Clinical Immunology 161 (2015) 120–127

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

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

Marjolein W.J. Wentink [a,1], Annechien J.A. Lambeck [b,1], Menno C. van Zelm [a], Erik Simons [a], Jacques J.M. van Dongen [a], Hanna IJspeert [a], Elisabeth H. Schölvinck [c], Mirjam van der Burg [a,*]

[a] Dept. of Immunology, Erasmus MC, University Medical Center Rotterdam, Wytemaweg 80 3015 CN, Rotterdam, The Netherlands
[b] Dept. of Laboratory Medicine, Medical Immunology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9700 RB, Groningen, The Netherlands
[c] Dept. of Pediatrics, Beatrix Children's Hospital, University of Groningen, University Medical Centre Groningen, Hanzeplein 1,9700 RB, Groningen, The Netherlands

ARTICLE INFO

Article history:
Received 4 July 2015
Received in revised form 17 August 2015
Accepted with revision 21 August 2015
Available online 30 August 2015

Keywords:
CD21
CD19
CD81
Hypogammaglobulinemia
Primary antibody deficiency

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.

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 antigenic 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

* Corresponding author at: Erasmus MC, Dept. of Immunology, Wytemaweg 80, 3015 CN Rotterdam, The Netherlands.
E-mail address: m.vanderburg@erasmusmc.nl (M. van der Burg).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.clim.2015.08.010
1521-6616/

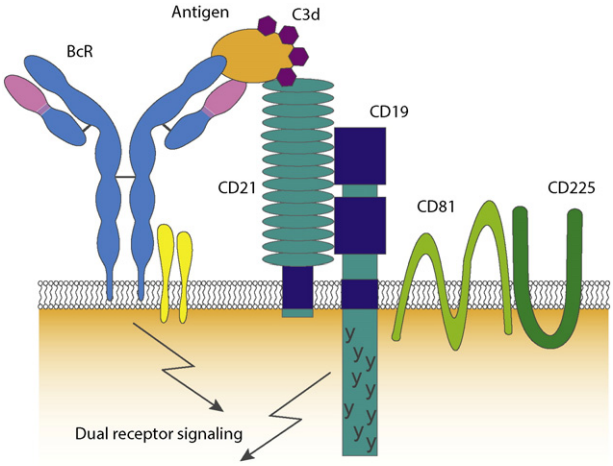

Fig. 1. Schematic representation of the B cell co-receptor complex, in which CD81 is vital for CD19 expression on the cell membrane [15], CD19 has an intracellular tail with multiple tyrosine-kinase residues [11,16] and CD21 is able to connect to the BR *via* antigen bound complement [18–21]. Co-activation 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 Cγ (3′Cγ-CH1, [37]) and Cα (IGHA-R, [38]) primers. The