Interleukin-21: a double-edged sword with therapeutic potential

Rosanne Spolski and Warren J. Leonard

Abstract | Interleukin-21 is a cytokine with broad pleiotropic actions that affect the differentiation and function of lymphoid and myeloid cells. Since its discovery in 2000, a tremendous amount has been learned about its biological actions and the molecular mechanisms controlling IL-21-mediated cellular responses. IL-21 regulates both innate and adaptive immune responses, and it not only has key roles in antitumour and antiviral responses but also exerts major effects on inflammatory responses that promote the development of autoimmune diseases and inflammatory disorders. Numerous studies have shown that enhancing or inhibiting the action of IL-21 has therapeutic effects in animal models of a wide range of diseases, and various clinical trials are underway. The current challenge is to understand how to specifically modulate the actions of IL-21 in the context of each specific immune response or pathological situation. In this Review, we provide an overview of the basic biology of IL-21 and discuss how this information has been — and can be — exploited therapeutically.

Interleukin-21 (IL-21) is a pleiotropic cytokine that is composed of four α-helical bundles and produced primarily by natural killer T (NKT) cells, T follicular helper (T_{FH}) cells and T_H17 cells (FIG. 1), with lower levels of production by numerous other populations of lymphohaematopoietic cells[1,2]. IL-21 signals via heterodimers of the IL-21 receptor (IL-21R)[2,3] and the common cytokine receptor γ-chain, γ_c (REF. 4) (encoded by *IL2RG*) (FIG. 2), which is mutated in individuals with X-linked severe combined immunodeficiency (X-SCID)[5] and is also shared by the receptors for IL-2, IL-4, IL-7, IL-9 and IL-15 (REF. 6). X-SCID is a disease that is characterized by profoundly decreased numbers of T and NK cells, resulting from defective IL-7 and IL-15 signalling, respectively; B cells develop but they are not functional[6], as IL-21 is crucial for their function (as discussed below).

Functional IL-21R is broadly expressed on lymphohaematopoietic populations, including on myeloid cells[1]. Correspondingly, IL-21 exerts its effects on a broad range of cell types (FIG. 1). Given the breadth of immunomodulatory targets and the pleiotropic actions of IL-21, IL-21 and IL-21R are attractive targets for therapeutic manipulation; indeed, antibodies against IL-21 and IL-21R (see the clinical trials listed in TABLE 1) as well as IL-21 antagonists[7] have been developed (FIG. 2). As discussed herein, IL-21 itself is under evaluation in Phase I and II clinical trials for its anticancer activity, whereas blocking IL-21 has been evaluated in Phase I clinical trials for rheumatoid arthritis and is being evaluated in Phase I trials for systemic lupus erythematosus and Crohn's disease. These treatments may also be a promising way to control a range of autoimmune and potentially other diseases.

Laboratory of Molecular Immunology, National Heart, Lung, and Blood Institute (NHLBI), US National Institutes of Health, Bethesda, Maryland 20892, USA.
e-mails: spolskir@nhlbi.nih.gov; wjl@helix.nih.gov
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Signalling by IL-21

IL-21 signals via the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) signalling pathway, the mitogen-activated protein kinase (MAPK) signalling pathway and the phosphoinositide 3-kinase (PI3K)–AKT signalling pathway (FIG. 2). Like other γ_c family cytokines, IL-21 activates JAK1 and JAK3. Interestingly, in T cells IL-21 activates STAT3 more potently and in a more sustained fashion than STAT1, STAT5A and STAT5B[8]. In studies of the regulation of mouse B lymphocyte-induced maturation protein 1 (BLIMP1; a transcription factor encoded by the *Prdm1* gene), an IL-21 response element was shown to be composed of a bipartite element that binds both interferon regulatory factor 4 (IRF4) and STAT3 (REF. 9). Unexpectedly, when analysed by chromatin immunoprecipitation linked to next-generation sequencing (ChIP–seq), such bipartite response elements were found in a genome-wide fashion and were globally involved in the regulation of many IL-21-responsive genes[9].

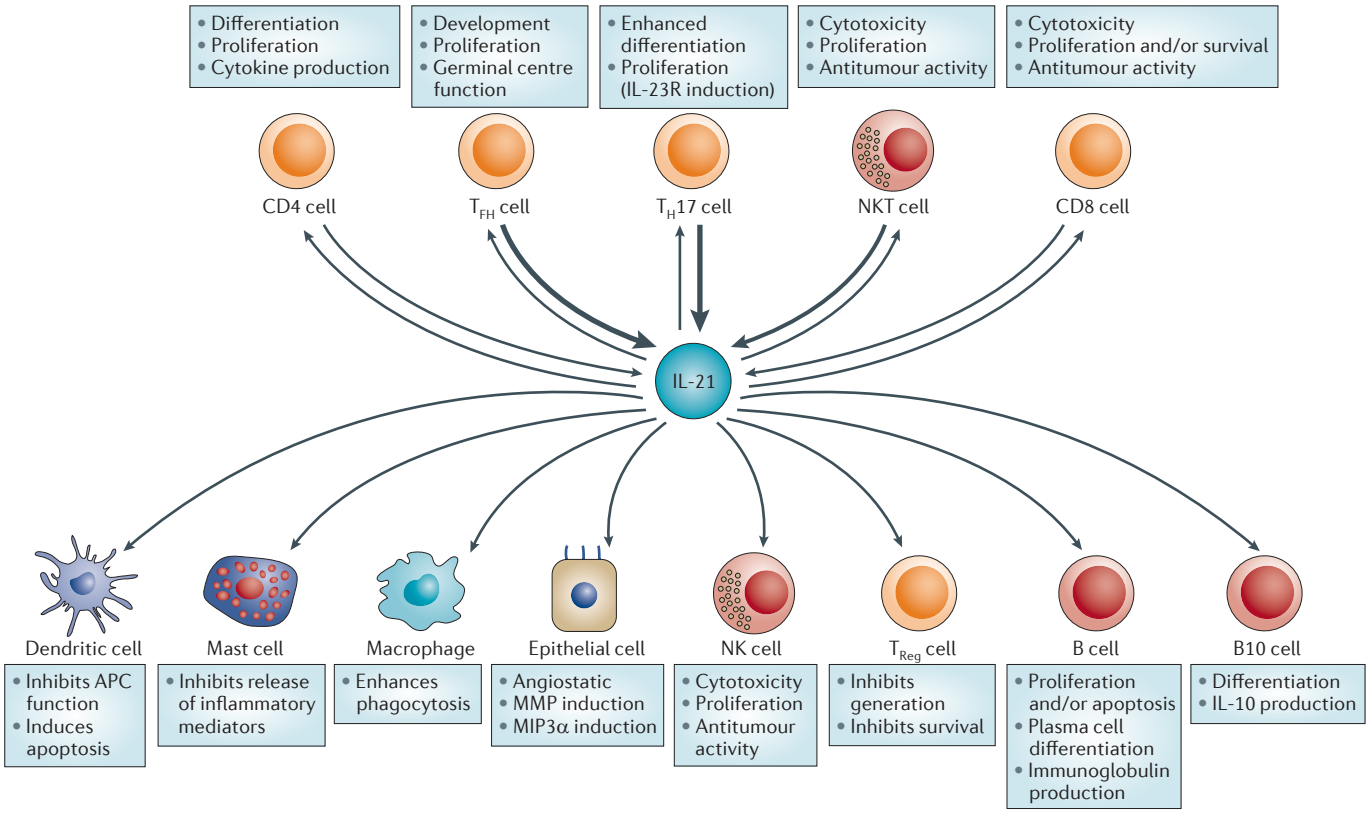

Figure 1 | **Sources of IL-21 and its cellular targets.** Interleukin-21 (IL-21) is produced by $CD4^+$ T cell populations, with the highest production by T follicular helper (T_{FH}) cells and T_H17 cells, and slightly lower levels produced by natural killer T (NKT) cells (see bold arrows). $CD8^+$ T cells can also produce IL-21. IL-21 exerts actions on multiple lymphoid and myeloid populations as well as on epithelial cells. The consequences of IL-21 signalling in each cell type are listed. APC, antigen-presenting cell; IL-23R, IL-23 receptor; MIP3α, macrophage inflammatory protein 3α; MMP, matrix metalloproteinase; T_{Reg}, regulatory T.

An added complexity for IL-21 signalling in T cells was identified by studies demonstrating that IRF4 binds to AP-1–IRF composite elements (AICEs) in T cells, whereas it binds to ETS–IRF composite elements (EICEs) in B cells[10–12]. These latter sites are bound by PU.1–IRF4 complexes, whereas the former are bound by basic leucine zipper transcription factor ATF-like (BATF)–JUN–IRF4 complexes[10–12]. Therefore, in T cells, many target genes of IL-21 are regulated through BATF, JUN, IRF4 and STAT3. These transcription factors are also potential targets through which IL-21 signalling might be modulated in T cells. Importantly, a JAK3 inhibitor, tofacitinib (Xeljanz; Pfizer), which inhibits signalling by IL-21 as well as by other γ_c family cytokines, has now been approved by the US Food and Drug Administration (FDA) for the treatment of rheumatoid arthritis[13]. However, tofacitinib can also inhibit JAK1 and JAK2, so its effects in autoimmune disease may be caused by the inactivation of multiple JAKs or by blocking signalling from other cytokines (in addition to IL-21)[14].

Regulation of humoral responses by IL-21

Immunoglobulin production. Studies in both human and mouse systems have revealed that IL-21 has a major role in the development of B cell immunoglobulin responses. Although IL-21 is not required for B cell development in either the bone marrow or the periphery, *Il21r*-knockout mice have substantially reduced antigen-specific immunoglobulin G1 (IgG1) responses; however, surprisingly, IgE levels are significantly increased in these animals[15]. The elevated levels of IgE are dependent on IL-4, as *Il4*$^{-/-}$ *Il21r*$^{-/-}$ double knockout mice have significantly reduced levels of all immunoglobulin isotypes, including IgE, which is typical of the pan-hypogammaglobulinaemia observed in patients with X-SCID and suggests that both IL-4 and IL-21 are crucial for normal B cell differentiation[15]. Interestingly, following haematopoietic cell transplantation in patients with X-SCID, if donor B cells do not engraft, the *IL2RG*-deficient host B cells can respond to IL-4 and IL-13 *in vitro*, presumably via the γ_c-independent type 2 IL-4R that consists of IL-4Rα and IL-13Rα1. However, the same host B cells cannot respond to IL-21 (REF. 16). If donor B cells expressing γ_c are successfully engrafted, these cells are able to respond to IL-21 as well as IL-4 *in vitro*[16].

