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CD21 and CD19 deficiency: Two defects in the same complex leading to different disease modalities



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ABSTRACT

Purpose: Deficiencies in CD19 and CD81 (forming the CD19-complex with CD21 and CD225) cause a severe clinical phenotype. One CD21 deficient patient has been described. We present a second CD21 deficient patient, with a mild clinical phenotype and compared the immunobiological characteristics of CD21 and CD19 deficiency. **Methods:** CD21 deficiency was characterized by flowcytometric immunophenotyping and sequencing. Real-time PCR, *in vitro* stimulation and next generation sequencing were used to characterize B-cell responses and affinity maturation in CD21^{-/-} and CD19^{-/-} B cells.

Results: A compound heterozygous mutation in *CD21* caused CD21 deficiency. CD21^{-/-} B cells responded normally to *in vitro* stimulation and *AID* was transcribed. Affinity maturation was less affected by CD21 than by CD19 deficiency.

Conclusions: Both CD21 and CD19 deficiencies cause hypogammaglobulinemia and reduced memory B cells. CD19 deficiency causes a more severe clinical phenotype. B-cell characteristics reflect this, both after *in vitro* stimulation as in affinity maturation.

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1. Introduction

Genetic defects leading to primary antibody deficiencies (PAD) have been described in both the B cell co-receptor complex and the complement cascade [1,2]. Previously 12 patients have been described with deficiencies in CD19 and CD81, both part of the B cell co-receptor complex [3–9]. Thus far, only a single patient with a deficiency in CD21, also part of this complex, has been described [10]. All of these patients exhibit hypogammaglobulinemia and impaired vaccination responses and suffer from recurrent infections. However, age of disease onset and severity of infections are variable.

CD19, CD81 and CD21 form, together with CD225, the B cell co-receptor complex (Fig. 1) that enhances B-cell receptor (BR) mediated signaling [11–14]. In this complex, CD81 (a tetraspanin) is essential for CD19 expression on the B-cell membrane [4,15]. CD19 has a cytoplasmic tail with multiple tyrosine-kinase residues, needed for intracellular signaling [11,13,16,17]. CD21 is also known as complement receptor 2 (CR2) or EBV receptor [18–20]. It is expressed on both B

cells and follicular dendritic cells [21]. The 145 kDa protein consists of 15 short consensus repeats, a transmembrane domain and a short cytoplasmic tail [20,22]. The 19 exons of the *CD21* gene are encoded on chromosome 1q32 [20]. CD21 facilitates complement binding via C3d-opsonized immune complexes and responses to low dose antigens [18,20]. Functioning as a complement receptor, CD21 is involved in antigen uptake and presentation, clearance of immune complexes and apoptotic cells, induction of tolerance, generation of immunological memory, and survival, activation, and differentiation of B cells [21,23,24]. Studies in mice have been done with CD21/CD35 knock-out models, since these proteins are encoded by the same *Cr2* locus [25]. Results from these studies indicate that CD21/CD35 deficiency leads to decreased specific antibody responses [26–29] and increased susceptibility to autoimmune diseases [30]. In several immunological diseases such as HIV and autoimmune disorders increased CD21^{low/-} B-cell populations can be found [31–34].

Since CD81 is required for expression of CD19 on the plasma membrane, patients with CD19 and CD81 deficiencies show a highly similar phenotype with recurrent ear-nose-throat and respiratory infection starting early in childhood [3–9]. Most patients develop accompanying skin and gastro-intestinal infections. Upon flowcytometric analysis, all patients have normal B-, T- and NK-cell numbers, but reduced transitional and memory B-cell numbers. Both CD81 deficiency and CD19

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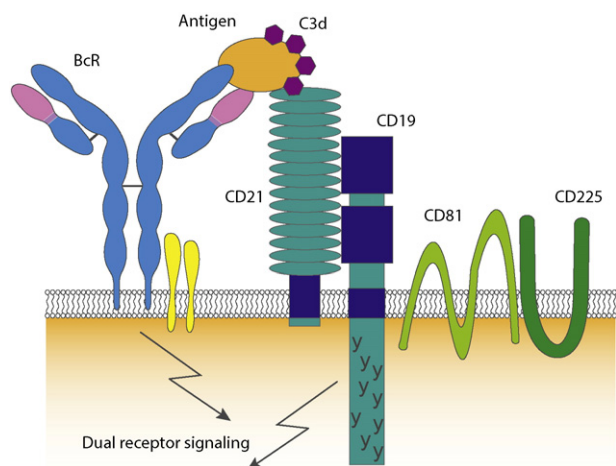


Fig. 1. Schematic representation of the B cell co-receptor complex, in which CD81 is vital for CD19 expression on the cell membrane [15], CD19 has an intracellular tail with multiple tyrosine-kinase residues [11,16] and CD21 is able to connect to the BR via antigen bound complement [18–21]. Co-activation of the BR and B cell co-receptor complex, leads to dual receptor signaling [45].

deficiency result in reduced frequencies of somatic hypermutations (SHMs) [7]. BR activation upon antigenic *in vitro* stimulation is impaired in CD19 and CD81 deficient cells. This emphasizes the necessity of CD19 and CD81 in the co-receptor complex to enable BR signaling.

