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Immunoglobulin Kappa and Lambda Light Chain Dual Genotype Rearrangement in a Patient with Kappa-secreting B-CLL

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Abstract—*The cytogenetics, immunophenotype and immunoglobulin gene rearrangements were analysed in a consecutive series of 56 patients with lymphoma or lymphatic leukaemia. One patient with B-CLL showed monoclonal rearrangements of the constant μ, κ and λ genes. The immunophenotype was κ-secreting indicating expression of one immunoglobulin light κ allele. Clones of κ producing B-cells usually show germline λ genes. The present case may thus be interpreted as a failure of the mechanisms controlling isotypic exclusion at the gene level. Further studies are needed to determine the frequency of such events and whether the patients show distinct clinical or other features.*

INTRODUCTION

IMMUNOGLOBULIN genes in germ-line configuration are organized as discontinuous families of segments that must be brought together during B-lymphocyte differentiation as a necessary prerequisite for gene expression. For immunoglobulin heavy chain genes (IGH) one of several diversity (D) segments is fused to one of several joining (J) segments located 5′ of several constant (C) genes; thereafter, one of many variable (V) segments becomes associated with the DJ rearranged segment. Immunoglobulin light chain (IGL) genes lack D segments. Hence, recombination preceding IGL gene expression only reassociates one V with one J segment. Somatic recombination of IG genes starts first in one allele and if it is successful in giving rise to a productive gene, the other allele cannot start or continue rearrangement. On the other hand, if the first allele fails to produce a functioning gene, the second allele undergoes recombination. In both cases only one allele is expressed and the other is silent (allelic exclusion) [1, 2].

In human cells, the two different immunoglobulin light chain isotypes (κ and λ) are known to be encoded by one pair of IGLκ alleles and by six pairs of IGLλ alleles, respectively [3, 4]. The analysis of rearrangements involving these genes in murine and human B-cell malignancies has shown that productive rearrangements of IGLκ genes are almost always associated with IGLλ genes in germline configuration. Conversely, productive rearrangements of IGLλ genes are almost always associated with aberrant rearrangements or deletions of IGLκ genes [5, 6]. It has been proposed that somatic recombination of IGL genes follows a hierarchical order with κ gene rearrangements preceding those of λ genes, and with λ genes undergoing recombination only in those cells that failed to produce a productive κ allele [6]. The available experimental evidence supports this hierarchical model of rearrangement [7–9]. However, there is not much information about variations in the sequence of events predicted by the model. We report here one case of human B-cell chronic lymphatic leukaemia (B-CLL) which besides being κ-secreting and having a productively rearranged IGLκ allele also had a rearrangement of one IGLλ gene. This example suggests that successful rearrangement of IGLκ genes does not always prevent IGLλ from undergoing recombination.

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MATERIALS AND METHODS

Case report

The patient is a 56-year-old male who presented

in February 1985 with enlarged lymph nodes in the neck, supraclavicular fossae, axillae and groin. The lymph nodes were about 2 cm in diameter. Blood haematologic values were: haemoglobin 129 g/l, white blood cell count 343.0 × 10^9/l, (differential: 97% small lymphocytes and 3% granulocytes) and platelet count 191 × 10^9/l. A bone marrow differential showed 70% small lymphocytes.

A biopsy of a lymph node in the neck revealed a histology of a small lymphocytic lymphoma. A diagnosis of chronic lymphocytic leukaemia was made. Chlorambucil 6 mg daily was started in April 1985 and the dose reduced to 2 mg daily in October 1985.

In October 1985 the haemoglobin was 143 g/l, white blood cell count 343.0 × 10^9/l, (differential: 93% small lymphocytes and 7% granulocytes) and platelets 119 × 10^9/l. The lymph nodes were approximately the size as in February 1985. His condition has since remained essentially unchanged.

Methods

Blood samples for lymphocyte characterization and cytogenetic analysis were obtained on four occasions between October 1985 and May 1987. The cells were used for surface marker analysis, chromosome banding analysis and extraction of DNA for Southern hybridization analysis.

Surface marker analysis. Immunologic marker studies were done by fluorescence analysis using FACS IV (Becton Dickinson) or by immunoperoxidase staining. The monoclonal antibodies used were: OKT-11 for CD2, anti-kappa and anti-lambda (Ortho Pharmaceutical Corporation, Raritan, NJ, U.S.A.), and B1 for CD20 (Coulter Electronics, Luton, U.K.).