In vitro experiments with CD40-stimulated naive human B cells have demonstrated that IL-21 can induce the production of IgG1 and IgG3 by inducing class switch recombination, and this effect is enhanced by IL-4 (REF. 17). Although IL-21 alone cannot induce IgE production in human B cells, it considerably enhances IgE induction by IL-4 (REF. 17). In contrast to these

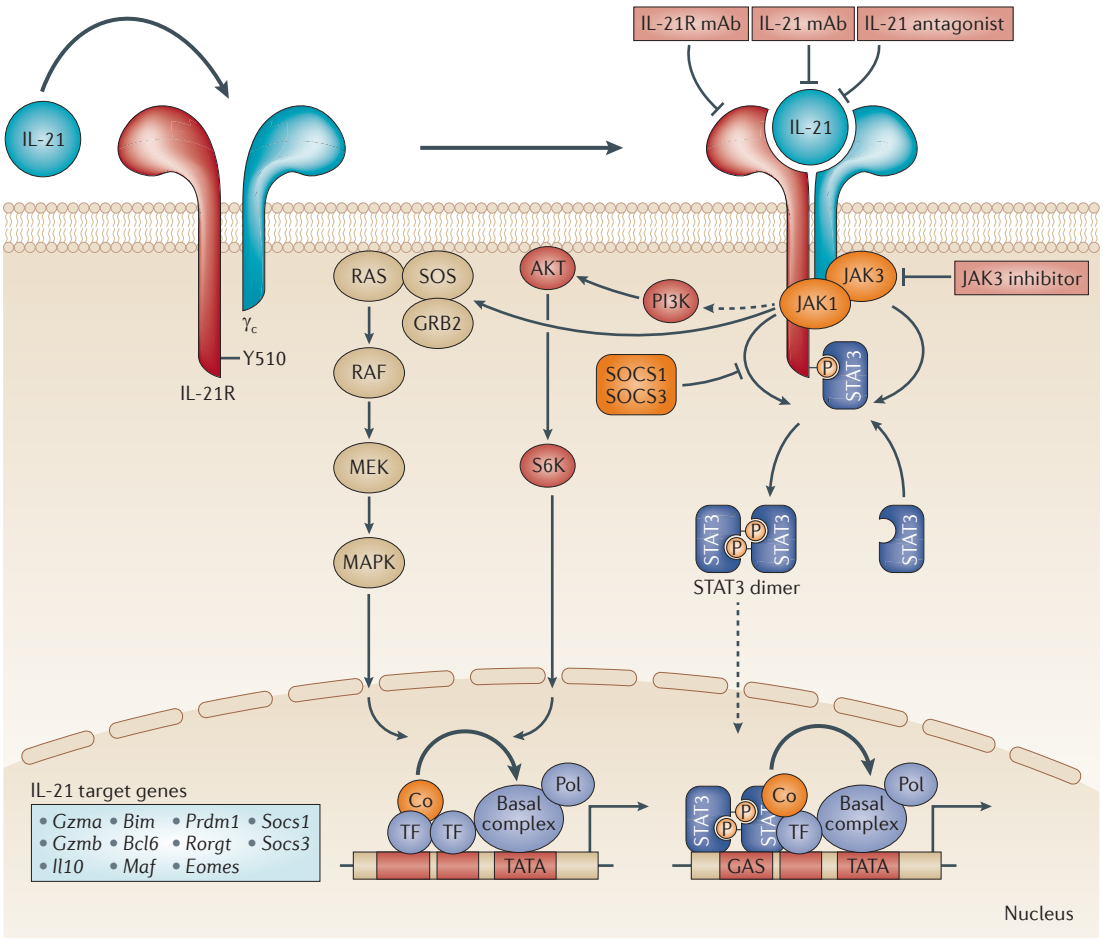

Figure 2 | **IL-21 signals through IL-21R and utilizes the JAK–STAT, MAPK and PI3K pathways.** Interleukin-21 (IL-21) binding stabilizes the complex between the IL-21 receptor (IL-21R) and the common cytokine-γ chain, γ_c, leading to the activation of Janus kinase 1 (JAK1) and JAK3, which allows the recruitment and phosphorylation of signal transducer and activator of transcription (STAT) proteins (predominantly STAT3, but also STAT1 and STAT5). These STAT proteins dimerize, enter the nucleus and activate a transcription programme that includes some of the target genes shown. IL-21 binding to IL-21R can also activate the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) signalling pathways. IL-21 induces the transcription of the suppressor of cytokine signalling 1 (SOCS1) and SOCS3 proteins, which downregulate the JAK–STAT pathway. Various sites are shown at which IL-21 signalling can be interrupted, including by IL-21- or IL-21R-blocking monoclonal antibodies (mAbs), IL-21R–Fc fusion proteins, an IL-21 antagonist or by a JAK3 inhibitor. *Bcl6*, B cell lymphoma 6; *Bim*, BCL-2 interacting mediator of cell death; Co, transcriptional cofactor; *Eomes*, eomesodermin; GAS, gamma activated site; GRB2, growth factor receptor-bound protein 2; *Gzma*, granzyme A; *Gzmb*, granzyme B; MEK, MAPK/ERK kinase; Pol, DNA polymerase; *Prdm1*, gene encoding BLIMP1 (B lymphocyte-induced maturation protein 1); *Rorc*, gene encoding retinoic acid receptor-related orphan receptor-γ (RORγ) and RORγt; S6K, p70 S6 kinase (also known as ribosomal protein S6 kinase); TF, transcription factor.

complementary actions of IL-21 and IL-4, opposing actions of IL-21 and IL-4 have been observed in some *in vitro* assays of B cell immunoglobulin production[18]. For example, IL-21 can induce CD40-stimulated human naive B cells to undergo isotype class switch to IgA, but this induction is inhibited by IL-4 (REF. 19). Some conflicting data regarding the interaction of IL-21 and IL-4 may reflect the ability of IL-4 to inhibit the effects of IL-21 in naive but not memory B cells[20]. IL-21 can also interact synergistically with transforming growth factor-β (TGFβ) to induce IgA isotype switching as well as homing to mucosal sites[21]. These data collectively suggest that the effects of IL-21 on immunoglobulin production *in vivo* may be specific to the stage of B cell differentiation and the site of action as well as the presence or absence of other interacting factors. The elucidation of these interactions will be important, for example, for the design of vaccine strategies directed at various pathogens.

IL-21 induces B cell apoptosis. Although IL-21 can stimulate B cell proliferation and differentiation in the context of a co-stimulatory T cell signal[2], this cytokine can, nevertheless, also potently induce B cell apoptosis, either in the absence of a T cell signal or in the presence of a Toll-like receptor (TLR) signal[22–24]. The pro-apoptotic activity of IL-21 is caspase-dependent and results from

Table 1 | **Clinical trials of therapies targeting the IL-21 pathway**

Treatment	Disease	Phase	Status	Clinical trial details*	Outcome
IL-21	Metastatic melanoma	I	Complete	NN028-1614	Elevated biomarkers[186]
IL-21	Metastatic melanoma and renal cell carcinoma	I	Complete	NCT00095108	• Metastatic melanoma: 1 CR; 11 SD; 24 total • Renal cell carcinoma: 4 PR; 13 SD; 19 total[95]
IL-21	Metastatic melanoma	II	Complete	NCT00514085	ORR: 22.5%[187]
IL-21	Melanoma (stage IV)	IIa	Complete	NCT00336987	ORR: 8.3%[94]
IL-21 plus ipilimumab	Melanoma	I	Active; not recruiting	NCT01489059	-
IL-21 plus PD1-specific antibody	Solid tumours	I	Recruiting	NCT01629758	-
IL-21-expanded NK cells	Acute myeloid leukaemia	I/II	Not yet recruiting	NCT01787474	-
IL-21 plus sunitinib	Renal cell carcinoma (stage IV)	I	Study terminated	NCT00617253	Therapeutic dose not tolerated[188]
IL-21 plus rituximab	Non-Hodgkin's lymphoma	I	Complete	NCT00347971	84% decrease in size target lesion[119]
IL-21 plus sorafenib	Metastatic renal cell carcinoma	I	Complete	NCT00389285	ORR: 21%[189]
IL-21 plus liposomal doxorubicin	Ovarian cancer	II	Complete	NCT00523380	Not yet available
IL-21 plus cetuximab	Colorectal cancer (stage IV)	I	Study terminated	-	No MTD determined[96]
IL-21-specific antibody NNC0114-0006	Systemic lupus erythematosus	I	Recruiting	NCT01689025	-
IL-21-specific antibody NNC0114-0000-0005	Rheumatoid arthritis	I	Complete	NCT01208506	Not yet available
IL-21-specific antibody (NNC0114-0006)	Rheumatoid arthritis	-	Complete	EudraCT-2011-005376-42	Not yet available
IL-21R-specific antibody (ATR-107)	Normal volunteers	I	Complete	NCT01162889	75% anti-drug antibodies[190]
IL-21-specific antibody	Crohn's disease	II	Ongoing	NCT01751152	-
IL-21-specific antibody	Systemic lupus erythematosus	I	Complete	NCT01689025	Not yet available

CR, complete response; IL-21, interleukin-21; IL-21R, IL-21 receptor; MTD, maximum tolerated dose; NK, natural killer; ORR, overall response rate; PD1, programmed cell death protein 1; PR, partial response; SD, stable disease. *All trials with NCT numbers can be found at the ClinicalTrials.gov website.

the induction of BCL-2 interacting mediator of cell death (BIM), a pro-apoptotic mitochondrial protein. IL-21-induced B cell apoptosis can potentially serve as a mechanism for eliminating inappropriately activated autoreactive B cells, which is perhaps analogous to the role of IL-2 in the activation-induced death of T cells. Interestingly, a number of classes of non-Hodgkin's lymphoma undergo apoptosis in response to IL-21 (as discussed below) — an activity that can potentially be exploited therapeutically.