The first patient described with a CD21 deficiency [10] suffered from recurrent upper respiratory tract infections in early childhood. In his early twenties, this patient developed recurrent infections, including respiratory tract and gastrointestinal infections. He had hypogammaglobulinemia mainly affecting immunoglobulin (Ig)G; IgA levels were slightly reduced and IgM levels were normal. Vaccination responses to protein antigens were normal, but the response to pneumococcal polysaccharide vaccination was moderately impaired. Flowcytometric analysis revealed normal B-T and NK-cell numbers, but reduced memory B cells. BR mediated signaling was affected in a complement-dependent manner in case of sub-optimal stimulation but unaffected upon strong stimulation. This underlines the complement receptor function of CD21 as an enhancer of B cell co-receptor signaling.

Here we describe a second patient (13-year old male) with a compound heterozygous CD21 deficiency resulting in hypogammaglobulinemia. We compared the clinical and immunobiological characteristics of CD21 deficiency with CD19 deficiency. We show that CD21 deficient B cells have a normal BR mediated signaling upon maximal stimulation *in vitro*, but slightly reduced SHM frequency and class switch recombination (CSR).

2. Methods

2.1. Cell samples and ethical approval

Peripheral blood was obtained from the CD21 deficient patient, age-matched healthy controls and both parents of the patient with informed consent and according to the guidelines of the local Medical Ethics Committees.

2.2. Flowcytometric immunophenotyping

Eight-color flowcytometric immunophenotyping of peripheral blood was performed on a Canto II (BD BioSciences, San Jose, CA, USA). Data were analyzed using FACS Diva (BD BioSciences) and Infinicyt software (Cytognos, Salamanca, Spain). The following

antibodies were used: CD19-PerCP-Cy5.5 (SJ25C1), CD21-PECy7 (B-ly4), IgD-biotin (IA6-2), CD27-APC (L128), CD38-APC-H7 (HB7; all from BD Biosciences), CD24-PB (SN3; Exbio, Prague, Czech Republic), CD45-PO (HI30; Invitrogen, Life Technologies, Carlsbad, CA, USA), IgG-PE (K0103-41437) and IgA-FITC (IS11-8E10; both Miltenyi, Bergisch Gladbach, Germany), polyclonal IgD-FITC and IgM-PE (Southern Biotechnologies, Birmingham, AL, USA). CD21 absence was determined using the following four CD21 antibodies: CD21-APC (CR2; BD Biosciences), CD21-PB (LT21; Exbio), CD21-PE (LB21; Serotech, Hercules, CA, USA), and CD21-PerCP (Bu32; BioLegend, San Diego, CA, USA).

2.3. Molecular analysis

DNA was isolated from blood granulocytes after separation using Ficoll Hypaque (GE Healthcare, Little Chalfont, UK) according to the manufacturer's instructions. Sequence analysis of *CD21* was performed following PCR-amplification of the coding regions with TaqGold™ (Life Technologies), followed by direct sequencing on an ABI Prism 3130 XL fluorescent sequencer (Applied Biosystems, Bleiswijk, The Netherlands). Primer sequences are available upon request. Sequences were analyzed with CLC DNA-workbench software (CLCbio, Aarhus, Denmark) and compared to the NCBI reference sequence (NG_013006).

2.4. Sorting of B-cell subsets and Ig transcript analysis

Naive, natural effector and memory B cells from 4 healthy controls and the patient were sorted from post-Ficoll mononuclear cells on a FACS Aria I (BD Biosciences) using the following antibodies: CD19-PerCP-Cy5.5 (SJ25C1), CD27-APC (L128), CD3-FITC (SK7; all from BD Biosciences) and polyclonal IgD-PE (Southern Biotechnologies). mRNA was extracted using Genelute mammalian total RNA Kit (Sigma-Aldrich, Saint Louis, MO, USA) and converted into cDNA. Taqman based RQ-PCR with gene specific primers and probes (sequences available upon request) was used to measure *CD19*, *CD21*, *CD81*, *CD79A* and *PAX5* transcript levels as described before [3,4]. Expression levels were normalized to *ABL* and *PAX5*.

