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B cell receptor light chain repertoires show signs of selection with differences between groups of healthy individuals and SLE patients

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ABSTRACT

We have developed a microarray to study the expression of L-chain V genes (V_L genes) in healthy and SLE patient peripheral κ- and λ-sorted B cells. In all repertoires tested, one V_L gene accounts for over 10% of all gene V_L expression, consistent with positive selection acting on L-chains. While a few V_L genes were highly expressed in all individuals, most V_L genes were expressed at different levels. Some V_L genes (5 out of a total of 78) were not detected. We attribute their absence from the repertoire to negative selection. Positive selection and negative selection were also found in SLE repertoires, but expression of V_L genes was different; the differences point to less regulation of V_L gene repertoires in SLE. Our data shows that V_L gene expression is variable and supports a model where the L-chain repertoire is generated by both positive and negative selection on L-chains.

1. Introduction

B cell receptors are formed by pairing of Ig heavy chain (H-chain) and light chain (L-chain) that together determine the specificity of the receptor. Thus, the initial repertoire diversity is due to combinatorial association of H-chains and L-chains as well as combinatorial joining of V, D and J gene segments (Kawasaki et al., 2001, 1997; Matsuda et al., 1998). Additional diversity arises at the junction of V, D and J gene segments by N-nucleotide additions, P-nucleotide additions and nibbling (Weigert et al., 1980; Wu and Kabat, 1970). Selection acts on the B cell repertoire through both tolerance mechanisms and antigen selection. Tolerance selects B cells during development when autoreactive receptors are formed and acts on individual B cells through receptor editing, a process which replaces the originally rearranged L-chain with a newly recombined L-chain (Gay et al., 1993; Halverson et al., 2004; Radic et al., 1993; Tiegs et al., 1993). In the case of antigen selection, H-chain and L-chain pairs present in the pre-immune repertoire undergo clonal expansion. We are interested in the L-chain repertoire of B cells that have undergone selection.

Previous repertoire analysis has used a variety of approaches such as isoelectric focusing, Southern blotting and PCR amplification (Bentley, 1984; de Wildt et al., 1997; Klobeck et al., 1984; Mattson et al., 1982; Meffre et al., 2001, 2000, 2004; Walker et al., 1983; Williams et al., 1987; Dorner et al., 1999, 2002, 2001; Farner et al., 1999; Foster et al., 1997; Girschick and Lipsky, 2002; Heimbacher et al., 2001; Jacobi et al., 2002; Kaschner et al., 2001; Lee et al., 2004, 2000; Wardemann et al., 2004; Wrammert et al., 2008). However, these studies have limitations, including culture efficiency, PCR biases and cost. To examine L-chain repertoires, we developed a microarray that surveys all human V_L genes. There are several advantages to the microarray method: it is not subject to PCR primer bias; the microarray is highly specific, permitting resolution of members within multi-gene families; and it allows comparison of individual or group repertoires. Because of these features, the microarray allows for complete repertoire analysis on a large scale.

The repertoires of κ and λ B cells from healthy individuals were determined using this method. We also studied L-chain repertoires in patients with SLE, a disease in which tolerance is broken. High expression of V_L genes was observed in each of the repertoires tested. A small, but substantial number of V_L genes could not be detected in the repertoires, individually and in some cases collectively. Within healthy and SLE groups, expression of individual V_L genes varied from one individual to the next, and comparison of healthy and SLE patient L-chain repertoires identified several differences in Vκ and Vλ gene expression.

2. Materials and methods

2.1. Healthy and SLE blood samples

Twelve milliliter (12 mL) of peripheral blood was collected in BD vacutainer blood collection tubes with EDTA (Becton Dickinson)

Abbreviations: L-chain, light chain; H-chain, heavy chain; V_L gene, light chain V gene; SLE, systemic lupus erythematosus.

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from 10 healthy donors and 10 SLE patients with evidence of active disease (identified by low complement (C3 or C4) levels or clinical signs of active SLE) after informed consent. The use of human blood was approved by the University of Chicago, Institutional Review Board (protocol 14801B). The blood was immediately processed for cell isolation and subsequent cell sorting.

2.2. FACS sorting

Blood was diluted 1:1 in PBS immediately after collection, and Lymphocyte Separation Medium (Mediatech) was used for isolation of mononuclear cells according to the manufacturer's protocol. Cells were resuspended in RPMI supplemented with 10% FBS at a concentration of 10^8 cells/mL. Cells were stained using the following antibodies for FACS sorting at the concentrations recommended by the manufacturer: anti-CD138 APC (Dako), anti-Igκ FITC (BD Pharmingen), anti-Igλ PE (BD Pharmingen),anti-CD20 PE (BD Pharmingen), anti-CD20 PE (BD Pharmingen), anti-CD27 APC (eBiosciences) and anti-CD38 PE-Cy7 (eBiosciences). Eight samples from each group were phenotyped for CD27 and CD38 levels. Sorting was performed using MoFlo (Dako-Cytomation) and FACSAria (BD Biosciences) machines using the single cell sort purity mode. Lymphocytes were gated according to forward- and side-scatter profiles and doublets were excluded using forward-scatter pulse width and pulse area profiles. One-thousand events in the CD20+CD138−Igκ+Igλ− and 1000 events in the CD20+CD138−Igκ−Igλ+ gates were sorted into RNALater (Ambion) and stored at −20 °C. Although rare in peripheral blood, plasma cells were specifically excluded in the sorting strategy based upon the following: forward- and side-scatter gates were set to exclude plasma cells; CD138+ cells were not gated; and plasma cells have absent or low levels of surface CD20, κ and λ.

