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Persistence of a rheumatoid factor (RF)-producing B cell clone with a somatically mutated Igκ chain in a patient with rheumatoid arthritis

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SUMMARY

The VκIV gene encoding the light chain of an IgA RF has been shown to have undergone 31 somatic mutations compared with the single existing VκIV germ-line gene. We now show the persistence of the rearranged and mutated DNA coding for this RF over a period of 5 years in the peripheral blood lymphocytes (PBL) of the patient with rheumatoid arthritis (RA). The sequence of the RF has been conserved to identity over this period. These results raise the possibility that the particular antigenic stimulus leading to RF production in this RA patient is active over a long period of time.

Keywords rheumatoid factor rheumatoid arthritis immunoglobulin genes somatic mutation

INTRODUCTION

RF are IgM, IgG, IgA or IgE antibodies that bind to the Fc region of autologous IgG [1]. RF are found in normal individuals [2], transiently during infectious diseases [3,4] and after certain vaccinations [5]. Characteristically, these 'physiological' RF are of the IgM isotype, and display a low affinity for the Fc fragment of IgG. They are, in general, encoded by a restricted set of V-region genes in virtually unmutated (germ-line) configuration, and are selectively rearranged early in fetal development. They have a low-affinity binding for multiple apparently unrelated antigens (reviewed in [6]). Several investigators propose a physiological role for these RF. They are thought to promote the clearance of circulating immune complexes (reviewed in [7]). RF-expressing B cells are considered to act as highly efficient antigen-presenting cells for low concentrations of immune-complexed antigen [8].

In 60–80% of patients with rheumatoid arthritis (RA), elevated levels of RF can be found [9]. Monoreactive, high-affinity RF, and RF of IgG and IgA isotype, are common. The cross-reacting idiotypes (CRI) and V-genes often found to be involved are distinct from those expressed in the naturally occurring RF [6]. There is evidence that the pathological RF in RA undergo affinity maturation and are the product of an antigen-driven response [10–14]. Especially IgG RF isolated from synovial tissue have been shown to be encoded by somatically mutated V-region genes [11,14] and thus resemble RF characterized in the MRL/lpr mouse model of RA [15]. However, the question of how long single RF-producing clones persist in RA patients has not been addressed so far.

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Previously we were able to characterize a human heterohybridoma derived from peripheral blood lymphocytes (PBL) of a patient with RA which was producing an IgA RF [16]. The V-region genes encoding the heavy and light chains of this RF were sequenced. The V_H gene is related to two other RF-encoding V_H genes [17,18] that belong to the recently described V_H7 gene family [19] which is closely related to the V_H1 family. Therefore, the V_H gene of the RF was initially classified as a member of the V_H1 family. The light chain is encoded by a VκIV gene which is known to be the only member of the VκIV gene family [20,21]. VκIV germ-line polymorphism could be excluded by comparing the RF-encoding κ gene with the VκIV germ-line gene analysed in the same patient [13]. We were able to show that the RF light chain had undergone 31 somatic mutations [13,16]. Ten of these mutations were arranged in blocks of two mutations next to each other. This phenomenon of block mutations was recently described as characteristic of somatically mutated κ genes [22].

In an attempt to follow up the persistence of this RF-producing B cell clone we examined the PBL of the RA patient 3 and 5 years after isolating PBL for producing the IgA RF-secreting hybridoma, which was established in 1987.

MATERIALS AND METHODS

DNA preparation and amplification

Peripheral blood mononuclear cells (PBMC) were prepared from 20 ml heparinized blood by centrifugation on Ficoll–Hypaque. Genomic DNA was prepared and purified [23]. The following oligonucleotide primers were used for polymerase chain reaction (PCR) amplification: $V_H1/7$ 5′CTA-GGTCGACCCTCAGTGAAGGTYTCCTGCAAGGC3′; J_H4