2.5. Ca^{2+} flux analysis

Post-Ficoll PBMCs from the CD21-deficient patient and a healthy control were used to determine free intracellular Ca^{2+} levels before and after stimulation with anti-IgM as described previously [35]. Ca^{2+} influxes for the CD19 deficient cells were determined previously (Patient CD19-1.1) [3,4].

2.6. *In vitro* stimulation

PBMCs were cultured in 24-well plates (2×10^6 PBMCs per well) in 1 ml of IMDM culture medium, supplemented with 10% FCS, penicillin (100 U/ml) and streptomycin (100 µg/ml), at 37 °C and stimulated with anti-IgM (10 µg/ml) and anti-CD40 (10 µg/ml) and either hIL-4 (10 ng/ml) or hIL-10 (10 ng/ml) as described previously [7]. After 3 and after 6 days of culture cells were harvested and RNA was isolated. This RNA was used to synthesize cDNA. Taqman based RQ-PCR with gene specific primers and probes (sequences available upon request) was used to measure *CD19*, *AID* and *ABL* transcription levels as described before [7]. *AID* levels were normalized to *ABL* and *CD19* levels and compared to expression levels in unstimulated cells to calculate the fold increase in transcript levels.

2.7. Analysis of SHM and CSR

cDNA was prepared following RNA isolation of post-Ficoll PBMCs of patients and age-matched healthy controls. This cDNA was used to amplify *IGA* and *IGG* transcripts using VH1-6 consensus BIOMED-2 primers [36] and $C\gamma$ (3'C γ -CH1, [37]) and $C\alpha$ (IGHA-R, [38]) primers. The

primers were adapted for 454 sequencing by adding the forward A, or the reverse B adaptor, the 'TCAG' key and multiplex identifier (MID) adaptor. PCR products were purified by gel extraction with the QIAQuick gel extraction kit (Qiagen, Valencia, CA, USA) and Agencourt AMPure XP beads (Beckman Coulter, Fullerton CA, USA). Subsequently, the PCR concentration was measured using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen). The purified PCR products were sequenced on the 454 GS Junior instrument using the GS Junior sequencing kit XL+, sequencing kit and PicoTiterPlate kit (454 Life Sciences, Roche, Bradford CT, USA) according to the manufacturers recommendations. Using the Antigen Receptor Galaxy Tool [39] sequences were demultiplexed based on their MID sequence and quality checked. FASTA files were uploaded in IMGT HighV-Quest [40]. For all sequences the subclass of constant region was determined. Uniqueness of sequences was determined by V gene usage, amino acid sequence of the CDR3 and C gene usage. Only unique, productive sequences were used to calculate the frequency of mutated nucleotides in the V_H gene (from CDR1 until FR3).

In addition, IGG and IGA transcripts were amplified and analyzed as described previously [41,42]. The mutation frequency was determined for the V_H segment, with exclusion of FR1, of each unique transcript.

2.8. Statistical analysis

Differences in mutation frequencies in SHM were analyzed using the nonparametric Mann–Whitney *U*-test (1 tailed) and transcript expression differences were analyzed by the two-tailed *T* test for independent samples (*P* < 0.05 was considered significant) in the GraphPad Prism program (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Clinical and immunological presentation/case report

Our patient was a 13-year old boy, the second child of non-consanguineous Dutch parents without family history of recurrent infections or autoimmune diseases. He was referred to the department of Pediatrics of the University Medical Centre Groningen for evaluation hypogammaglobulinemia, which was discovered in the work-up for a possible auto-immune origin of myalgia and rigidity. He did not have a history of recurrent infections. No abnormalities were found upon physical examination. Total serum IgG was 4.4 g/l (ref. 7–16 g/l for age). IgG subclass analysis showed reduced IgG1 (2.5 g/l), reduced IgG2 (0.5 g/l) and absent IgG4 (<0.1 g/l), whereas IgG3 was normal (0.2 g/l). IgA levels were reduced (0.3 g/l (ref. 0.7–4 g/l)), as were IgM levels (0.3 g/l, (ref. 0.4–2.3 g/l)). Serum IgE levels were in the high normal range (109 g/l). He had been vaccinated according to the Dutch National Vaccination Program and his antibody titers were within the normal range, however additional booster vaccinations with subsequent determination of antibody levels have not been done. Autoimmune antibodies were negative. A C1q binding test was performed to analyze presence of immune complexes; no abnormalities were found. Analysis of B- and T- cell subsets revealed normal B–T- and NK-cell

numbers with slightly increased naive B cells and reduced memory B cells (Table 1). However, none of the B cells expressed CD21 (Fig. 2A).

After three years of follow-up, now 16 years old, the patient is in good health and has not experienced any (serious) infection. There has been no further reduction in his Ig levels.

