD40-induced activation [30,33]. However, IL-21 acts as both a forward and reverse regulator in B cell generation, differentiation, maturation and apoptosis, mediated by activated or co-stimulation signals [33–38]. Somewhat paradoxically, IL-21, directly or indirectly, can suppress activation and maturation of the antigen-presenting function of DCs [6], induce the apoptosis of B cells and NK cells, and suspend or reduce B cell generation with *anti*-IgM and IL-4 stimulation [3,8,11,33,38]. These pleiotropic effects, involved in both innate and adaptive immune responses, suggest that IL-21 is a key point in complex immune networks and might be a convincing therapeutic target for treating autoimmune disorders.

## 3. IL-21 signaling mechanism

### 3.1. The regulation of IL-21 secretion

Evidence has shown that secretion of IL-17 and IL-21 can be mediated by ROCK2, one of the two Rho kinases, through the RhoA-ROCK pathway [39]. In the same pathway, IRF4, a member of the interferon regulatory factor family, drives high production of IL-17 and IL-21 via the phosphorylation of ROCK2 [40,41]. Additionally, the cytokines IL-6, IL-7 and IL-15, rather than IL-4, induce IL-21 expression [42]. Maf has the capacity to upregulate IL-21 transcription [43]. Calcium signaling is sufficient to regulate IL-21 induction resulting from the binding of nuclear factor of activated T cells (NFAT) [44]. NFAT or NFATc2 directly activate the transcription of IL-21, whereas T-bet inhibits IL-21 expression by repressing NFATc2 binding to the promoter of IL-21 [45]. Similarly, TGF-β strongly reduces both IL-21 and IL-22 secretion. Additionally, the production of IL-21 and IL-22 is mediated via B7/CD28 co-stimulation [46]. Moreover, the aryl hydrocarbon receptor nuclear translocator-like 2 (Arntl2) gene reduces IL-21 expression directly by binding to the RNA polymerase II of the IL-21 promoter [24]. Lack of MALAT-1 significantly downregulates IL-21 transcription in primary monocytes from systematic lupus erythematosus patients [47]. These results suggest that IL-21 is an essential node in immune networks and is regulated by multiple factors, mainly via the RhoA-ROCK and NFAT pathways.

### 3.2. Downstream of the IL-21-IL-21R signaling pathway

It has been well documented that analogous type I cytokines, IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 execute their functions through the JAK/STAT pathway [2,18,19,48]. The ligation of IL-21 and IL-21R can activate JAK1 and JAK3, which belong to Janus family of tyrosine kinases, and subsequently phosphorylate STAT3 and STAT1, while also phosphorylating both STAT5a and STAT5b to a lesser degree. The activation of STAT leads to protein translocation to the nucleus, subsequently resulting in connections with the regulatory elements of target genes [2,4,8,11,17–19]. However, IL-2 and IL-15 both strongly activate STAT5 but not STAT3, which notably differentiates IL-21 from IL-2 and IL-15 [49]. It has been reported that IL-21 phosphorylates STAT1 and STAT3, instead of STAT5, mediated by tyrosine 510 (Y510) [17]. IL-21 acts in an autocrine fashion when produced by Tfh and Th17 cells and sustains or promotes Tfh and Th17 cell lines through STAT3 activation; additionally, IL-21 regulates itself in the same way [11,42,48]. Furthermore, IL-21 facilitates the PI3K/AKT and MAPK pathways, which both contribute to IL-21 signal transmission [2,8,17]. By activating these pathways, IL-21 reaches its target genes. IL-21 modulates the expression of HLA-B, -C, -E, FZD6, WNT5a, and B lymphocyte induced maturation protein-1 (Blimp-1) and upregulates CCR7, CXCR5 and CXCL10 transcription [6,23], as well as strongly suppressing the expression of suppressor of cytokine signaling (SOCS)-1 and SOCS-3 [40]. IL-21 significantly enriches TET2 in the Bcl-6 promoter region in a STAT3-dependent manner [15]. Additionally, IL-21 reduces the upregulation of CD72 enhanced by the combination of IL-10 and BAFF with CD40L and increases CD11c expression on naive B cells [50,51]. Genes activated or suppressed by IL-21 are associated with responses to tumors and viruses and are involved in regulating immune cell proliferation, differentiation and programmed death [23]. IL-21 acts on immune responses via the JAK/STAT, MAPK, and PI3K pathways, as well as a large number of downstream genes.

IL-21 regulation is mediated by the RhoA-ROCK pathway, and the phosphorylation of ROCK2 is induced by IRF4, an interferon regulatory factor. Maf upregulates the transcription of IL-21. Calcium ions regulate IL-21 by connecting nuclear factor of activated T cells (NFAT) and NFATc2, while NFAT is inhibited by T-bet, promoting the transcription of IL-21. In combination with the RNA polymerase II of the IL-21 promoter, aryl hydrocarbon receptor nuclear translocator-like 2 (Arntl2) downregulates IL-21 expression directly. Fig. 1.

IL-21R consists of the common γ-chain (CD132) and an IL-21 specific α-chain. IL-21R is expressed on both lymphatic and extra-lymphatic cells, such as T cells, B cells, NK cells, DCs, macrophages, keratinocytes, thyroid cells and synovial fibroblasts. The ligation of IL-21 and IL-21R activates JAK1 and JAK3, which are both Janus family tyrosine kinases, with subsequent phosphorylation of STAT3 and STAT1 regulated by tyrosine 510 (Y510); however, the phosphorylation of STAT5 is weak. The activated STATs transfer proteins into the nucleus, subsequently acting on regulatory elements of target genes, for example, HLA-B, HLA-C, HLA-E, FZD6, WNT5a, Blimp-1, CCR7, CXCR5, CXCL10, SOCS-1, SOCS-3, Bcl-6 and IL-21. Additionally, the PI3K/AKT and MAPK pathways contribute to IL-21 signal transmission. Fig. 2.

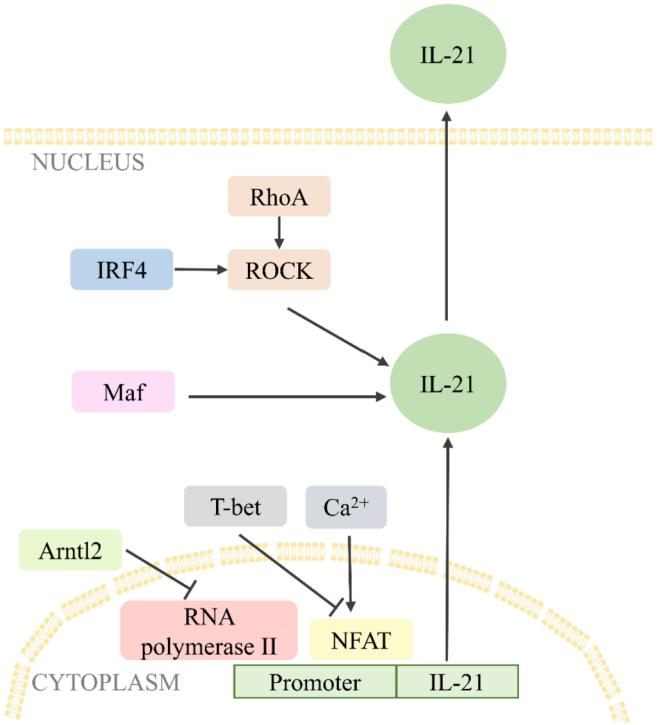


**Fig. 1.** The regulation of IL-21.

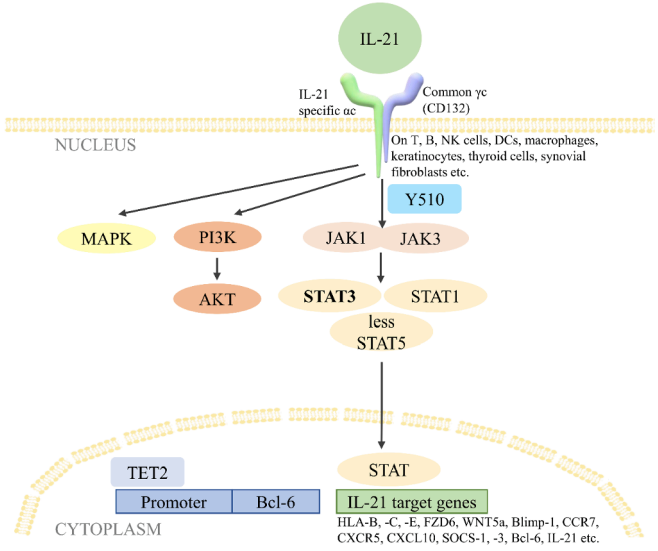


**Fig. 2.** Downstream of the IL-21 signaling pathway.

## 4. IL-21 and T cells

With stimulation, $CD4^+$ T cells can differentiate into distinct T cell subsets, including Th1, Th2, Th17, Treg and Tfh cells, and the balance of T cell subsets is affected by cytokine networks [13,52,53]. Both IL-21 and IL-21R are irreplaceable members of such cytokine networks. In human resting T cells, IL-21R mRNA expression levels are low and are rapidly elevated after T-cell receptor (TCR) stimulation [54]. IL-21 expression at high levels not only boosts immune responses but also induces autoimmune disturbance. As a pleiotropic cytokine, IL-21 plays a momentous role in the polarization or generation of effector T cell subsets and mediates innate or adaptive immune responses in autoimmune diseases and infections and anti-tumor responses [19,29,52,53].

