



B-cell memory and primary immune deficiencies: interleukin-21 related defects

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Purpose of review

The purpose of this study is to describe recent advances in our understanding of the role of interleukin-21 (IL-21) in B-cell maturation, and how defects in IL-21 receptor (IL-21R) signalling pathways (IL-21R/γc/JAK3/STAT3) are related to primary immune deficiencies.

Recent findings

Abnormal signalling through IL-21R/γc/JAK3/STAT3 pathway has been related to decreased specific antibody responses following vaccination, and to increased susceptibility to encapsulated bacterial infections. This is manifested in the hyper-IgE syndrome, X-linked and JAK3-related severe combined immunodeficiency (SCID) and loss-of-function mutations in the *IL-21R* gene. Common variable immunodeficiency is associated with impaired in-vitro development of peripheral blood mononuclear cells or purified B-cells into memory or CD38⁺ B-cells following addition of IL-21.

Summary

IL-21 is a key cytokine in development of B-cells into immunoglobulin-secreting cells. Abnormal signalling through the IL-21R/γc/JAK3/STAT3 pathway leads to defective humoral immune responses to both T-dependent and T-independent antigens and impairs the establishment of long-lasting B-cell memory. Studies involving patients with hyper-IgE syndrome demonstrated the nonredundant role of STAT3 in B-cell production of high-affinity specific antibodies, while total serum immunoglobulins could be maintained through STAT3-independent activation of AID (activation-induced cytidine-deaminase). IL-21 related defects may also be associated with reduced natural killer (NK)-cell cytotoxicity and T_H17 cytokine production, indicating that abnormalities in the IL-21-IL-21R pathway have profound effects on crucial immune responses.

Keywords

B-cell memory, common variable immune deficiency, humoral immune response, interleukin-21, primary immune deficiency

INTRODUCTION

Adequate protection against bacterial pathogens depends on our capacity to build a functional adaptive immune response in which B-cells interact with both the innate immune system and helper T cells. Primary humoral immune deficiencies are characterized by intrinsic defects in the quantitative and/or qualitative aspects of this response and by insufficient production of high-affinity specific antibodies. Affected patients often suffer from recurrent oto-sino-pulmonary infections and carry an increased risk of chronic lung disease and bronchiectasis [1]. Anomalies occurring at different stages of B-cell development have been described. These abnormalities may occur very early, such as at the pre-B-cell stage in X-linked agammaglobulinemia [2*]. Other defects affect later stages in B-cell development, as in the case of CD40L mutation and

hyper-IgM syndrome, wherein B-cells have limited capacity to enter and trigger germinal centre reactions in secondary lymphoid tissues. For many clinical syndromes, the cause remains unknown [3]. The purpose of this review is to describe recent advances in our understanding of the role of interleukin-21 (IL-21) in B-cell maturation pathways, and how defects in IL-21 receptor (IL-21R) signalling

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KEY POINTS

- IL-21 is a key cytokine in B-cell development in secondary lymphoid organs.
- Abnormal signalling through the IL-21R/ γ_c /JAK3/STAT3 is associated with impaired specific antibody responses and defective establishment of B-cell memory.
- IL-21 contributes to both normal homeostasis and pathological states and should be considered as a novel therapeutic target alone or in combination with other modalities that modulate B-cell development.

(IL-21R/ γ_c /JAK3/STAT3) can lead to primary immune deficiencies.