3.2. Flowcytometric immunophenotyping

The absence of CD21 expression on B cells was confirmed using different antibody–fluorochrome combinations with multiple CD21 clones. Furthermore, following fixation and permeabilization, CD21 could not be detected intracellularly either (data not shown). Analysis of parental peripheral blood showed a decreased expression of CD21 on the surface of B cells in both parents, as compared to healthy controls (Fig. 2A). Expression of CD19 on the cell membrane was increased in the CD21-deficient patient and to a lesser extent in his parents (Fig. 2B). CD81 expression was normal in both the patient and his parents, as was CD35 (complement receptor 1) expression (data not shown).

3.3. Molecular analysis

Sequence analysis of all 19 exons and splice sites of the *CD21* gene revealed the presence of two heterozygous mutations (Fig. 2C, D): a nonsense mutation in exon 2 (c.424C>T; p. Arg142Stop), and a two-nucleotide deletion in exon 15 leading to a frame shift and a premature stop codon (c.2777_2778delTA; p.lle926SerfsX14). Both mutations lead to premature truncations of the protein in such way that the protein lacks its transmembrane and cytoplasmic domain. Analysis of the parents revealed that the mother was heterozygous for the mutation in exon 2 and the father was heterozygous for the exon 15 mutation (Fig. 2C). We concluded that these two truncating mutations lead to the absence of CD21 expression on the cell membrane and in the cytosol of B cells.

3.4. Transcript levels of CD19, CD81 and CD21

To study the nature of the increased membrane expression of CD19 on CD21-deficient cells, we determined the transcript levels of CD19, CD21 and CD81 in CD27-IgD+ naive, CD27 + IgD + natural effector and CD27 + IgD – memory B cells of 4 healthy controls and the patient (Fig. 3A). In all subsets from the patient, *CD21* transcripts were severely reduced. *CD19* transcript levels in all the patient's subsets were comparable to normal. *CD81* and *CD79A* (anchor protein for the BR) transcript levels in the patient, were in normal ranges in all subsets. The increased expression of CD19 did not seem to result from altered transcriptional regulation.

3.5. BR signaling

To study the effects of CD21 absence on BR signaling, we analyzed Ca²⁺ influx following stimulation of the patient's B cells with anti-IgM. CD21-deficient B cells showed a normal initial Ca²⁺ flux from the rough endoplasmic reticulum into the cytoplasm, which is indicative

Table 1
Distribution of B-cell subsets in our CD21 deficient patient at 13 years of age.

B cell subsets	%	Absolute count (cells/μl)	Normal values (10–16 yr)	
Transitional B (CD38 ^{high} /CD24 ^{high})	9.5	52	4–108	Within CD19 ⁺ gate
Naive Mature (CD38 ^{dim} /CD24 ^{dim} /IgD ⁺ /CD27 ⁻)	81.0	446	87–390	–
MZ/Natural effector (CD38 ^{dim} /CD24 ^{dim} /IgD ⁺ /CD27 ⁺)	5.3	29	7–90	–
Memory (CD38 ^{dim} /CD24 ^{dim} /IgD ⁻ /CD27 ⁺)	1.6	9	10–76	–
IgM	23.0		5–32%	Within memory B-lymphocytes
IgG	39.5		25–74%	–
IgA	37.5		14–47%	–
Plasmablasts	0.3			Within CD19 ⁺ gate

Numbers indicated in bold are deviating from the normal values.