IL-21 induces plasma cell differentiation. Corresponding to their increased immunoglobulin levels, IL-21-transgenic mice have increased numbers of plasma cells[24]. *In vitro*, the combination of IL-21 and a B cell receptor (BCR) signal can directly induce the differentiation of naive B cells into plasma cells, and this probably results from the ability of IL-21 to induce the transcription of BLIMP1 (REFS 9,24), which functions as a master switch for plasma cell differentiation[25]. IL-21 plus BCR or CD40 signals can induce human naive cord blood B cells or memory cells to express BLIMP1 and to differentiate into plasma cells[18]. Activation of STAT3 in human B cells appears to be crucial for the induction of BLIMP1 expression and plasma cell differentiation, and BLIMP1 protein can be induced by STAT3 activation even when B cell lymphoma 6 (BCL-6) is overexpressed, although plasma cell differentiation is diminished in this setting[26]. Although IL-21 activates both STAT1 and STAT3 in B cells, naive B cells from patients who are deficient in STAT3 cannot differentiate into plasma cells in response to IL-21, whereas B cells from patients who are deficient in STAT1 can respond functionally to IL-21 (REF. 27). *STAT3* mutations also result in reduced numbers of antigen-specific memory B cells, demonstrating an intrinsic B cell requirement for STAT3 signalling[27]. In addition to its role in driving plasma cell differentiation, IL-21 derived from human T_{FH} cells inhibits the apoptosis of tonsil-derived plasma cells *in vitro*[28].

Recent studies have demonstrated that, in the presence of a BCR signal but the absence of T cell co-stimulation by the CD40 ligand, IL-21 can induce human B cells (but not mouse B cells) to differentiate into granzyme B-expressing cells that have cytotoxic activity towards tumour cells[29,30]. Moreover, human plasma B cells produce granzyme B in response to IL-21 in a STAT3-dependent manner[31]. These results suggest that the regulation of B cells by IL-21 in the early stages of the immune response involves not only apoptotic signals for the B cell but also the activation of a cytotoxic programme that may function in the context of tumours or early viral infections.

Inhibitory effects of IL-21 on dendritic cells

IL-21 has potent inhibitory activity towards the activation and maturation of granulocyte–macrophage colony-stimulating factor (GM-CSF)-induced dendritic cells (DCs)[32]. Strikingly, it can potently induce the apoptosis of conventional DCs by a STAT3- and BIM-dependent mechanism, but it has, at most, a modest effect on plasmacytoid DCs[33]. GM-CSF primarily activates STAT5 instead of STAT3 and inhibits the induction of BIM, serving to block the IL-21-mediated apoptosis of DCs. Thus, IL-21 and GM-CSF exhibit cross-regulatory actions that regulate the number of conventional DCs and thereby the magnitude of the immune response, as DCs are important antigen-presenting cells (APCs). IL-21-induced apoptosis of DCs may contribute to the development of tolerance.

Effects of IL-21 on CD4$^+$ T cell differentiation

T_H17 cells. Although IL-21 is not required for CD4$^+$ T cell development, it importantly contributes to the functional differentiation of several CD4$^+$ T cell subsets. T_H17 cells are responsible for the pathogenic inflammatory responses that develop in a number of disease states[34]. IL-21 is produced by T_H17 cells, and although TGFβ and IL-6 can still stimulate the development of mouse T_H17 cells (albeit weakly) even in the absence of IL-21, the production of IL-21 by T_H17 cells can further stabilize and expand this population of cells[35–37]. IL-21 acts by augmenting IL-23R expression, which then allows increased cellular responsiveness to IL-23. IL-21 also induces the expression of retinoic acid receptor-related orphan receptor-γt (RORγt; encoded by *RORC*), a transcription factor that functions as a master regulator of the T_H17 phenotype[38]. Several studies have found that although IL-21 can promote T_H17 differentiation *in vitro*, the development of T_H17 cells in response to experimental allergic encephalitis is similar in wild-type and *Il21r*-knockout mice, which indicates that there are potentially redundant pathways for the induction of these cells in mice[39,40].

T_{FH} cells. T_{FH} cells reside primarily in B cell follicles and are important for the development of a fully functional germinal centre B cell antibody response[41] (FIG. 3). Most T_{FH} cells express CXC-chemokine receptor 5 (CXCR5), inducible T cell co-stimulator (ICOS) and programmed cell death protein 1 (PD1), and secrete high levels of IL-21. Although germinal centres can develop in the absence of IL-21 signalling, the number of germinal centre B cells is reduced. Interestingly, the B cells that do develop in the absence of IL-21 signalling are short-lived and have reduced somatic mutations in their heavy-chain variable (V_H) regions, demonstrating an important role for IL-21 in affinity maturation[42–44]. IL-21 signalling is also required for the efficient development of T_{FH} cells, as IL-21 upregulates the transcription factors BCL-6 (REF. 24) and MAF[45], which are central to the transcriptional programme of the T_{FH} cell[46,47]. Interestingly, IL-6 can not only directly induce T_{FH} cell differentiation[48] but it also influences T_{FH} cell differentiation via its induction of IL-21 production[49].

It remains controversial whether IL-21 signalling in the T_{FH} cell is absolutely required for the development of an antibody response. Some studies have demonstrated that IL-21 signalling is intrinsically required in the germinal centre B cell to either induce or maintain BCL-6 expression as part of the B cell maturation programme[43,44], but is not required for T_{FH} cell development or function. Interestingly, the development of an antibody response to a helminth infection was shown to depend on IL-21 signalling in B cells, even though BCL-6 levels in germinal centres were not affected by the loss of IL-21 signalling[50]. A key role for IL-21 has been suggested by a study that found that *Il21r*-knockout CD4$^+$ T cells were unable to function as T_H cells for the development of a long-lived antiviral antibody response[51].

Although IL-21 was initially considered to be the hallmark cytokine for T_{FH} cells, these cells are now known to also secrete various additional cytokines, including IL-4 and interferon-γ (IFNγ)[52,53], allowing for the induction of specific immunoglobulin isotypes. Recent studies have identified multiple populations of circulating T_{FH} cells in human peripheral blood[54] and mouse mucosal tissues that are CC-chemokine receptor 9-positive (CCR9$^+$)[55] and that express typical T_{FH} markers, including ICOS, BCL-6 and MAF. These cells can also provide help to B cells. Various human diseases are associated with increased levels of circulating T_{FH} cells, and the number of these cells has been correlated with disease severity in some cases (as discussed below). T_{FH} cells and plasma B cells are controlled by the transcription factors BCL-6 and BLIMP1, respectively, which are known to negatively cross-regulate each other[25,56]. Interestingly, activated plasma cells have been found to suppress the expression of BCL-6 and IL-21 by T_{FH} cells, and excessive plasma cells can limit the ability of T_{FH} cells to generate germinal centre B cells[57].

A small population of forkhead box P3-positive (FOXP3$^+$) follicular regulatory T (T_{FR}) cells found in germinal centres share phenotypic markers with both T_{FH} and regulatory T (T_{Reg}) cells[58,59]. T_{FR} cells were shown to suppress the *in vivo* generation of both T_{FH} and germinal centre B cells[58,59]. It remains to be determined whether IL-21 controls the development or function of these T_{FR} cells. A study of peripheral T_{FH} cells in patients with HIV who had been immunized against the H1N1 influenza virus showed that the patients who responded to the vaccine had higher levels of peripheral T_{FH} cells (that secreted high levels of IL-21) than the patients who

did not respond to the vaccine[60]. A better understanding of the regulation of T_{FH} cell generation and persistence will be important for the development and optimization of vaccine strategies utilizing IL-21.

IL-21 and immunosuppression by CD4⁺ T cells. IL-21 has both positive and negative effects on immunosuppression. Numbers of T_{Reg} cells, which express the transcription factor FOXP3, are increased in both

