IMMUNOLOGY ORIGINAL ARTICLE

Characterization of a new V gene replacement in the absence of activation-induced cytidine deaminase and its contribution to human B-cell receptor diversity

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Summary

In B cells, B-cell receptor (BCR) immunoglobulin revision is a common route for modifying unwanted antibody specificities via a mechanism called VH replacement. This *in vivo* process, mostly affecting heavy-chain rearrangement, involves the replacement of all or part of a previously rearranged *IGHV* gene with another germline *IGHV* gene located upstream. Two different mechanisms of *IGHV* replacement have been reported: type 1, involving the recombination activating genes complex and requiring a framework region 3 internal recombination signal; and type 2, involving an unidentified mechanism different from that of type 1. In the case of light-chain loci, BCR immunoglobulin editing ensures that a second V-J rearrangement occurs. This helps to maintain tolerance, by generating a novel BCR with a new antigenic specificity. We report that human B cells can, surprisingly, undergo type 2 replacement associated with κ light-chain rearrangements. The *de novo IGKV-IGKJ* products result from the partial replacement of a previously rearranged *IGKV* gene by a new germline *IGKV* gene, in-frame and without deletion or addition of nucleotides. There are *wrcy/rgyw* motifs at the 'IGKV donor–IGKV recipient chimera junction' as described for type 2 *IGHV* replacement, but activation-induced cytidine deaminase (AID) expression was not detected. This unusual mechanism of homologous recombination seems to be a variant of gene conversion-like recombination, which does not require AID. The recombination phenomenon described here provides new insight into immunoglobulin locus recombination and BCR immunoglobulin repertoire diversity.

Keywords: *AICDA*; B-cell receptor immunoglobulin repertoire diversity; gene conversion-like recombination; immunoglobulin κ; immunoglobulin genes; IGKV replacement.

Introduction

Developing B cells generate a vast antibody repertoire, by multistep somatic recombination events involving immunoglobulin genes(*IG*), also known as V-(D)-J rearrangements. As a consequence, a limitless number of non-self antigens can be recognized. However, this mechanism can also produce autoreactive B cells that, secondarily, may be tolerated by various mechanisms, such as B-cell receptor (BCR) editing.[1] During this process, the stimulation of autoreactive BCR induces an initiation signal for secondary V-J rearrangement at light-chain loci. Such secondary rearrangements replace (genotypic editing) or outcompete (phenotypic editing) the primary light-chain rearrangements, thereby modifying autoreactive BCR specificity.[2–4] Another process, BCR revision, occurs in mature B cells and involves the modification of self-reactive heavy-chain V-D-J rearrangements by *IGHV* gene replacement.[5–8] Two different types of replacement have been described. The first is mediated by the recombination-activating genes (RAG) complex, using the 3′-recombination signal (3′V-RS) of

an incoming germline *IGHV* (donor) as the substrate and an internal or cryptic RS (cRS), in the opposite orientation, present in the FR3 (framework region 3) of the rearranged VH (recipient).[9–15] These type 1 *IGHV* replacements use the same mechanism as conventional RAG-dependent V-D-J rearrangement and the new junctions formed characteristically display nucleotide deletions and P and N additions. The entire *IGHV* coding region, except for a short conserved native CDR3 (complementarity-determining region 3) sequence (six to seven nucleotides) downstream from the internal RS, is replaced with a new germline *IGHV*. This recombination increases the length of the V-D junction and may result in a functional product if the reading frame is conserved. The second type of *IGHV* replacement was first described by Wilson *et al.*[16] and Itoh *et al.*[17] in normal human peripheral B cells and in B cells from patients with rheumatoid arthritis. Darlow and Stott[18,19] recently named this new '*IGHV* chimera' as type 2 VH replacement. Unlike type 1 replacement, no addition or deletion of nucleotides is observed, and the reading frame is not affected. This phenomenon resembles homologous recombination between a rearranged *IGHV* gene and a germline *IGHV* gene. These potentially non-reciprocal homologous recombination events occur at perfectly matched coding regions in the two *IGHV* genes, such that the exact break point of the recombination cannot be identified. The same resulting sequence signatures are observed in various species, including birds and rabbits, in which gene conversion is used to produce the antibody repertoire and its diversity.[20,21] During gene conversion, a segment of DNA is replaced by copying from a *cis* or *trans* V sequence, thereby generating a 'V chimera segment' without alteration of the donor template.[22] This process of gene conversion is initiated by the *AICDA* gene product, activation-induced cytidine deaminase (AID),[23] a key enzyme in somatic hypermutation[24,25] and in class switch recombination[26,27]; its target sequences contain hot spot (a/t)(a/g)$\underline{c}$(c/t)/(a/g)$\underline{g}$(c/t)(a/t) motifs (*wrcy/rgyw*).[28] These hotspot motifs are found, within the chimera gene, on either side of the perfectly matched sequence corresponding to identical regions in the two fused *IGHV* genes. Gene conversion, in the context of lymphocyte repertoire diversification, has not been observed in humans, but it has been suggested that type 2 *IGHV* replacement provides evidence of gene conversion-like events,[29,30] involving AID. However, the direct implication of AID in this process has never been proved. Most of the published sequences of type 2 replacement products were obtained from human B cells expressing *AICDA*, but such products have also been identified in immature B cells (which are theoretically AID-negative).[31] It therefore remains unclear whether AID is actually involved in this newly described V gene replacement process. We tried to resolve this issue, by

