Clinical Immunology 161 (2015) 190–196

Contents lists available at ScienceDirect

Clinical Immunology

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

High-throughput sequencing reveals an altered T cell repertoire in X-linked agammaglobulinemia

Manish Ramesh [a], Noa Simchoni [b], David Hamm [c], Charlotte Cunningham-Rundles [b,d,*]

[a] Montefiore Medical Center, Bronx, NY, United States
[b] Icahn School of Medicine at Mount Sinai, New York, NY, United States
[c] Adaptive Biotech, Seattle, WA, United States
[d] Department of Pediatrics, The Immunology Institute, Box 1089, Icahn School of Medicine at Mount Sinai, 1425 Madison Avenue, New York, NY 10029, United States

ARTICLE INFO

Article history:
Received 26 July 2015
accepted with revision 1 September 2015
Available online 7 September 2015

Keywords:
XLA
T cell receptor
High throughput sequencing
Junctional diversity
Amino acid sequence

ABSTRACT

To examine the T cell receptor structure in the absence of B cells, the TCR β CDR3 was sequenced from DNA of 15 X-linked agammaglobulinemia (XLA) subjects and 18 male controls, using the Illumina HiSeq platform and the ImmunoSEQ analyzer. V gene usage and the V–J combinations, derived from both productive and non-productive sequences, were significantly different between XLA samples and controls. Although the CDR3 length was similar for XLA and control samples, the CDR3 region of the XLA T cell receptor contained significantly fewer deletions and insertions in V, D, and J gene segments, differences intrinsic to the V(D)J recombination process and not due to peripheral T cell selection. XLA CDR3s demonstrated fewer charged amino acid residues, more sharing of CDR3 sequences, and almost completely lacked a population of highly modified Vβ gene segments found in control DNA, suggesting both a skewed and contracted T cell repertoire in XLA.

1. Introduction

With the increasing use of B cell depleting therapies in human autoimmune diseases, the impact of loss of B cells on the T cell repertoire has been of broad interest [1]. Examining the T cell repertoire of subjects with X-linked agammaglobulinemia (XLA), who lack B cell development, has revealed that broad alterations of the T cell compartment can be observed in these subjects, including decreased numbers of peripheral blood memory CD 45RA cells, effector memory CD4 + T cells, and follicular helper T cells [2–4]. These changes could potentially be due to the abnormal germinal center formation characteristic of this immune defect [5]. As seen in subjects treated with anti-CD20 monoclonal B cell depleting antibody [6], circulating Th17 cells also appear diminished in subjects with XLA [7], further suggesting that loss of B cells affects the T cell compartment. Testing cellular immunity in XLA has, however, generally demonstrated preserved global T cell responses, with normal proliferation to influenza vaccine, tetanus toxoid, and non-specific mitogens [8–10], and with development of T cell memory after hepatitis B vaccination [3]. In contrast, impaired delayed type hypersensitivity (DTH) reactions, impaired anti-meningococcal T cell immunity [11], and, in an older study, skewing to a Th1 cytokine response [12] have been observed in XLA. To examine the T cell repertoire in XLA more closely, in this study we have used high throughput sequencing to examine the structure of the XLA T cell receptor as compared to age appropriate normal male subjects. Our results show that relative to controls, subjects with XLA have skewed V–J gene usage, fewer deletions and insertions, differential use of amino acids, increased CD3 hydrophilicity, and lack of a population of T cell receptors with more extensive nucleotide deletions from V genes.

2. Methods

2.1. Demographics and clinical characteristics

Fifteen male children and adults with X-linked agammaglobulinemia defined by congenital agammaglobulinemia and a mutation in the *Btk* gene and/or family history of congenital agammaglobulinemia were recruited for this study (Table 1). Two subjects were brothers, and two sets of cousins were included. Eighteen banked control male PBMC DNA samples were obtained from the Department of Genetics and Genomics at the Icahn School of Medicine and used as controls. The age ranges for XLA subjects (2 years to 54 years) and controls (3 years to 42 years) were not significantly different (p-value = 0.12). All XLA patients were receiving replacement immunoglobulin at time of study participation; none were ill or on immunomodulatory or immunosuppressive medications at the time of blood collection. This study was approved by the Institutional Review Board of Mount Sinai Hospital, and written informed consent was obtained from all patients or their parents.

* Corresponding author at: Departments of Medicine and Pediatrics, The Immunology Institute, Box 1089, Icahn School of Medicine at Mount Sinai, 1425 Madison Avenue, New York, NY 10029, United States.

http://dx.doi.org/10.1016/j.clim.2015.09.002
1521-6616/

Table 1
Clinical information.