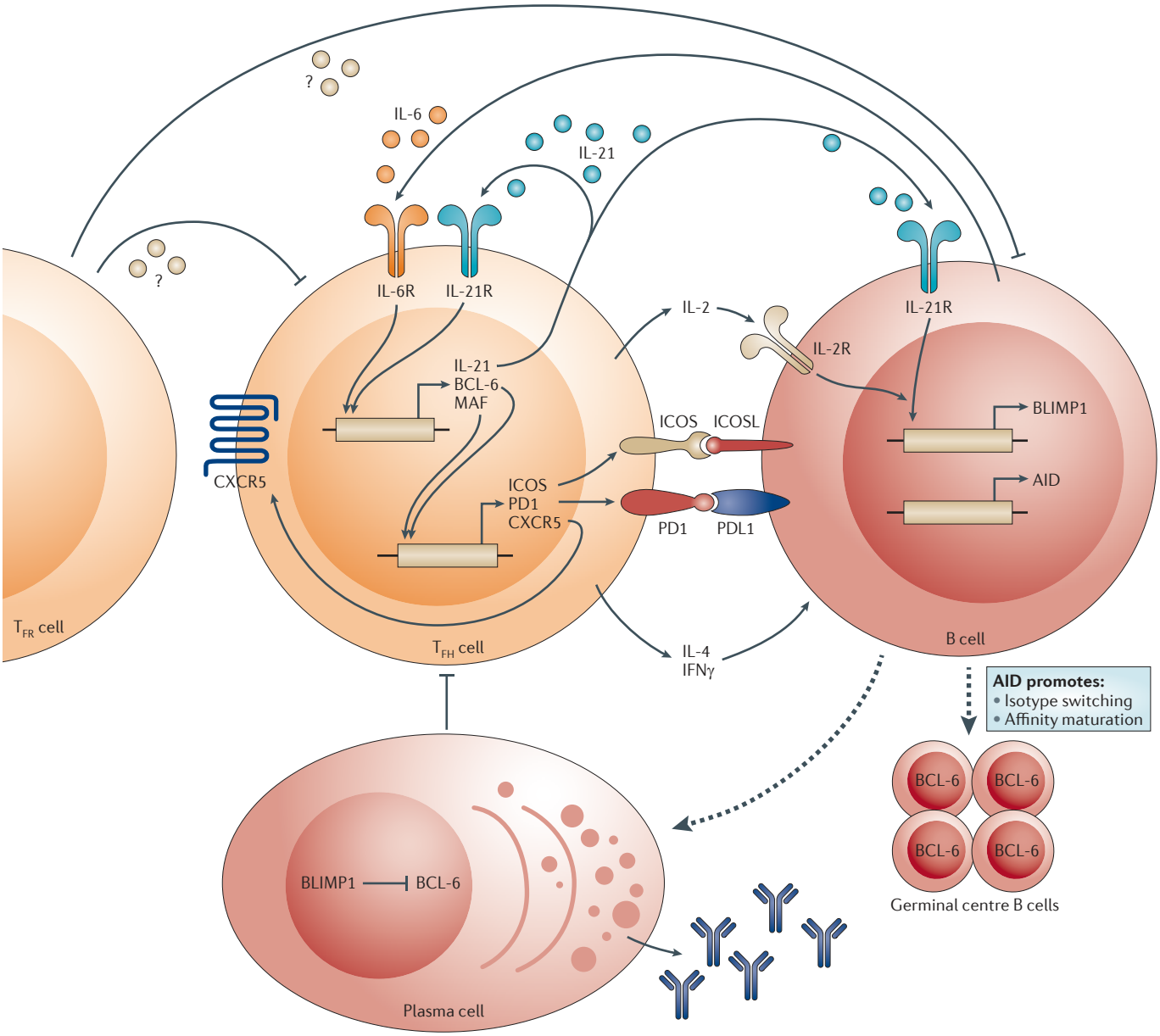

Figure 3 | **IL-21 has a key role in B cell differentiation and germinal cell development.** T follicular helper (T_{FH}) cell differentiation can be driven by either interleukin-6 (IL-6) or IL-21, leading to the upregulation of B cell lymphoma 6 (BCL-6) and MAF transcription factors, which then orchestrate a transcriptional programme that upregulates genes encoding inducible T cell co-stimulator (ICOS) and programmed cell death protein 1 (PD1). These proteins interact with the B cell surface proteins ICOS ligand (ICOSL) and PD1 ligand 1 (PDL1). The transcriptional programme orchestrated by MAF and BCL-6 also upregulates the secretion of cytokines (including IL-21, IL-4, interferon-γ (IFNγ) and IL-2). CXC-chemokine receptor 5 (CXCR5) expressed on T_{FH} cells leads to their localization in the follicle via its interaction with CXC-chemokine ligand 13 (CXCL13). T_{FH} cell-derived IL-21 acts on naive B cells in conjunction with co-stimulatory signals to drive the differentiation of either germinal centre B cells or plasma cells subsequent to isotype switching and affinity maturation. The balance between the IL-21-induced transcription factors B lymphocyte-induced maturation protein 1 (BLIMP1) and BCL-6 determines differentiation to either plasma cells or germinal centre B cells. The germinal centre reaction can be modulated by plasma cell feedback inhibition or by the interaction with follicular regulatory T (T_{FR}) cells, which share phenotypic markers with both T_{FH} and regulatory T (T_{Reg}) cells. T_{FH} populations ($CXCR5^{-}BCL6^{-}$ cells) that exert IL-21-dependent B cell help can also be found in peripheral blood. Mucosal T_{FH} cells ($CCR9^{+}$ cells) secrete IL-21 that acts predominantly on $CD8^{+}$ T cells. AID, activation-induced cytidine deaminase.

Il21- and *Il6*-knockout mice, as TGFβ signalling favours the generation of T_{Reg} cells over T_H17 cells in the absence of either IL-21 or IL-6 (REFs 35,61). Consistent with this, IL-21 can directly inhibit the generation of T_{Reg} cells *in vitro*. However, the ability of IL-21 to inhibit the generation of T_{Reg} cells *in vivo* may be an indirect effect, resulting from IL-21-mediated inhibition of IL-2 production, which decreases the viability of T_{Reg} cells[61]. Infection with lymphocytic choriomeningitis virus (LCMV) can induce the proliferation of T_{Reg} cells, but this expansion is antagonized by IL-21 in a cell-intrinsic manner[62], which suggests that IL-21 might promote $CD8^+$ T cell antiviral responses by diminishing the number of suppressive T_{Reg} cells. Although the inflammatory disease that develops in *Il2*-knockout mice was presumed to result from reduced T_{Reg} cell numbers, when *Il2*$^{-/-}$*Il21r*$^{-/-}$ double knockout mice were examined there was a marked decrease in the inflammatory response even though T_{Reg} cell numbers were unchanged[63]. Thus, IL-21 has inflammatory effects that are independent of its actions on T_{Reg} cells.

Although inhibiting T_{Reg} cell expansion should enhance immune responses, IL-21 can also be immunosuppressive because of its ability to induce IL-10 in naive $CD4^+$ T cells, $CD8^+$ T cells and B cells[64]. Cells that are polarized to either T_H1 or T_H17 populations in the presence of IL-21 also produce higher levels of IL-10 (REF. 64). Another subset of regulatory cells is the immunosuppressive T regulatory 1 (T_R1) cells, which lack FOXP3 expression and produce IL-10 (REF. 65). The initial stimulus for these cells is the production of IL-27 by DCs, which leads to MAF-dependent induction of IL-21; IL-21 then induces the production of IL-10 (REF. 65).

IL-21 effects on $CD8^+$ T cells

IL-21 is not required for $CD8^+$ T cell development but promotes their proliferation and functional responses. Although IL-21 alone has minimal effects on $CD8^+$ T cell proliferation, it strongly synergizes with either IL-7 or IL-15 to induce both proliferation and IFNγ production[66].

A study comparing the effects of IL-2, IL-15 and IL-21 on $CD8^+$ T cell function revealed that whereas IL-2 and IL-15 can induce a mature effector phenotype ($CD44^{hi}CD62L^{low}$) with enhanced cytolytic activity, IL-21 preserves a less activated effector phenotype ($CD44^{low}CD62^{high}$) with decreased cytolytic activity[67]. However, following the transfer of these populations of cytokine-primed $CD8^+$ T cells into tumour-bearing mice, cells that are primed *in vitro* in the presence of IL-21 have characteristics of memory T cells and exhibit enhanced persistence *in vivo*[67].

Although the effects of exogenous IL-21 on $CD8^+$ T cell function have been appreciated for some time, it has more recently been discovered that $CD8^+$ T cells can also produce IL-21. For example, stimulation of $CD8^+$ T cells with IL-27 can induce the production of IL-21, which then leads to autocrine induction of granzyme B[68]. Production of IL-21 by $CD8^+$ T cells has also been found in HIV long-term non-progressors[69].

HIV long-term non-progressors
Individuals who have been infected with HIV for long periods of time but can control the infection without the need for antiretroviral therapy. Their viral loads are under 10,000 copies per ml of blood and their $CD4^+$ T cell counts are normal, although they may undergo a slow progression to lower CD4 counts.

Although the mechanisms through which IL-21 expression is induced by $CD8^+$ T cells *in vivo* remain to be identified, the distinctive effects of IL-21 on $CD8^+$ T cells as well as its production by these cells help to explain the antitumour activity of this cytokine, as described below.

Lessons from immunodeficient patients

Importantly, individuals with loss-of-function mutations in *IL21R* as well as *STAT1* and *STAT3* have been identified[70–74]. Studies of these patients have revealed both similarities and differences in the phenotypes associated with the homologous mouse mutations. Patients with autosomal dominant hyper-IgE syndrome (AD-HIES) resulting from *STAT3* mutations have reduced numbers of T_{FH} cells, leading to a reduction in the number of memory B cells and plasma cells[75]. In addition, their T_H17 responses are reduced and they have recurrent candidiasis as well as staphylococcal infections of the skin and lung[76]. Although primary T cell responses to viral infections are not impaired, patients have a high rate of reactivation of varicella zoster virus and Epstein–Barr virus (EBV), resulting from a reduced ability of T cells to develop into central memory cells[74,77]. *STAT1*-deficient patients are susceptible to viral infections, but patients with AD-HIES respond normally to primary viral infections. A direct comparison of $CD8^+$ T cells from *STAT3*- versus *STAT1*-deficient patients demonstrated that cytotoxic function in response to IL-21 was dependent on STAT3 signalling but not on STAT1 signalling, and that co-stimulation through the T cell receptor could overcome the requirement for STAT3 (REF. 74). Interestingly, $CD8^+$ T cell proliferative responses to IL-21 are not impaired in either the *STAT1*- or *STAT3*-deficient cells, which suggests that other signalling pathways besides STAT1 and STAT3 can mediate these responses[74].

Loss-of-function mutations in the *IL21R* gene were identified in a group of patients who presented with chronic cryptosporidiosis[71]. As expected, cells from these individuals have defective IL-21 signalling, with defective IL-21-induced proliferation, cytokine production, NK cell-mediated cytotoxicity and immunoglobulin class switching; in addition, analogous to the *Il21r*-knockout mice, levels of IgE in these patients were markedly elevated. The affected individuals also presented with recurrent pneumocystis pneumonia and had chronic cholangitis and liver disease[71]. Unlike these patients, *Il21r*-knockout mice are less susceptible to liver fibrosis than wild-type mice[78].

Role for IL-21 in cancer treatment

Because of the ability of IL-21 to enhance the cytotoxic activity of both $CD8^+$ T cells and NK cells, it has obvious potential as an antitumour agent. As discussed below, studies using mouse *in vivo* tumour models as well as clinical trials in patients with advanced solid tumours have demonstrated that IL-21 can function as a potent antitumour agent[79] (TABLE 1).