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                  25                30 *******CDR1********                   40
VH-P61B27       T TCT GGA TAC ACG TTC ACA AAC TAT GCT CTG AAT TGG CTG CGA CAG GCC
VH-CONS.SEQ.90  - --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                41                                  50    *****************56
VH-P61B27       CCT GGA CAA GGC CTT GAG TGG ATG GGA TGG ATC AAC ACC AAC ACT GGA
VH-CONS.SEQ.90  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                ***CDR2******60*********************                70      72
VH-P61B27       AAA GCA ACA TAT GCC CAG GCC TTC ACA GGA CGC TTT GTT TTC TCA CTG
VH-CONS.SEQ.90  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                73                          80                              88
VH-P61B27       GAC ACC TCT GTC AGT ACG ACA TAT CTA CAG ATC AGC AGC CTA AAG GCT
VH-CONS.SEQ.90  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                    90                                  ****100**CDR3*****
VH-P61B27       GAA GAC ACC GCC GTC TAT TTC TGT GCG AGA GAT AGG TGG AAT GA
VH-CONS.SEQ.90  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --
```

Fig. 1. Consensus nucleotide sequence (V_H-CONS.SEQ.90) of four individual V_H clones identical to the published sequence of the V_H7 heavy chain (V_H-P61B27) of the IgA RF-producing hybridoma P61B27 [14] derived from peripheral blood lymphocytes (PBL) of the same patient in October 1990.

5′CGTCGTCAACCACGGTCCTTGGCCCCAGTAG3′; VκIV 5′CGTCGTCGACAGACTCCCTGGCTGTGTCTCTGGGC3′; Jκ1 5′CGTCGTCGACTCACGTTTGATTTCCACCTTGGTC3′; Jκ2 5′CGTCGCGACTCCAGCTTGGTCCCCTGGCCAAAAG3′; Jκ3 5′CGTCGTCGACGTTTGATATCCACTTTGGTCCCAG3′; Jκ4 5′CGTCGTCGACGTTTGATCTCCACCTTGGTCCCTCC3′; Jκ5 5′CGTCGTCGACGCTTACGTTTAATCTCCAGTCGTG3′.

```
                17          20 FR1          *******CDR1**27**+***+***+***+***+****+*
Vκ4gl           GAG AGG GCC ACC ATC AAC TGC AAG TCC AGC CAG AGT GTT TTA TAC AGC TCC 99
P61B27          --- --- -T- --- G-- --- --- --- -TG --- --- --- --- --- C-- --- ---
CONS.SEQ.90     --- --- -T- -#- G-- --- --- --- -TG --- --- --- --- --- C-- --- ---
CONS.SEQ.92     --- --- -T- --- G-# --- --- --- -TG --- --- --- --- --- C-- --- ---
92KL51B         --- --- -T- --- G-- --- --- --- --- --- --- --- --- --- C-- --- ---

                *28******30*****************                FR2  40
Vκ4gl           AAC AAT AAG AAC TAC TTA GCT TGG TAC CAG CAG AAA CCA GGA CAG CCT CCT 150
P61B27          --T --A C-- --- --- --- --- --- -T- --- --A --C --- --- --- --- ---
CONS.SEQ.90     --T --A C-- --- --- --- --- --- -T- --- --A --C --- --- --- --# ---
CONS.SEQ.92     --T -#A C-- --- --- --- --- --- -T- --- --A --C --- --- --- --- ---
92KL51B         --T --A C-- --- --- --- --- --- -T- --- --A --C --- --- -G- --- ---

                                    *50**CDR2*******************            60
Vκ4gl           AAG CTG CTC ATT TAC TGG GCA TCT ACC CGG GAA TCC GGG GTC CCT GAC CGA 201
P61B27          --A --C --- --- --T --- --T --- G-- --A C-- --- --- --- --- -C- ---
CONS.SEQ.90     --A --C --- #-- --T --- --T --- G-- --A C-- --- --- --- --- -C- ---
CONS.SEQ.92     --A --C --- --- --T --- --T --- G-- --A C-- #-- --- --- --- -C- ---
92KL51B         --A --C --- --- --T -A- --T --- G-- --A C-- --- --- --- --- -C- ---

                                                 70     FR3
Vκ4gl           TTC AGT GGC AGC GGG TCT GGG ACA GAT TTC ACT CTC ACC ATC AGC AGC CTG 252
P61B27          --- -TG --- --- --- --- --- --- --A --- -G- --- --- --- --- --- ---
CONS.SEQ.90     --- -TG --- --- --- --- --- #-- --A --- -G- --- --- --- --- --- ---
CONS.SEQ.92     --- -TG --- --- --- --- --- --- --A --- -G- --- --- --- --- --- ---
92KL51B         --- -TG --- --- --- --- --- --- --A --- -G- --- --- --- --- --- ---