### 4.1. IL-21 and Tfh cells

The phenotypic and functional features of Tfh cells are the expression of CXCR5, ICOS, PD-1, Bcl-6 and IL-21, regulation of GC formation or responses, and B cell activation [11,15]. Since Tfh cells are a main source of IL-21, they act as IL-21 target cells as well [2]. However, the production of IL-21 is not associated with the high expression of Bcl-6 or CXCR5. IL-21 and IL-6 redundantly induce Tfh cell differentiation, while the absence of IL-21 results in decreased Tfh polarization and reduces Bcl-6 expression [25]. In many reports, Tfh cells promote aberrant GC responses via the development of autoreactive memory B cells and enhance autoantibody production derived from plasma cells, partly in an IL-21-dependent manner [12,13,15]. Tfh cells can support Ig subset class switching and initiate and maintain memory B cells and long-lived plasma cells in response to immune stimulation [55]. It has been shown that after treatment with *anti*-IL-21, the frequency of Tfh cells derived from B6. Sle1. Yaa (Yaa means Y-linked autoimmune acceleration) mice did not change, but interferon-γ (IFN-γ)$^+$ IL-21$^+$ Tfh cell numbers were reduced, indicating IL-21 blockade altered Tfh cell phenotypic and functional properties [12]. Consistently, SM934 (an analogue of artemisinin with potent immunosuppression in an autoimmune therapy model) has been found to impede IL-21 signaling through STAT3, diminish IL-21-producing $CD4^+$ T cells in vitro, and decrease the production of morbigenous antibodies without downregulating the number of GC B cells [11]. Accumulating evidence demonstrates that IL-21 and Tfh cells contribute to humoral immune responses and the production of abundant antibodies and thereby play a critical part in the development of autoimmune pathologies such as SLE, autoimmune diabetes, and RA [15,56,57].

### 4.2. IL-21 and Th17 cells

Th17 cells, which express high amounts of IL-17A, IL-17F, IL-21, and IL-22, are supposed to be proinflammatory effector T cells, with vital functions in autoimmunity induction and mediating tissue inflammation [11,14,16,58]. Compared with Th1 or Th2 cells, Th17 cells show approximately fivefold higher levels of IL-21 mRNA and protein [14,16]. Several molecular pathways are impacted by the differentiation of Th17 cells, including the principal IL-6/TGF-β/RORγt pathway, the subordinate IL-21/TGF-β/RORγt pathway and the amplified IL-23/IL-22 pathway. In the presence of IL-6, IL-21 is dispensable for the differentiation of Th17 but serves as a regenerative feedback mechanism for Th17 cell expansion and suppresses Foxp3 expression [11,14,16,59]. The frequency of Th17 cells is dramatically reduced in IL-21 or IL-21R KO mice. Similarly, Th17 responses are defective in IL-21R knockout T cells, which might be due to IL-21-mediated IRF4 involvement [14,16,40]. Proinflammatory Th17 cells are effective B-cell helpers because they not only arouse strong B cell generation, particularly that of antigen-specific B cells, but also trigger antibody class switching, preferentially to IgG1, IgG2a, IgG2b, and IgG3. Conversely, blockade of IL-17 results in barren GCs in both number and size and reduces humoral responses in mouse models [58,60]. Autoimmune mouse models such as BXD2 mice show high IL-17 levels and aberrant development of germinal centers, suggesting that IL-17 motivates autoimmune responses by driving the formation of spontaneous GCs [60]. In addition, IL-21 participates in immune responses by subordinately inducing Th17 cell proliferation, regulating their functions and regulating IL-17 production by Th17 cells.

### 4.3. IL-21 and Treg cells

$CD4^+$ $CD25^+$ Treg cells play a central role in the counterbalance of immune responses, especially in neutralizing pathogenic immune responses, by regulating the excitation, proliferation and cytokine secretion of autoreactive cells [2,61]. Numerous references in the literature confirm that Treg cells act as immunological protectors in peripheral tissue by suppressing immune responses to maintain self-tolerance, and this function is modulated by IL-21 in vitro and in vivo [13,62,63]. IL-21 enhances the ability of $CD4^+$ T cells to resist the suppression of Treg cells, which is associated with high levels of IL-21 in vivo, rather than inhibiting Treg cell activity without enhancing CD28 or decreasing Foxp3 [53,62,63]. IL-21 decreases the absolute number of Treg cells and differentiation indirectly by decreasing IL-2 production in T cells in a STAT3-and STAT5-dependent manner, thereby antagonizing the protective suppression of Treg cells [29,63]. The counteracting suppression of IL-21, which is exerted by acting on classic T cells and is associated with the inhibition of Bcl-6 and IL-21R expression, results in the suppression of Treg responses to IL-2 [2,24,29,62,63]. In the absence of IL-2, IL-21 substitutes for IL-2 as a growth factor so that conventional T cell responses are unimpaired, and the Treg cell compartment becomes defective and is unable to be replaced by IL-21 [63]. In IL-21 KO mice, the approximately fourfold increase in the population of $Foxp3^+$ $CD4^+$ T cells demonstrates the negative effects of IL-21 [14]. Interestingly, TGF-β regulates Foxp3 and induces the generation of Treg cells, whereas IL-21 reduces TGF-β-induced $Foxp3^+$ Treg cells to induce immune tolerance and resistance to immunosuppression [2,16,59,61]. Superabundant IL-21 breaks the immune tolerance and protection exerted by Treg cells by restraining the generation and development of Treg cells and enhancing $CD4^+$ T cell resistance to Treg cell suppression.

### 4.4. IL-21 and Th1, Th2, and $CD8^+$ T cells

Compared with Th1 cells, Th2 cells express IL-21 preferentially both in vitro or in vivo. IL-21, as a Th2 cell cytokine, functions during the differentiation of naive T helper precursors and can inhibit Th1 cell

polarization [26]. However, in another study, human IL-21 was secreted mainly by IFN-γ-producing Th1 cells instead of IL-4-producing Th2 cells, while mouse IL-21 was detected in Th2 cells [27]. It was reported that IL-15 has synergistic effects with IL-21 to potently augment the production of IFN-γ and promote the generation of naive and memory $CD8^+$ T cells in vitro. The expression patterns of genes have shown that IL-15 plus IL-21 upregulates the expression of granzyme B, IL-4R, IL-21R, Jun and Adam19 by more than double while downregulating CD28, CXCR4 and Itgae. Moreover, IL-21 alone has been found to induce the expression of granzyme A, Bcl-3, JAK3, and IL-17R [64]. As expected, IL-21 enhances $CD8^+$ effector T cell proliferation and increases IFN-γ secretion and cytotoxic function in an allogeneic mixed lymphocyte reaction [3,64]. During antigen-specific donor $CD8^+$ T cell responses to the host, IL-21 signaling is essential for cytotoxic T lymphocyte (CTL) effector maturation, and both the granzyme B/perforin and Fas/FasL killing pathways are impaired without IL-21R and recover with IL-21 treatment. Consistently, neutralization of IL-21R restrains CTL responses and is accompanied by limited B cell hyperactivity and downregulated autoantibody production [31].

### 4.5. IL-21 and new T cell subsets

IL-21 enhances the generation of responder T (Tresp) cells in Tresp/Treg cocultures, inhibits the secretion of IL-4 from cells derived from myasthenia gravis patients or healthy controls, and reduces IL-17A only in cells from healthy controls, which demonstrates that IL-21 induces the proliferation of Tresp cells in the presence of Treg only [13]. Recently, a new $CD4^+$ T cell subset named 'peripheral helper' T (Tph) cells was reported provisionally. This new population is defined as PD-$1^+$ $CXCR5^-$, accompanied by the expression of IL-21, ICOS, CXCL13 and MAF, enabling Tph cells to help B cells [28,29]. Tph cells functionally resemble PD-$1^+$ $CXCR5^+$ Tfh cells. After activation, both Tfh and Tph cells upregulate co-stimulatory factors including IL-21, PD-1 and ICOS, which are essential for supporting B cell proliferation and differentiation and promoting autoantibody production or B cell responses without upregulating Bcl-6 or Blimp-1 [28,65]. The mutual conversion of Tph and Tfh subsets has not been detected in vitro, indicating that Tph cells induce B cell responses uniquely in pathological situations [28]. A new $CD4^+$ T cell subset, characterized as Blimp-$1^+$ $Foxp3^+$ $CXCR5^+$ PD-$1^+$ $CD4^+$ T cells, has been observed in germinal centers and described as follicular regulatory T (Tfr) cells. The phenotypic characteristics of Tfr cells are similar to those of Tfh and conventional Treg cells but distinct from either cell type [66]. Tfr cells limit Tfh cell production and the secretion of cytokines, leading to the suppression of germinal center B cell-mediated antibody production, while IL-21 is able to inhibit Tfr cells and resist the suppression of Tfh and B cells mediated by Tfr cells [66,67]. However, the mechanisms underlying the interactions between these new T subsets and IL-21 are largely unknown.

## 5. IL-21 and B cells

B cells have an irreplaceable role in humoral immunity and the development of immune responses, constituting a complex immune network including B cell receptor (BCR) antigen recognition, antigen presentation, cytokine secretion, and antibody-producing plasmablast and plasma cell differentiation [68]. With BCR stimulated by foreign antigens or autoantigens, the subsequent combination of CD40 on B cells and CD40L on T cells can induce extrafollicular generation and ephemeral plasmablast differentiation into plasma cells or migration into germinal centers [35,69]. IL-21 is an essential cytokine in the B cell regulatory network. IL-21R is expressed by naïve and germinal center B cells and is even more highly expressed by activated B cells but is not expressed by memory or plasma cells [30,33,34,69].