B-CELL DEVELOPMENT

B-cell development takes place in the bone marrow from pluripotent stem cells. During the initial, antigen-independent phase, there is successful rearrangement of light and heavy chains leading to the expression of the μ B-cell receptor (BCR) on the surface of transitional B-cells that leave the marrow for the systemic circulation [4]. Further B-cell development occurs after antigen stimulation in secondary lymphoid tissues. Foreign pathogens are carried by cells of the innate immune system to lymphoid organs wherein adaptive immune responses begin. During these responses, naive B-cells (CD19⁺CD27⁻IgM⁺) [2[■],5] undergo maturation, including BCR affinity maturation and immunoglobulin (Ig) class-switch recombination. The mature B-cell pool consists of marginal zone B-cells (CD19⁺CD27⁺IgM⁺) [2[■],5], which are believed important for early antibody responses, and follicular B-cells that differentiate during germinal centre reactions to class-switched memory B-cells (CD19⁺CD27⁺IgM⁻) [2[■],6] and to specialized immunoglobulin-secreting cells (ISC) called plasmablasts (CD19^{low}CD27⁺⁺CD38⁺⁺⁺) [5]. Only a low number of plasmablasts become long-lived plasma cells (CD19⁻CD27⁺⁺CD38⁺⁺⁺CD138⁺) [5] that transit back to the bone marrow. During germinal centre reactions, three signals are required for optimal B-cell expansion, which occurs at the immunological synapse between B-cells and T-cells. Signal 1 is related to activation of the different BCR components, while signal 2 depends on the positive interaction of coreceptors, mainly CD40/CD40L. CD40 is a phosphorylated glycoprotein that belongs to the tumour necrosis factor (TNF) receptor superfamily on B cells, although its ligand (CD40L or CD154) is found on activated T cells. The CD40/CD40L interaction activates a signal transduction cascade involving nuclear factor κ B essential

modulator, which can be responsible for a primary immune deficiency syndrome if disrupted, named NEMO deficiency. Other important costimulatory signals can be provided by the inducible T cell costimulator (ICOS), the transmembrane activator and calcium modulator cyclophilin ligand interactor (TACI) on B cells and the B cell activating factor receptor (BAFF-R). Signal 3 relies on the influence of the surrounding cytokine milieu that polarizes the humoral immune response (Fig. 1). IL-21 has been identified as a key cytokine for inducing B-cell proliferation and differentiation into high-affinity ISC that is critical for establishment of long-lasting immune responses [7,8[■]].

INTERLEUKIN-21

IL-21 was first described in 2000 by Parrish-Novak *et al.* [9] and Ozaki *et al.* [10]. The human *IL-21* gene was mapped to chromosome 4 (4q26-q27), close to interleukin-2 (IL-2) and interleukin-15 (IL-15) [9]. IL-21 folds into a four-helix-bundle structure and binds to class I cytokine receptors (IL-21R) found on B, T and natural killer (NK) cells. The receptor is formed of two chains: the IL-21R chain and the common gamma-chain (γ_c), also a crucial component of the IL-2, IL-4, IL-7, IL-9 and IL-15 receptors. The receptor recognition module is composed of two pairs of conserved cysteine residues and an important WSXWS [11[■]] motif, while the intracellular part of the receptor transduces signals through the Janus kinase/signal transducers and activators of transcription (JAK/STAT) apparatus [12]. Although IL-21 can bind its receptor without the γ_c component, the absence of γ_c results in insufficient phosphorylation of the targets JAK 1 and JAK 3 and STAT1, STAT3 and STAT5 (Fig. 1) [10,13,14[■]].

ROLE OF INTERLEUKIN-21 IN B-CELL DEVELOPMENT AND DIFFERENTIATION

IL-21 can have a variety of influences on the fate of B-cell development (Fig. 1). First, IL-21 stimulation may induce B-cell apoptosis [9,15] when it occurs in the absence of BCR engagement and T-cell help. This may assist in eliminating autoreactive B-cells. Second, early in the immune response (day 0–3) when IL-21 and BCR stimulation can occur in the absence of complete T-cell expansion and without CD40 ligation, B-cells may be driven to produce and secrete granzyme-B proteases [16[■],17,18]. Granzyme-B positive B-cells may potentially play a role in the early phase of the immune response against viral pathogens, in autoimmunity and in cancer surveillance. Finally, IL-21 promotes B-cell proliferation, differentiation and class-switching into memory and ISC/plasma cells in the presence of Toll-like receptor