establishing a model of the recombination, *de novo*, of immunoglobulin V genes in the absence of *AICDA*. We successfully induced, *in vitro*, type 2 V replacement in the immunoglobulin κ (*IGK*) light-chain locus in human pre-B cells, and we report evidence that *AICDA* is not expressed in this model.

Materials and methods

Cell line

The human pre-B cell line 697 was purchased from DSMZ. Cells were cultured at 37°, under a humidified atmosphere containing 5% CO_2, in RPMI-1640 medium (Gibco, Paisley, UK) supplemented with 10% heat-inactivated fetal calf serum (PAA Laboratories, Yeovil, UK), 50 U/ml each of penicillin and streptomycin and 2 mM L-glutamine.

Cell treatment

After overnight serum deprivation, 697 cells cultured at a density of 0.5×10^6 cells/ml were induced by incubation for 7 days with 10 ng/ml of the cytokine interleukin-1α (IL-1α) (Tebu-Bio, Le Perray-en-Yvelines, France) or with 1 μg/ml lipopolysaccharide (LPS, Sigma, St Louis, MO). After treatment, a PCR-based test was used to check for the absence of mycoplasma in the cultures.

Genomic DNA extraction

Induced and control cells ($\sim 5 \times 10^6$) were harvested and washed, and QIAamp spin column technology (Qiagen, Hilden, Germany) was used to extract genomic DNA. The concentration of isolated genomic DNA preparations was measured with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Multiplex PCR, cloning and sequencing

Genomic *IGKV*1, *IGKV*2, *IGKV*3 to *IGKJ*5 rearrangements were amplified by multiplex PCR with 500 ng of genomic DNA, using *IGKV*1f/6, *IGKV*2f and *IGKV*3f forward primers and a Jκ5 reverse primer. AmpliTaq Gold and ABI Gold Buffer (Applied Biosystems, Foster City, CA) were used as recommended in the standardized BIOMED-2 PCR protocol.[32] PCR amplification conditions were as specified in the BIOMED-2 protocol and PCR was run for 40 cycles. PCR products were subjected to electrophoresis in a 1% agarose gel in 1× TAE buffer for 15 min at 100 V. PCR products were purified with the MinElute gel extraction kit (Qiagen) and cloned with the Topo TA Cloning kit (Invitrogen, Carlsbad,

CA). The Nucleospin Plasmid kit (Macherey Nagel, Düren, Germany) was used to purify the plasmids, and the inserts were sequenced with the Big Dye Terminator sequencing kit and subjected to electrophoresis on an Applied Biosystem ABI 3500 sequencer. Both strands were analysed with internal pCR®2.1-TOPO® forward and reverse M13 primers (Invitrogen). Three independent experiments were performed and PCR was carried out three times for each experiment. IMGT®, the international ImMunoGeneTics information system® (http://www.imgt.org)[33] and the Basic Local Alignment Search Tool (BLAST) database were used to analyse sequence data for more than 130 clones. Selected *IGKV* type 2 sequences were submitted to EMBL-EBI (European Nucleotide Archive, http://www.ebi.ac.uk/): Accession numbers: HF674720, HF674721, HF674722, HF674723, HF674724, HF674719 and HF674725.

