Clinical Immunology 163 (2016) 1–9

Contents lists available at ScienceDirect

Clinical Immunology

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

B cell development in chromosome 22q11.2 deletion syndrome

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ARTICLE INFO

Article history:
Received 5 November 2015
accepted with revision 8 December 2015
Available online 10 December 2015

Keywords:
DiGeorge syndrome
Follicular helper T cells
Somatic hypermutation
TBX1
Immunoglobulin
22q11.2 deletion syndrome
Switched memory B cells

ABSTRACT

Chromosome 22q11.2 deletion syndrome is a common immune deficiency associated with thymic hypoplasia. Most patients did not survive until the mid-1980s and now there is a growing adult population. B cell and immunoglobulin defects have been described and appear to be increased in the adult population. We used flow cytometry, B cell stimulation and repertoire analysis to understand B cell function. B cell production at early stages appeared to be normal in patients but adult patients exhibited a deficit of switched memory B cells. Follicular helper T cells were present at higher percentages in patients and they exhibited a more activated phenotype in patients compared to controls. In spite of that, somatic hypermutation was decreased in patients compared to controls at all ages. Fewer mutations per clone were seen, strongly implicating aberrant T cell help. Therefore, patients with chromosome 22q11.2 deletion syndrome have a progressive decrease in switched memory B cells and evidence of compromised T cell help. In children, evidence of compromised T cell help is limited to decreased somatic hypermutation. With age, greater manifestations become apparent even though a minority of patients have hypogammaglobulinemia. As this population ages, this has important implications for management.

1. Introduction

Chromosome 22q11.2 deletion syndrome (22q11.2DS) is a common primary immune deficiency with an estimated frequency of one in 4000 live births [1,2]. Due to advances in cardiac care in the 1980s, the population frequency is increasing because larger numbers of patients are reaching adulthood and reproducing. The diagnosis is typically established after the identification of a clinical constellation that can include conotruncal cardiac defects, hypocalcemia, palatal abnormalities, developmental delay, dysmorphic facial features, and diminished T cell numbers [3]. The immune deficiency in 22q11.2DS can be extremely diverse, with some patients having no evidence of an immune deficiency and some patients having a phenotype consistent with severe combined immune deficiency [4–6]. Most patients have a mild to moderate decrement in their T cell numbers and at least initially have preserved T cell function [7]. Over time, with homeostatic pressure on the T cell compartment, T cell function can decline [8,9]. Evidence of T cell exhaustion occurs in adulthood [10,11]. In addition, there can be repertoire imbalances that also arise as a consequence of the homeostatic pressure on a limited number of founder cells [7,9,12,13]. Clinical manifestations of the immune deficiency include recurrent infections and autoimmune disease [11,14,15].

While the defect in T cell production has been well characterized and is known to be secondary to thymic hypoplasia, there is less information about 22q11.2DS and B cell function. Several studies have identified low IgA or low IgG levels [14,16,17]. While vaccine responses and antibody production are largely preserved, there are now emerging data suggesting that the B cell compartment can be compromised, particularly in adulthood [8,14,16,18]. Unlike the T cell compartment, the B cells do not appear exhausted [19]. Antibody defects have been associated with a significant infection history and autoimmunity [14,16]. A greater understanding of the etiology of these B cell defects is critical as this population increases in size and grows older.

Our data have previously demonstrated diminished numbers of switched memory B cells in adults with 22q11.2DS and a large registry study has identified hypogammaglobulinemia in approximately 6% of patients [8,17]. This current study was undertaken to understand whether the abnormalities in the B cell compartment and antibody production were intrinsic to the B cells themselves or secondary to diminished T cell help. The gene felt to be responsible for the majority of the phenotypic features in 22q11.2DS is *TBX1* [20–23]. *TBX1* is expressed in early embryologic development and elicits its major effect through promoting growth of mesodermal cells and neural crest cells in the pharyngeal arches. When the pharyngeal arches fail to develop properly, there can be abnormalities in cell mass or migration of the parathyroid glands, the thymus, and the heart. It has not been thought that *TBX1* is expressed in adult bone marrow. However, it is possible that early

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http://dx.doi.org/10.1016/j.clim.2015.12.004
1521-6616/

in development, haplosufficiency for *TBX1* could alter the differentiation program for B cell precursors. This study was undertaken in an effort to define adequacy of T cell help for B cell production of antibodies.

2. Methods

2.1. Subjects

This study was approved by the Institutional Review Board at The Children's Hospital of Philadelphia. Subjects were recruited from clinic patients at The Children's Hospital of Philadelphia and Dalhousie University/IWK Health Center. A total of 27 adults and 44 children with 22q11.2DS were examined. The diagnosis for patients was established by fluorescent in situ hybridization, MLPA, or single nucleotide polymorphism array confirmation of a chromosome 22q11.2 deletion. Adult and pediatric controls were from The Children's Hospital of Philadelphia and the University of Pennsylvania. The demographic data of patients and controls are given in Table 1. Not all patients and controls were included in each study due to limitations imposed by blood volumes. The N for each experiment is given in the figure legend.

2.2. Immunologic assays

Flow cytometric analysis of T cell subsets and B cell subsets was performed with fixation with 1% paraformaldehyde. Samples were run on an LSR Fortessa (BD Biosciences, San Jose, CA) and analyzed using FlowJo software version 8.8.7 (TreeStar, Ashland, OR). B cells were defined by physical parameters and CD19 expression and CD4 T cells were defined by physical parameters and CD4 expression. We defined follicular helper T cells (TFH) as CD4+, CXCR5+, and ICOS+. Active TFH were defined as CD4+, ICOS+, CXCR5+, CCR7lo, PD1hi or CD4+, CXCR5+, CCR7lo, PD1hi. We defined transitional B cells as CD27-CD38++, non-switched memory B cells as CD27 + IgM+, switched memory B cells as CD27 + IgM- and naïve B cells as CD27-IgM+. Plasmablasts were defined using two different gating schemes, as either CD27++ and CD38++ or CD38++ and IgM-.

