Extending the clinical and immunological phenotype of human interleukin-21 receptor deficiency

Combined immune deficiencies (CID) are defined by severely impaired adaptive immunity leading to increased susceptibility to opportunistic infections, immune dysregulation and malignancies. CID of moderate severity may not lead to death in infancy but still carry a high burden of morbidity and mortality during childhood. Here we report a deleterious homozygous mutation in the Interleukin-21 receptor (IL-21R) in a Palestinian girl of consanguineous descent presenting with hypogammaglobulinemia and *Pneumocystis jirovecii pneumonia* corroborating the previously reported profound CID in IL-21R deficiency.[1]

The early development of the 8-year old index patient was unremarkable but at 2-years old she presented with recurrent otitis media. At 5-years old she was admitted with severe interstitial pneumonia that rapidly progressed to acute respiratory distress syndrome requiring prolonged mechanical ventilation. All cultures were sterile. Laboratory investigations revealed reduced IgG and IgA, while IgM was increased. Other routine laboratory parameters [T-, B-, NK- and naïve CD4 T-cell numbers, proliferative response to mitogens phytohemagglutinin (PHA), Concanavalin A (ConA) and pokeweed mitogen (PWM), T-cell receptor Vβ repertoire and T-cell receptor excision circle (TREC) numbers] were normal. Due to persistent panhypogammaglobulinemia she was treated with monthly immunoglobulin infusions. At 6-years old she presented with significant respiratory distress and hypoxia due to *Pneumocystis jirovecii* infection, indicating a severe T-cell dysfunction. Trimethoprim-sulfamethoxazole and steroids were administered and the patient recovered; however, chest computed tomography (CT) disclosed bronchiectasis at the bases of both lungs. Her liver presented normal in ultrasound and by laboratory evaluation, rendering liver disease in the previously reported patients most likely a sequela of cryptosporidial infection and not of the IL-21R deficiency itself, resembling the experience in CD40 ligand (CD40L) deficiency.[2] Thus, like the other IL-21R-deficient patients, our patient presented during later childhood with recurrent respiratory tract infections, bronchiectasis and *Pneumocystis jirovecii* pneumonia, but without liver disease or gastrointestinal manifestations, which had been observed in the first patient with IL-21 deficiency.[3]

The homozygous mutation chr16:27455957 G>A, p. Arg201Gln in the IL21R gene had been discovered by

Table 1. Immunological parameters of the IL-21R-deficient patient.

	Cells/µl	Normal values (cells/µl)	% of parental population	Normal values (%)
Lymphocytes	3700	1800-5000[a]		
CD4 T cells	**1565**	641-1453	**42.3**	26.5-41.4[a]
Naïve	**1201**	375-1096	**77.3**	55.6-75.8[a]
Total memory	355	216-497	**22.7**	24.0-43.4[a]
RTE of naïve	n.d.	n.d.	**92.8**	61.0-84.2[a]
Treg	77	18-86	4.9	2.3-7.7[a]
Circulating TFH	33	11-43	9.2	3.9-11.5[c]
CD8 T cells	973	200-1700	26.3	13-47[b]
Naïve	342	42-1300	35.1	16-100[b]
Central memory	26	6-43	2.7	1-6[b]
Effector memory	228	45-410	23.4	5-100[b]
Terminally diff. effector	**12**	57-340	**1.2**	15-41[b]
NK T cells	n.d.		0.016	0.007–0.23[c]
γδ T cells	n.d.		**18.2**	<10[c]
NK cells	115	70-590	3.1	2-31[b]
B cells (bone marrow)	n.d.		**26.4**	6-25[c]
Pro B cells	n.d.		3.7	2-7.4[c]
Pre BI cells	n.d.		12.1	8-27[c]
Pre BII cells	n.d.		**38.7**	39-62[c]
Immature	n.d.		**50.3**	15-43[c]
B cells (peripheral blood)	381	296-784	10.3	8.5-20.2[a]
Transitional	**73**	13-63	**19.2**	3.4-9.0[a]
Naïve	288	154-413	**75.6**	47.8-69.8[a]
IgG switched	**1**	13-74	**0.03**	2.7-14.0[a]
IgA switched	**0**	5-35	**0**	1.1-6.1[a]
IgM Memory	**8**	24-135	**2.1**	6.3-22.0[a]
Serum immunoglobulins	g/l	Normal values (g/L)		
IgG	**2.21**	5.4-13.4		
IgA	**0.27**	0.3-1.88		
IgM	**3.07**	0.42-1.7		
IgE	60	<100IU/mL		