RNA extraction and RT-PCR

Total cellular RNA was isolated from ~5 × 10⁶ cells, with RNeasy Mini kits (Qiagen), and treated with DNAse. We reverse-transcribed 1 μg of RNA by the High-Capacity cDNA Reverse Transcription protocol (Applied Biosystems). AmpliTaqGold DNA polymerase (Applied Biosystems) was used for PCR amplification of the *AICDA* and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) genes from 100 ng of cDNA, and the PCR products were analysed by electrophoresis in 1% agarose gels containing ethidium bromide and visualized with the Chemidoc system (Bio-Rad, Hercules, CA). The primers used were: *AICDA* forward 5′-cactggactttggttatcttcgc-3′; *AICDA* reverse 5′-cgt aagtcatcaacctcatacagg-3′; *GAPDH* forward

5′-tcggagtcaac ggatttggtcg-3′; *GAPDH* reverse 5′-tcagtg-tagcccaggatgcct-3′.

Results

Induction of immunoglobulin κ (*IGK*) locus rearrangement in human B cells

In the mouse B-cell model, immunoglobulin κ (*IGK*) locus recombination is induced by treatment with LPS[34,35] or IL-1.[36,37] There are several Toll-like receptor 4-positive and IL1-R1-positive human pre-B-cell lines. We chose to use the RAG1/2-positive 697 cell line for this study. We confirmed that treatment with either IL-1 or LPS successfully induced *de novo* *IGKV-IGKJ* rearrangements without altering RAG1/2 mRNA production (data not shown). The 697 cell line was chosen because of its particular configuration of *IGK* haplotypes: one *IGK* locus contains a native unmutated *IGKV1-8-IGKJ4* rearrangement, and the five *IGKJ* genes have been deleted from the second locus by *IGKV3-7-KDE* rearrangement. These particular locus configurations facilitate the exploration of *de novo* *IGKV-IGKJ* rearrangements with a single *IGKJ*5 antisense primer for their detection and for PCR product isolation (Fig. 1). As the single genes of each of the *IGKV*4, *IGKV*5 and *IGKV*7 subgroups (IMGT®; http://www.imgt.org> IMGT Repertoire >Locus representation) have been deleted, *de novo* *IGKV-IGKJ*5 rearrangements can only involve genes of the *IGKV*1, *IGKV*2 and *IGKV*3 subgroups. Aliquots of 500 ng of genomic DNA isolated from 10⁵ cells were used for multiplex PCR and electrophoresis of the heterogeneous products of PCR amplification yielded a smear.
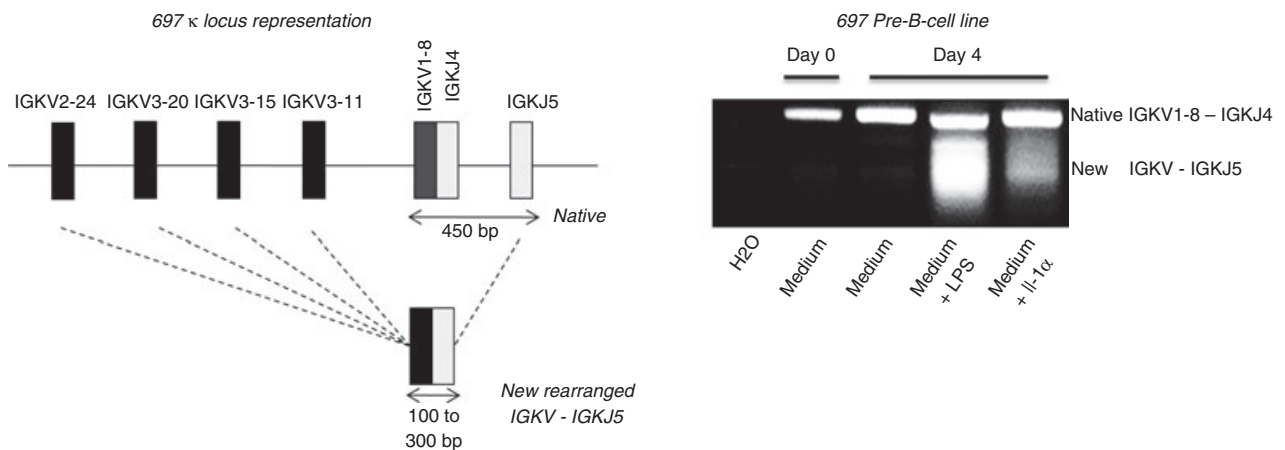