IL-21 exerts both positive and negative effects on B cell proliferation, differentiation, maturation and apoptosis, depending on the activation signals and co-stimulation [30,33,34]. IL-21 has been found to amplify B cell proliferation induced by anti-CD40 or *anti*-IgM plus anti-CD40 by increasing the expression of CD11c. IL-21 enhances the proliferation of splenic naïve, memory and germinal center B cells, which are excessively induced by IL-4, IL-10, and IL-13, but restrains generation induced by *anti*-IgM only [1,30,33,50]. IL-21 also results in reduced quantities of B cells and induces apoptosis when B cells are activated by innate toll-like receptor (TLR) ligands, such as LPS or CpG DNA and BCR alone, correlating with the downregulated expression of Bcl-2 and Bcl-xL [33,34,38]. Both Bcl-2 and Bcl-xL are Bcl-2 family members and play antiapoptotic roles. IL-21 can upregulate the expression of Bim and Apaf-1, which are proapoptotic factors [8,11].

IL-21, a strong exciter of Bcl-6 and Blimp-1, the latter of which is a transcriptional controller that mediates the terminal polarization from B cells to plasma cells, also has the ability to promote the differentiation of B cell subsets [2,8,38]. When IL-21 signaling is defective, the response to antigens, the formation of splenic and bone marrow plasma cells, and the persistence and function of GCs are profoundly affected, and the proliferation and transition into memory or mature B cells are reduced. In IL-21 KO or IL-21R KO mice, memory B cells carry significantly decreased numbers of mutated $V_H$ genes and exhibit less selection or mutation compared with that of C57BL/6 mice [37].

Research both in vivo and in vitro has uncovered that IL-21 plays an essential role in differentially regulating the development or apoptosis of B cells, depending on various costimulatory signals.

### 5.1. IL-21 and plasma cells

Tfh cells, known to be an abundant source of IL-21, induce the differentiation of B cells into plasma cells by mediating serum levels of IL-21 [70]. By mediating the IL-21 and IL-22 pathways, Th17 cells induce β-galactoside α2,6-sialyltransferase 1 expression in newly polarizing antibody-secreting cells and determine the glycosylation profile of immunoglobulin (Ig)G (mainly IgG1 and IgG3) subsequently secreted by the plasma cells [38,71]. Some subgroups of T helper cells such as $CCR9^+$ and $CXCR5^+$ T helper cells, which express more IL-7Rα and show elevated proportions of secreted IL-21, IL-4, IL-10, IL-17, and IFN-γ, potently demonstrate higher competence to induce IgG production than $CCR9^-$ $CXCR5^-$ T helper cells. Moreover, $CCR9^+$ T cells produce increased IL-21 and IL-10 compared with that of $CXCR5^+$ T cells [72]. Furthermore, IL-21 can induce T helper cells and enhance plasma cells indirectly.

IL-21 has a central role in the extensive proliferation, differentiation, and class switch recombination (CSR) of plasmablasts and plasma cells, with CD40 co-stimulatory engagement [35,68]. The above effects require STAT3. Although IL-21 signaling activates both STAT1 and STAT3, STAT3 deficiency dramatically reduces the number and differentiation of plasma cells in response to IL-21, while STAT1 deficiency has no effect [73,74]. The combination of IL-21 and BCR in vitro directly activates naïve B cell differentiation into plasma cells via the transcription of Blimp-1 induced by IL-21 [35,38,69]. Blimp-1, a transcription factor, serves as a master trigger for the terminal polarization program of B cells to plasma cells. Surprisingly, IL-21 also induces the expression of Bcl-6, another transcription factor involved in B cell differentiation [38].

Plasma cell differentiation and accumulation from murine splenic B cells directly induced by IL-21 plus *anti*-IgM enhances Syndecan-1 (CD138) and IgG1 in vitro. In normal mature murine B cells, IL-21 has been found to downregulate CD23 expression obviously and decrease CD21 expression slightly [38]. Additionally, IL-21 increases the proportion of B lymphocytes and the plasma levels of IgG in an MRL-$Fas^{lpr}$ mouse model with anti-CD40 or anti-CD40 plus *anti*-IgM [75].

Corresponding to its effects in mice, IL-21 is also an important component in the differentiation of human B cells [70]. IL-accompanied by anti-CD40 and anti-CD40 plus *anti*-IgM (not *anti*-IgM alone) promotes the proliferation of plasma cells from memory B cells. Furthermore, human naive cord blood B cells and post-switch memory B cells are induced to polarize the largest numbers of plasma cells, which result from IL-21 cross-linking to BCR or CD40 signaling [35]. IL-21 efficiently induces the proliferation of vast numbers of immunoglobulin-secreting cells and results in the secretion of enormous quantities of IgM, IgG and IgA from murine or human naïve B cells, with indispensable CD40 or BCR co-stimulation [35,69,70].

Some cytokines highlight the potency of IL-21 as a master factor in B cell generation and differentiation during immune responses. For instance, IL-2 enhances the abilities of IL-21, and testing IL-2 and IL-21 individually and in combination suggests that these factors induce optimal plasmablast differentiation with dominant CD40L stimulation. IL-21 also upregulates IL-2RA expression on normal or STAT1-deficient B cells but has no effect on STAT3-mutant B cells, suggesting that IL-21 mediates IL-2RA via the STAT3 pathway directly [35,68,73]. Another ambivalent cytokine, IL-4, promotes the production of multiple immunoglobulins in cooperation with IL-21 [33,38], whereas IL-4 alone inhibits the differentiating effect of IL-21 on B cells by reducing the expression of Blimp-1 [35,70]. Similarly, IL-21 reduces IgE production by human PBMCs stimulated by IL-4 and mitogen in vitro [70]. If both IL-6 and IL-21 are neutralized, approximately 90% of circulating antiviral IgG will be lost [25]. With BAFF persistence, IL-21 synergizes to stimulate splenic memory B cell differentiation into plasma cells [68].

Transgenic mice overexpressing IL-21 exhibit amplified plasma cell propagation, hypergammaglobulinemia with increased levels of IgG and Syndecan-1, and a high proportion of class-switched Ig [38]. IL-21 blockade remarkably restrains the differentiation of plasma cells and Ig production mediated by PD-1$^+$ CXCR5$^-$ cells and PD-1$^+$ CXCR5$^+$ cells, and SLAMF5 (a T-B cells interactive factor) may be involved [28]. IL-21R knockout mice show normal formation of germinal centers. However, after immunization, these IL-21R KO mice show substantially lower plasmatic expression of antigen-specific IgG1 but higher IgE than wild-type mice. Doubly deficient IL-4 and IL-21R transgenic mice exhibit significantly impaired IgG responses with dysgammaglobulinemia [76].

Mounting evidence indicates that IL-21 produced mainly by Tfh and Th17 cells during T cell-dependent immune responses is a primary contributor to the triggering and maintenance of long-term humoral immunity.

### 5.2. IL-21 and GCs

GCs are specialized sites working as secondary lymphoid tissues for developing B cell follicles, where antigen-specific B cells improve their affinity for antigen, undergo somatic hypermutation, proliferate and differentiate into antibody-secreting plasma cells as well as long-lived memory B cells. GC B cells are well-documented precursors of antigen-specific B cells and long-lived plasma cells [11,37,68]. The interactions between T and B cells along with cytokines are irreplaceable components of immune responses.

Tfh cells are present in GCs and provide co-stimulatory or specific signals for the generation, maturation and class switching of GC B cells; specifically, Tfh cells directly orchestrate physiological GC B cell responses [11,12,36,65] by producing CD40L, IL-21, IL-4, ICOS, Bcl-6, CXCL13 and upregulating the transcription factor Blimp-1 [28,77,78]. The proliferation of GC B cells is reduced, followed by the reduction of CD40L expression levels on Tfh cells [12]. Tfh cells sorted from Thy1.2$^+$, IL-21-Kat/$^+$, IL-4-GFP/$^+$ mice were injected into Thy1.1$^+$ B6 mice, which were then immunized, and the transferred cells, producing both IL-21 and IL-4, induced higher levels of Bcl-6 in GCs and collaboratively mediated GC B cell responses [78]. As major secretors of IL-21 and IL-17, Th17 cells have a distinct role in the incitement and development of GC reactions in a STAT3-dependent manner [11]. Tfh and Th17 cells share IL-21 with a positive autocrine feedback loop, and other cytokines are also needed for the irritation and persistence of GCs.

IL-21 acts as a controller of GC B cell formation and function, and IL-21 signaling is important for B cell isotype switching and response to foreign antigens or autoantigens by maintaining the high levels of Bcl-6 in GC B cells [11,36,37]. If only IL-21 is neutralized, the maturation and antibody production of GCs are subsequently reduced, while the formation of early memory B cells is unsuppressed, although memory B cell development is still impaired. Furthermore, the frequencies of B220$^+$ GL7$^+$ CD95$^+$ TCRβ$^-$ GC B cells and B220$^-$ CD138$^+$ TCRβ$^-$ plasmablasts are notably reduced with IL-21 neutralization [12,25,36]. Consistently, IL-21, IL-2, and BAFF have been shown to induce CD27$^+$ memory B cell activation and differentiation into plasmablasts [68]. In contrast, the absence of IL-6 has no impact on GCs [25].

Taken together, IL-21 is a master regulator of GC responses and B cell formation and function, directly or indirectly, with help from T helper cells and the involvement of T-B cell interactions.

## 6. IL-21 and innate immune cells

With the exception of the effects of acquired immune cells, IL-21 acts on innate immunocytes such as NK cells and DCs. IL-21R KO mice show no impairment in terms of the numbers and activation of NK cells with undetectable responses to IL-21, indicating IL-21 is unnecessary for the generation and development of NK cells with low levels of IL-21R expression [3,79]. IL-21 enhances the abilities of murine activated NK cells, including their cytotoxic activity and the expression of IFN-γ, but decreases the duration of NK cell viability as well as blocks the amplification of resting NK cells mediated by IL-2 and IL-15, which induces the translation of IL-21R [3,8,79].