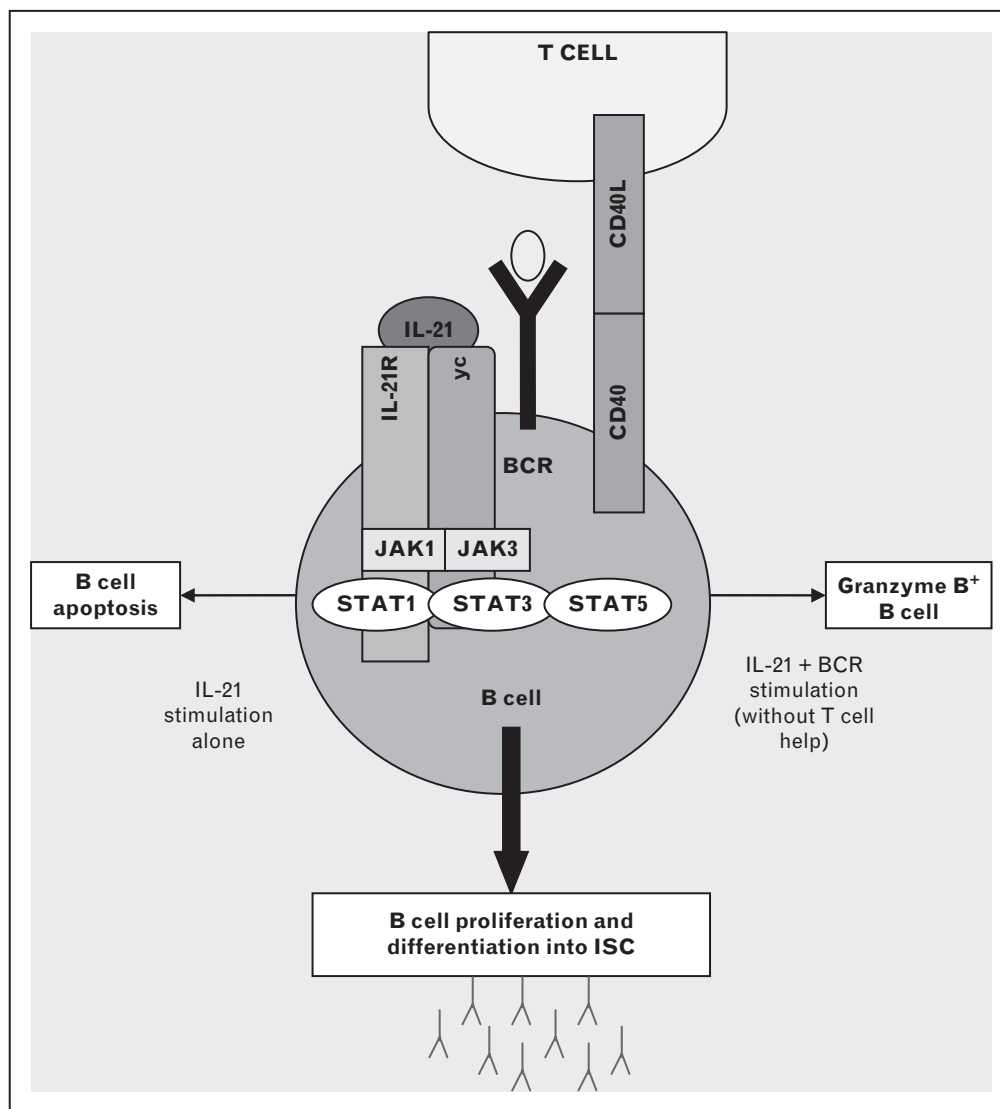


FIGURE 1. Interleukin-21 (IL-21) is a key cytokine in secondary lymphoid organs B-cells development. IL-21 stimulation induces B-cell proliferation and differentiation into immunoglobulin-secreting cells (ISCs) when occurring in association with B-cell receptor (BCR) stimulation and T-cell help (CD40L/CD40 interaction) through the JAK/STAT signalling pathway. Alternatively, IL-21 stimulation alone appears to induce B-cell apoptosis, while IL-21 with BCR engagement without T-cell help enhances granzyme B⁺ B-cells. γ_c , common gamma-chain; IL-21R, interleukin-21 receptor; JAK1, Janus kinase 1; JAK3, Janus kinase 3; MHC, major histocompatibility complex; STAT 3, signal transducers and activators of transcription 3; STAT1, signal transducers and activators of transcription 1; STAT5, signal transducers and activators of transcription 5.

signal (CpG) [19[¶]] or T-cell interaction [9,15,19[¶],20–22] with or without BCR activation. IL-21 can also upregulate the B-cell maturation protein A (BCMA), a cell surface receptor for BAFF with a prosurvival effect on CD38⁺ plasmablasts [23[¶]].