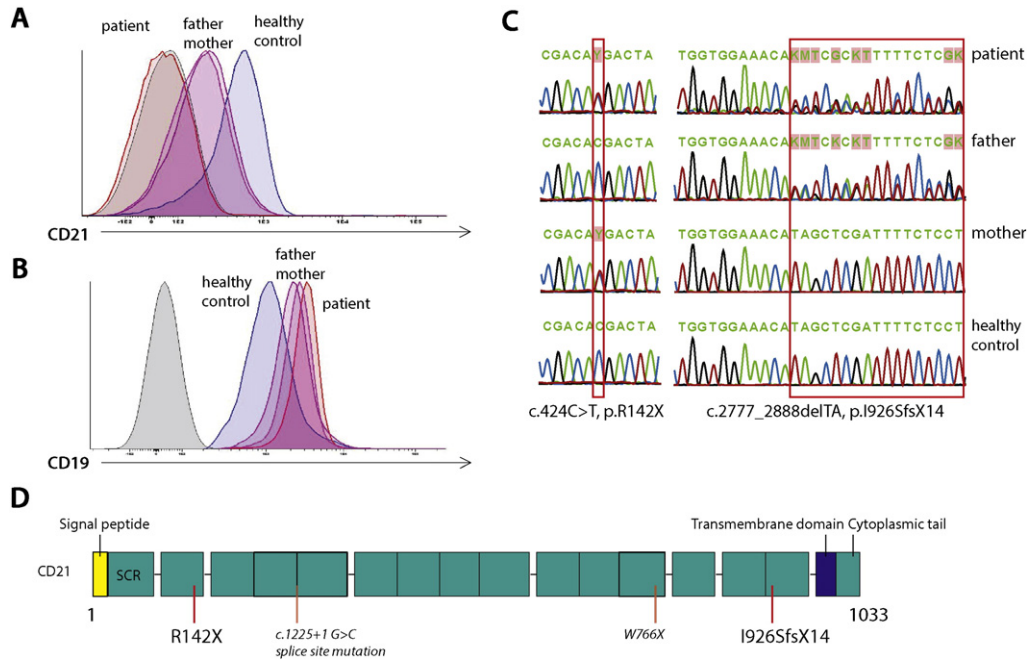


Fig. 2. CD21 deficiency due to a compound heterozygous mutation. A. CD21 membrane expression on B cells of the patient (in red), his parents (in purple), a healthy control (in blue) and CD19 negative lymphocytes from the healthy control (in gray). B. CD19 membrane expression on B cells of the patient (in red), his parents (in purple), a healthy control (in blue) and CD19 negative lymphocytes from the healthy control (in gray). C. Compound heterozygous mutations in the *CD21* gene of the patient. Both parents carry one of both mutations. D. Schematic representation of CD21 with 15 short consensus repeats (SCR). Position of the mutations found here (R142X and I926SfsX14) and the mutations found previously (c.1225 + 1 G > C and W766X, in Italics) [10] are depicted.

for a normal response upon stimulation with high amounts of anti-IgM (Fig. 3B). In contrast, cells from CD19-deficient patients showed a defective Ca^{2+} flux after maximal stimulation (Fig. 3B, [3,4]). Thus, signaling via BR upon stimulation is normal in CD21 deficiency, but impaired in CD19 deficiency.

3.6. *In vitro* stimulation

We studied whether the absence of CD21 affected the induction of AID transcription, since AID is a major regulator in affinity maturation after antigenic stimulation [43]. We stimulated total PBMCs from the CD21-deficient patient *in vitro* for 3 or 6 days with anti-CD40, anti-IgM and either hIL-4 or hIL-10 to induce transcription of AID. Using real-time quantitative PCR the levels of AID transcripts were determined after stimulation and compared to unstimulated cells. To correct for the amount of B cells present in each sample, we normalized for CD19 transcripts. AID transcription was upregulated in B-cells of healthy controls after 3 days of stimulation and was further enhanced after 6 days of stimulation. Induction of AID transcription in B cells from the CD21-deficient patient was less than the normal control after 3 days of stimulation. After 6 days of stimulation, the patient cells reached the same levels in AID transcripts as the healthy control cells after 3 days (Fig. 3C). These results show that AID transcription can be induced in CD21-deficient B cells, but at slower pace.

3.7. SHM analysis

To study the effect of CD21-deficiency on the frequency of SHM, we prepared PBMC derived cDNA from the CD21-deficient patient, age-matched CD19-deficient patients (6–12 years) and age-matched healthy controls (6–14 years). IGG and IGA transcripts were amplified in a PCR reaction with specific primers and PCR products were sequenced using next-generation sequencing. The proportion of unique productive sequences in the CD21 deficiency is comparable to the healthy controls but lowered in the CD19 deficiency (data not shown). SHM frequencies in transcripts of the CD21-deficient patient were

slightly lower than those of age-matched controls (Fig. 4A), but significantly higher than in CD19-deficient patients.

These findings are in line with previous analysis of these transcripts in CD19 deficiency [3,4].

3.8. Analysis of CSR

To study Ig CSR, we determined the IGG and IGA constant region subclass usage in amplified IGG and IGA transcripts prepared from PBMC derived cDNA. Compared to the healthy control, usage of downstream IGG regions (IGG2 and IGG4) was reduced in CD21 deficiency, resulting in increased IGG1 usage. In CD19 deficiency this increased IGG1 usage is even more pronounced (Fig. 4B). IGA usage in CD21 deficiency was comparable to normal, while in CD19 deficiency both IGA1 and IGA2 transcripts were overall strongly reduced and could only be amplified by conventional cloning and sequencing (Fig. 4B).

4. Discussion

We describe the second patient with a CD21 deficiency caused by two compound heterozygous mutations in the *CD21* gene. Both mutations cause a premature stop-codon resulting in truncated proteins both lacking their transmembrane and cytoplasmic domains.