IL-21 directly inhibits the maturation and function of bone-marrow-derived DCs and maintains increased phagocytotic activities as well as decreases DC antigen presentation, which leads to the limitation of antigen-specific T cell responses. IL-21 forcefully stimulates the expression of SOCS-1 and SOCS-3; suppressor of cytokine signaling is well known to suppress DC functions [6].

IL-21 significantly drives macrophages producing IL-4Rα and IL-13Rα1 and alternatively activates macrophages. Co-stimulation with IL-4 and IL-13 and pretreatment IL-21 induces significant upregulation of the translation of the arginase-1 and FIZZ1 genes, which are both involved in fibrosis development [80].

## 7. IL-21R structure and expression

The heterodimer of IL-21R consists of the common γ-chain (CD132) and an IL-21-specific α-chain, which differentiates IL-21R from the α-chain (CD25) and β-chain (CD122) of IL-2R and IL-15R [18,49,81]. The γ-chain, as half of IL-21R, has been localized on human chromosome Xq13, overlapping with the locus of X-linked severe combined immunodeficiency (X-SCID) [82]. X-SCID contributes to γ-chain mutation and defects in cytokine signaling paths, which are associated with the reduction of T and NK cells as well as the defective function of B cells [82,83].

IL-21R was first observed to be expressed on T, B, and NK cells and was subsequently detected on dendritic cells (DCs), macrophages and keratinocytes [1,5,6,8,33,80,84,85]. The expression of IL-21R has been detected on naïve B cells and germinal center (GC) B cells and on some subsets, such as CD11c$^+$ B cells and CD5$^+$ B cells, instead of memory or plasma cells. The highest levels of IL-21R have been observed on activated B cells [30,50,86,87], indicating that the IL-21-IL-21R pathway is profoundly involved in autoimmune diseases with B cell dysfunction or

autoantibody production. Recent studies have shown that IL-21R is expressed on extra-lymphatic cells and organizations, such as patients' thyroid cells, keratinocytes in skin lesions, synovial tissues and synovial fibroblasts [7–10], indicating a role for the IL-21-IL-21R pathway in nonlymphocytic cells and its contribution to autoimmune disorders.

## 8. IL-21 and autoimmune diseases

Aberrations in T helper cells, plasmablasts, plasma cells, and antibody or autoantibody levels are often observed in autoimmune diseases and play a pathogenic role [68]. IL-21, acting on both lymphoid and nonlymphoid cells and as a master regulator in humoral immune responses, is responsible for the alterations to T helper cells such as Tfh, Th17, and Treg cells and B cell subsets, including memory B, naive B, and antibody-secreting cells [15,31,50,68,88–91], whose pathogenesis also has been described in most autoimmune diseases and connected with the high expression of IL-21 or IL-21R. Compared with normal controls, elevated levels of IL-21 mRNA or protein have been found in serum, peripheral blood mononuclear cells (PBMCs) and impaired tissues from patients with autoimmune diseases and correlated with disease initiation and development. These diseases include systematic lupus erythematosus (SLE) [1,45], rheumatoid arthritis (RA) [92–94], immune thrombocytopenia (ITP) [57,69], type 1 diabetes (T1D) [95], primary Sjogren's syndrome (pSS) [65,96], autoimmune thyroid disease (AITD) [7,97], psoriasis [10] and others. In this case, manipulations to neutralize or decrease IL-21 signaling may lead to a novel practical direction for treatment and improved clinical outcomes. Table 1.

### 8.1. IL-21 and SLE

SLE, a systemic chronic inflammatory autoimmune disease characterized by loss of tolerance to nuclear components, detected serologic titers of circulating autoantibodies and the formation of circulatory immune complexes, arouses serious damage to various organs or tissues, including skin, joints, vessels, kidneys and central nervous system [12,98]. Hallmarks of SLE are the disturbed proportions of T helper cells, memory B cells, plasmablasts, and plasma cells and high levels of autoantibody expression [15,31,50,86,91].

Antibody-secreting cells express IL-21R and BAFF-R in higher proportions and show lower IL-6 receptor complex expression in lupus patients [86]. B cells express significantly upregulated CXCR4 in SLE, especially in patients with nephritis. IL-21 restores CXCR4 expression downregulated by IgM and CD40L, the PI3K/AKT, JAK/STAT signaling pathways and CD63 gene defects might be involved in this dysregulation [99]. In SLE patients, estrogen promotes IL-21 secretion by $CD4^+$ T cells in a dose- and time-dependent manner via MAPK signaling pathways. When co-cultured with SLE $CD4^+$ T cells treated with estrogen, B cells produce more immune globulin, which can be abrogated by an IL-21 blocking antibody [100]. $CD11c^+$ B cells are a B cell subgroup that is increased in SLE patients, particularly in serious patient groups with a higher SLE Disease Activity Index (SLEDAI) (9 or above), and coincides with nephritis and malar rash activity. The frequency of $CD19^+$ $CD38^+$ $CD27^+$ plasma cells and serum autoantibody levels display positive correlations with increased percentages of $CD11c^+$ B cells in SLE. Unexpectedly, IL-21 potently promotes $CD11c^+$ B cells expansion and differentiation into autoreactive plasma cells, with the combination of *anti*-IgM and anti-CD40 [50]. Another small subset of B cells, featuring two distinct B-cell-antigen-receptors, is $B_{2R}$ cells. The frequency of $B_{2R}$ cells is increased in SLE patients and the MRL-$Fas^{lpr}$ mouse model, a classical model of lupus. $B_{2R}$ cells show better proliferation with T cell-dependent antigen co-stimulation, and their homeostasis depends on IL-21 [89]. Correspondingly, both $CD27^-$ naive B cells and IL-$21R^+$ B cell subpopulations are significantly elevated in SLE [68]. Otherwise, lack of IL-21R can limit B cell hyperactivity and secretion of autoantibodies, such as IgG1, IgG2, and *anti*-dsDNA, while levels of IgM and IgE are unaffected [31,75], indicating that IL-21 and IL-21R result in the occurrence and development of SLE.

IL-21 levels are elevated in lupus $CD4^+$ T cells and correlate with the increased ratio of Th17 and memory B cells, which are positively associated with Bcl-6 expression [15,90]. High expression of IL-21 can significantly increase Bcl-6 transcription levels by enriching TET2 on the Bcl-6 promoter [15]. In lupus patients, the high concentration of IL-21 positively associates with elevated numbers of $CD4^+$ $CXCR5^+$ $PD\text{-}1^+$ Tfh cells, $CD4^+$ $CXCR5^+$ $PD\text{-}1^+$ $Bcl\text{-}6^+$ populations [15], circulating IL-$21^+$ Tfh-like cells, $CD27^+$ B cells, SLEDAI, C3, and the erythrocyte sedimentation rate (ESR) [91]. Surprisingly, only $CD4^+$ $CXCR5^+$ $PD\text{-}1^+$ $Bcl\text{-}6^+$ cTfh cells, rather than the $CD4^+$ $CXCR5^+$ $PD\text{-}1^+$ subpopulation, are proportional to SLEDAI and *anti*-dsDNA antibody levels [15,86]. Tfh-like cells positively correlate with $CD19^+$ $CD5^+$ $CD1d^+$ B cells [91]. Inflammatory cells infiltrating into the kidney are characteristic of lupus nephritis, with B cells and Tfh cells located in close proximity, indicating that T-B cell interactions play key roles in the mechanism underlying glomerulonephritis and renal dysfunction [12]. IL-21 mRNA levels are significantly increased in primary monocytes of SLE patients and are profoundly mediated by metastasis associated lung adenocarcinoma transcript 1 (MALAT-1) [47].

IL-21 is markedly accumulated in autoimmune-prone mice based on the augmentation of TFH cells, including the lupus-prone B6. Sle1. Yaa mice [12], BXSB-Yaa strain [38,88,101], SLE-like MRL-$Fas^{lpr}$ mice [75], and graft-versus-host disease (GVHD) mice [102].

IL-21R-deficient and IL-21R-competent BXSB-Yaa mice have been compared to determine the parameters of autoimmune responses. BXSB-Yaa mice with normal IL-21R levels show numerous abnormalities, including high autoantibody expression, reduction of marginal zone B cells, monocytosis, hypergammaglobulinemia, premature morbidity, and renal impairment, including a thick glomerular basement membrane, neutrophilic infiltrates, glomerular sclerosis, and immune deposits [101]. Nevertheless, no abnormal characteristics of lupus in IL-21R-competent BXSB-Yaa mice have been observed in IL-21R-deficient mice with decreased frequencies of $CD11b^+$ monocytes or $CD11c^+$ $CD11b^+$ dendritic cells and with downregulated activation of Ly6a/e

**Table 1**
The role of IL-21 in immune cell subsets.

| Cell | Effects | Ref. |
|---|---|---|
| Tfh cells | ↑Proliferation<br>↑Development<br>↑Germinal center function and formation | [2,12,13,15,25] |
| Th17 cells | ↑Autocrine<br>↑Proliferation<br>↑Differentiation (subordinate) | [11,14,16,59] |
| Treg cells | ↓Generation<br>↓Differentiation (indirect)<br>↓Protective suppression | [13,29,53,62,63] |
| $CD8^+$ T cells | ↑Proliferation<br>↑Maturation<br>↑Cytotoxicity<br>↑Production of cytokines | [3,31,64] |
| B cells | ↑Proliferation or apoptosis (dependent on co-stimulation)<br>↑Plasma cell differentiation<br>↑Ig production and CSR | [2,8,30,33–35,38,68,71] |
| NK cells | ↑Cytotoxicity<br>↑Production of cytokines<br>↓Viability | [3,8,79] |
| DCs | ↑Phagocytosis<br>↓Maturation<br>↓Antigen presentation | [6] |
| Macrophages | ↑Activation<br>↑Production of cytokines | [80] |