IL-21 in solid non-haematopoietic tumours. Early studies in mice, using systemic expression of IL-21 by plasmid-mediated *in vivo* delivery, have revealed that IL-21 can inhibit the growth of large melanomas and fibrosarcomas.

Experiments in which select populations of cells were eliminated have shown that most of the antitumour activity of IL-21 is mediated by NK cells, with a smaller contribution from $CD8^+$ T cells[80]. NK cell-mediated cytotoxic activity towards tumours targets the NKG2D (NK group 2, member D) ligand on tumour cells, and antibodies targeting NKG2D can decrease the observed cytotoxic activity[81]. Importantly, treatment of mice with IL-21 does not cause the *in vivo* toxic effects of vascular leak syndrome that have been observed after treatment with IL-2, even when IL-21 is administered at high concentrations[82].

In a study comparing the activity of intraperitoneally delivered IL-2, IL-15 and IL-21 against syngeneic tumours in mice, IL-21 had the most potent antitumour activity, resulting in substantially enhanced long-term survival[83]. This IL-21-mediated survival is dependent on a persistent $CD8^+$ T cell memory population. Strikingly, the potency of IL-21 was enhanced when it was combined with IL-15, resulting in regression of a subset of melanomas and long-term survival in the majority of mice[66]. This dual treatment led to the accumulation of a population of $CD8^+$ T cells with enhanced production of IFNγ, which is consistent with the ability of the IL-21 and IL-15 combination to potently drive the expansion of $CD8^+$ T cells *in vitro*[66].

Adoptive cell transfer of *in vitro* expanded tumour-specific $CD8^+$ T cells has been used to increase the number of cytotoxic cells, and IL-2 has been used to expand tumour-infiltrating lymphocytes (TILs) *in vitro* before their infusion into patients with melanoma[84]. However, IL-2-mediated expansion leads to the accumulation of effector $CD8^+$ T cells with reduced longevity *in vivo* as well as the expansion of T_{Reg} cells[85,86]. Experiments comparing the antitumour activity and persistence of tumour-specific $CD8^+$ T cells that undergo expansion in response to IL-2, IL-15 or IL-21 have demonstrated that IL-21 confers a distinctive differentiation programme, with less apparent effector function *in vitro* but enhanced antitumour activity upon *in vivo* transfer into mice with established melanomas[67]. Importantly, treatment of $CD8^+$ T cells with IL-21 *in vitro* resulted in their enhanced longevity *in vivo*, even after regression of the primary melanoma. Consistent with these findings, when TILs from primary human tumours were expanded in the presence of APCs that artificially expressed IL-2, IL-15 or IL-21, although IL-2 led to the greatest *in vitro* expansion, the IL-21-expanded TILs had a 'young', less-well-differentiated phenotype, with longer telomeres and higher CD27 and CD28 expression[87], and this phenotype was associated with stem-cell-like increased longevity and antitumour activity after adoptive transfer[88]. An important distinction between the IL-2- and IL-21-mediated expansion of TILs is that IL-21 does not induce the expansion of T_{Reg} cells, which impair the activity of the expanded antitumour $CD8^+$ T cells[87].

Combination therapy with IL-21. Based on the diversity of its effects on both innate and adaptive immune cells, IL-21 has been used in combination with other cytokines or antibodies for the treatment of cancer, both in mouse models and in clinical trials. IL-21 is known to enhance the ability of NK cells to lyse antibody-coated tumour cells (through antibody-dependent cell-mediated cytotoxicity (ADCC))[89], and the combination of IL-21 with tumour-specific antibodies might enhance antitumour activity. Another mechanism by which IL-21 can enhance tumour-directed antibody therapy is illustrated by studies in mice using antibodies against death receptor 5 (DR5; also known as TRAILR2), which is a receptor for TNF-related apoptosis-inducing ligand (TRAIL); the TRAIL–DR5 ligand–receptor pair controls apoptosis. In this system, a DR5-specific monoclonal antibody was shown to inhibit tumour growth, leading to the apoptosis of tumour cells[90]. Importantly, subsequent treatment with IL-21 resulted in the expansion of $CD8^+$ cytotoxic cells that had been primed by these tumour cells, leading to enhanced suppression of tumour metastasis and enhanced memory responses to subsequent tumour challenge[90].

IL-21 is also known to affect both the growth and cytolytic activity of NKT cells. When tumour-bearing mice were treated with the CD1d-reactive glycolipid α-GalCer, which activates NKT cells, subsequent treatment with IL-21 resulted in synergistic inhibition of tumour metastasis[91]. When the α-GalCer glycolipid was delivered by DCs and combined with IL-21 treatment, established metastatic tumours decreased in size[91].

Although IL-2 has been used for the treatment of metastatic melanoma and renal cell carcinoma, its efficacy has been limited in part owing to toxicity. The combination of IL-2 with IL-21 was therefore tested in a mouse model of melanoma and resulted in synergistically enhanced and longer-lived responses than those seen with either cytokine alone[92]. The combination of IL-15 with IL-21 has also been tested in mouse models of melanoma and it not only increases the cytotoxic activity of cytotoxic T lymphocytes and NK cells but also results in long-lived antitumour responses[66,93].

Clinical trials against solid tumours. IL-21 has been tested in Phase I and Phase II clinical trials as a single agent for melanoma, renal cell carcinoma and metastatic colorectal cancer (TABLE 1). Favourable therapeutic responses have been observed, with one patient in a Phase I trial achieving complete remission, and 9 out of 29 achieving stable disease[94,95]. IL-21 has also been combined with cetuximab (Erbitux; Bristol-Myers Squibb/Eli Lilly), an antibody targeting epidermal growth factor receptor (EGFR) that has been used with some success in triggering ADCC against tumours[96]. In Phase I trials to evaluate the combination of IL-21 and cetuximab against stage IV colorectal cancer, stable disease was achieved in 60% of patients, but these clinical trials were subsequently terminated[96]. Interestingly, subsequent studies in mice (as described below) suggested a potentially deleterious role for IL-21 in colorectal cancer[97].

Role of IL-21 in tumours of haematopoietic origin. Both lymphoid and myeloid cells can express IL-21R, and IL-21 can have either pro- or anti-apoptotic effects on these cells, depending on the stage of development and co-stimulatory factors. Chronic lymphocytic leukaemia (CLL) is a malignancy of B cell origin, and CLL cells have been found to express variable amounts of IL-21R.

Several biological effects of IL-21 have been described for these cells. *In vitro*, IL-21 directly promoted the apoptosis of CLL cells via upregulation of the pro-apoptotic protein BIM[98], but in another study IL-21 did not promote CLL cell apoptosis[99]. IL-21 was also shown to induce granzyme B production in B-CLL cells, contributing to the apoptosis of tumour cells by a fratricide mechanism[100]. More recent studies have shown that T_{FH} cell numbers are elevated in patients with CLL and that IL-21, in combination with IL-4, can promote the proliferation of CLL cells[101]. Interestingly, IL-21 expression led to an increase in a chemotherapy-resistant subpopulation of cells[101]. In addition, an IL-21-induced gene signature was identified in lymph node samples from patients with CLL[102]. These results suggest that the targeting of IL-21 and other signals from T_{FH} cells in the lymph node might have a therapeutic effect in CLL.

Diffuse large B cell lymphoma (DLBCL) is the most prevalent subtype of non-Hodgkin's lymphoma. Most DLBCL cells express IL-21R, and IL-21 can induce caspase-dependent apoptosis in DLBCL cell lines[103]. This form of apoptosis is dependent on STAT3-mediated upregulation of MYC, which leads to decreased expression of the anti-apoptotic BCL-2 and BCL-X_L proteins.

Follicular lymphomas, although typically indolent, can become clinically aggressive and transform into DLBCL. A survey of the expression of IL-21R on follicular lymphoma cells shows that high-level IL-21R expression correlates with the presence of more aggressive DLBCL in the biopsy specimen[104]. Although initial studies found that IL-21 could induce the apoptosis of follicular lymphoma cells *ex vivo*[105,106], later studies have suggested that *in vivo* access to IL-21 from T_{FH} cells within the follicle can actually serve to increase the growth of follicular lymphoma cells[104]. Studies correlating the prognosis of follicular lymphoma with different cell populations within the tumour concluded that patients with a higher number of follicular $CD4^+$ T cells had a worse prognosis[107], which suggests that blocking IL-21 *in vivo* might be a therapeutic option for the treatment of follicular lymphoma.

Multiple myeloma is a heterogeneous disease that is characterized by the accumulation of long-lived malignant plasma cells. IL-21 was reported to be a growth factor for multiple myeloma cells[108], but subsequent studies have shown that this effect of IL-21 is restricted to the $CD45^-$ subset of these cells[109]. The IL-21-mediated growth-promoting effect results from the induction of insulin-like growth factor 1 (IGF1), which has autocrine growth effects as well as additive effects in combination with IL-21 on multiple myeloma cells[109].

Hodgkin's lymphomas are composed of malignant cells that are derived from germinal centre B cells in addition to multi-nucleated Reed–Sternberg cells. The majority of Hodgkin's lymphoma cell lines express IL-21R as well as IL-21 (REF. 110), and blocking IL-21 signalling was shown to decrease the proliferation of a Hodgkin's lymphoma cell line.

Angioimmunoblastic T cell lymphoma (AITL) is a systemic disease that presents clinically with B cell symptoms[111]. AITL tumour cells seem to express a large number of T_{FH} cell markers, including BCL-6, PD1, CXCR5, ICOS, signalling lymphocytic activation molecule-associated protein (SAP) and MAF[112–117]. Gene expression profiling has revealed that IL-21 is produced by AITL cells[118], but its role in the progression of AITL has not been evaluated.

The effect of IL-21 on haematopoietic malignancies depends on the particular cell type and stage of malignancy. In CLL, follicular lymphoma, DLBCL and mantle cell lymphoma, the apoptotic effects of IL-21 make it an attractive candidate for use as a single agent as well as for combined treatment with tumour-specific antibodies. Indeed, IL-21 has been used in combination with rituximab (a CD20-specific antibody) in Phase I clinical trials, with clinical responses seen in 8 out of 19 patients[119] (TABLE 1). However, the growth-promoting effects of IL-21 in multiple myeloma, Hodgkin's lymphoma and other haematological malignancies suggest that blocking the IL-21 signalling pathway might be of therapeutic value in these settings; blockade of IL-21 signalling could be achieved by targeting the extracellular interaction with IL-21R-specific antibodies, with an IL-21R–Fc fusion protein or via inhibition of the JAK–STAT pathway.