Figure 1. *IGKV-IGKJ*5 multiplex PCR with DNA from the human pre-B cell line 697 after induction with lipopolysaccharide (LPS) or interleukin-1 (IL-1). Genomic DNA was isolated and *IGKV-IGKJ* rearrangements were amplified by PCR with the *IGKV*1f/6, *IGKV*2f and *IGKV*3f forward primers and the Jk5 reverse primer.[32] A schematic diagram of the organization of the native haplotype carrying the *IGKV*1-8-*IGKJ*4 rearrangement in the 697 cell line is shown on the left (EMBL accession number HF674726). New *IGKV-IGKJ*5 rearrangements result in a smear migrating between 120 and 300 bp (right). After longer periods of induction, the signal for the native *IGKV*1-8-*IGKJ*4 disappeared and the signal from the new *IGKV*3-*IGKJ*5 became more intense (see Supplementary material, Fig. S1).

V replacement in κ locus rearrangement

After cloning and sequencing, we analysed 220 sequences obtained from several independent experiments, with IMGT® and Ig-BLAST tools and associated databases. In total, 130 of the 220 clones analysed had different sequences, and 28 of these clones were mutated. Comparisons of the sequences we obtained with the IMGT/V-QUEST[38] tool indicated that about 22% of the individual *IGKV-IGKJ*5 rearrangements induced *de novo* carried somatic mutations, including 18 with at least two mutations or about 14% of the independent sequences analysed. Comparisons between *IGKV-IGKJ*5 sequences carrying a single mutation and germline *IGKV* genes were not considered in this study. Alignments of apparently multiple mutated rearrangements with germline reference *IGKV* sequences revealed an '*IGKV* chimera gene' and led to the first identification of *IGKV-IGKJ* sequences with

type 2 *IGKV* replacement (Fig. 2). We chose seven chimera sequences with clear characteristics of *IGKV* type 2 replacement for further study. As shown in Fig. 2, *IGKV* replacement can take place at various sites in the V-REGION, from the FR3-IMGT (e.g. HF674720) to the CDR3-IMGT (HF674724). The use of an *IGKV* gene as both recipient and donor sequence is illustrated by the HF674719 and HF674720 sequences. An example of internal *IGKV* replacement is provided by the HF674725 sequence. A comprehensive alignment of full sequences is provided in the Supplementary material (Fig. S3). Type 2 replacement was observed in *de novo IGKV-IGKJ*5 rearrangements, irrespective of the *IGKV* subgroup involved (*IGKV*1, *IGKV*2 or *IGKV*3), although *IGKV*2-*IGKJ*5 recombinations were found preferentially. *IGKV* replacement occurred only between donor and recipient *IGKV* genes from the same subgroup. Within the same sub-

Figure 2. Hybrid *IGKV* sequences from multiplex PCR *IGKV-IGKJ*5. Examples of sequences from the cloned PCR products obtained from interleukin-1α (IL-1α) or lipopolysaccharide (LPS) -treated human pre-B 697 cells. Seven chimeric sequences were selected to illustrate different examples of *IGKV* type 2 replacement in terms of localization, in particular: from the FR to CDR targeted regions, internal replacement and *IGKV* gene usage as either a recipient or donor sequence. IMGT/V-QUEST[33,38] was used for alignment with *IGKV* germline genes. *IGKV-IGKJ*5 rearrangements were identified as follow: on the alphabetic sequences of the cloned PCR products, dark green corresponds to *IGKV* genes, *IGKJ*5 sequences are shown in blue, and P- and N-diversity are shown by orange and red letters, respectively. The figures in brackets indicate the number of nucleotides deleted during the initial *IGKV-IGKJ* rearrangement. The lower aligned reference sequences are represented as dashes in cases of a perfect match and as letters in cases of discordance. The *IGKV* genes initially rearranged with the *IGKJ*5 gene (recipient *IGKV* gene) are shown in green and the donor *IGKV* genes replacing the initial rearranged recipient *IGKV* gene are shown in blue. Superimposed blue and green sequences indicate regions of sequence identity (not assigned). Potential AID hotspots (WRCY/RGYW motif with W:A/T; R:A/G; Y:C/T) are underlined and in bold.