Tfh, follicular helper T; Th17, T helper 17; Treg, regulatory T; Ig, immune globulin; CSR, class switch recombination; NK, natural killer; DCs, dendritic cells.

and CD69, as well as prolonged survival within expected parameters [101]. In BXSB-Yaa mice, the expression of IL-21R is increased on $B220^+$ B cells, such as dominant follicular B, marginal zone B and GC B cells, as well as $CD8^+$ T cells, while there is no contribution to the elevated IL-21R expression on $CD4^+$ T cells, the primary producers of IL-21. This suggests that BXSB-Yaa murine B and $CD8^+$ T cells have enhanced responses to IL-21 [88]. Additionally, $Ighm^{-/-}$ B cell-absent mice survive and are associated with lower levels of $CD4^+$ T and $CD11b^+$ cells, compared to the 20% survival rate of WT BXSB-Yaa mice [88]. Selective silencing of IL-21R on B cells, $CD8^+$ T or all T cells on the BXSB-Yaa mouse background showed that IL-21R on B subsets is essential for the progression of all classical characteristics of lupus manifestations and is required for high serum levels of total Ig, *anti*-ssDNA and ANA. In addition, IL-21 signaling sustains the augmentation of central memory cells and $CD8^+$ T cells [88]. A chronic lupus-like GVHD model has been established by injecting $CD4^+$ T cells from either B6 wild-type (WT) or B6 IL-$21R^{-/-}$ donors and injecting $CD4^+$ T cells into WT or IL-$21R^{-/-}$ hosts. IL-21 mRNA is upregulated by 30 or 28-fold in chronic GVHD (cGVHD) models (WT donors into B6D2DF1 mice or B6 donors into C57BL/6 J mice, respectively) compared with that observed in controls, and moreover, donor $CD4^+$ T cells have significantly higher expression of IL-21 than host $CD4^+$ T cells, suggesting that IL-21 plays an important role in the progression of cGVHD [31,102]. Donor naïve $CD4^+$ T cells are engrafted in cGVHD, after which naïve T cells polarize into $CD4^+$ $ICOS^+$ $CXCR5^+$ Tfh cells with upregulated transcription factor Bcl-6 and settle down in GCs, particularly in B cell areas showing T-B cell interactions. Additionally, the transcription of IL-17A and RORγt required for Th17 cell polarization is increased in cGVHD mice and depends on upregulated IL-21 mRNA levels [102]. cGVHD mice injected with IL-$21R^{-/-}$ donor cells have smaller GCs, a lower proportion of GC B cells, downregulated levels of *anti*-ssDNA antibodies, alleviated glomerular sclerosis, crescent formation and lupus-like nephritis in the long term relative to WT cells from induced cGVHD mice [102], indicating that IL-21/IL-21R is irreplaceable for maintaining Tfh and Th17 cell quantities, aggravating lupus-like renal disease, and inducing and sustaining optimal germinal centers and plasma cells, as well as autoantibody production [102]. In another lupus model, sanroque mice develop high titers of autoantibodies, similar to the pathology of SLE, which are positively associated with elevated quantities of Tfh cells and the overproduction of IL-21 [103].

On the background of IL-21R knockout models, the production of anti-nuclear antibodies is significantly reduced, kidney disease is absent, and the survival ratio is increased [88,101–103]. This suggests that neutralizing IL-21 might have a profound therapeutic effect. In mouse models of SLE, there are abnormal elevated serum levels of IL-21; moreover, neutralizing IL-21 or blocking IL-21 signaling improves clinical symptoms and disease activity of SLE [42]. Thus, targeting IL-21 cytokine levels and IL-21-producing cells are considered promising therapies for SLE.

B6. Sle1. Yaa mice (Yaa means Y-linked autoimmune acceleration), a lupus-prone animal model, were intraperitoneally treated with 30 mg/kg *anti*-IL-21 monoclonal antibody or saline 2–3 times per week [12]. *Anti*-IL-21 antibody-treated mice showed decreased titers of anti-chromatin or *anti*-dsDNA antibodies, inhibited B cell generation with downregulation of the proliferative marker Ki-67, deferred development of glomerulonephritis, abrogated renal-infiltrating Tfh and Th1 cells and lower levels of immune complex deposition or serum creatinine, in contrast to the glomerulus crescent formation and nephritis observed in control mice [12]. *Anti*-IL-21 treatment surprisingly improved the survival rate: almost all *anti*-IL-21-treated mice survived to six months old, while more than half of control mice died at that time. Additionally, numbers of GC B cells, plasmablasts, and accumulated $IL\text{-}21^+$ IFN-$\gamma^+$ Tfh cells were obviously decreased with *anti*-IL-21 treatment, which portends long-term improved effects [12]. Nonetheless, treatment of older B6. Sle1. Yaa mice had no significant improvement on impaired kidney function at later stages [12]. Therapy with an *anti*-IL-21 antibody suppressed the excessive accumulation of IL-21-secreting T helper cells and reduced downstream cytokines, while their frequencies were unaffected [12]. However, *anti*-IL-21 antibody treatment in the B6. Sle1. Yaa model inhibited the generation of GC B cells, $CD138^+$ plasmablasts, populations of autoantibodies and IFN-γ-dependent IgG2c, showing again that IL-21 is no substitute for the pathogenesis of lupus [12].

In line with those results, compared with saline and *anti-E. tenella*, lupus-prone MRL-$Fas^{lpr}$ model mice were treated with soluble IL-21R-Fc fusion protein for 10 weeks and showed an improved lupus phenotype and disease severity [75], including deferred proteinuria, remarkable decreased IgG deposition in the glomeruli without the thickened glomerular basement membrane with incrassation that was observed in control groups, lower titers of IgG1 and IgG2b dsDNA autoantibodies, which were elevated in the plasma starting at 6 weeks of age, and initiation of pathogenesis and mitigation of skin rashes or lymphadenopathy [75,104]. Treatment abated renal damage by suppressing the formation of immune deposits, while IL-21R-Fc protein did not affect perivascular inflammatory infiltrates or glomerular cellularity. Additionally, blocking IL-21R resulted in fewer splenic $CD4^+$ or $CD8^+$ T lymphocytes in vivo and impacted the function of splenic B cells producing antibodies in response to IL-21 in vitro, suggesting that blocking the IL-21 pathway affects both T and B cell responses [75].

Another SLE mouse model, NZB/NZW, was tested with a novel treatment involving IL-21R blockade by neutralizing antibodies, followed by immunization with sheep red blood cells (SRBCs), and humoral and cellular immune responses were measured. Treatment of NZB/NZW mice completely inhibited nephritis onset and dramatically reduced splenomegaly and lymphocyte activation [105]. Blockading IL-21R signaling specifically suppressed IgG responses following SRBC immunization. In mouse models with preexisting SLE, treatment with an *anti*-IL-21R antibody delayed development, overturned mortality and partly recovered nephritis. Furthermore, pausing the therapy did not result in rapid relapse of lupus [105].

The BXSB-Yaa mouse model has SLE-like autoimmune syndromes, and increased serum IL-21 is observed when the characteristics of autoimmunity first become evident. These mice were treated with an IL-21R-Fc fusion protein for 24 weeks to interdict the IL-21 pathway. Unexpectedly, the results indicated a notable biphasic effect of IL-21: blocking IL-21 increased the severity of disease early on while improving the survival rate at later stages [106]. Since IL-21 activates $CD8^+$ T suppressor cells, it is conceivable that IL-21R blockade increases the risk of viral infections and cancer [88]. Additionally, neutralization of IL-21 also reduces IgG1 plasma levels and proteinuria levels. Speculatively, IL-21 enhances $CD8^+$ T suppressor cells, which exert protective effects in the early phase of lupus and later induce humoral immune responses [106].

ATR-107 is a complete human monoclonal *anti*-IL-21R antibody whose tolerability and safety has been tested in a double-blind phase 1 study in healthy volunteers compared with a placebo-control. With 3–300 mg subcutaneous (SC), 30–120 mg intravenous (IV), or placebo single treatment, the study showed a long-term pharmacodynamics effect (at least 42 days) assessed by IL-21R occupancy (maximal occupancy maintained with more than 60 mg ATR-107 IV), a high immunogenicity with ascending single-dose administration, and pharmacokinetics properties with low bioavailability (approximately 30%) and rapid clearance [107]. Routine laboratory parameters, urinalysis, and physical examinations showed no significant difference. Hypersensitivity reactions and viral upper respiratory tract infection occurred in the 300 mg *anti*-IL-21R antibody SC group, while the placebo, other SC doses and IV treatment cohorts showed general tolerability and safety. Additionally, more than 75% of *anti*-IL-21R antibody-treated healthy

subjects had antidrug antibodies, which limits further clinical applications, and a new therapeutic strategy should be exploited to take full advantage of *anti*-IL-21R antibodies [107].

A phase 1 clinical trial (ClinicalTrials.gov Identifier: NCT01689025) was initiated to investigate the tolerability and safety of NNC0114-0006 (*anti*-IL-21 monoclonal antibody) in clinical active SLE patients with stable background therapies by administering four doses of *anti*-IL-21 subcutaneously every other week. However, no results have been published yet.

As IL-21 has as an essential role in the occurrence and progression of SLE, treatment with neutralizing antibodies to restrain IL-21 signaling shows amazing improvement of clinical parameters and outcome measurements. Furthermore, the bidirectional effect of IL-21 exerts a negative or positive impact on the new-onset or terminal phase of SLE. Despite their dissatisfactory bioavailabilities and clearance rates, the advantages and safety of human *anti*-IL-21 antibodies should be considered and exploited to the fullest.

### 8.2. IL-21 and RA

RA is a systemic chronic autoimmune disease affecting more than 1% of the population that is characterized by cartilage and bone destruction, synovial inflammation, and hyperplasia of synovial tissues [28,108].