INTERLEUKIN-21 SIGNALLING DEFECTS AND PRIMARY IMMUNE DEFICIENCIES

Defective signalling through the IL-21R/ γ_c /JAK3/STAT3 pathway has been associated with several different primary immune deficiency syndromes.

The absence of normal IL-21 signalling leads to qualitative defects in B-cell function.

HYPER-IMMUNOGLOBULIN E SYNDROME

Autosomal dominant hyper-IgE syndrome (AD-HIES) is a multisystemic disorder characterized by dermatitis, boils, coarse facial features and bone and tooth abnormalities. Patients suffer from recurrent mucocutaneous infections or pneumatocoles involving *Staphylococcus aureus* and/or *Candida albicans*, cold abscesses with absent or inappropriately poor

elevation of inflammatory markers. The hallmark of the disorder is markedly elevated serum IgE with minimal allergic diatheses [24]. Approximately 60% of cases are caused by heterozygous mutations in STAT3. Dominant-negative mutations of STAT3 are associated with abnormal STAT3 proteins, leading to decreased numbers of functional STAT3 dimers, impaired signalling and diminished initiation of transcription [25]. The multisystem involvement of HIES is explained by the vast number of pathways in which STAT3 is implicated. STAT3 is found in more than 25 cytokine signalling pathways, including IL-6 acute-phase responses. It is also implicated in the development of IL-17 producing T-cells (T_H17) and in the osteoblasts/osteoclasts differentiation process, conferring, respectively, increased susceptibility to *Staphylococcus aureus* and *Candida albicans* [26] and bony abnormalities [25].

In the eighties, it was documented that patients with HIES also have increased susceptibility to encapsulated bacteria, such as *Streptococcus pneumoniae*, *Haemophilus influenzae* and group A *Streptococcus*. This was associated with abnormal functional humoral responses following immunization with T-dependent (tetanus toxoid) and T-independent (capsular polysaccharide of *H. influenzae*) vaccines, despite the presence of normal serum IgG levels [27]. Twenty years later, Avery *et al.* [14[■]] described the critical and nonredundant role of STAT3 in B-cell signalling through the IL-21 receptor responsible for generation of intrinsic long-term humoral immune memory. More precisely, they demonstrated that STAT3 mutant (STAT3_{MUT}) B-cells have an intrinsic survival and proliferative defect and fail to differentiate into CD27⁺CD38⁺ ISC when stimulated *in vitro* with CD40L and IL-21. This defect resulted in a 100 to 1000-fold decrease in immunoglobulin secretion (IgM, IgG and IgA). In contrast, they established that the class-switch recombination enzyme AID (activation-induced cytidine-deaminase) could still be expressed through STAT3-independent pathways (e.g. BCR, CD40L, BAFF, TLR ligands/CgG, cytokines IL-4 and IL-15 [28,29]), explaining why these patients have normal serum immunoglobulin levels. Although polyclonal immunoglobulin can be produced, the amount of specific antibodies against tetanus toxoid was decreased more than 30-fold, suggesting that the IL-21R/ γ_c /JAK3/STAT3 machinery is crucial for quality humoral immune responses and generation of long-term B-cell memory. The increased serum IgE in HIES is still not well understood. Interestingly, IL-21 receptor knockout mice (IL-21R^{-/-}) also exhibit dysregulated IgE [7]. One controversial hypothesis is that IgE may follow a different maturation programme compared with other immunoglobulins [30]. IgE production is

highly dependent on IL-4, IL-13 and STAT6 signalling [31] and it is possible that STAT3 mutations result in the loss of an important negative regulatory feedback loop. Recently, Wesemann *et al.* [32] reported that maturity of B-cells could dictate the choice between class-switching to IgE versus IgG₁, as immature B-cells displayed a preference to switch directly from C _{μ} to C _{ϵ} instead of undergoing a transitional step through C _{γ_1} (C _{μ} to C _{γ_1} to C _{ϵ}). This propensity may partially explain why immune deficiencies with abnormal B-cell development frequently are associated with high, dysregulated IgE levels.