Amino acid (AA) changes (HF674720)

IMGT labels	V-REGION	FR1-IMGT	CDR1-IMGT	FR2-IMGT	CDR2-IMGT	FR3-IMGT	CDR3-IMGT
Nb of positions including IMGT gaps (AA)	110	–	–	17	10	39	44
Nb of AA	60	–	–	15	3	36	6
Nb of identical AA	56	–	–	15	3	35	3
Nb of AA changes	4	–	–	0	0	1	3

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                        FR2-IMGT        CDR2-IMGT              FR3-IMGT                CDR3 - IMGT
                        (39-55)          (56-65)               (66-104)

                  40          50        60          70        80        90        100
                  .|.........|....   ....|....   ....|.........|.........|.........|....
HF674720          ..WLQQRPGQPPRLLIY  KI.......S  NRFSGVP.DRFSGSG..SGTDFTLKISRVEAEDVGVYYC  MQGTHW
X12684 Homsap IGKV2-24*01 F  LSWLQQRPGQPPRLLIY  KI.......S  NRFSGVP.DRFSGSG..AGTDFTLKISRVEAEDVGVYYC  MQATQFP
                                                                     S                              G HW
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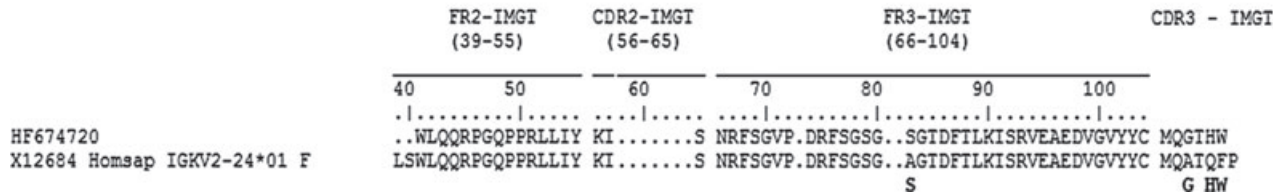
Figure 3. V-region protein display from a hybrid sequence. Amino-acid sequence of the HF674720 clone from lipopolysaccharide (LPS) -treated human pre-B cell line 697. HF674720, a chimeric sequence, is shown as an example of amino-acid changes resulting from *IGKV* type 2 replacement. IMGT/V-QUEST[33,38] was used and provides a V-REGION alignment together with the number of amino acid changes. Sequence alignment with the *IGKV*2-24 donor gene is shown, together with the amino acid changes resulting from *IGKV*2-30 replacement.

group, no difference was observed between *IGKV* genes: *IGKV*2-30 could be replaced with *IGKV*2-24 and vice versa. No cryptic RS in the *IGKV* recipient gene was identified to account for the localization of replacement sites. Following type 2 replacement, the chimera sequences encode new Vκ domains displaying amino acid changes when aligned with the donor sequence or the recipient sequence (Fig. 3). Unlike type 1 *IGHV* replacement, in which the 3′V-RS of the *IGHV* donor gene rearranges with the internal RS in the FR3 of the *IGHV* recipient gene with a resulting longer sequence, no change in the length of type 2 V chimera *IGKV* genes was observed. The '*IGKV* chimera genes' were all in-frame, without the addition or deletion of nucleotides. The alignment of *IGKV-IGKJ*5 sequences with *IGKV* germline sequences, using IMGT/V-QUEST[38] made it possible to assign particular regions to specific *IGKV* genes accurately (Fig. 2). However, at the junctions of these assigned regions, there are short sequences of 10–20 nucleotides, the origin of which is unclear because they are identical to the sequences of both the *IGKV* receiver gene and the *IGKV* donor gene. These short unassigned regions correspond to stretches of strict identity between the donor and recipient genes. As found for type 2 *IGHV* replacements, AID motifs are present at sites flanking these strictly identical sequences.