2.3. cDNA preparation

Total RNA for each sample was isolated using TRIZOL (Invitrogen). The RNA concentration and quality was determined using a NanoDrop (Thermo Scientific), and only RNA with 260/280 greater than 1.75 were used for amplification. Twenty ng (20 ng) of total RNA was used for cDNA amplification using Ovation RNA Amplification System V2 (Nugen) and purified using DNA Clean and Concentrator – 25 columns (Zymo research) according to Nugen protocols. If necessary, samples were stored at −80 °C until the day of hybridization. Purified cDNA used for the microarray was labeled by adding 4 μg of cDNA in 20 μl to 10 μl of Ulysis Alexa-Fluor 647 (Invitrogen) and heating to 80 °C for 20 min. Labeled cDNA was purified using Bio-Spin 6 SSC columns (Bio-Rad) after washing the columns 3 times with water. A NanoDrop was used to quantify the cDNA amount and amount of Alexa-Fluor 647.

2.4. Syndecan-1 (CD138) PCR

Primers were designed based on NCBI reference sequence for syndecan (NM_002997.4). The forward primer (AAATGGCAAAGGAAGGTGGATGGC) and reverse primer (ATACACTCCAGGCAGAAAGTCGCA) were synthesized by IDT DNA, and the PCR steps were: 95 °C for 5 min; 30 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 50 s; and 72 °C for 10 min. Reagents were used at the recommended concentrations for the polymerase, Taq DNA Polymerase (Invitrogen).

2.5. Microarray probe design

Light chain V region sequences used for probe design were obtained from publications on the human *IGK* and *IGL* loci (Kawasaki et al., 2001, 1997; Schable and Zachau, 1993). Control genes were included on the microarray; sequences for *ACTA* (NCBI reference sequence: NM_001100), *ACTB* (NM_001101), *AKR1B1* (NM_001628.2), *B2M* (NM_004048.2), *CD19* (NM_001770), *GAPDH* (NM_002046), *IGKC* (J00241.1), *LDHA* (NM_005566), *MS4A1* (NM_152866) and *NONO* (NM_001145409) were downloaded from the National Center for Biotechnology Institute gene database (http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene). Probe sequences were determined by filtering V_L gene sequences for: probe length (65–74 bp) aligning to any region of the V_L gene, uniqueness compared with all V_L genes (BLAST), self-binding (Smith and Waterman, 1981), complexity, melting temperature (69.0 ± 1.5 °C) and distance from 3′ end. Probes were named and are reported using the original nomenclature to be consistent with the microarray data files. Seven pairs of Vκ genes were identical or nearly identical, and probes meeting the design criteria correspond to identical regions in the pair. For these Vκ genes, expression is reported together (e.g. *O8* and *O18* are detected by the probe O8/O18). For the expression levels by cluster or position, estimates of expression for each gene within the pair were assumed to be equal. Appendix Table A.1 lists sequences for the V_L gene probes. Probes were manufactured by Integrated DNA Technologies.

2.6. Reference sample

A reference sample containing the reverse-complement of all V_L gene probe sequences at equal molar concentrations and 0.12 ng of each reference sequences was co-hybridized with every cDNA sample (Integrated DNA Technologies). The reference was labeled using Ulysis Alexa-Fluor 555 (Invitrogen). For the series of experiments used to estimate the amount of each gene present, the reference was labeled using the Ulysis Alexa-Fluor 647 dye (Invitrogen).

2.7. Estimation of expression level

The complementary reverse sequence of two Vκ genes (B2 and O2/O12) and two Vλ genes (2–13 and 1–19) were hybridized using the same techniques and methods as the cDNA samples. The four genes were chosen from different gene families (Fig. A.2). The effect of increasing DNA concentration on signal intensity was also determined using these four genes by adding Alexa Fluor 647-labeled Vκ and Vλ targets to Alexa Fluor 647-labele reference sample at known concentrations (see Section 2.11 and Fig. A.3), which was then hybridized along with the Alexa Fluor 555-labeled reference sample. Vκ B2 was tested at 12.2%, 21.7% and 41.0%; Vκ O2/O12 was tested at 5.2% and 10.0%; Vλ 2–13 was tested at 10.6%, 19.2% and 37.3%; and Vλ 1–19 was tested at 19.2%, 8.7% and 4.6% (percent refers to the molar amount of the gene present in the sample). Each V_L gene and concentration was hybridized two times. The second hybridization of V1–19 at 4.6% had a scratch across a portion of the array and was not included in the analysis. Normalized expression values for each of these hybridizations (reference subtracted – see Section 2.11 for data analysis and normalization) were compared with the concentration of the genes in the hybridized sample. The Curve Fitting Tool in Matlab was used to identify the best-equation for this data. This equation was then used to estimate expression levels for all of the cDNA samples.

2.8. Microarray spotting

Microarrays were spotted using a GeneMachines OmniGrid 100 (Genomic Solutions) onto SuperAmine 2 slides (ArrayIt). Each oligonucleotide probe was spotted twelve times per array, and the print layout was such that these twelve replicates were spotted by four different pins. In addition to the human oligonucleotides, the microarrays also had mouse oligonucleotides spotted on the array. After printing, microarrays were dehydrated following the