                    80                                  *****90***CDR3***********95
Vκ4gl           CAG GCT GAA GAT GTG GCA GTT TAT TAC TGT CAG CAA TAT TAT AGT ACT CCT 303
P61B27          --- --- --- --- --- --- --- --- --- --- --A --- --- --- GA- T-- A-C --T -
CONS.SEQ.90     --- --- --- --- --- --- --- --- --- --- --A --- --- --- GA- T-- A-C --T -
CONS.SEQ.92     --- --- --- --- --- --- --- --- --- --- --A --- --- --- GA- T-- A-C --T -
92KL51B         --- --- --- --- --- --- --- --- --- --- --A --- --- --- GA- T-- A-C --T -
Jκ2gl                                                                           TAC A
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Fig. 2. Consensus nucleotide sequences of individual VκIV clones (CONS.SEQ.90 and CONS.SEQ.92; $n = 7$ and $n = 8$, respectively) identical to the published sequence of the somatically mutated VκIV light chain of the IgA RF-producing hybridoma P61B27 [14] derived from peripheral blood lymphocytes (PBL) of the same patient in October 1990 and in December 1992. They are compared with the RF sequence (P61B27) [14], with the VκIV germ-line sequence (Vκ4gl) [17] and with clone 92KL51B. Nucleotide exchanges that were each found in only one of the clones that were sequenced, most probably due to misincorporation by the Taq polymerase, are indicated by #. Identities are indicated by dashes. Numbering is according to Klobeck [17], numbers at the ends of the lines designate nucleotides, numbers above the sequence designate amino acids, + designates additional amino acids in CDR1 characteristic for the VκIV family.

Each PCR contained 1 μg genomic DNA, 6·25 pmol of each primer, 0·2 mM dNTP, 50 mM KCl, 10 mM Tris-Cl pH 8·4, 2·5 mM $MgCl_2$ and 2·5 U Taq polymerase (Boehringer, Mannheim, Germany) in a total volume of 50 μl. The enzyme was added after a preincubation of 5 min at 95°C, followed by 3 min at 63°C and 90 s at 72°C. The PCR programme consisted of 35 cycles of 80 s at 95°C, 30 s at 63°C and 90 s at 72°C. The synthesis was finished with 5 min of incubation at 72°C.

Cloning and sequencing

The PCR was controlled by electrophoresis in a 2% agarose gel and prepared by electrophoresis in 2% low melting agarose (FMC NuSieve, Biozym, Oldendorf, Germany). The excised band was isolated with a geneclean kit (BIO 101) according to the recommendations of the manufacturer. For dG-tailing, the purified band was incubated with 140 mM cacodylic acid, 30 mM Tris-HCl pH 6·8, 1 mM $MnCl_2$, 67 nM dGTP, 0·1 mM DTT with TdT (Pharmacia, Freiburg, Germany) for 20 min at 37°C. dG-tailed DNA was annealed to a PstI cut and dC-tailed pTZ19U vector [24]. *Escherichia coli* strain DH5 alpha was transformed with the annealed plasmid using the procedure of Hanahan [25]. The plasmids were screened based on restriction sites which were characteristic and present in the originally described clone [16]. One of the somatic mutations of the light chain has created a new EcoR1 restriction site, and the heavy chain differs from the most related sequences by an MboII restriction site. Clones bearing the expected restriction site were expanded for further examinations. dsDNA sequencing was carried out with Sequenase Version 2.0 (USB), using M13 universal and reverse sequencing primer.

RESULTS

Detection of the RF heavy and light chain in PBL from 1990

After amplifying and cloning the VH1/7-JH4 heavy chain rearrangements, the inserts of eight of 25 prepared plasmid clones were digestible with MboII. The sequences of four clones containing an MboII restriction site were 100% identical to the published RF heavy chain sequence (Fig. 1) [16]. Three clones of the remaining four MboII-digestible clones were nearly 100% homologous to an unrelated V_H1 gene.

Performing the same procedure for the VκIV-Jκ2 light chain rearrangements, 16 of 20 examined plasmid clones contained an EcoR1 restriction site. Figure 2 shows the consensus sequence of seven independent clones. It is identical to the published RF light chain sequence [13,16]. In almost 2000 bp sequenced, there were only four individual nucleotide differences at different positions. There was one exchange from C to T (nucleotide position 59), one exchange from T to C (nucleotide position 147), and two from A to G (nucleotide postions 160 and 223). The most likely explanation for these deviations is artefacts due to infidelity of the Taq polymerase. The corresponding calculated Taq polymerase error rate is 6×10^{-5}, which is similar to that described by others [26].