In comparison with healthy control, RA patients have obviously elevated frequencies of circulating naive B cells, activated B cells, and Tfh cells and serum IL-21 levels. Furthermore, the percentage of Tfh cells is positively correlated with the ratio of $CD95^+$ B cells and accompanied by the expression of anti-cyclic citrullinated peptide (*anti*-CCP) antibodies [92–94]. Surprisingly, after one month of drug therapy, the accumulation of $CD3^+$ $CD4^+$ $PD\text{-}1^+$ $CXCR5^+$ Tfh cells and $CD19^+$ $CD86^+$ B cells was significantly decreased and associated with evidently lower levels of IL-21 in 9 of 13 treatment-responding RA patients [94].

$CD4^+$ $PD\text{-}1^+$ memory T cells have elevated expression of IL-21, CXCL13, IFN-γ, MAF, SAP, and Blimp-1, but not IL-2 or Bcl-6, which is similar to that of synovial fluid $PD\text{-}1^+$ cells. As expected, $CD4^+$ $PD\text{-}1^+$ T cells isolated from RA synovial fluid show sky-high expression of IL-21 mRNA—more than 100-fold—compared to that of $PD\text{-}1^-$ T cells, as well as higher CXCL13, IFN-γ and IL-10. MAF, an IL-21 promoter, is upregulated in both $PD\text{-}1^+$ $CXCR5^-$ and $PD\text{-}1^+$ $CXCR5^+$ cells and may be involved [28]. Compared with osteoarthritis (OA) patients, upregulated IL-21R is observed in synovial tissues as well as fibroblast-like synoviocytes (FLS) of RA patients. As IL-21 induces the proliferation of FLS and the secretion of IL-6 and tumor necrosis factor α (TNF-α) via the STAT3 and PI3K/AKT pathways, IL-21R-Fc interdicts IL-21 signaling and attenuates the release of IL-6 and TNF-α [109]. Total RNA extracts and inflamed synovial tissue of RA patients expressed higher levels IL-21R compared with those of OA patients, which expressed none or only minimal IL-21R; interestingly, in the same samples, IL-21 was undetectable by real-time PCR or immunohistochemical staining in synovial tissue [8,9,32]. When culturing synovial tissue in vitro, IL-21R-Fc suppressed the secretion of proinflammatory cytokines such as TNF-α, IL-6 and IL-1β from culture medium [110].

Compared with psoriatic arthritis, the proportion of synovial fluid $CD4^+$ $IL\text{-}21^+$ T cells increases significantly in RA patients, and peripheral blood $CD4^+$ $IL\text{-}21^+$ T cells positively associate with disease activity score 28 (DAS28), anti-CCP antibodies, and rheumatoid factor (RF) [111]. IL-21 induces osteoclastogenesis in a PI3K/AKT-dependent manner, accompanied by receptor activation via the nuclear factor-κB ligand (RANKL) pathway, and promotes the expression of calcitonin receptor (CTR) and cathepsin K [112]. Multiple inflammatory cytokines make a difference in the onset and progression of RA. The concentration of IL-21 is remarkably higher in RA than in osteoarthritis (OA) patients and HCs and positively correlates with ESR and disease activity [93,94,108]. In line with these results, IL-21 is increased in the synovium and serum of collagen-induced arthritis (CIA) mice [113]. Importantly, when stimulated by IL-21 plus anti-CD3, isolated T cells from the peripheral blood or synovial fluid of RA patients secrete incredibly higher amounts of TNF-α and IFN-γ and induce more CD69 expression than controls [32], suggesting IL-21 has the ability to upregulate proinflammatory cytokines and contributes to the worsening of disease.

Neutralizing both IL-21 and TNF leads to significantly lower levels of matrix metalloproteinase (MMP) produced by FLS, suggesting that *anti*-IL-21 combined with anti-TNF could be a feasible therapeutic strategy in RA patients to improve inflammation or joint destruction induced by MMP [111]. IL-21 induces the migration and invasion of RA fibroblast-like synoviocytes and promotes the production of MMP-2, MMP-3, MMP-9, and MMP-13, which involves the PI3K, STAT3 and extracellular signal-regulated protein kinase 1 and 2 pathways [111,114]. *Anti*-IL-21 autoantibodies are detected in RA, and the concentration is prominently elevated in the serum of RA patients compared to that of OA patients and HCs. There are positive correlations between anti-21 autoantibody levels and IgG, IgA, IgM, and disease activity; however, there is no association between *anti*-IL-21 autoantibody titers and IL-21 levels [108].

CIA mice are a classical RA model whose oncome and development are prevented in IL-21R KO background mice. IL-21R-deficient mice also show the impairment of chicken type II collagen (CII)-specific IgG, and GC B cells in the draining lymph nodes are reduced; nevertheless, the responses of CII-specific Th1 and Th17 cells and the number of CII-specific Tfh cells are unaffected [115]. $IL\text{-}21R^{-/-}$ K/BxN mice, another RA model mainly involving inflammatory responses mediated by autoantibodies, completely reverses the development of spontaneous arthritis and shows lower proportions of Tfh, IgG1 memory B and autoantigen-specific IgG1 plasma cells but more Th17 cells. Additionally, low expression levels of receptor activator of NF-B ligand (RANKL) are observed in the synovia of IL-21R KO mice, indicating that IL-21 plays an essential role in the oncome and development of autoimmune arthritis via IL-21-dependent Tfh cell proliferation, autoreactive B cell maturation and RANKL induction, rather than Th17 cell function [113,116].

Collagen-induced arthritis (CIA) animals are a recognized RA model. The CIA model is induced by immunizing DBA/1 mice with bovine type II collagen. After observing inchoate arthritis symptoms, the induced mice are treated with murine IL-21R-Fc fusion protein or *anti-E. tenella* as a control [117]. IL-21R-Fc treatment decreases the histologic signs in CIA mice and clinical symptoms with a dose-response relationship, and 400 μg of IL-21R-Fc shows greater improvement than 200 μg and reduces serologic titers of nonspecific IgG1, IL-6 and IL-6 mRNA in the paws while increasing IFN-γ mRNA in the paws [117]. In another adjuvant-induced arthritis model, Lewis rats are immunized with Freund's complete adjuvant, followed by the administration of IL-21R-Fc protein when articular inflammation peaks, which results in a remarkable dose-dependent amelioration of swollen joint scores; conversely, clinical signs are fully recovered when IL-21R-Fc is administered up to 6 mg/kg. Additionally, the histologic phenotypes of synovitis and cartilage damage scores are remarkably reduced with IL-21R-Fc treatment [117], indicating that blocking the IL-21 pathway would be a promising strategy to reduce the infiltration of inflammatory cells and cartilage erosion, even in established disease models. An IL-21 blockade strategy has been tested in a randomized, double-blind, placebo-controlled trial. NNC0114-0005, a human recombinant *anti*-IL-21 monoclonal antibody, has been assessed for safety, pharmacodynamics and pharmacokinetics in RA patients and healthy subjects [118]. Participants have been randomly divided into single intravenous (IV) or subcutaneous (SC) dose ($\leq$25 mg/kg IV; $\leq$ 4 mg/kg SC) treatment groups or a placebo group and assessed for more than 12 weeks. After treatment of 20 RA patients (active and with methotrexate treatment) and 44 healthy subjects with the *anti*-IL-21 monoclonal antibody, the main side effects were headache and nasopharyngitis, and there was no

skin reaction to injection. Furthermore, the adverse effects rate in the treatment group was not higher than that of the placebo group (44% of the drug-treated group and 63% of the placebo-treated group), suggesting that a single dose is fully tolerated in both types of subjects. The study indicated linear pharmacokinetics of the *anti*-IL-21 monoclonal antibody, with 2–3 weeks of mean terminal half-life, and RA activity was reduced with at least 25 mg/kg NNC0114-0005. In the NNC0114-0005 treated group, there was no significant change in the percentage and IL-21R expression of lymphocyte subsets [118].

Additionally, two closely linked clinical trials were presented at the Annual Meeting of American College of Rheumatology (no study results posted). The clinical trials both are randomized, double-blind, placebo-controlled, and are attempting to evaluate the safety and efficacy of an *anti*-IL-21 antibody (NNC0114-0006) in active RA patients with a methotrexate treatment background and healthy subjects [119,120]. One is a phase 1 trial including 32 RA patients who have been randomized to SC administration (at four doses of 0.05, 0.25, 1 or 4 mg/kg every other week for six weeks) of NNC0114-0006 or placebo [120]. The other is a phase 2 trial in which 62 RA patients have been randomized to NNC0114-0006 (12 mg/kg IV, n = 41) or placebo, and two doses have been administered six weeks apart [119]. The incidence of adverse events between treatment and placebo patients is equal in both trials. However, increased risks of infection and skin responses have been observed in patients with high-level *anti*-IL-21 antibody treatment compared with other doses and the placebo group [119,120]. Additionally, anti-drug antibodies have not been detected, and NNC0114-0006 has been identified as safe and tolerable [119,120]. A significant reduction in DAS28 and c-reactive protein (CRP) has been found in the *anti*-IL-21 antibody group, as well as large improvements in swollen and painful joints, although the changes have no statistical significance at the endpoint [119].

Clinical trial and animal experiments have proven that *anti*-IL-21 antibodies or IL-21R-Fc protein could be promising drugs to help RA patients and also improve the activity or joint symptoms of RA, although such treatments may increase the risks of infection and skin disorders. More advantages and disadvantages of *anti*-IL-21 and IL-21R-Fc in RA should be researched for applications in clinical practice.

### 8.3. IL-21 and T1D

T1D or insulin-dependent diabetes, a primarily T cell–driven autoimmune disease, is characterized by the destruction of pancreatic β-cells and the production of islet autoantibodies. B cells have been demonstrated to play a pathogenic role in T1D, and the abnormal activation of autoreactive B cells is largely dependent on Tfh cells [56,121].