X-LINKED AND JAK3 SEVERE COMBINED IMMUNODEFICIENCY

SCID is a paediatric clinical emergency, presenting with severe recurrent opportunistic infections, failure to thrive and evidence of abnormal B-cell and T-cell function. X-linked SCID (XL-SCID) is caused by mutations in the IL-2 receptor gene (*IL2RG*) encoding γ_c , while JAK3 mutations are responsible for autosomal recessive SCID. Ineffective signalling through the γ_c affects the IL-2R, IL-7R and IL-15R pathways and results in abnormal development of T-cell and NK-cells, although total numbers of B-cells are preserved (T⁻ B⁺ NK⁻ SCID phenotype). Haematopoietic cell transplantation with or without preconditioning is potentially curative [33]. One of the issues complicating this treatment is that although the T-cell and NK-cell compartment is usually reconstituted, B-cells stay of host origin in two-thirds of cases (incomplete chimerism) [34]. These patients often exhibit abnormal humoral function after transplant and require long-term IgG replacement therapy [34] to prevent infections. Recher *et al.* [8[■]] have shown that this persistent B-cell defect in incomplete chimerism was related to poor B-cell response to IL-21 through the mutated γ_c of IL-21R. They established that patients with less than 5% donor B-cells had significantly less PBMC differentiation into plasmablasts (CD19⁺CD27⁺⁺CD38⁺⁺⁺), and significantly less antibody secretion (IgM and IgG) after 4 days of culture with CD40L and IL-21 compared with patients with more than 5% B-cell engraftment or healthy controls. In affected individuals, the immunoglobulin production was at the level of untransplanted JAK3 or XL-SCID patients. They demonstrated that *IL2RG* mutated PBMC-derived B-cells failed to proliferate in response to CD40L and IL-21, but proliferation was intact for B-cells stimulated through γ_c -independent pathways [8[■]]. These results highlight the crucial role of IL-21 in B-cell development and maturation into ISC and add an important clinical point regarding the use of IL-21 in assays

to better predict the need for immunoglobulin replacement therapy or initiation of protective immunization for transplanted patients.

LOSS-OF-FUNCTION MUTATIONS IN THE INTERLEUKIN-21 RECEPTOR GENE

Homozygous loss-of-function mutations in the *IL-21R* gene were recently identified in two unrelated families [11[¶]]. Affected patients had recurrent upper respiratory and gastrointestinal infections as well as systemic invasive infections with *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Pneumocystis jirovecii*, *Candida albicans* and mycobacteria. A consistent finding was the occurrence of chronic purulent cholangitis with cryptosporidia evolving to end-stage liver disease if untreated, as seen in HIV or hyper-IgM syndrome patients [35]. These patients had mild and variable abnormalities of T, B and NK-cells along with normal or slightly decreased IgG and increased IgE. One patient also had decreased class-switched memory B-cells (CD27⁺IgG⁺). The syndrome was caused by misfolded, mutant IL-21R proteins (IL-21R^{Arg201Leu} or IL-21R^{Cys81del}) that were retained in the endoplasmic reticulum and were unable to traffic to the cell surface. Decreased phosphorylation of STAT1, STAT3 and STAT5 was observed following addition of recombinant IL-21. Using in-vitro PBMC cultures with CD40L and IL-21, the patients' B-cells exhibited decreased proliferative capacity, defective upregulation of AID and limited surface expression of IgA and IgG compared with healthy controls. As with HIES and SCID, specific antibody responses to diphtheria and tetanus toxoid and to *H. influenzae* type-B were abrogated. Finally, similarly to IL-21R^{-/-} mice [9,36], they exhibit reduced NK-cells cytotoxicity and/or T-cell proliferation in response to specific antigens, with decreased production of T_H17 cytokines, which may explain their susceptibility to cryptosporidium infection.