In contrast to the first described CD21-deficient patient, this patient has no remarkable infections so far, despite his hypogammaglobulinemia. However, at the age of 13, the previously described patient was asymptomatic as well, developing recurrent infections only during early adulthood [10]. Both patients exhibit hypogammaglobulinemia, with normal vaccination responses. This indicates that their immune system is capable of mounting a seemingly normal immune response. With time, however, infections have occurred in the first patient, resulting in the need for intravenous immunoglobulin (IVIg) substitution. This in contrast with CD19 and CD81 deficiencies leading to (severe) recurrent infections already in childhood, requiring frequent hospital admission, surgery for chronic sinusitis and IVIg at early age [3–9]. The CD19-deficient patients often

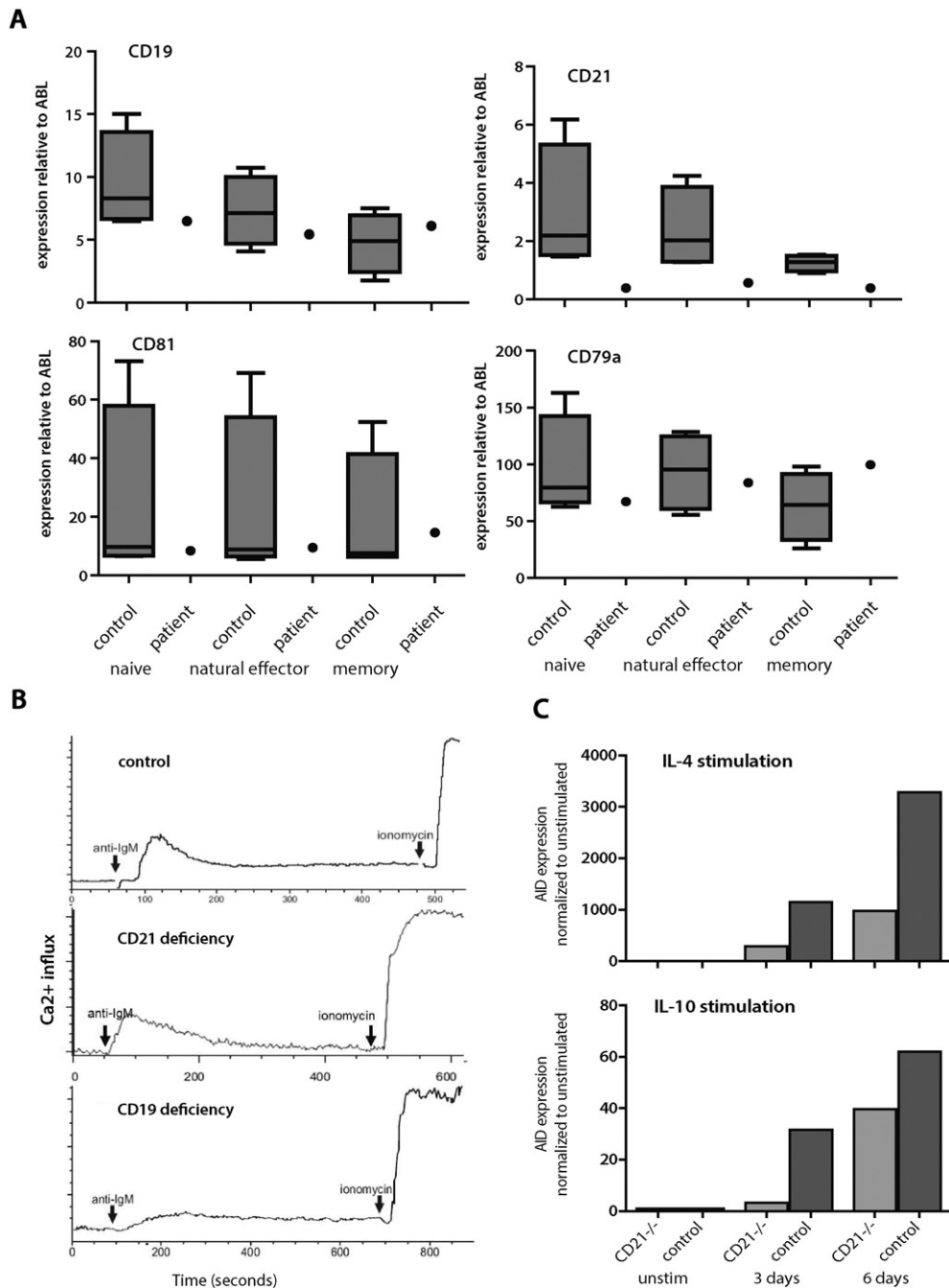


Fig. 3. Transcription of CD19-complex members and response to *in vitro* stimulation. **A.** Expression levels of *CD19*, *CD21*, *CD81* and *CD79A* in naive, natural effector and memory B cells of controls ($n = 4$) and the CD21-deficient patient, normalized to *ABL*. **B.** Ca²⁺ influx in CD21 deficient B cells resembles influx in healthy control cells upon maximal stimulation with anti-IgM. Ca²⁺ influx in CD19-deficient cells is severely reduced upon maximal stimulation with anti-IgM. Response to ionomycin is the same in all samples. Ca²⁺ influx for the CD19 deficiency was performed by Van Zelm et al., previously [3]. **B.** *AID* transcription in CD21-deficient B cells and healthy control B cells after *in vitro* stimulation with anti-IgM, anti-CD40 and either hIL-4 or hIL-10. Expression after 3 and 6 days was normalized to *ABL* and compared to unstimulated cells from the same samples. *CD19* expression levels were used to correct for the amount of B cells, present in the sample. In CD21-deficient B cells, *AID* transcription can be induced.

have splenomegaly and impaired vaccination responses, indicating that defects in CD19-complex can seriously impair B-cell function.

CD21 deficiency, like CD19 deficiency, does not lead to a decrease in absolute B-, T- and NK-cell numbers. CD21 deficiency leads to increased naive mature B cells and slightly decreased memory B cells, whereas CD19 deficiency and CD81 deficiency result in a more prominent decrease in transitional (CD81) and memory B cells (both CD19 and CD81). It seems that in CD21 deficiency an immune response is mounted upon encounter of an antigenic stimulus, but with reduced memory formation, whereas in CD19 and CD81 deficiency the immune

response is severely hampered. This is in line with the observation that in CD19 and CD81 deficiency, signaling upon BR stimulation is impaired, while calcium influx upon maximal stimulation is unaffected in CD21 deficiency. Previously, Thiel et al. showed that calcium influx in CD21 deficiency is impaired only in a complement dependent manner [10]. Thus, hypogammaglobulinemia in CD19 and CD81 deficiency can, at least partially, be explained by defective BR signaling, whereas this is intact in CD21 deficiency.

Interestingly, the CD21-deficient B cells show an increase in CD19 expression on the plasma membrane which was not reflected on