Effects of IL-21 on responses to viral pathogens

Given its role in the functional responses of $CD4^+$ T cells, $CD8^+$ T cells and B cells, IL-21 has major effects on the development of adaptive immunity to acute viral infections. Recent studies have delineated a role for IL-21 in the maintenance of $CD8^+$ T cell memory and in the prevention of immune exhaustion in the context of a range of viral infections. Initial studies indicated that *Il21r*-knockout mice showed decreased responses to infection with vaccinia virus[66,120], but a later study did not find differences in the responses of *Il21r*-knockout mice to acute infection with either vaccinia virus or influenza virus; the study indicated equivalent clearance of the virus in knockout versus wild-type mice[121].

Studies investigating the role of IL-21 in the immune response to LCMV infection have shown significant differences in the immune responses mounted during acute versus chronic viral infections[121–123]. Strong production of IL-21 accompanies the primary response to acute LCMV infection, whereas lower IL-21 levels are seen during the primary response to chronic LCMV infection. Chronic LCMV infection in the absence of IL-21 results in severe functional 'exhaustion' of *Il21*-knockout $CD8^+$ T cells, which is characterized by reduced expression of IL-2, tumour necrosis factor (TNF) and IFNγ, along with increased surface expression of the inhibitory PD1 protein. Studies using bone marrow chimeric mice have revealed that the expression of IL-21R on $CD8^+$ T cells is required for effective viral responses[124]. Treatment of *Il21*-knockout mice with IL-21 during the initial response to LCMV infection can restore a normal antiviral response, but unfortunately this treatment is associated with the immunopathology related to a heightened immune response[124]. IL-21 and IL-10 are both required for the formation of memory precursor $CD8^+$ T cells, resulting in the induction of the transcription factors BCL-6, eomesodermin (EOMES; also known as

Fratricide
The induction of apoptotic death in nearby cells, which normally occurs via a death receptor and its ligand. This occurs naturally in the immune system and other systems, and it can also be induced by chimeric ligands.

Table 2 | **Diseases in which manipulation of *in vivo* IL-21 levels may have desirable therapeutic consequences**

Disease	Approach	Desired outcome
Hepatitis B	Increase IL-21 levels	Boost T_{FH} cells to increase virus-specific $CD8^+$ T cell and B cell responses in children
Hepatitis C	Increase IL-21 levels	Reverse $CD8^+$ T cell exhaustion and decrease T_{Reg} cell numbers
HIV-1	Increase IL-21 levels	Combine IL-21 with blockade of inhibitory receptors to boost antiviral responses
Systemic lupus erythematosus	Block IL-21	Inhibit expansion of autoreactive B cells
Rheumatoid arthritis	Block IL-21	Reduce T_H17 cell-mediated joint inflammation and inhibit osteoclastogenesis
Type 1 diabetes	Block IL-21	Combine IL-21 with pancreatic islet transplantation to repair pancreatic function and allow tolerance development
Multiple sclerosis	Block IL-21	Reduce T_H17 cell-mediated neural inflammation
Multiple sclerosis	IL-21-mediated expansion of B10 regulatory cells *ex vivo*	Use IL-21 to expand B regulatory cells *in vitro* prior to adoptive transfer into patients with multiple sclerosis to inhibit inflammatory response *in vivo*
Uveitis	Block IL-21	Reduce T_H17 cell-mediated ocular inflammation
Inflammatory bowel disease	Block IL-21	Reduce recruitment of inflammatory T_H1 and T_H17 cells to mucosal tissue to prevent tissue damage
Psoriasis	Block IL-21	Reduce IL-21-mediated $CD4^+$ T cell recruitment and proliferation of keratinocytes

IL-21, interleukin-21; T_{FH}, T follicular helper; T_H, T helper; T_{Reg}, T regulatory.

TBR2) and BLIMP1 (REF. 125). Collectively, these results demonstrate that acute responses to LCMV infection do not necessarily require IL-21 but that the quality of the memory response depends on the presence of IL-21 during the primary response.

IL-21 and hepatitis. Serum levels of IL-21 have been found to correlate with the severity of liver disease after hepatitis B virus (HBV) infection[126]. Patients with acute HBV infection had a higher number of HBV-specific $IL\text{-}21^+CD4^+$ T cells than patients with chronic HBV infection, and this correlated with the number of HBV-specific $CD8^+$ T cells[127]. High serum levels of IL-21 after antiviral therapy also correlated with the development of HBV-specific antibody responses[128]. Collectively, these data suggest that IL-21 has an important role in the adaptive responses to HBV infection and that increasing IL-21 levels in HBV infection might be beneficial (TABLE 2).

HBV is cleared in most adults, but chronic infection and liver disease can often develop in children. Using a mouse model of human HBV, adult mice were shown to have higher HBV-specific IL-21 production in the liver than young mice[129]. The lower level of IL-21 in young mice results from inefficient development of T_{FH} cells, and this reduced IL-21 leads to lower frequencies of virus-specific $CD8^+$ and B cell responses. These results have been confirmed in samples taken from patients with HBV, as patients with acute infection have higher levels of IL-21 than patients with chronic HBV infection[129]. Results from this model system also suggest that T cell priming to HBV occurs in the liver and that the HBV-specific IL-21-producing T_{FH} cells also reside in the liver[130]. Although IL-21 has a role in the adaptive immune response to HBV, it is possible that it may also exacerbate the development of HBV-induced liver cirrhosis. IL-21 levels are elevated in patients with HBV who also have liver cirrhosis, and IL-21 has been shown to directly induce hepatic stellate cells to secrete proteins involved in liver fibrosis[131].

IL-21 also has an important role in the response to acute hepatitis C virus (HCV) infection. Acute-resolving HCV infection is associated with increased levels of plasma IL-21 as well as increases in the number of IL-21- and IL-17-producing T_H17 $CD4^+$ cells[132]. Conversely, HCV persistence is associated with increased levels of exhausted T cells expressing the inhibitory receptors PD1 and TIM3 (T cell immunoglobulin and mucin domain-containing protein 3), as well as with the expansion of T_{Reg} cells that directly inhibit the proliferation and function of HCV-specific $CD4^+$ and $CD8^+$ T cells. Taken together, these studies indicate that IL-21 may serve to limit viral persistence in both HBV and HCV (TABLE 2).

IL-21 and HIV-1. $CD4^+$ T cells are the major producers of IL-21 and also the main targets of HIV-1 infection. Given the roles of IL-21 in the function of B cell antibody responses as well as in the cytotoxic function of $CD8^+$ T cells and NK cells, lower IL-21 production could be a contributing factor to the compromised immune response of patients with HIV-1 infection. Studies of different groups of patients with HIV-1 infection have shown that the amount of IL-21 production correlates with viral load in the early stage of infection, but IL-21 plasma levels are substantially reduced when the viral load reaches 20,000 copies per ml, at which point peripheral blood $IL\text{-}21^+CD4^+$ T cells are not detectable[133,134]. HIV-1 infection of $CD4^+$ T cells *in vitro* leads to a reduction in the expression of MAF, which promotes IL-21 transcription[133]. Interestingly, elite controllers of HIV-1 infection can maintain normal levels of $IL\text{-}21^+CD4^+$ T cells despite the decreased proportions of IL-2- and IFNγ-secreting $CD4^+$

Elite controllers
Individuals who are infected with HIV but have extremely low viral loads (< 50 copies of RNA per ml). These individuals are believed to have a strong and persistant anti-HIV immune response.

T cells[133–135]. Stimulation of HIV-1-specific $CD8^+$ T cells in the presence of IL-21 leads to enhanced degranulation and cytotoxic effector function[135]. Although both IL-21 and IL-15 can induce perforin production in $CD8^+$ T cells from patients with HIV, IL-21 does not lead to cellular activation, which suggests that IL-21 might have a therapeutic role in modulating cytotoxic function in patients with HIV[136]. Interestingly, HIV-1-specific $CD8^+$ T cells can produce IL-21 after primary infection, and this population of cells is expanded in patients who are elite controllers of HIV-1 infection[69].

Levels of viraemia in infected patients are associated with an expansion of HIV-1-specific T_{FH} cells and germinal centre B cells, which might account for the hypergammaglobulinaemia present during HIV-1 infection[137]. Although the elevated numbers of T_{FH} cells and plasma cells might predict functional antibody responses, patients with HIV-1 infection do not develop high-affinity neutralizing antibodies, which suggests that factors other than IL-21 are involved in the generation of such antibodies, and the ability of these expanded HIV-1-specific T_{FH} cells to provide help to B cells is impaired[138]. Germinal centre B cells from HIV-1^+ lymph nodes express high levels of PD1 ligand 1 (PDL1), and PD1 ligation by these B cells results in reduced T_{FH} cell activation, which leads to a reduction in the secretion of IL-21, IL-4 and IL-10 by these T_{FH} cells[138]. Thus, although T_{FH} cell numbers are increased during HIV-1 infection, the cells are functionally inadequate. These studies suggest that PD1 blockade could enhance HIV-1-specific antibody responses.

Although IL-21 has not been administered to patients with HIV-1/AIDS, administration of IL-21 to rhesus macaques that were chronically infected with simian immunodeficiency virus (SIV) led to enhanced function of $CD8^+$ T cells (characterized by higher levels of perforin and granzyme B), NK cells and B cells, but did not diminish the viral load[139]. This treatment also resulted in increased numbers of intestinal T_H17 cells and reduced levels of virus-induced mucosal inflammation[140]. Thus, it remains possible that IL-21, if combined with the blockade of inhibitory receptors, might result in more effective immune responses to HIV-1 infection, which is a promising area for future investigation (see TABLE 2).