*Relative values refer to the respective parental subpopulations. Reference values were taken from a) van Gent et al.[7] b) Schatorje et al.[8] or are c) internal reference values. *Of CD45RA^{neg}/CD4^{pos}/CD3^{pos} T cells. Abnormal values are printed in bold. RTE: recent thymic emigrants; Treg: regulatory T cells; TFH : T-follicular helper cells; NK T cells: natural killer T cells; NK cells; by natural killer cells. Circulating TFH cells were defined as CXCR5^{pos}/PD-1^{pos}/CXCR3^{neg}/CD45RA^{neg}/CD4^{pos}/CD3^{pos} cells.*

whole exome analysis (*Online Supplementary Appendix, Online Supplementary Table S1* and *Online Supplementary Figure S1A*) and segregated within the family. Interestingly, a homozygous mutation affected the same codon, Arg201Leu, in one kindred of the originally described IL-21R-deficient patients, and was implicated in deficient glycosylation and transport of the protein to the cell surface.[1] While the new missense mutation did not interfere with normal mRNA expression level (*Online Supplementary Figure S1B*), surface protein expression and its upregulation after CD40 stimulation[4] could not be detected by flow cytometry as had been the case in the healthy control (*Online Supplementary Figure S1C*). The absent surface expression was associated with absent phosphorylation of the IL-21R signaling module of STAT1, STAT3 and STAT5 after IL-21 stimulation (Figure 1A), while STAT phosphorylation was readily detectable in the patient's peripheral blood mononuclear cells after stimulation through IFN-γ, IL-6 and IL-2, respectively (Figure 1B), indicating a complete loss of function of the IL-21R.

Immune phenotyping showed unremarkable circulating lymphocyte populations in the patient, except of slightly increased CD4 T cells, due to increased naïve CD4 T cells at the relative expense of memory CD4 T cells, and elevated γδ T cells. CD45RA^{pos}/CCR7^{neg}/CD27^{neg} terminal effector CD8 T cells were decreased, as previously reported for 3 IL-21R-deficient patients.[5] Interestingly, in contrast to inducible T-cell co-stimulator (ICOS)-deficient and CD40L-deficient patients,[6] circulating T-follicular helper (TFH) cells were within the reference range (Table 1 and Figure 1C),