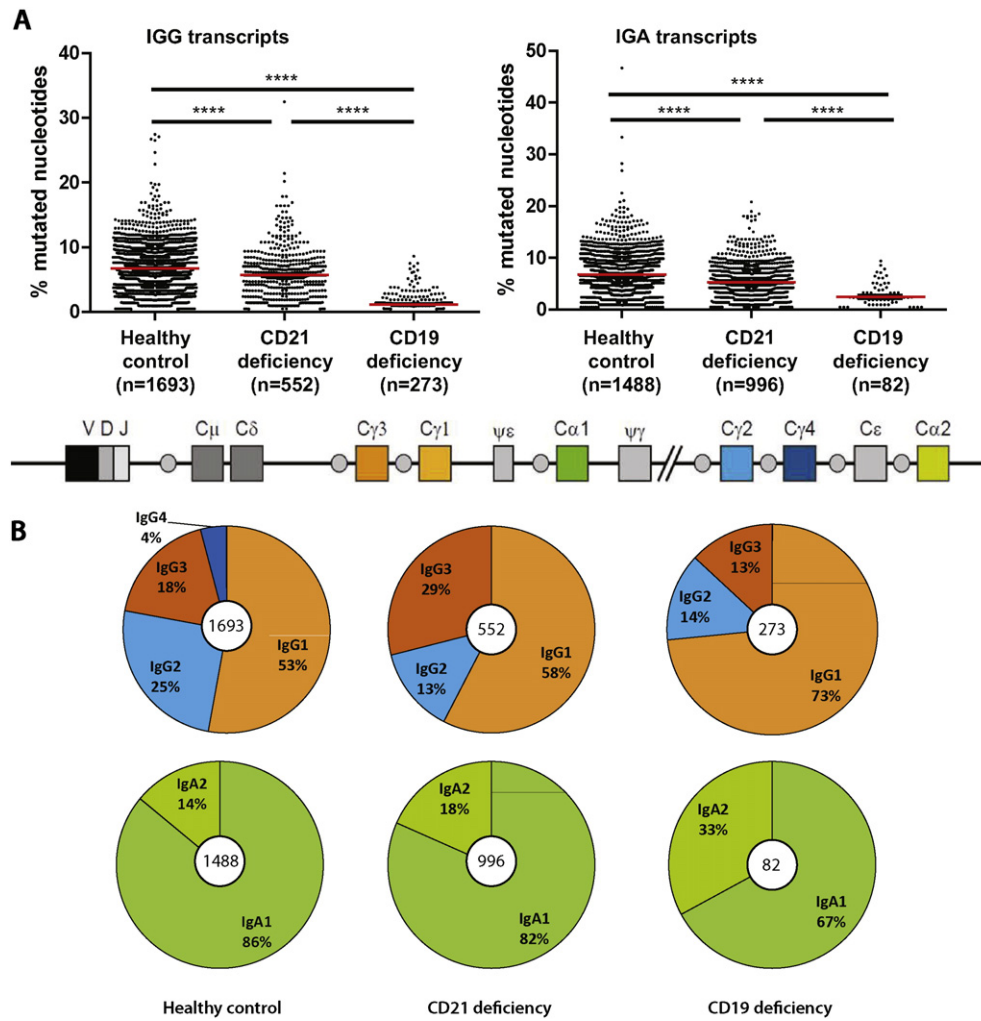


Fig. 4. SHM and CSR in CD21 and CD19 deficiency compared to in aged matched donors (all samples between 6 years and 14 years of age), data from multiple donors was combined for controls (n = 4) and CD19 deficiency (n = 4). A. SHM frequency determined in unique productive sequences N represents the number of unique productive sequences that were analyzed. B. Usage of IG constant gene segments determined for unique productive sequences. In CD19 deficiency no NGS data for IgA was available, sequences were all derived from cloning and sanger sequencing. The number in the center of the plots represents the number sequences that were analyzed.

transcript level. This increased CD19 expression on the membrane is also seen in CD21⁻ cells found in auto-immune diseases [32–34] and on CD21^{low/-} cells in healthy individuals, although in health very few of those cells can be detected. Increased CD19 expression was also found in CD21/CD35 deficient mice [44–46]. In contrast, in CD19 deficiency, CD21 expression is decreased on the plasma membrane. This suggests that CD19 and CD21 expression levels are inter-connected. The formation of a regulatory loop between CD19, CD21 and C3 has previously been postulated in mouse studies, which also showed that CD19 can function independent of complement activation [44,45]. Our results indicate that this is not due to deregulated transcription. Possibly, the increased CD19 expression is compensating for the loss of CD21 whereas in the case of the loss of CD19, CD21 cannot compensate and might even be down regulated.

CD21 deficient B cells respond normal to maximal stimulation, but in the patient we see a profound hypogammaglobulinemia and a reduction in class switched memory B cells. After *in vitro* stimulation, *AID* transcription could be induced in patients B cells (largely naive B cells). The delay that we see in the patient sample could be caused by the reduced number of memory B cells in the patient sample compared to the healthy control, which show fast induction of *AID* after stimulation. It seems that overstimulation or prolonged exposure to antigenic stimuli can induce a proper response, but short or weak stimulation does not evoke a response. This would be consistent with CD21 acting

as a receptor for complement: lowering the threshold for a response to stimulation [18–21,47].