COMMON VARIABLE IMMUNODEFICIENCY

Common variable immunodeficiency (CVID) is characterized by hypogammaglobulinemia, impaired specific antibody responses and increased susceptibility to bacterial infections, autoimmune diseases and malignancies due to dysregulated immunity [37–39]. Although CVID is the most frequently encountered clinically relevant humoral defect, it represents a syndrome rather than a single diagnostic entity, as a molecular defect has been identified in only 10–15% of cases [40]. One frequent finding in patients with CVID is a decrease in

mature, isotype class-switched memory B-cells [6], which tends to correlate with a more severe disease course [41,42]. In light of what has been described for their role in B-cell development, IL-21 and IL-21R are among the potential candidate genes identified in research on familial CVID [43]. In our cohort of CVID patients and healthy controls [44], we found that CVID patients have a decrease in class-switched memory B-cells (CD19⁺CD27⁺IgM⁻) and plasmablasts (CD19⁺CD27⁺⁺CD38⁺⁺⁺IgM⁺), though the percentage of IgM⁺ memory B-cells (CD19⁺CD27⁺ IgM⁺) was preserved. Following 7 days in culture, PBMC from CVID patients failed to develop memory B-cells (CD19⁺CD27⁺) in response to anti-CD40 and IL-21 or anti-CD40, IL-4 and IL21 compared with healthy controls. This was associated with impaired IgG production in culture supernatants. Although these results were preliminary, they are in line with those of Clemente *et al.* [19[¶]] who demonstrated that purified B-cells from CVID patients have lower differentiation into CD38⁺ cells and produce less IgG and IgA following anti-CD40+ IL-21± anti-IgM stimulation. However, our results differ from another study [45] suggesting that immunoglobulin production could be restored by IL-21. This disparity might be explained by their use of Elispot counting ISC versus our measurement of actual protein by cytometric bead array [19[¶]] or ELISA [44]. Further studies are required to understand the signalling and/or activation defects observed upon anti-CD40 and IL-21 (±IL4) stimulation in CVID patients and the exact role that the IL-21R/γc/JAK3/STAT3 pathway is playing in this syndrome.

PERSPECTIVES

IL-21 has a broad influence on the immune system with actions not only on B-cells but also on T [46[¶]] and NK-cells. A growing literature has found associations between excess IL-21 and autoimmune conditions, such as systemic lupus erythematosus [47], Behçets [48] and Graves' disease [49]. These findings have led to generation of therapeutic antihuman IL-21 neutralizing mAbs [50]. The role of anti-IL-21 in the therapy of autoimmune or inflammatory diseases still needs to be assessed. Moreover, recombinant IL-21 has been used in phase I clinical trials as an adjuvant therapy to enhance the immune response against metastatic colorectal cancer [51] and in haematological malignancies [52]. In light of these novel applications of IL-21 in medicine, more studies are required to improve our understanding of how IL-21 modulates immune cells, contributes to pathological states and can be used as a novel therapeutic target alone or in combination with other modalities

that modulate B-cell development, such as BAFF-neutralizing antibodies [23[¶]].

CONCLUSION

IL-21 as a member of the γ_c -receptor family is a major player in B-cell secondary lymphoid organ development and maturation. Intact signalling through the IL-21R/ γ_c /JAK3/STAT3 pathway is essential for proper specific antibody responses to both T-dependent and T-independent antigens and for establishment of long-lasting memory immune response. Specific mutations in this pathway have been now related to many primary immune deficiencies, including HIES, XL and JAK3-SCID and loss-of-function mutations in the *IL-21R* gene with significant clinical implications.

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Conflicts of interest

Dr Mazer is a William Dawson Scholar of McGill University. Dr Desjardins received the Richard & Edith Strauss Fellowship in Medicine in collaboration with McGill University.

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