2.3. B cell stimulation

B cells were isolated from PBMCs with the Dynal Human Untouched B cell Kit and cultured for 7 days. Cells were stimulated with anti-CD40 at 2 μg/ml, anti-IgM at 7.5 μg/ml, and IL-21 at 50 ng/ml. Anti-IgG at 7.5 μg/ml was the negative control. The percent of CD80 positive cells is reported after flow cytometric analysis gating on CD19 positive cells.

Table 1
Subject demographics.

	Number	Age
Flow cytometry of fresh samples		
Adult 22q11.2DS	27	25 ± 7
Adult Control	35	29 ± 7
Child 22q11.2DS	44	9 ± 6
Child Control	32	8 ± 5
B cell stimulation		
Adult 22q11.2DS	3	22 ± 8
Adult Control	7	29 ± 7
Child 22q11.2DS	7	10 ± 7
Child Control	14	6 ± 4
TREC and KREC		
Adult 22q11.2DS	11	26 ± 4
Adult Control	14	28 ± 7
Child 22q11.2DS	21	8 ± 6
Child Control	15	9 ± 6

Table 1. Demographic characteristics of the study population. The numbers of patients and their ages are given for each subset of data.

2.4. RT-PCR, TREC and KREC analysis

RT-PCR was performed using ABI primers for IL-21 on a TaqMan 9600. TRECs and KRECs were measured as previously described [24]. The assay was performed as described by Sottini with additional calculations as described by van Zelm [25,26]. PBMC DNA was isolated using the Gentra Puregene kit (Qiagen). 50 ng of DNA was used in each PCR reaction. Duplex PCR reactions were performed on a SDS 7500 Fact Realtime PCR system. Primer sequences are as follows:

KREC forward- 5′ TCAGCGCCATTACGTTTC 3′.
KREC reverse- 5′ GTGAGGGACACGCAGCC 3′.
KREC probe- 5′ 56-JOEN/CCAGCTCTTACCCTAGAGTTTCTGCACGG 3′ BHQ-1.
TREC forward- 5′ CACATCCCTTTCAACCATGCT 3′.
TREC reverse- 5′ GCCAGCTGCAGGGTTTAGG 3′.
TREC probe- 5′ 56-FAM/ACACCTCTGGTTTTTGTAAAGGTGCCCACT 3′ Tamra.
TRAC forward- 5′ TGGCCTAACCCTGATCCTCTT 3′.
TRAC reverse- 5′ GGATTTAGAGTCTCTCAGCTGGTACAC 3′.
TRAC probe- 5′ 56-JOEN/TCCCACAGATATCCAGAACCCTGACCC 3′ BHQ-1.
ACTIN forward- 5′ CCGGCGCTGTTTGAACC3'.
ACTIN reverse- 5′ CGGCCGCGTTATTACCATAAA 3′.
ACTIN probe- 5′ Cy5/ACGCCTCCGACCAGTGTTTG 3′ BHQ-2.

Numbers of TRECs, TRACs, and KRECs were calculated using a standard curve from known copy numbers. The plasmid with the insets was supplied by Imberti [27]. Actin was used to assess the overall quality of the DNA. Samples with poor actin amplification were excluded. Calculations were:

(mean of TRECs or KRECS number)/(mean of TRACs number/2) $* 10^6$.

2.5. IgH sequencing

Immunoglobulin heavy chain family-specific PCRs were performed on genomic DNA samples from peripheral blood CD19+ B cells of healthy controls and subjects with 22q11.2DS. Five children and five adults from the control and patient sets were sequenced (N = 20 subjects total). Three multiplexed mixes were employed with forward primers matching VH family leader sequences and reverse primers matching JH gene segment sequences, using a similar strategy to that described in Meng et al. [28]. Heavy chain gene rearrangements (VDJ) were amplified with AmpliTaq Gold (Life Technologies, Carlsbad, CA) and 10× buffer at a final concentration of 1.5 mM $MgCl_2$, 0.2 mM dNTPs and 0.4 mM of each primer cocktail. The primers each contained an appropriate adaptor sequence for subsequent Illumina sequencing. Amplicons were purified by QIAquick PCR purification kit (Qiagen) followed by size selection with Pippin prep (Sage Science, Beverly MA). Library quality was evaluated using Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA) and loaded onto an Illumina MiSeq using a 2 × 300 bp paired end kit (Illumina MiSeq Reagent Kit v3, 600 cycle).

2.6. IgH data analysis

Fastq files were obtained after sequencing and the PRESTO suite of tools (website: http://clip.med.yale.edu/presto/, [29]) was used to perform Quality score trimming using Q30 10-nucleotide sliding window and single nucleotide <Q30, to replace low quality nucleotide calls with N-nucleotides. (N′s are not counted as somatic hypermutations). All sequences were length trimmed (any sequence with fewer than 60 nt was discarded). Clonally related sequences were collapsed, allowing for up to 3 nucleotides to be N values when matching sequences. Quality filtered sequences were then assigned to germline IGHV gene segment alleles, based on alignments to a database of known IGHV alleles, using the IMGT/HighV-QUEST server [30]. Mutation levels were analyzed on the basis of "100 − % of VH identity to the nearest germline allele in the IMGT database" obtained from