IL-21 and PVM. IL-21 has been shown to have a role in the response to a respiratory infection that elicits a strong inflammatory response. Pneumonia virus of mice (PVM) is a pathogen that initiates an infection with similar characteristics to severe human respiratory syncytial virus (RSV) infection. IL-21-expressing $CD4^+$ T cells accumulate in the lungs of mice infected with PVM, and *Il21r*-knockout mice have significantly diminished recruitment of neutrophils to the lung compared with wild-type mice[141]. IL-21 has a deleterious role in the response to this infection, as *Il21r*-knockout mice have a survival advantage after PVM infection. Moreover, when IL-21 signalling was blocked with an IL-21R–Fc fusion protein in wild-type mice, survival after infection with PVM was enhanced[141]. Thus, blocking IL-21 may interrupt the immunopathology that occurs following PVM infection and perhaps other respiratory infections as well.

MRL–*Fas*^{lpr} mice
A strain of mice that are deficient for the FAS receptor, which is involved in the induction of cell death. This strain spontaneously develops an autoimmune disease that resembles the human disease systemic lupus erythematosus.

IL-21 promotes autoimmune disease

As discussed above, IL-21 has a major role in driving terminal B cell differentiation to plasma cells and it also has major effects on T cell function. These actions indicate a potential role for IL-21 in the promotion of autoimmune disease. In fact, genome-wide association studies have identified risk variants in the *IL21* gene for systemic lupus erythematosus (SLE)[142], type 1 diabetes[143], inflammatory bowel disease[144], coeliac disease[145], psoriasis and psoriatic arthritis[146]. Initial studies in animal models have begun to elucidate the mechanisms by which IL-21 promotes autoimmune disease, and these studies are now being validated in patients with such diseases.

Role of IL-21 in systemic autoimmune disease. SLE is a chronic autoimmune disease that is characterized serologically by the presence of autoreactive antibodies, particularly double-stranded DNA (dsDNA)-specific antibodies that can form pathogenic immune complexes. In the BXSB-*Yaa* mouse model of SLE, there is an accumulation of T_{FH} cells expressing high levels of IL-21 (REFs 147,148), and serum levels of IL-21 correlate with the severity of disease. When BXSB-*Yaa* mice were crossed onto the *Il21r*-knockout background, there was a significant reduction in the production of anti-nuclear antibodies, which was accompanied by the absence of kidney disease and increased survival[148]; this suggests that blocking IL-21 might have a therapeutic effect. Indeed, treatment of the lupus-prone MRL–*Fas*^{lpr} mice with an IL-21R–Fc fusion protein as a blocking agent lowered levels of circulating autoantibodies and diminished glomerular immune complexes[149]. A subsequent study treating BXSB-*Yaa* mice with the IL-21-blocking agent showed biphasic results, as this agent increased disease severity if administered early in the course of the disease, but reduced severity if administered at later time points[191]. Experiments using mixed bone marrow chimeric mice to identify the IL-21 target cells responsible for disease development showed that IL-21 was a 'double-edged sword': it can promote the expansion of autoreactive B cells while inhibiting disease progression through its effects on suppressive $CD8^+$ T cells[147].

An examination of $CD4^+$ T cell populations in patients with systemic lupus erythematosus has revealed an expansion of IL-21-producing $CXCR5^-CD4^+$ cells in these patients, which correlates with increased numbers of T_H17 cells and decreased numbers of T_{Reg} cells; by contrast, an expansion of $IL\text{-}21^+CXCR5^+CD4^+$ T_{FH} cells correlates with alterations in germinal centre B cell proliferation[150]. Overall, these studies suggest that blocking IL-21 could have therapeutic effects, with the caveat that IL-21 is also required for functional immune responses in these patients.

Rheumatoid arthritis is a systemic autoimmune disease that is characterized by an inflammatory process in joints and the destruction of bone and cartilage. Autoreactive T cells that accumulate in the synovial tissue express T_H1 and T_H17 cytokines, which amplify the inflammatory process. Studies in several mouse models have suggested that IL-21 has an important role in the development of rheumatoid arthritis. When K/BxN

mice, which normally develop autoantibody-mediated arthritis, were crossed with *Il21r*-knockout mice, the mice failed to develop autoantigen-specific immunoglobulins, and no animals developed spontaneous arthritis[151]. When K/BxN mice or mice with collagen-induced or adjuvant-induced arthritis were treated with the IL-21R–Fc fusion protein, this delayed the onset and severity of arthritis[151]. IL-21 is highly expressed in both sera and synovial fluid from patients with rheumatoid arthritis, and this expression correlates with levels of IL-17 in these fluids, which is consistent with the role of IL-21 in the development of T_H17 inflammatory cells[152]. Patients with rheumatoid arthritis also have increased levels of T_{FH} cells in the peripheral blood, which correlate with increased levels of the cyclic citrullinated peptide (CCP)-specific antibody — a diagnostic biomarker for rheumatoid arthritis[153]. Interestingly, IL-21 in the K/BxN model of autoimmune arthritis is produced by T_{FH} cells and not by T_H17 cells[154].

One of the inflammatory targets in rheumatoid arthritis is IL-6, and serum levels of both IL-6 and IL-21 are elevated in patients with rheumatoid arthritis. Clinical trials have shown that tocilizumab, a humanized antibody against IL-6R, is highly effective in the treatment of patients with rheumatoid arthritis[155]. Interestingly, a study of the mechanism of action of tocilizumab in patients with rheumatoid arthritis has revealed that blocking the IL-6 pathway inhibits IL-21 production, leading to a reduction in CCP-specific antibodies[156], which suggests that blocking IL-21 might be very effective as well. In fact, mechanistic studies of the role of IL-21 in the progression of rheumatoid arthritis have shown that IL-21 can promote osteoclastogenesis in patients with rheumatoid arthritis, which contributes to bone destruction[157]. Targeting IL-21 may therefore inhibit the progressive bone loss in patients with rheumatoid arthritis, and Phase I clinical trials using an IL-21-specific monoclonal antibody have recently completed (NCT01208506 and EudraCT-2011-005376-42).

Role of IL-21 in autoimmune diabetes. Type 1 diabetes is characterized by the infiltration of immune cells into the pancreas, followed by the destruction of insulin-producing β-cells. Non-obese diabetic (NOD) mice have been used to analyse the mechanisms involved in the progression of autoimmune diabetes. Multiple susceptibility loci for diabetes have been identified, including insulin-dependent diabetes susceptibility 3 (*Idd3*), which contains the genes encoding IL-2 and IL-21. Levels of IL-21 are higher in NOD mice than in diabetes-resistant strains of mice[158], and NOD mice lacking the IL-21 signalling pathway do not develop diabetes[159,160]. Several studies have indicated that changes in APCs or $CD4^+$ T cell populations are required for the development of the autoimmune response that initiates pancreatic destruction. One study showed that APCs from NOD mice were more efficient than wild-type APCs in inducing the differentiation of T_H17 $CD4^+$ T cells — an effect that required IL-21R on the APCs, through which IL-21 directly induced the production of the mediators that drive T_H17 cell development[161]. In a cell transfer model of diabetes, IL-21R expression on APCs was required for disease development, and when $IL\text{-}21R^+$ DCs were transferred into *Il21r*-knockout mice, these mice were no longer resistant to the development of diabetes[162].

Another study identified a population of $IL\text{-}21^+CCR9^+$ T_H cells that were enriched in the pancreas of NOD mice with diabetes, wherein IL-21 produced by these cells acted on $CD8^+$ T cells, which expanded in the pancreas and exhibited cytolytic activity against pancreatic islet cells[55]. Cytokine priming with IL-21 plus IL-15 enabled autoreactive $CD8^+$ T cells to respond more robustly to weak antigens in a mouse model of diabetes[163]. Thus, in the NOD mouse model system, IL-21 is essential for the development of diabetes and can act on multiple cell populations, which suggests that blocking the actions of IL-21 might inhibit progression of this disease. However, although treatment with an IL-21R–Fc fusion protein reduced immune infiltration into the pancreas at an early stage of disease, it was ineffective in the treatment of mice once diabetes had developed[164]. Encouragingly, however, the combination of IL-21 blockade and syngeneic islet transplant successfully reversed diabetes in mouse models, even in the context of a short-term blockade[164]. These studies suggest that IL-21 blockade could be a rational component of a combinatorial strategy to repair pancreatic function and simultaneously induce tolerance.

Role for IL-21 in neuroinflammatory diseases. Multiple sclerosis is a severe neuroinflammatory demyelinating disease of the central nervous system (CNS). A role for IL-21 was initially analysed in experimental autoimmune encephalitis (EAE), which is a widely used mouse model of multiple sclerosis induced by immunization with myelin protein. In several studies, *Il21r*-knockout mice have exhibited reduced EAE disease progression and reduced numbers of T_H17 cells, which demonstrates that IL-21 is required for the induction and expansion of these inflammatory cells[35,36]. Consistent with this, administration of IL-21 before induction of the disease increased the severity of EAE via the activation of NK cells[165]. However, other studies have suggested that IL-21 is not required for the development of EAE and may even have a protective effect[39,40].

Genetic analysis has associated polymorphisms in the *IL21R* locus with the development of multiple sclerosis in humans[166]. In addition, both IL-21 and IL-21R proteins are expressed by $CD4^+$ T cells in acute as well as chronic multiple sclerosis lesions[167]. IL-21 and IL-21R were also detected in cortical neurons in tissue samples from the brains of patients with multiple sclerosis, which suggests that IL-21 can contribute to the inflammatory response in these lesions, leading to neurodegeneration[167]. Interestingly, patients with progressive multiple sclerosis have a high number of $CD4^+$ T cells in their cerebrospinal fluid, and these cells express increased IL-21 and ICOS as well as elevated IL-17, which suggests the involvement of T_{FH} and/or T_H17 cell-mediated inflammation in the pathogenesis of the disease[168].

Clinical trials have shown that treatment with alemtuzumab, an antibody directed against lymphoid and myeloid CD52, in patients with early-stage relapsing–remitting multiple sclerosis results in greater amelioration

of disease than does treatment with IFNβ[169]. However, a subset of treated patients developed secondary autoimmunity, such as autoimmune thyroiditis or idiopathic thrombocytopaenic purpura, and these patients had high levels of serum IL-21 (REF. 169).