AICDA expression after LPS or IL-1 induction

We investigated the expression of *AICDA* in 697 pre-B cells after stimulation. Cultures of 697 pre-B cells were
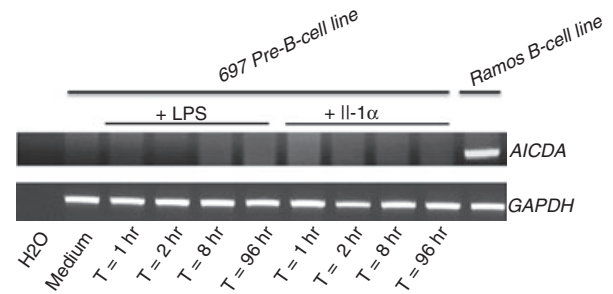

Figure 4. *AICDA* expression in the human pre-B cell line 697 after induction with lipopolysaccharide (LPS) or interleukin-1 (IL-1). Total RNA was reverse-transcribed to generate cDNA and *AICDA* expression was analysed by PCR. The controls used were: *GAPDH* as a positive control for RT-PCR and the Ramos mature B-cell line as a positive control for *AICDA* expression. The results shown are representative of three independent experiments.

treated with LPS or IL-1. Genomic DNA was extracted for the analysis of *IGKV-IGKJ*5 rearrangements and total RNA was isolated and tested for *AICDA* expression. The AID-positive Ramos cell line was used as a positive control. No *AICDA* mRNA was detected in 697 cells, after either 2 hr or 4 days of induction or at intermediate times. Moreover, the treatment of 697 cells with three *AICDA* inducers – the synthetic analogue of dsRNA, Poly (I:C), which acts as a ligand of Toll-like receptor 3; the Toll-like receptor 9 agonist CpG and the CD40 ligand – did not induce *AICDA* expression, as assessed by RT-qPCR (data not shown). Neither LPS nor IL-1 induced *AICDA* gene expression (Fig. 4). Consequently,

this enzyme cannot be considered to be an inducer of, or even be involved in, the type 2 *IGKV* replacement observed in these cells.

Discussion

In this study, we demonstrated that type 2 V replacements, previously described in IGH rearrangements, also occur in V-J rearrangements of the κ locus in human pre-B-cell lines. We also showed that they could be induced *in vitro*. We focused our study on a human B-cell line able to generate new *IGKV-IGKJ* rearrangements after LPS or IL-1 treatment. Due to the particular configuration of the two κ haplotypes, pre-B cells of the 697 line can generate *de novo IGKV-IGKJ* rearrangements only from a single haplotype already harbouring a native *IGKV1-8-IGKJ4* rearrangement. As all *IGKJ* genes other than *IGKJ5* have been deleted (by *IGKV1-8-IGKJ4* rearrangement), any subsequent *de novo IGKV-IGKJ* rearrangements must use *IGKJ5* as the junction gene for *IGK* recombination. Alignments of sequences corresponding to new recombination products showed that most *IGKV-IGKJ5* rearrangements were not mutated, but that a minority (about 10–15%) carried more than two mutations. We considered only the *IGKV-IGKJ5* sequences carrying two or more mutations in subsequent analyses of type 2 *IGKV* replacements. These *IGKV* replacements, at the *IGKV-IGKJ5* rearrangements induced *de novo*, occurred in the 5′ upstream region of the *IGKV* recipient, resulting in a new, in-frame sequence for the antigen-binding Vκ domain. Indeed the V chimera domain generated could carry, for example, the *IGKV* donor sequence from the FR1 to CDR2 and the native sequence from the FR3 to CDR3, so contributing to the diversity of the BCR immunoglobulin repertoire. Remarkably, the length of the replaced sequences was identical to that of potential germline *IGKV* sequences. Hence, the upstream replacements of the rearranged *IGKV* genes were not the result of the repair of RAG-induced double-strand breaks at a putative cryptic RS,[39] as described in type 1 replacement. The replacement of the inner region of the *de novo* rearranged *IGKV* segments, as observed for the HF674725 sequence, could be explained by a mechanism of homologous recombination from a *cis* or *trans* donor sequence with double cross-overs affecting a very short sequence. However, homologous recombination does not seem to be a plausible mechanism for such short regions because double cross-overs generally occur in conditions in which the sequence exchanged is longer than 3 kb.[22] The observation of inner replacements, as described for HF674725, also confirms that the type 2 *IGKV* replacements observed in this study were not PCR artefacts. As *IGKV2-24* was deleted during *IGKV2-30-IGKJ5* recombination, it is possible that *IGKV* genes from the excision circle, are used as the template for type 2 replacements either while still in circular form, or after the reintegration of the excision circle into