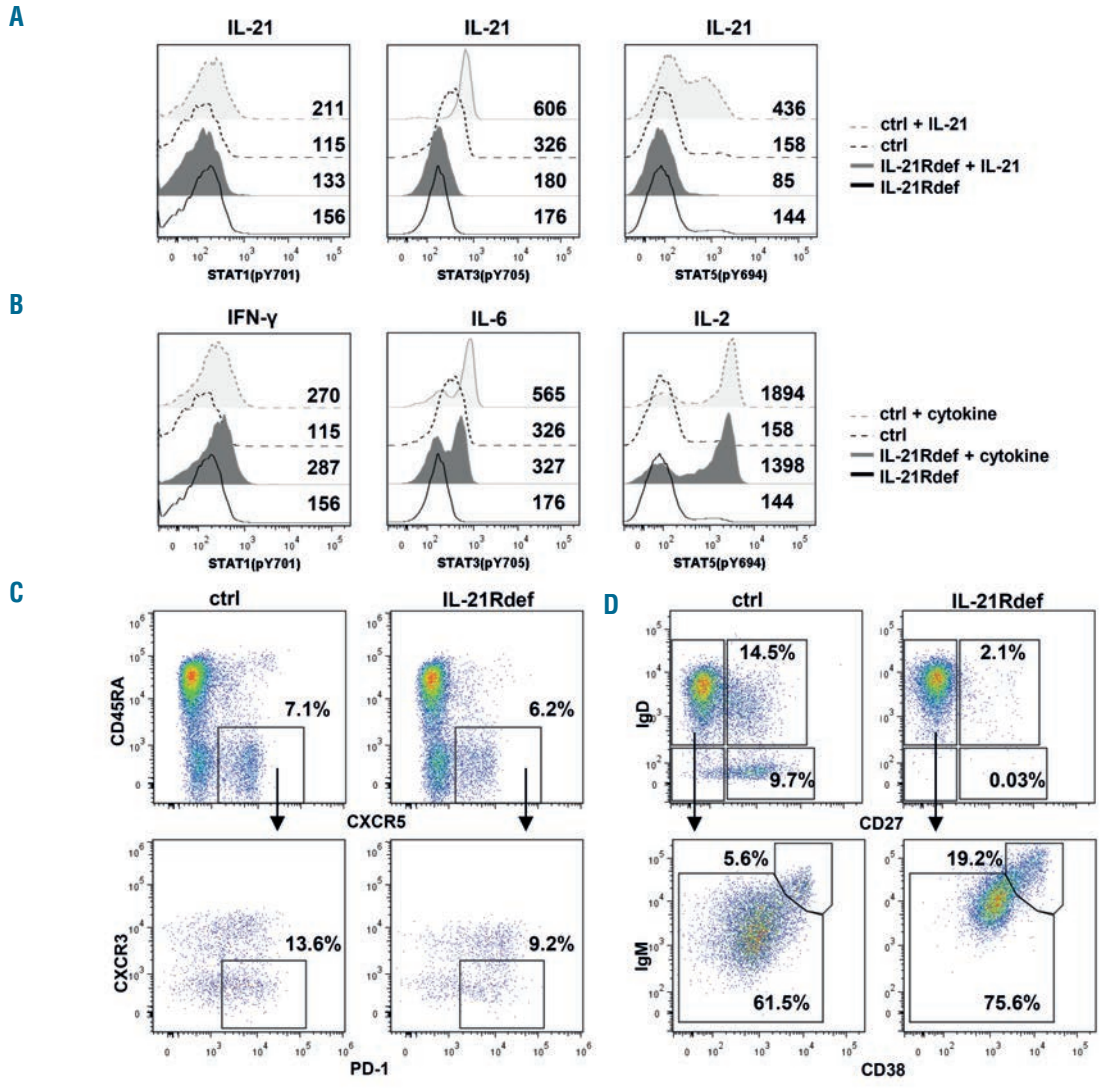

Figure 1. Abrogated IL-21R signaling is associated with normal TFH cells but disturbed peripheral B-cell differentiation. (A) Phosphorylation of STAT5 in CD4, STAT3 in CD8 T cells and STAT1 in B cells after stimulation with IL-21. (B) Phosphorylation of STAT5 after IL-2, STAT1 after IFN-γ and STAT3 after IL-6 stimulation in the respective subpopulations. Mean fluorescence intensities (MFI) are indicated. (C) TFH cells in the patient and a 5-year old control after gating on CD4pos T cells. CXCR5^{pos}/CD45RA^{neg} T cells refer to CD4 T cells. CXCR5^{pos}/PD-1^{pos}/CXCR3^{neg}/CD45RA^{neg} TFH cells are shown after gating on CXCR5^{pos}/CD45RA^{neg} T cells. The percentage of CD45RA^{neg} CD4 T cells is indicated. (D) Switched memory (IgD^{neg}/CD27^{pos}) and IgM memory B cells (IgD^{pos}/CD27^{pos}) after gating on CD19^{pos} B cells in the IL-21R-deficient patient and a 5-year old healthy control (upper panels). CD38^{high}/IgM^{pos} transitional B cells after gating on IgD^{pos}/CD27^{neg} B cells (lower panels).

suggesting that the development of germinal centers (GC) and the differentiation of TFH cells, the main source of IL-21 in GC[9] might be preserved in IL-21R deficiency. Peripheral B cells contained nearly no class-switched memory B cells (Figure 1D) as had been reported previously in IL-21R deficiency and in the IL-21-deficient patient,[1,3,10] confirming the central role of IL-21 in the differentiation of class-switched memory B cells during the GC response.[11] Also IgM memory B cells were reduced (Figure 1D). As outlined in the described alterations of the B-cell homeostasis in IL-21 deficiency,[3] the expansion of transitional B cells (Figure 1D) might indicate a previously unknown role of IL-21/IL-21R signaling in early peripheral B-cell maturation, while the B-cell development in the bone marrow was unaffected except for a relative increase in immature B cells (Table 1).

Addressing B-cell function in more detail, we observed an increase of CD25 (Figure 2A) and CD86 expression (*data not shown*) in naïve B cells of a healthy control after stimulation with IL-21 but not in IL-21R-deficient B cells. After co-stimulation with anti-CD40 or anti-IgM the expression of CD25 and CD69 was strongly augmented by IL-21 in the control but not in the patient (Figure 2A). Proliferation of IL-21R-deficient B cells was absent after CD40/IL-21 stimulation, but normal after IL-4/anti-IgM/anti-CD40 stimulation (Figure 2B). Furthermore, *in vitro* differentiation of plasmablasts and immunoglobulin secretion were completely abrogated after IL-21/CD40 stimulation compared to immature and naïve B cells from healthy cord blood, revealing the severe defect in T-cell dependent plasma cell differentiation. Interestingly, plasma cell differentiation and IgM and IgA secretion after CpG stimulation was also reduced in IL-21R-deficient B cells compared to cord blood-derived B cells, suggesting a possible role of IL-21/IL-21R in