Recent studies using the EAE mouse model have shown that IL-21 also induces the expansion and function of a suppressive population of regulatory B cells that produce IL-10 (B10 cells)[170]. When these cells were expanded *in vitro* and transferred into mice with ongoing EAE, symptoms were reduced even after the disease had been initiated[170]. These studies collectively suggest that IL-21 can have both protective and exacerbating roles in the development and progression of CNS autoimmune diseases.

Autoimmune uveitis in humans includes a group of intraocular inflammatory diseases. Experimental autoimmune uveitis (EAU), a disease that is induced in mice by immunization with retinal protein, has been used to study the mechanisms of the development and progression of uveitis. $CD4^+$ T cells expressing IL-21 accumulate in the retinas of mice with EAU, and *Il21r*-knockout mice develop less severe disease, with lower IL-17A and IL-1β expression in the draining lymph nodes of these mice than in wild-type mice[171]. These results suggest that IL-21 has a key role in the development of this disease and suggest that blocking IL-21 may have therapeutic value in the treatment of uveitis.

Role of IL-21 in inflammatory bowel disease and colorectal cancer. Inflammatory bowel disease (IBD) is a chronic inflammatory disease of the gastrointestinal tract. The tissue damage that occurs in Crohn's disease or ulcerative colitis results from a multifactorial process, encompassing both genetic and environmental factors, which leads to the intestinal pathology that is characteristic of these diseases[172]. Genome-wide association studies have revealed that polymorphisms in the human *IL2–IL21* genetic locus are associated with ulcerative colitis[144], Crohn's disease[144] and coeliac disease[145]. High levels of IL-21 were found in mucosal biopsy samples taken from patients with either Crohn's disease or ulcerative colitis, when compared to controls[173,174]. Studies using the dextran sodium sulphate (DSS) mouse model of colitis showed that treatment with DSS led to increased levels of IL-21 in gut tissue, and *Il21*-knockout mice treated with DSS had reduced gut pathology with lower cellular infiltration and lower IL-17 production than wild-type mice[175].

In addition to the role of IL-21 in ulcerative colitis and Crohn's disease, increased numbers of IL-21-producing cells were found in biopsy samples taken from paediatric patients with coeliac disease, even in mild lesions[176]. These $CD4^+$ T cells co-expressed IL-21 and IFNγ but not IL-17A. By contrast, in biopsy samples of the most severe lesions from adult patients with coeliac disease, $CD4^+$ T cells co-expressing IL-21 and IL-17A were found, which suggests that although IL-21 might have a role in early disease progression, IL-17A may be expressed only after extensive tissue damage has already occurred[176].

Although IL-17A may be involved in tissue damage, there is evidence that IL-21 acts directly on gut epithelial cells to induce the production of macrophage inflammatory protein 3α (MIP3α; also known as CCL20), a chemokine that attracts both T_H1 and T_H17 cells expressing CCR6 (REF. 177). IL-21 also induces the secretion of matrix metalloproteinases from epithelial cells, and these proteins are known to be responsible for mucosal tissue damage[178].

Chronic inflammatory bowel diseases have been associated with an increased incidence of colon cancer. Interestingly, *IL21* mRNA and IL-21 protein were both highly expressed in human colon cancer samples[97,179]. The role of IL-21 in the development or progression of colon cancer was therefore assessed using a mouse model of colitis-associated colon cancer. Importantly, *Il21*-knockout mice were resistant to the induced development of colon cancer, and mice that were treated with an IL-21-blocking agent had reduced numbers of colon tumours[97,179]. Thus, IL-21 appears to have a major role in promoting the inflammation-induced development of colon cancer. A clinical trial using IL-21 combined with cetuximab in the treatment of stage IV colorectal cancer was initiated before the above studies were published, but the trial was subsequently terminated[96].

Allergic disease and IL-21

Airway allergic responses. IL-21 is known to have an important role in B cell differentiation and immunoglobulin production, and it was therefore expected to have a crucial role in allergic responses. Interestingly, *Il21r*-knockout mice produced higher levels of serum IgE[15], analogous to the enhanced production of IgE in *IL21R*-deficient patients[71]. In keeping with the suspected inverse relationship between IL-21 and IgE, administration of IL-21 during the sensitization phase of an antigen response was shown to prevent antigen-specific IgE production[180]. However, *Il21r*-knockout mice developed less eosinophil airway inflammation in response to antigen than wild-type mice[181]. A potential therapeutic role for IL-21 in allergic rhinitis was demonstrated in a study wherein intranasal administration of IL-21 to mice significantly reduced the levels of antigen-induced IgE and alleviated the symptoms of allergic rhinitis[182].

Cutaneous allergic reactions. The immediate hypersensitivity reaction involves the production of allergen-specific IgE in the initiation phase and then an IgE-mediated stimulation of mast cells upon subsequent exposure to antigen. An analysis of the role of IL-21 in a mouse model of allergic cutaneous responses has revealed that systemic administration of IL-21 during the sensitization phase leads to a suppression of the allergic response through its suppression of the production of allergen-specific IgE[183]. However, administration of IL-21 after the sensitization phase does not decrease IgE production, although it does suppress mast cell degranulation. *In vitro* experiments have shown that IL-21 has a direct effect on the release of histamine and other inflammatory mediators of allergic responses from mast cells[183]. These studies contrast with another study reporting that *Il21r*-knockout mice have reduced allergic cutaneous inflammation in response to tape-stripping epicutaneous sensitization[184].

Tape-stripping epicutaneous sensitization
A method for inducing allergic skin inflammation that involves the application of antigen combined with tape stripping, which mimics scratching, leading to skin injury and a heightened allergic response.

IL-21 and psoriasis. Psoriasis is a chronic inflammatory disorder of the skin that manifests as plaques caused by the hyperproliferation of keratinocytes in the epidermis and infiltration of inflammatory cells. Genome-wide association studies identified the region encoding *IL2* and *IL21* as a susceptibility locus for psoriasis[146]. Skin lesions from patients with psoriasis have higher levels of IL-21 than areas of normal non-lesioned skin from the same patients[185]. Keratinocytes express IL-21R, and IL-21 can induce the proliferation of keratinocytes *in vitro* via the MAPK pathway[185]; skin thickening occurs following intradermal injection of IL-21 as a result of keratinocyte proliferation and infiltration of inflammatory cells[185] (see TABLE 2). A role for IL-21 in this hyperplasia has been confirmed in a human psoriasis xenograft SCID model, in which an IL-21-blocking antibody decreased epidermal thickness and reduced the number of inflammatory cells[185]. Further studies have shown that IL-21 acts by recruiting $CD4^+$ T cells that produce IFNγ, with IFNγ then inducing the epidermal hyperplasia.

Conclusions

Since the discovery of IL-21 and IL-21R in 2000, a tremendous amount has been learned about the actions of IL-21 on a broad array of target cells. Although IL-21 was originally thought to act primarily on haematopoietic cells, it is clear that non-haematopoietic cells in mucosal and neural tissues can also respond to IL-21, and so our understanding of the breadth of target cells for this cytokine is likely to still be incomplete. It also remains largely unknown how IL-21 signalling to a cell at a specific stage of differentiation is integrated together with the range of other environmental cues, either in the homeostatic state or in response to infection, inflammation or transformation. The diverse effects of IL-21 on different cell types also make it difficult to determine a priori whether IL-21 will have a net positive or negative effect; the integration of multiple signals determines the net signal and also determines whether the signal induces or represses differentiation, promotes growth or induces apoptosis. The complexity of the responses to IL-21 under different conditions indicates that blocking or augmenting IL-21 may not suffice and that combinatorial therapy may be required for optimal responses in many diseases (TABLE 2).

There are two general areas where the positive effects of IL-21 are important. First, in cancer immunotherapy there has been a rapid progression from animal studies to clinical trials for several classes of solid tumours. A challenge of these studies is to develop optimal *in vivo* combination therapies to take advantage of the diverse actions of IL-21 on $CD8^+$ T cells, NKT cells and NK cells. In addition, there is substantial evidence that the priming of tumour-specific $CD8^+$ T cells *in vitro* by IL-21 alone or in conjunction with other cytokines leads to the formation of superior antitumour cells for adoptive immunotherapy. This has not yet been exploited clinically but is a promising frontier. Second, although the use of IL-21 has not advanced to the clinic for the treatment of infectious agents such as viruses, it is clear that this cytokine contributes to the development of strong memory responses to various viral pathogens. Boosting levels of IL-21 at the time of vaccination or during the treatment of infections in which the immune system has become exhausted are rational strategies that should be explored preclinically.

Conversely, IL-21–IL-21R blockade may also have therapeutic benefit in the treatment of autoimmune diseases and inflammatory conditions. Stemming from preclinical experiments related to the blockade of IL-21 activity, we know that timing may be critical: IL-21 can have diverse actions in the course of a single disease, and understanding this will require additional investigation, including in animal model systems. Blocking IL-21 alone may not suffice to reverse disease or may not be as effective as combinatorial strategies. Moreover, it may be challenging to effectively deliver blocking agents at effective doses to the crucial sites *in vivo*, given the partial effects seen in some of the animal models. Should treatment with IL-21 and/or by blocking IL-21 prove to be effective, one must certainly be wary of untoward effects. For example, one concern is that the administration of IL-21 (such as in cancer) might necessarily exacerbate underlying autoimmune disease, whereas blocking IL-21 (such as in an autoimmune disease) might promote the development of a malignancy. Nevertheless, such risks are intrinsic in many therapies for severe diseases and are not necessarily contraindications but reasons for thoughtful development of therapeutic regimens and careful monitoring.

Despite the inherent complexity in understanding the conundrum posed by the pleiotropic actions of IL-21, we have now begun to tap the therapeutic potential of this cytokine system. The challenge now is to better understand the breadth of actions of IL-21 to optimize the beneficial effects that may result from augmenting or inhibiting its actions *in vivo*.

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Competing interests statement
The authors declare no competing interests.

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