Cytogenetic study. The cells were cultured for 3 days in the presence of pokeweed mitogen (PWM; 100 μg/ml, P-L Biochemicals, Milwaukee, WI, U.S.A.) or 12-*O*-tetradecanoylphorbol-13-acetate (TPA; 2 μg/ml, Sigma, St. Louis, MO, U.S.A.), or without any mitogen. Our methods for fixation and G-banding have been described previously [10].

Southern hybridization analysis. DNA was extracted from leukaemic mononuclear cells separated from whole blood by one-step density gradient centrifugation in Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden). To obtain control DNA, fibroblast cultures were initiated from a skin biopsy of the patient.

DNA samples (5 μg) were digested overnight with 30–40 U of the appropriate restriction endonuclease. Assay buffers were those suggested by the suppliers (Promega, Madison, WI, U.S.A.; Gibco-BRL, Gaithersburg, MD, U.S.A.). Digested DNA fragments were separated in 0.8% or 0.6% agarose gels and capillary blotted to nitrocellulose membranes (Schleicher & Schuell, Dassel, F.R.G.). Prehybridizations were carried out overnight at 65°C in a mixture of 4 × SSC, 10 × Denhardt's solution, 0.1 M NaH_2PO_4 and 50 μg/ml denatured herring sperm DNA. Probes were made radioactive by nick translation and hybridizations were performed at 65°C for 20–24 h in the above solution. Filters were washed for 30 min with 2 × SSC, 0.1% SDS, followed by two washes of 30 min each in 1 × SSC, 0.1% SDS and 0.1 × SSC, 0.1% SDS, respectively. All washes were at 65°C. Autoradiograms were exposed for 24–72 h at −70°C using intensifying screens. The probes employed were: a 1.3 kb EcoRI–EcoRI fragment homologous to the Cμ heavy-chain gene [11], a 2.5 kb EcoRI–EcoRI fragment homologous to the Cκ light-chain gene [12], a 3.5 kb EcoRI–HindIII fragment homologous to the $C\lambda_{1-6}$ light-chain genes [4], and a 2.5 kb BamHI–HindIII fragment designated κ-deleting (κde). This probe detects deletions of Cκ genes [13].

RESULTS

Surface marker analysis

Surface marker analysis revealed kappa light chain clonality in the blood. The percentage of kappa positive cells was 85% whereas that of lambda positive cells was 5%. The proportions of B1 and OKT-11 positive cells were 90% and 10%, respectively.

Cytogenetic study

Chromosome studies were performed on three successive blood samples. In October 1985 a normal karyotype was found in all 35 PWM-stimulated cells but two out of 11 mitoses stimulated with TPA revealed a clonal chromosome abnormality. One year later only cells with a normal karyotype were observed in both PWM- and TPA-stimulated cultures. In May 1987 a clonal abnormality similar to that encountered 2 years earlier was found in four out of 20 mitoses stimulated with TPA.

The chromosome number of the abnormal clone was 46. Both homologous chromosomes 11 and one homologue of chromosomes 4, 6 and 12 were abnormal. The karyotype could be interpreted as 46,XY,t(4q−;11q+),6q−,11q−,12p?− (Fig. 1A). The breakpoints involved in the t(4;11) translocation were most likely 4q12 and 11q13. Thus, the breakpoints were presumably different from those observed in the B-cell/myeloid mixed leukaemia-specific t(4;11)(q21;q23) translocation. However, a breakpoint in 11q13 is seen in t(11;14)(q13;q32) occurring in B-CLL. The 6q− and 11q− chromosomes were metacentric and resulted from translo-