the genome.[40] This possibility is supported by one of the interesting features of this model: type 2 replacement was not detected in any of the native *IGKV1-8-IGKJ4* sequences after IL-1 or LPS stimulation (see Supplementary material, Fig. S2), suggesting that type 2 replacement and V-J rearrangement may be chronologically linked. As a more conventional model, in the case of reintegration of the excision circle, it is possible that, at the time of V-J rearrangement, the spatial chromatin conformation promotes the juxtaposition of donor and recipient genes, thereby facilitating the exchange of DNA sequences. We cannot exclude the possibility of the use of *IGKV* genes from the second allele for κ type 2 replacement, by interchromosomal recombination. Indeed, all of the 30 or so sequences with a type 2 replacement analysed displayed the use, as the donor, of an *IGKV* gene and allele present on both loci. Furthermore, both *IGK* haplotypes in the 697 cell line contain exactly the same IGKV genes, with the exception of the *IGKV3-7* gene, which is present in the second haplotype only. Hence, whatever the *IGKV* gene used as the donor, it is present simultaneously on both strands (in both *cis* and *trans*). The 697 cell line is therefore not the most appropriate model for distinguishing between *cis* (intra-allelic) and *trans* (inter-allelic) exchanges of DNA. However, it could be argued that the absence of *IGKV* replacement in the *IGKV1-8-IGKJ4* native rearrangement may be a result of the chromatin conformation and epigenetic organization of the *IGK* haplotype, which remains inaccessible to the DNA replacement machinery in non-rearranging loci but acquires a permissive conformation in the locus undergoing *de novo IGKV-IGKJ* rearrangements,[41] which may promote DNA replacement events. The hypothesis that inter-allelic DNA exchange is responsible for type 2 replacement is theoretically possible, but this would require the simultaneous availability of both κ loci, which would be incompatible with the phenomenon of allelic exclusion.

As previously described for type 2 VH replacements, (a/t)(a/g)c(c/t)/(a/g)g(c/t)(a/t) hotspot motifs (*wrcy/rgyw*) were identified on either side of the short, unassigned region showing strict identity between the donor and recipient *IGKV* genes. However, many other AID motifs[42] can also be identified throughout the *IGKV-IGKJ5* sequences, both within and outside the unassigned region. The short unassigned region may ensure matching between recipient and donor *IGKV* sequences. After matching, the flanking AID hotspots or other DNA motifs[22,43] may then trigger double-strand breaks and the process of type 2 *IGKV* replacement. In species using gene conversion for the generation of their immunoglobulin repertoire diversity, such AID motifs are essential for homologous recombination. As previously suggested,[16–19] type 2 replacement may result from a mechanism related to the 'gene conversion-like' mechanism involving AID. Surprisingly, no *AICDA* expression was detected in our pre-B-cell model either before or after treatment with

IL-1 or LPS, even after 8 hr of stimulation, the time-point corresponding to the beginning of the rearrangement process, or after 4 days, corresponding to the end of stimulation. By contrast, previous studies have shown that LPS treatment greatly increases *AICDA* expression in mature B cells.[44,45] However, in this study, we used κ recombination inducers (IL-1 and LPS) that can cause type 2 *IGKV* replacements in precursor B cells in the absence of *AICDA* expression. Hence, AID is not required for type 2 recombination processes in this context.

Type 2 *IGKV* and *IGHV* replacements cannot both be due to a phenomenon of AID-dependent gene conversion.[46] Instead, they may both involve a new unidentified mechanism of homologous recombination (gene conversion-like recombination). Presumably, proteins other than AID are involved in this mechanism: possible candidates include error-prone DNA polymerases with spontaneous mutagenic activity on undamaged DNA[47,48] and other members of the APOBEC family.[49,50] As native *IGKV*1-8-*IGKJ*4 does not undergo type 2 *IGKV* replacements, this phenomenon of homologous recombination seems to be tightly associated with the V-J rearrangements process. These new *IGKV* and *IGHV* replacements independently contribute to the broadening of B-cell repertoire diversity, by taking part in BCR immunoglobulin editing/revision in an AID-independent manner. We are currently investigating the relationships between this new replacement process, the stimulation of κ enhancers and B-cell transcription factors.

Acknowledgements

Disclosure

The authors declare that they have no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. The native rearrangement of 697 cells disappears after a long period of induction with interleukin-1.

Figure S2. Identical sequence for the all native 450 bp PCR products from treated 697 cells.

Figure S3. Comprehensive alignments of full hybrid *IGKV* sequences.