Tfh cells are increased in the progression of T1D; both the $CD4^+$ $CXCR5^+$ $PD\text{-}1^+$ $ICOS^+$ Tfh and $CD4^+$ $CXCR5^+$ $PD\text{-}1^+$ $ICOS^-$ Tfh subsets are activated ex vivo and produce high levels of IL-21, which efficiently induces naive B cells and positively correlates with multiple islet autoantibodies [56,121]. In line with these results, the serum levels of IL-21 and IL-17 are obviously higher in T1D patients [95]. There are higher frequencies of $CD4^+$ $IL\text{-}21^+$ T cells and $IL\text{-}21^+$ $CD45RA^-$ memory T subsets in T1D patients and upregulated expression of Tfh cell markers, including CXCR5, ICOS, PD-1, Bcl-6 and IL-21, compared with those in HCs [121,122].

Nonobese diabetic (NOD) mice are a predisposed autoimmune model for spontaneous T1D, and the IL-21 pathway is irreplaceable in the oncome and progression of the NOD model [24,123,124]. The diabetes-modified insulin-dependent diabetes 3 (Idd3) site is also found at the IL-2/IL-21 locus, and significantly increased production of IL-21 is observed in NOD mice [124,125]. IL-21 is overexpressed in pancreatic β-cells and leads to the secretion of inflammatory cytokines and chemokines, such as IL-17A, IL-17F, IFN-γ, monocyte chemoattractant protein-1 and -2 (MCP-1 and MCP-2), and interferon-inducible protein-10 (IP-10), resulting in leukocytic infiltration and islet destruction and even inducing spontaneous T1D in diabetes-resistant C57Bl/6 mice [124]. The IL-21R-deficient NOD mice are resistant to spontaneous T1D, including decreased production of insulin autoantibodies and reduced lymphocytic infiltration into the pancreas [124,125]. In $IL\text{-}21R^{-/-}$ NOD mice, there is an obvious defect in autoreactive effector T cells, while the lymphoid compartment is normal, and the regulatory T cell ratio and effector cytokine responses show no change [124], suggesting that the IL-21 pathway contributes to diabetes by augmenting the proliferation of T cells. Conversely, when IL-21R-sufficient DCs are transfected into IL-21R KO NOD mice, the insulin-dependent diabetes resistance is broken. Antigen-presenting DCs in the pancreas need IL-21R to catch the chemokine receptor CCR7 and immigrate to draining lymph nodes [85].

Accumulating evidence shows that IL-21 induces pancreas destruction, inflammatory infiltration, and the production of islet autoantibodies and inflammatory factors in T1D. In other words, blocking IL-21 would be a feasible strategy to recover impaired islets and resist hyperglycemia. The NOD model has higher plasma levels of IL-21 corresponding to the destruction of islets and initiation of diabetes [126]. Moreover, increased IL-21 expression may predict subsequent clinical diabetes. Blockade of IL-21 signaling in NOD mice evidently inhibits diabetes. The IL-21R-Fc chimeric protein blocks IL-21 at different stages of the disease process. The effects of short-term treatment at the early phase are reversible and have a limited impact on disease incidence. After 11 days of treatment at the late prediabetic phase, the use of IL-21R-Fc prolongs the onset and decreases morbidity to 40% compared with 90% for control group at 40 weeks [126]. NOD mice treated with either IL-21R-Fc or IL-21 neutralizing antibody show improved insulitis only at the preclinical diabetes stage. The early effects of IL-21R-Fc are lower islet infiltration of $CD4^+$ T, $CD8^+$ T and $B220^+$ B cells, while $CD8^+$ T and B cells, instead of $CD4^+$ T cells, maintain lower levels in the long term. Neutralizing IL-21 reduces IL-21-producing cells and attenuates the number of activated lymphocytes in islets [126]. Because IL-21 suppresses $CD8^+$ T cell effects, neutralization of IL-21 combined with islet transplantation significantly recovers autoimmune diabetes, hyperglycemia, and reduce islet allograft rejection [126].

Homoplastically, the use of an *anti*-IL-21 monoclonal antibody significantly postpones or prevents diabetes in both NOD and NOD. scid adoptive transfer models in a dose-dependent manner [123]. Excitingly, an *anti*-IL-21 antibody combined with the GLP-1R agonist liraglutide effectively reverses preexisting diabetes, and enhanced efficacy is observed particularly in severely hyperglycemic mice. Combined treatment is better than either monotherapy to reverse established autoimmune diabetes. After therapy withdrawal, the majority of model mice still retain stable normoglycemia [123].

Fundamental research and data from disease models provide evidence that IL-21 is of vital importance in mediating the immune and inflammatory responses and induces autoimmune damage to the pancreatic islets. Either *anti*-IL-21 or IL-21R-Fc alone or combined with other treatments can recover glycemia and maintain euglycemia in the long term, which would be a profound strategy for the treatment of insulin-dependent diabetes, especially insulin-resistant patients.

### 8.4. IL-21 and ITP

ITP, an autoimmune bleeding disorder, is connected with impaired platelet production and increased platelet destruction, both of which are thought to be mediated by antiplatelet autoantibodies dependent on T-B cell interactions [57]. Additionally, cytokines are supposed to contribute to the pathogenesis of ITP.

The frequency of Tfh cells and the secretion of serum IL-21, IL-4 and BAFF are distinctly elevated in ITP compared with that in controls [57,69]. A high level of IL-21 induces the generation of abnormal plasma cells in ITP patients, unrelated to BCR signaling, and this effect does not happen in NC cells. IL-21, *anti*-IgM and CD40L promote the expression of phosphatase and tensin homolog (PTEN) protein, an

activator of B cells mediated by PI3K signaling, to regulate the proliferation and function of B cells [69]. Tfh cells show a convincing negative correlation with platelet count and a positive connection to GPIIb/IIIa and GPIb/IX; both react against self-antigen autoantibodies produced by autoreactive B cells. Additionally, the mRNA levels of Bcl-6 and c-Maf on $CD4^+$ T cells are forcefully increased, while Blimp-1 is decreased in ITP patients [57]. After treatment, the elevated percentage of Tfh cells, serum levels of IL-21 and IL-4, and transcription of Bcl-6 mRNA in newly diagnosed ITP patients return to the same levels as those in NCs, suggesting that Tfh-dependent IL-21 and Bcl-6 are involved in the onset and development of ITP [57].

This research demonstrates that aberrant Tfh cells, IL-21 and IL-4 may contribute to the immunopathogenesis of ITP [57,69].

### 8.5. IL-21 and pSS

pSS, a systemic autoimmune disease, is characterized by lymphocytic infiltration of T and B cells and the involvement of the implicate lacrimal and salivary glands, leading to dry eyes and mouth [65]. pSS also involves extra-glandular tissues and organs such as musculoskeletal tissues, lung and skin [4,65]. Hallmarks of pSS are Tfh-induced dysfunctional GC formation and B cell hyperactivity with hypergammaglobulinemia and autoantibody secretion [65].

Patients with pSS show a high frequency of Tfh cells and significant overexpression of IL-21, the signature cytokine positively correlated with IgG, especially IgG1 levels [65,96]. With abatacept treatment, the high serum levels of IL-21 and elevated numbers of Tfh cells and plasmablasts recover to normal levels [65]. In the labial salivary glands (LSGs), that expression of IL-21 is higher, while that of IL-21R is low in the damaged areas in pSS patients comparing with those in control LSGs, which display minimal expression of both molecules [96]. pSS patients have elevated percentages of transitional and mature B cells and decreased primary memory B cell ratios, which are associated with granzyme B and IL-21R expression on $CD19^+$ B cells [87]. Enhanced IL-21R expression is mainly produced by $CD19^+$ $CD5^+$ B cells rather than $CD19^+$ $CD5^-$ B cells and induces granzyme B production and regulates $CD19^+$ $CD5^+$ B cell functions, indicating that IL-21 signaling presumably acts as a looped regulator and has a crucial role in the progression of pSS [87].

There is a distinct association between IL-21 and pSS, and IL-21 is involved in the pathogenesis of pSS, which could indicate a direction for in-depth research.

### 8.6. IL-21 and pemphigus

Pemphigus is an autoimmune bullous disease targeting damaged skin and mucosal membranes [127]. Circulating *anti*-desmoglein (the cell adhesion molecules) 1 and 3 antibodies are biomarkers and mediate blister formation. Using immunohistochemistry and immunofluorescence staining, enormous quantities of B cells and T helper cell subsets, particularly Tfh and plasma cells, have been detected in skin lesions [127,128].

Infiltrated T lymphocytes are presents in pemphigus vulgaris lesions, and most of these cells are $CD4^+$ T helper cells that secrete IL-21 and IL-17a, instead of typical $CXCR5^+$ Tfh cells [127,129]. Consistently, IL-27 and IL-21-expressing T cells are involved in the nosogenesis of pemphigus, accompanied by increased plasma levels of IL-21 [128]. The above results suggest that IL-21 is an important cytokine that links morbigenous cells to pemphigus by promoting T helper cells and antibody-secreting cells and T-B cell interactions. A recent clinical trial (ClinicalTrials.gov Identifier: NCT03177213) in patients with pemphigus vulgaris tried to investigate the role of IL-21 by measuring serum IL-21 levels and comparing them with those of healthy subjects. Regrettably, there are no posted results.

### 8.7. IL-21 and AITD

AITD, predominantly comprising Graves' disease (GD) and Hashimoto's thyroiditis (HT), is an organ-targeting autoimmune disease [7]. There are higher plasma IL-21 and IL-21 mRNA levels in newly diagnosed GD and HT patients and elevated proportions of $CD3^+$ $CD8^-$ $IL\text{-}21^+$ T cells in HT patients compared with those in HCs. However, IL-21 shows no positive relation with clinical parameters, including FT3, FT4, TRAb, TgAb, and TPOAb [7,97].