Although the number of memory B cells is reduced in CD21 deficiency, they have a close to normal frequency of SHM and show a normal ability for CSR, although they seem to switch preferentially to the more upstream constant genes. This contrasts with the CD19 deficiency where SHM frequency is substantially lower and where the skewing towards *IGG1* is more pronounced. We hypothesize that CD21 negative B cells need strong or prolonged stimulation to evoke an antigenic response. However, when this stimulation is strong enough, the cells will respond with almost appropriate affinity maturation. CD19-deficient B cells cannot respond to even these strong stimuli, resulting in disturbed affinity maturation.

In our patient, IgE levels are opposed to IgA and IgG levels in a high-normal range. It seems that class-switching to IgE is not hampered, showing that class-switch machinery functions correctly and the reduced usage of downstream genes is rather a reflection of an impaired secondary germinal center response than inability of CD21 deficient cells to switch to distantly located constant regions.

Thus, in CD21 deficiency the hypogammaglobulinemia and impaired memory formation seem to result from a defect in signaling threshold rather than from an intrinsic inability to form memory cells whereas in CD19 and CD81 deficiency the clinical syndrome can be attributed

to a defect in B-cell memory formation already during an early phase of memory formation.

5. Conclusions

In conclusion, CD21 deficiency results in reduced numbers of memory B cells. However, the fraction of productive unique sequences is normal and SHM frequency is only slightly reduced. CSR is intact but shows a slight preference for switching to upstream genes. An increased signaling threshold seems to cause of an impaired immune response, resulting in hypogammaglobulinemia and reduced memory B cells. This might partially be restored by increased CD19 expression. Prolonged or repeated antigen exposure can probably result in normal antigen specific memory formation. In contrast, in CD19 deficiency impaired BR signaling is seen and therefore a defect in the early phase of memory formation with a more severe clinical phenotype. Whereas CD21 and CD19 belong to the same protein complex; the absence of CD21 gives a milder immunological and clinical phenotype than CD19 deficiency.

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