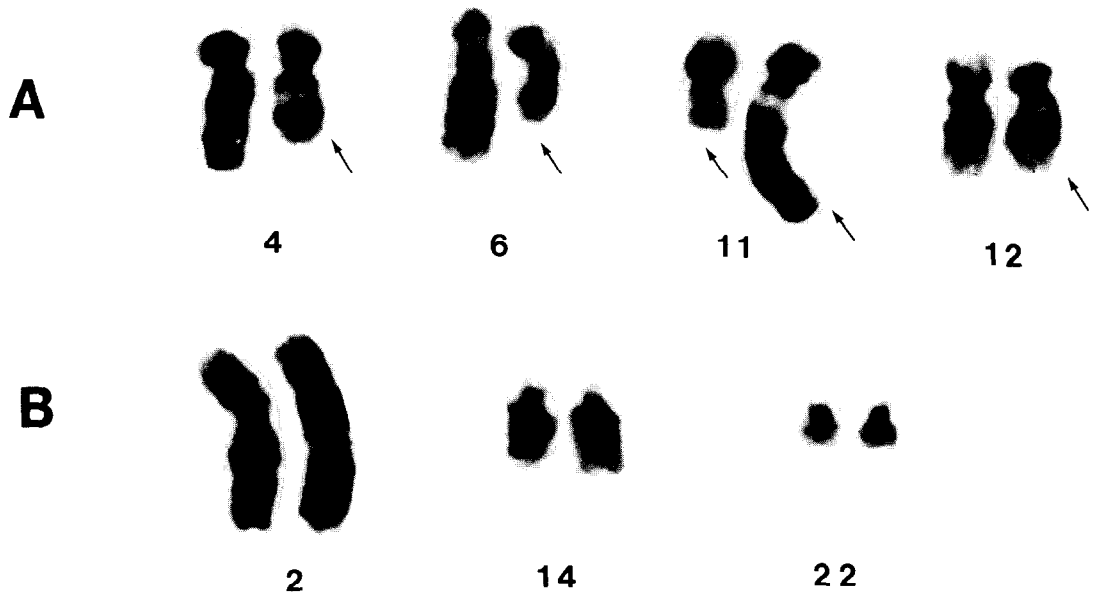

Fig. 1. Partial G-banded karyotype. Chromosomes involved in the clonal abnormality (A) and those containing the immunoglobulin genes (B). Arrows indicate the abnormal homologues.

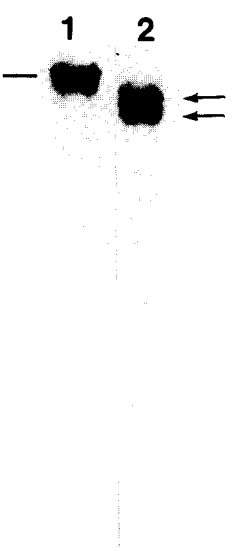

Fig. 2. BamHI digestion and hybridization with Cμ. Lanes 1 and 2: fibroblast and leukaemic DNA samples, respectively. The dash indicates a germ-line fragment. Arrows indicate rearranged bands.

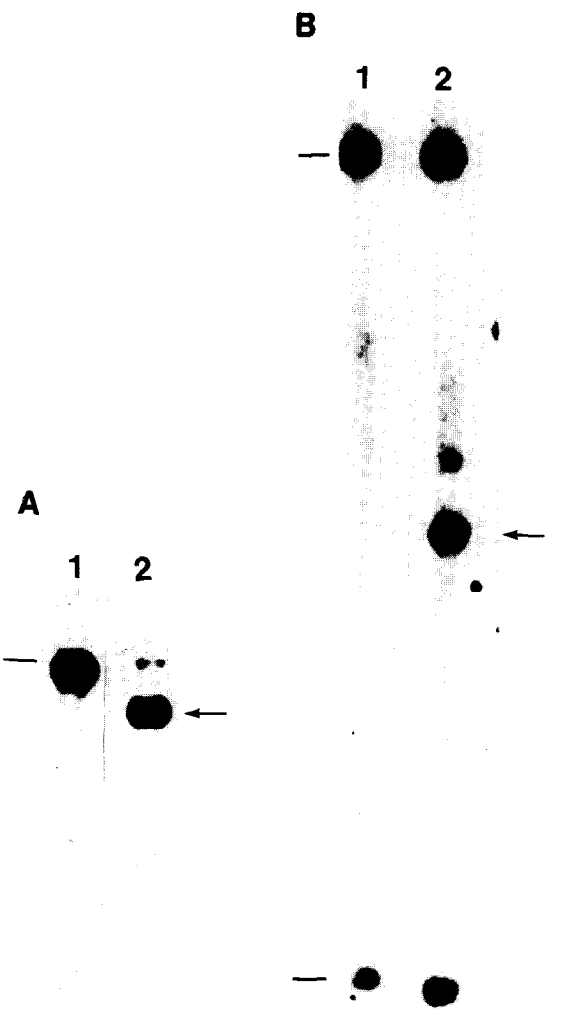

Fig. 3. A. BamHI digestion and hybridization with Cκ. Lanes 1 and 2: fibroblast and leukaemic DNA samples, respectively. B. BamHI digestion and hybridization with κde. Lanes 1 and 2: fibroblast and leukaemic DNA samples, respectively. Dashes indicate germ-line; arrows rearranged bands.