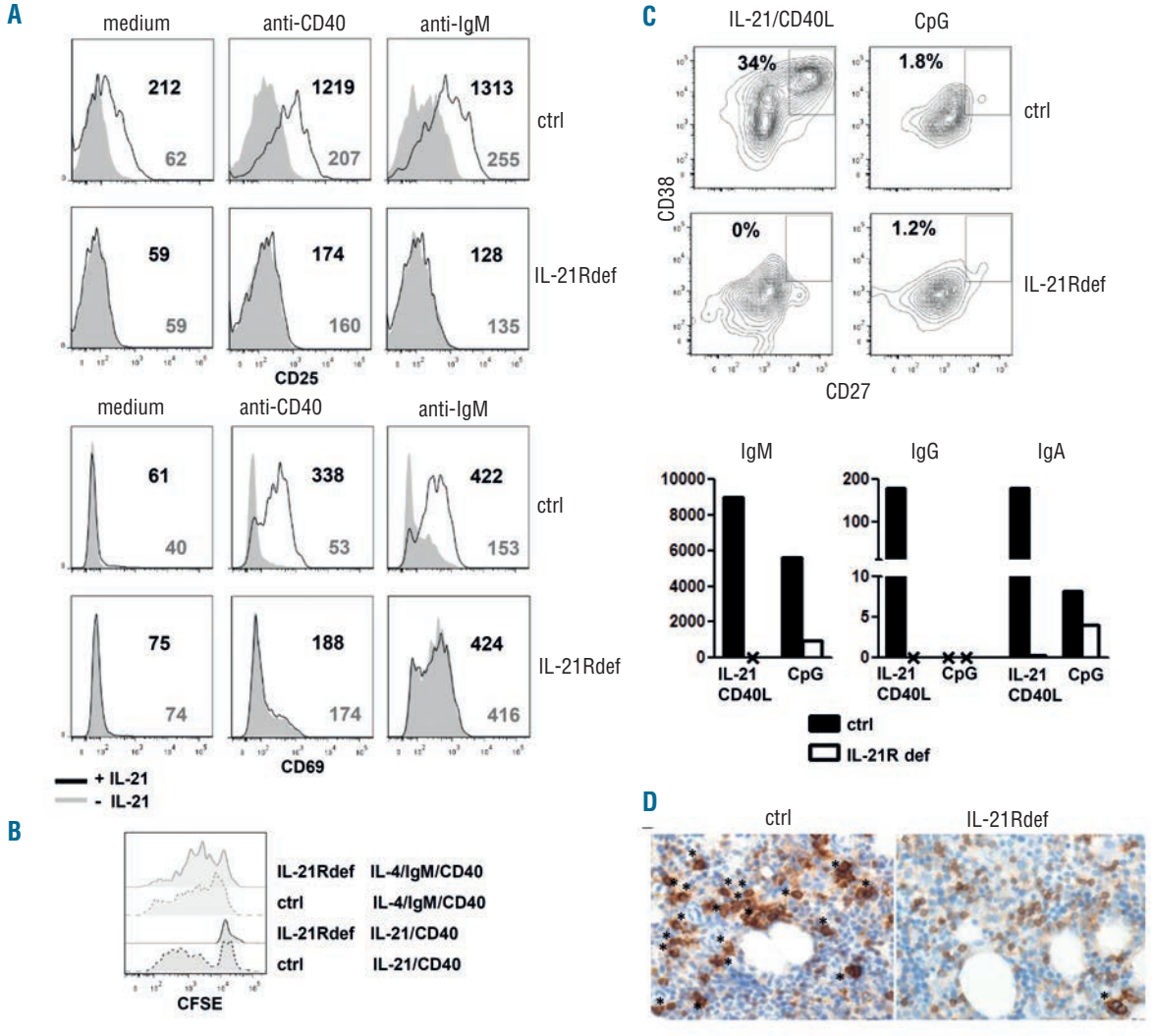

Figure 2. Impaired B-cell activation and terminal differentiation in IL-21R deficiency. (A) CD25 (upper row) and CD69 (lower row) expression in $CD27^{neg}/CD19^{pos}$ B cells in medium, after stimulation with anti-CD40 and anti-IgM with (black) or without IL-21 (gray). The MFI is indicated. (B) Proliferation of IL-21R-deficient and control B cells after stimulation with IL-21/anti-CD40 or with IL 4/anti-IgM/anti-CD40. (C) *In vitro* differentiation of plasmablasts after stimulation of isolated IL-21R-deficient B cells and B cells from healthy cord blood with IL-21/CD40L or CpG. ELISA for IgM, IgG and IgA in (ng/mL). (D) Higher density of $CD38^{high}$ (brown stain) plasma cells (marked by asterisks) in the bone marrow of an age-matched control (left) compared to solitary plasma cells in the IL-21R-deficient patient (right).

