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Immunoglobulin kappa variable region gene selection during early human B cell development in health and systemic lupus erythematosus

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ABSTRACT

The unique specificity of the B cell receptor is generated by an ordered sequence of gene rearrangement events. Once IGH genes have rearranged, rearrangement at the *IGK* locus is initiated followed by the *IGL* locus if functional *IGK* rearrangement is not achieved. Receptor specificity can subsequently be altered by secondary light chain editing based on the features of the heavy and light chain combination. The final profile of expressed genes is not random and biases in this profile are associated with several autoimmune diseases. However, how and when biases are created is not known.

To increase our understanding of the processes of selection and editing of *IGK* rearrangements, we compared four groups of rearrangements of IGK acquired by next generation sequencing. First, expressed rearrangements of *IGK* from cDNA of IGK expressing B cells. Second, productive rearrangements of *IGK* from DNA of the same kappa expressing B cells. Third, non-productive rearrangements of *IGK* from DNA of IGK and IGL expressing B cells, and fourth productively rearranged *IGK* from DNA of IGL expressing B cells. The latter group would have been rejected during B cell development in favour of rearrangement at the *IGL* locus and are therefore selected against.

We saw evidence that rearranged IGK segments can be selected at a checkpoint where the decision to rearrange the IGL locus is made. In addition, our data suggest that mechanisms regulating the expression or not of IGK rearrangements may also contribute to repertoire development and also that this latter component of the selection process is defective in SLE.

1. Introduction

The unique specificity of individual B cells for antigen is determined by the sequences of the expressed *IGH* and *IGK* or *IGL* light chain genes. The sequences are created by an ordered succession of rearrangement events starting with rearrangement of variable (V), diversity (D) and joining (J) segments of *IGH*. Successful expression of the pre-B cell receptor excludes recombination events on the second *IGH* allele and also activates enhancers that initiate rearrangement of the *IGK* locus. If functional rearrangement is not achieved on either allele at the *IGK* locus, then *IGK* is inactivated by deletional rearrangement with kappa deleting element (KDE) and gene rearrangement proceeds at the *IGL* locus (Hardy and Hayakawa, 2001; Santos et al., 2011).

The final shape and specificity of the BCR can potentially be altered many times during early B cell development by secondary editing rearrangements of segments upstream and downstream of an original *V-J* rearrangement of *IGK or IGL* (Wardemann et al., 2003; Yurasov et al., 2005b). Receptor editing may occur if the receptor as

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a whole is autoreactive, and development of autoimmune diseases may result from a failure to edit the BCR effectively (Panigrahi et al., 2008). Autoimmune diseases are frequently associated with biases in light chain gene segment usage, e.g., there is an over representation of *IGKV4*-1 in systemic lupus erythematosus (SLE) (Dorner et al., 1998; Woodward and Thomas, 2005), celiac disease (Steinsbø et al., 2014) and type 1 diabetes (Woodward and Thomas, 2005).

The *IGK* locus includes gene segments in forward and reverse orientations, relative to the downstream gene segments. For example, the *IGKV4-1* and *IGKV5*-2 genes most proximal to the J segments are in reverse transcriptional orientation compared to the other proximal *IGKV* genes (Foster et al., 1997; http://www.imgt.org/IMGTrepertoire/; Schoettler et al., 2012). When *IGKV4*-1 or *IGKV5*-2 recombine with *IGKJ* the DNA coils to permit alignment of the recombination signal sequences (RSS). After rearrangement, the intervening DNA between *IGKV* and *IGKJ* is not released as an episomal fragment, but instead the rearrangement is inserted in inverted orientation adjacent to or among the J segments. This rearrangement can be retained on the chromosome even after secondary editing rearrangements. It is therefore possible that KDE rearrangements to the intronic recombinant signal sequences (RSS) may not remove the edited gene rearrangements of *IGK* that have been selected against and cells may accumulate more than one unused rearrangement on a single chromosome. Therefore B cells can accumulate rearrangements that have been excluded from the functional repertoire. It is also possible that 'same orientation' secondary *IGK* rearrangements that excise existing rearrangements as episomes may still be amplifiable by PCR. This would be apparent as a high relative frequency of productive DNA rearrangements of an *IGK* gene segment compared to expressed sequences in cDNA and can be considered to be a consequence of accumulation of rearrangements in DNA that have been selected against during B cell development. Therefore, rearrangements of V genes that occur relatively more frequently in DNA compared to cDNA from the same cells may have been selected against.