Patient	Clinical information		Year of birth	T cells %# (normal range 55–89%)	CD4% (normal range 33–65%)	CD8% (normal range 10–41%)	B cells% (normal range 5–15%)
1	Pneumonia; brother died of ECHO virus	Base pair substitution C to T codon 582	1956	NA	NA	NA	<1
2	History of respiratory infections	Missense mutation exon 15	1976	84	57	25	0
3	History of respiratory infections; sinusitis; 2 affected nephews	ND; positive family history	1980	90	57	30	0
4	History of respiratory infections; joint pain	ND; positive family history	1971	83	64	19	<1
5	History of respiratory infections†	Exon 11 18-bp insertion	1982	NA	NA	NA	<1
6	Pneumonia in childhood‡	Missense mutation in the pleckstrin homology domain	1987	NA	NA	NA	<1
7	*Pseudomonas aeruginosa* bacteremia age 2†	Exon 11 18-bp insertion	1993	NA	NA	NA	<1
8	Pneumonia in childhood	2 kb deletion that removes exon 6 and 7	1996	93	60	30	<1
9	History of respiratory infections‡	Missense mutation in the pleckstrin homology domain	1997	89	69	24	0
10	Pneumonia in childhood†	Nonsense mutation exon 13	1999	92	55	32	1
11	*Pneumocystis carnii* pneumonia age 1†	Nonsense mutation exon 13	2006	94	69	24	0
12	Respiratory infections	Frameshift mutation leading to stop codon	2007	93	71	19	<1
13	Acute bacterial meningitis age 11 months	Nonsense mutation exon 13	2008	98	72	24	<1
14	Bacterial pneumonia age 2	Missense mutation exon 6; leads to stop codon	2008	92	65	25	<1
15	Upper respiratory infections age 1; RSV	ND; positive family history; 3 relatives	2010	95	71	32	<1

*, †, and ‡ are related patients.
NA = not available.
#, normal lymphocyte ranges are for subjects over age 10.

2.2. TCR β sequencing and analysis

PBMC DNA was prepared as described recently [13]. Equivalent amounts of control and CVID DNA were used for sequencing. A 60 bp sequence of the rearranged TCRβ CDR3 region was amplified and sequenced for all samples using the immunoSEQ™ assay, a high-throughput multiplex PCR assay for the re-arranged DNA of T cells (Adaptive Biotechnologies Inc.). Average number of sequence for patients was 95,129.47 (range of 18,049–185,067), and for controls 35,777.72 (range of 10,796–53956). PCR bias was controlled using synthetic templates [14]. For each unique nucleotide sequence, the V, D, and J gene usage, n-nucleotide insertions, base deletions, copy number, and frequency were determined. The predicted amino acid sequence of the productive sequences was determined. The mean CDR3 Kyte–Doolittle Hydropathy index was interpolated from this sequence.

2.3. Statistical methods

Statistical analyses and graphing were performed using the R statistical programming language (version 2.15.2) and GraphPad Prism (version 5.01). Normality was determined using histograms and the Shapiro–Wilk test. Unpaired t-tests were used for comparison of normally distributed numerical data, while nonparametric data were assessed with Wilcoxon tests. Pearson coefficients were calculated to test correlation. A p-value of <0.05 was regarded as significant. V, D, or J family and gene usage was compared using a two-way anova with Tukey HSD multiple comparisons test. Clustering analysis of V genes was performed using the *hclust* function in R using scaled data and with an algorithm using Manhattan distances and complete clustering.

3. Results

3.1. XLA patients have a unique pattern of V gene usage

The T cell receptor repertoire is determined by the V, D and J genes used and by subsequent recombination events that include nucleotide insertions and deletions. During V(D)J recombination, if the recombination event results in a sequence containing a stop codon or a frame-shift mutation, a second locus is rearranged. If this rearrangement results in a productive sequence the cell carries the productive and the previously rearranged non-productive sequence in its genome. Sequencing genomic DNA allowed us to examine both loci. We examined the distribution of individual V genes, J genes and, more specifically, V–J combinations in XLA as compared to controls. When assessing productive sequences, V gene usage and V–J combination use, but not J gene usage, were significantly different between XLA and normal controls (Fig. 1a,b). Anova with Tukey HSD correction for multiple comparisons; V–J combination p-value = 0.005, V genes p-value = 0.003, J genes p-value = 0.385). Similar differences were also seen when examining the VJ use of non-productive sequences (anova with Tukey HSD correction for multiple comparisons; V–J combination p-value of <0.0001). Non-biased clustering was performed on the basis of V gene usage. As seen in Fig. 1c, patients clustered independently of the control subjects.

3.2. Decreased V, D, and J deletions and n-nucleotide insertions in XLA

We next examined the structure of the CDR3 region of the XLA TCRs. XLA patients had significantly fewer total deletions from V, D, and J genes (XLA 14.95 ± 0.15; controls 15.84 ± 0.21; p = 0.002; Fig. 2a). There were fewer reciprocal insertions of non-templated nucleotides (XLA 7.998 ± 0.1254; 8.768 ± 0.1736; p = 0.002; Fig. 2b). CDR3 length was comparable between the groups (XLA 38.07 ± 0.05167; Controls 38.06 ± 0.06563; p = 0.91). Perhaps as a result of fewer deletions and insertions, we observed fewer non-productive sequences.

This skewing towards fewer deletions during recombination could conceivably be the result of a thymic or an extra-thymic selection event. To dissect the influence of initial recombination and subsequent selection, we examined non-productive sequences (the recombined sequences that resulted in stop codons or frameshift mutations) that are not expressed on the cell surface, and are thus exempt from peripheral selection. As for the productive sequences, the non-productive sequences also had fewer deletions (XLA 15.03 ± 0.1517; 16.13 ± 0.2131; p = 0.0003; Fig. 2c) and insertions (XLA 10.15 ± 0.1209; Controls 11.00 ± 0.1941; p = 0.0013; Fig. 2d). As such, this decrease in n-nucleotide addition and in base deletion is intrinsic to XLA T cell V(D)J recombination and is not a result of selection.