Detection of the RF light chain in PBL from 1992

The rearrangements between the VκIV gene and each Jκ segment (Jκ1–Jκ5) were amplified from the patient's PBL of 1992. As shown in Fig. 3, only the VκIV-Jκ2 product is partially digestible with EcoR1, generating two fragments of 213 bp and 116 bp. Accordingly, 16 of 75 prepared plasmid clones, derived from cloning the VκIV-Jκ2 PCR product, had an EcoR1 restriction site, consistent with the previously described mutation. Sequencing nine of these EcoR1-digestible clones yielded results similar to those obtained with the clones derived from the PBL of 1990. The consensus sequence of eight clones shown in Fig. 2 is identical to the published RF light chain sequence [13,16]. Altogether there were three nucleotide exchanges: one from C to T (nucleotide position 63), one from T to C (nucleotide position 184) and one from A to G (nucleotide position 104). As the nucleotide exchange rate is approximately 4×10^{-5}, it can again be assumed that they are caused by Taq polymerase errors (see above).

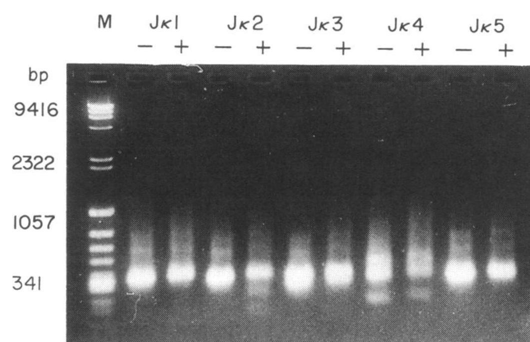

Fig. 3. EcoR1 digestion of the amplified VκIV rearrangements with each Jκ segment (Jκ1–Jκ5) of peripheral blood lymphocytes (PBL) from December 1992 from the rheumatoid arthritis (RA) patient. The Vκ-Jκ products for each Jκ segment are shown without (−) and after (+) EcoR1 digestion. Only the VκIV-Jκ2 product is partially digested with EcoR1, generating two new fragments of about 100 bp and 200 bp.

Of special interest is clone 92KL51B, also shown in Fig. 2. Its sequence is identical with the somatically mutated light chain gene with the exception that it lacks the TG-block mutation at the nucleotide positions 74 and 75, and has two additional nucleotide exchanges: one from A to G (nucleotide position 143) and one from G to A (nucleotide position 167).

DISCUSSION

The sequence analysis of the VκIV germ-line gene of the patient from whom the RF-producing hybridoma cell line had been established definitely proved that the gene encoding the VκIV chain of the RF is somatically mutated [13]. The demonstration of a somatic mutation of this gene was facilitated by the fact that there is only one member of the VκIV family [20,21]. In contrast, in large human V_H gene families it is very difficult to distinguish between polymorphism and somatic mutations. Thus, it is not surprising that only few other mutated RF factors have been reported, and that there is no study on the long-term follow up of a somatically mutated RF-producing B cell clone in PBL of a patient with RA.

The feasibility of studying somatic mutations in the one-membered VκIV family enabled us to investigate and demonstrate the long-term persistence of an RF-producing clone characterized by defined mutations in the VκIV gene. In addition, we were able to detect in samples of peripheral blood taken at an interval of 3 years the identical sequence of the gene encoding the RF heavy chain.

There are several possible explanations why a mutated B cell clone persists over a prolonged period of time without

undergoing further mutations: the respective B cell clone may produce an antibody with optimal or near-optimal affinity to a yet unknown antigen, and therefore have a reduced potential for further mutations. However, it may also be possible that the rate of proliferation of the B cell clone is very low, thus limiting the chance of additional mutations.

The persisting B cell clone described by us belongs most probably either to the long-lived, non-dividing, antigen-independent memory cells which have been described by Schittek & Rajewsky [27], or to the antigen-dependent long-lived memory cell which does not undergo further mutations, corresponding to a cell population that has recently been described in the mouse [28]. In the latter case it must be assumed that the particular antigenic stimulus giving rise to the respective B cell clone has persisted in the patient over a long period of time. As the 'precursor clone' of the described RF-producing B cell clone which lacks one block mutation (92KL51B) has also persisted for a long time, one can assume that it encodes an antibody with a comparable affinity to the putative antigen, because clones producing antibodies with inferior affinity can not compete for antigen, and die.

There is general consent that RF play a role in joint inflammation and synovial destruction in RA (reviewed in [6]). Therefore, the origin of these RF remains a question of major interest. The isolation of persisting B cell clones with somatically mutated RF-encoding genes in patients with RA may help to define specific antigens which play a causative role in the pathogenesis of RA.

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