Our aim was to analyse *IGKV* gene selection in humans and how this might be altered in SLE. To do this we have studied four groups of *IGK* sequences (1–4 in Fig. 1). The first group of sequences (1) was PCR amplified from cDNA of IGK expressing B cells. These are considered to be the gold standard indication of actual *IGKV* gene expression in the B cell population in our study. The second group of sequences (2) was productive *IGK* gene rearrangements from DNA of the same, sorted IGK expressing mature naïve B cell subset used for cDNA preparation. The third group of sequences studied (3) was the DNA rearrangements that were unproductive due to being out of frame or having a stop codon. The B cells expressing these genetic rearrangements of the *IGK* locus are not selected since they do not encode any protein. Their profile therefore reflects the intrinsic biases in the efficiency of rearrangement of *IGKV* gene segments determined, e.g., by transcriptional activity and RSS sequence and not the properties of the *IGKV* segment encoded (Foster et al., 1997). The fourth group (4) of sequences studied was productive DNA rearrangements of *IGK* from sorted IGL expressing B cells from the same donors as the IGK expressing cells. Since the B cells can change from rearrangement of *IGK* to rearrangement of the *IGL* locus at the pre-B stage, this group of productive rearrangements has been rejected during B cell development (Brauninger et al., 2001). This is summarised in Fig. 1.

By comparing these four groups of sequences we observed that some *IGKV* segments are consistently selected either for or against expression during early B cell development. We therefore compared the rearrangement profile of the four groups of sequences in six healthy controls and three patients with SLE. Whereas the overall imprint on repertoire selection at the DNA level is similar including the switch from *IGK* to *IGL* gene rearrangements, there are differences between health and SLE when comparing DNA and cDNA from IGK expressing B cells. This implies that mechanisms of gene silencing rather than detection of self-reactivity per se may be defective in SLE.

2. Materials and methods

2.1. B-cell isolation and cell sorting of naïve B cells

Peripheral blood mononuclear cells were isolated from six healthy donors, and three SLE patients using Ficoll-Paque Plus (GE

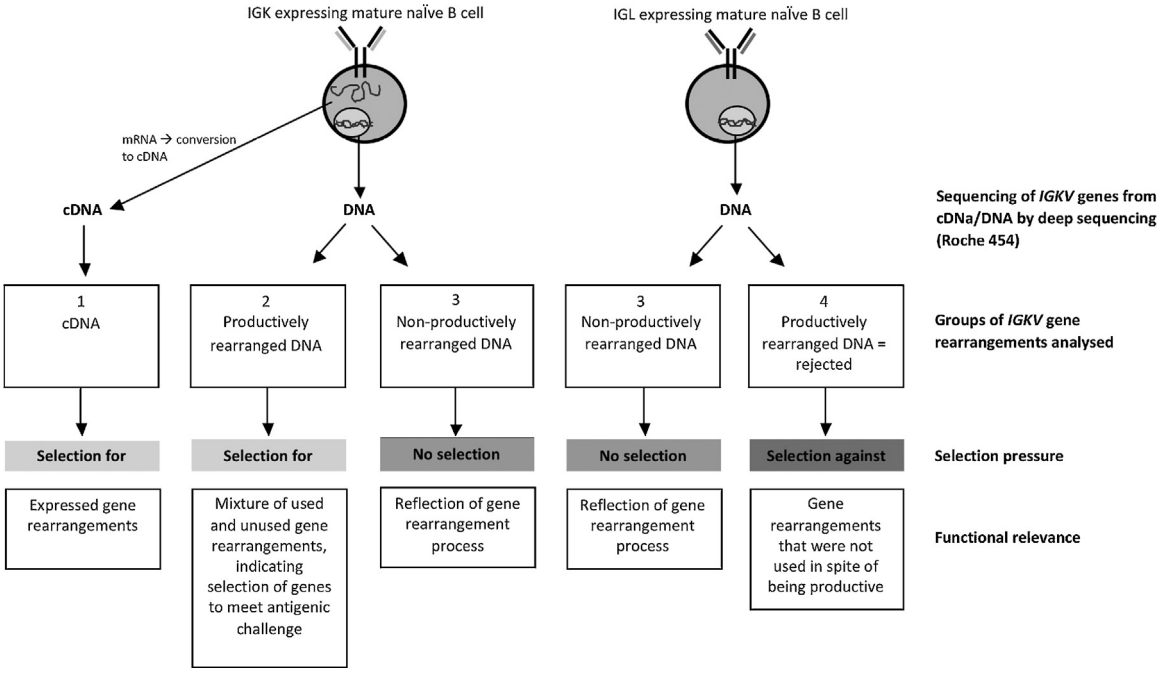

Fig. 1. Flow chart showing groups if IGKV gene rearrangements analysed and functional relevance. Genomic DNA and RNA were extracted from IGK and IGL surface expressing mature naïve B cells. Four groups of sequences are depicted and functional relevance of different subsets of IGKV gene rearrangements analysed are illustrated.