this context (Figure 2C). Compatible with the role of IL-21 in the differentiation of class-switched plasma cells,[11] our patient presented with low IgG and IgA serum levels (Table 1). However, strongly reduced serum IgG levels had previously been reported only in one patient, while 2 of 4 patients presented with slightly reduced and one even with normal levels. IgA was within the normal range in 2 of 2 reported patients and IgM even elevated in one of 2 reported patients,[1] suggesting that, in *in vivo* conditions, IL-21 independent pathways are sufficient for plasma cell differentiation. Supporting the special importance of IL-21/IL-21R signals for long-lived GC-derived IgG responses,[12] $CD38^{hi}$ plasma cell counts of the IL-21R-deficient patient were reduced in the bone marrow sample compared to an age-matched control (Figure 2D). Interestingly, all 4 previously reported IL-21R-deficient patients,[1] and to a lower extent the IL-21-deficient patient,[3] had increased levels of IgE, while serum IgE levels were within the normal range in our patient (Table 1). Thus elevated IgE serum levels are common in IL-21R/IL-21 deficiency but are possibly enhanced by cryptosporidial infection.[13]

In contrast to the overt impact of IL-21R deficiency on the humoral immune response, the alteration of the cellular immunity in IL-21R deficiency was less obvious. The upregulation of CD69, CD25, ICOS (*Online Supplementary Figure S2A*) and proliferation of IL-21R-deficient CD4 T cells was normal after anti-CD3+/-CD28 stimulation (*Online Supplementary Figure S2B*). In contrast to the decreased cytokine production by T cells of one of the previous patients,[1] our patient's memory CD4 T cells produced comparable amounts of IL-4, IL-17A and IFN-γ after PMA/ionomycin stimulation (*Online Supplementary Figure S2C*). These differences may be due to the different methodology and secondary changes during infection. While the global CD4 memory function was not significantly altered, subtle differences in antigen-specific T-cell memory responses cannot be ruled out. CD8 T cells of the IL-21R-deficient patient showed normal degranulation, cytotoxicity and proliferation (*Online Supplementary Figure S3A-C*). Regarding NK cells, we found a normal distribution of $CD56^{high}/CD16^{neg}$ and $CD56^{dim}/CD16^{pos}$ NK-cell subsets (*Online Supplementary Figure S4A*). Although 3 of 4 previously described IL-21R-deficient patients presented with decreased NK-cell cytotoxicity, NK-cell function of the fourth patient and the IL-21-deficient patient was reported normal, as in our patient; therefore, definite conclusions as to the effect of IL-21R deficiency on NK-cell function can not be drawn.[1,3]

While IL-21R deficiency clearly represents a form of CID, some patients may be misdiagnosed with common variable immunodeficiency before the onset of opportunistic infections. Interestingly, on top of the increased susceptibility to cryptosporidia and pneumocystis infections, CD40L deficiency and IL-21R deficiency share common features: normal or elevated IgM (3 of 3 analyzed IL-21R-deficient patients) and the absence of switched memory B cells (5 of 5).[14] On the other hand, IL-21R deficiency differs from CD40L deficiency not only in the pattern of inheritance, but also in often higher IgG levels[2] and detectable circulating TFH cells.[6]

The clinical diagnosis of combined immunodeficiency and of IL-21R deficiency is particularly difficult given the normal routine laboratory screening parameters in many of these patients. In our patient, given the genetic diagnosis of IL-21R deficiency, the presentation with *Pneumocystis jiroveci pneumonia*, and the poor outcome of the previously reported patients after cryptosporidial infection, we considered early hematopoietic stem cell transplantation (HSCT) to avoid poor outcome of transplantation after onset of severe secondary complications, as reported for CD40L deficiency.[15] Our patient underwent allo-HSCT from her fully matched healthy sister. Six months after transplantation and successful withdrawal of immunosuppressive therapy, she presented with 100% donor chimerism and normal immunoglobulins levels without any evidence of graft-*versus*-host disease.

In summary, IL-21R deficiency seems to affect the immunological, and especially B-cell memory, underlining its important role during the GC reaction. Our patient's history indicates that liver disease is probably not an intrinsic manifestation of the immunodeficiency, but is rather secondary to cryptosporidial infection. Given the poor reported prognosis after secondary complications, early genetic diagnosis and definite treatment with HSCT become important in the management of these patients.

Further information concerning Methods are available in the *Online Supplementary Appendix*.

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