Thyroid epithelial cells and lymphocytes from AITD patients express IL-21R. Using immunohistochemistry, IL-21R transcription on HT was shown to be stronger than that on GD, while normal thyroid samples were weakly stained [7]. In vitro, PBMCs cultured with rhIL-21 displayed enhanced IL-17A expression but inhibited IL-4 production by GD and promoted INF-γ secretion by HT [7]. The IL-21 gene has been located at chromosome 4q26-q27, which is connected to 4 autoimmune diseases: Celiac disease, T1D, GD and RA [130]. Although the role of IL-21 in GD onset and development is less well understood, there is a genuine relationship between IL-21 and AITD.

### 8.8. IL-21 and psoriasis

Psoriasis, a chronic inflammatory disease, is characterized by skin plaques as a result of keratinocyte hyperplasia in the epidermis and inflammatory cell infiltration [22]. Both protein and mRNA levels of IL-21 are higher in psoriatic lesions than in nonpsoriatic skin from the same patients and from healthy individuals. Among other cytokine family members, the mRNA expression of IL-15, rather than IL-17, is upregulated in skin lesions. The expression of IL-21R is detected in impaired skin keratinocytes from psoriasis patients, and patients have higher plasma levels of IL-21 as well [10].

Increased IL-21 induces the generation of keratinocytes via the MAPK pathway, and the IL-21-mediated activation of extracellular signal–related kinase-1 and 2 and mitogen-activated protein kinase is also involved. IL-21 intradermal injection results in epidermal hyperplasia and infiltration of inflammatory cells as well as upregulated mRNA levels of IFN-γ, IL-17A and IL-22. In a human psoriasis xenograft mouse model, an IL-21 blocking antibody reduces inflammatory cells, recovers keratinocyte proliferation and epidermal thickness, and decreases the transcription of IFN-γ and IL-17A [10], indicating that *anti*-IL-21 administration may become a brand-new treatment strategy for psoriasis.

## 9. Conclusion

In the past decade, the mechanism by which IL-21 affects the immune system has been intensively studied. Increasing numbers of studies have shown a critical pathogenic role of IL-21 in the initiation and progression of autoimmune diseases. However, more future studies are needed to uncover the underlying mechanisms. In addition, blocking the IL-21 signaling pathway with *anti*-IL-21 antibodies or IL-21R-Fc fusion proteins has shown a therapeutic role, impairing the symptoms of autoimmune diseases and improving prognosis in preclinical and clinical studies. However, this therapeutic efficiency varies in different diseases. Personalized treatments, such as the detection of IL-21 levels in circulation before treatment, are needed. Furthermore, safety and side effects should also be considered. For example, IV or SC treatment with high-level *anti*-IL-21 doses may increase the risk of viral infection or anaphylactic reaction. Moreover, without IL-21 signaling, B cell maturation and the effects of NK and $CD8^+$ T cells are restricted, in which case infection and tumors might be problematic. Therefore, doctors need to balance the risks and benefits of using systemic *anti*-IL-21 before treatment. Altogether, targeting IL-21 has provided us with new strategies to treat autoimmune diseases, especially those diseases mediated by B cells and those patients who are resistant to steroids and immune suppressants. Tables 2 and 3.

**Table 2**
Animal data on IL-21 in autoimmune diseases.

| Disease | Approach | Animal data | Ref. |
|---|---|---|---|
| Systemic lupus erythematosus | Block IL-21R | ↓Lupus manifestations in BXSB-Yaa mice and prolonged survival | [88,101] |
| | IL-21R$^{-/-}$ donors or IL-21R$^{-/-}$ hosts | ↓GC B cell proportion and plasma cell differentiation, autoantibody production in GVHD mice<br>Increased IL-21 expression associates with the differentiation of Tfh and Th17 cells | [31,102] |
| | *Anti*-IL-21 antibody | ↑Survival rate of B6.Sle1.Yaa mice<br>↓Glomerulonephritis, levels of anti-chromatin or *anti*-dsDNA antibodies | [12] |
| | IL-21R-Fc protein | ↓Proteinuria, immune deposition and disease severity in MRL-Fas$^{lpr}$ mice<br>↓Splenic T/B cells, autoantibodies | [75,104] |
| | IL-21R antibodies | ↑Survival rate of NZB/NZW mice<br>↓Nephritis and lymphocyte activation | [105] |
| | IL-21R-Fc protein | Anabatic disease severity at early stages in BXSB-Yaa mice and improved survival rate at later stages | [106] |
| Rheumatoid arthritis | Block IL-21R | ↓Ag-specific IgG, B cells in collagen-induced arthritis mice, accompanied by resistance to disease onset and development | [115] |
| | Block IL-21R | ↓Tfh, memory B, plasma B cells in K/BxN mice | [116] |
| | IL-21R-Fc protein | ↓Histologic signs, clinical symptoms, serologic titers of IgG1 and IL-6 in collagen-induced arthritis mice<br>↓Disease severity of adjuvant-induced arthritis rats | [117] |
| Type 1 diabetes | Increased IL-21 | ↑Inflammatory cytokines and chemokines in NOD mice, positively associated with islet destruction | [124,126] |
| | Block IL-21R | ↓Spontaneous T1D in NOD mice<br>↓Autoreactive effector T cells | [124,125] |
| | IL-21R-Fc protein or *Anti*-IL-21 antibody plus islet transplantation | ↓Morbidity, insulitis, islet infiltration<br>Recovers autoimmune diabetes and hyperglycemia and reduces allograft rejection | [126] |
| | *Anti*-IL-21 antibody or *anti*-IL-21 antibody plus liraglutide | Postpones or prevents diabetes in NOD mice and NOD.scid adoptive transfer mice | [123] |
| Psoriasis | IL-21-specific antibody | ↓Keratinocyte proliferation and inflammatory cells in a psoriasis xenograft model, downregulates IFN-γ and IL-17A | [10] |

GVHD, graft-versus-host disease; Ag, antigen; IgG, immune globulin G; Th17, T helper 17; Tfh, follicular helper T; NOD, Nonobese diabetic; T1D, Type 1 diabetes.

**Table 3**
Human data on IL-21 in autoimmune diseases and the safety or efficacy of *anti*-IL-21 treatment.

| Disease | Approach | Human data | Ref. |
|---|---|---|---|
| Systemic lupus erythematosus | Increased IL-21 | ↑Amplification of CD11c$^{+}$ B, memory B, Th17 cells and differentiation of autoreactive plasma cells, accompanied by Bcl-6 expression Positively associates with Tfh cells, SLEDAI, C3, ESR | [15,50,86,91] |
| | Block IL-21R | ↓B cell hyperactivity and production of autoantibodies | [31,75] |
| Rheumatoid arthritis | Increased IL-21 | In plasma and synovial fluid memory T cells<br>↑Osteoclastogenesis, expression of CTR and cathepsin K<br>Positively associated with DAS28, *anti*-CCP antibodies, RF and ESR | [28,93,94,108,112] |
| | IL-21 plus anti-CD3 | ↑TNF-α and IFN-γ from T cells | [32] |
| | *anti*-IL-21 plus *anti*-TNF | ↓MMP produced by FLS | [111,114] |
| | IL-21R-Fc protein | ↓TNF-α, IL-6 and IL-1β of synovial tissue | [109,110] |
| | Upregulated IL-21R | In inflamed synovial tissues and FLS | [9,32,109] |
| | Human *anti*-IL-21 monoclonal antibody (NNC0114-0005) | Randomized, double-blind, placebo-controlled trial<br>Fully tolerated in RA and healthy subjects and improves RA activity with linear pharmacokinetics | [118] |
| | Human *anti*-IL-21 antibody (NNC0114-0006) | Randomized, double-blind, placebo-controlled trials<br>Safety in RA and healthy subjects<br>Increased risks of infection and skin responses with high levels of *anti*-IL-21 antibody | [119,120] |
| Type 1 diabetes | Increased IL-21 | ↑Naive B cells and positively associated with islet autoantibodies | [56,95,121] |
| Immune thrombocytopenia | Increased IL-21 | ↑Abnormal plasma cells | [57,69] |
| Primary Sjogren's syndrome | Increased IL-21 | In plasma and damaged areas and positively associated with IgG levels | [65,96] |
| | Increased IL-21R | In CD19$^{+}$ CD5$^{+}$ B cells and induces cellular production of granzyme B | [87] |
| Autoimmune thyroid disease | Increased IL-21R | In thyroid epithelial cells and lymphocytes with increased IL-21 plasma levels | [7,97] |
| Psoriasis | Increased IL-21 | In plasma and psoriatic lesions | [10] |
| Healthy subjects | Human *anti*-IL-21R antibody (ATR-107) | A phase 1 study indicates a long-term pharmacodynamics effect, high immunogenicity with low bioavailability and rapid clearance because of antidrug antibodies | [107] |

Th17, T helper 17; Tfh, follicular helper T; SLEDAI, SLE Disease Activity Index; C3, Complement 3; ESR, erythrocyte sedimentation rate; CTR, calcitonin receptor; TNF, tumor necrosis factor; MMP, matrix metalloproteinase; FLS, fibroblast-like synoviocytes; DAS28, disease activity score 28; CCP, cyclic citrullinated peptide; RF, rheumatoid factor; RA, rheumatoid arthritis.

## Author's contributions

Di Long wrote the manuscript; Yongjian Chen, Haijing Wu, Ming Zhao and Qianjin Lu revised the manuscript.

## Conflicts of interest

The authors declare no conflicts of interest.

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