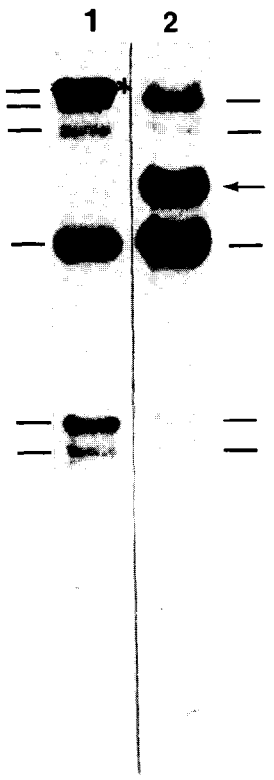

Fig. 4. EcoRI digestion and hybridization with Cλ. Lanes 1 and 2: fibroblast and leukaemic DNA samples, respectively. Dashes indicate germ-line; arrows rearranged bands. The 20 kb EcoRI fragment in fibroblasts that is not present in rearranged DNA is marked by an asterisk.

cations with undetermined other chromosomes rather than deletions, the breakpoints probably being at 6q13 and 11q13. Chromosomes 2, 14 and 22 containing the immunoglobulin genes were carefully studied. However, no abnormalities were observed in those chromosomes at the level of less than 300 bands/haploid set (Fig. 1B).

IG gene rearrangements

BamHI digestion and Cμ probing produced a germ-line fragment of 19.6 kb in the fibroblast DNA sample of the patient (Fig. 2, lane 1) while the B-CLL DNA sample showed two rearranged BamHI bands (Fig. 2, lane 2).

Fibroblast DNA hybridized with the Cκ probe exhibited a BamHI fragment of 12 kb (Fig. 3A, lane 1). The leukaemic DNA sample showed one weak germ-line and one rearranged allele (Fig. 3A, lane 2). After BamHI digestion and hybridization with the κde probe the leukaemic DNA exhibited 14 and 2.5 kb germ-line bands plus a rearranged band (Fig. 3B, lane 2). These data suggest the presence of one rearranged and one deleted Cκ allele. The very weak germ-line band observed with the Cκ probe was likely due to contamination with DNA from normal white cells.

The Cλ probe used detects EcoRI germ-line fragments at 16, 14, 8 and 5 kb resulting from cross-hybridization of the probe with $C\lambda_{1-6}$ genes (16, 14 and 8 kb bands) and a pseudogene (5 kb band) of unknown chromosome localization [4, 14]. Additional germ-line bands could also be seen at 4 kb and 20 kb in the fibroblasts (Fig. 4, lane 1). The 20 kb band is known to result from an EcoRI polymorphism of one of the Cλ genes contained in the 8 kb EcoRI fragment [4]. The leukaemic DNA sample showed a novel band of 9.6 kb, germ-line fragments of 16, 14, 8, 5 and 4 kb but no 20 kb fragment (Fig. 4, lane 2). This pattern suggests a rearrangement of the EcoRI 20 kb allele.

The rearrangements described above were identical on three subsequent occasions at 6 month intervals.

DISCUSSION

The CLL patient described here belonged to a consecutive series of 56 patients with lymphatic malignancies in whom the immunophenotype and immunogenotype were studied. Circulating malignant cells were of monoclonal origin as evidenced by the findings of one chromosomally abnormal clone, the expression of one light chain by surface marker analysis, and the presence of one clonal rearrangement by Southern hybridization. This suggests that the coincidence of a productive IGLκ rearrangement plus a non-productive IGLλ rearrangement was the result of a second rearrangement occurring in a productively rearranged B-cell that underwent malignant transformation.

B-Cells have the potential to assemble and express all IG genes. Yet, a given B-cell clone produces only one functional IGH gene and one functional IGL gene. There is now direct experimental evidence indicating the existence of a controlling mechanism by which the appearance of a functional V_HDJ_H rearrangement inhibits recombination in the other IGH allele [15, 16]. We assume that allelic and isotypic exclusion of IGL genes may also be mediated by a negative regulatory mechanism that prevents further IGL rearrangements after the occurrence of a functional VJ assembly.

The mechanisms controlling IG gene somatic recombination are effective. However, they are not totally error-free. Thirty per cent of non-T, non-B, acute lymphatic leukaemias and 10% of B-CLL leukaemias exhibit dual rearrangements of immunoglobulin and T-cell receptor (TCR) genes [17, 18]. This suggests a failure of the mechanisms by which a rearrangement of an IG gene blocks the rearrangement of TCR genes or vice versa.

The patient reported here exhibits dual rearrangements of IGLκ and IGLλ genes. Since the malignant clone was kappa-secreting, it may be considered as an example of failure of the mechanisms blocking further IGL gene rearrangements after the successful rearrangement of one IGL allele. In a study by Coleclough *et al.* [5], one cell line out of 14 κ-producing murine plasmacytomas revealed a non-productive rearrangement of one λ gene, while all the others had their λ genes in germline configuration. The frequency of κ and λ dual genotype rearrangement in κ-secreting human lymphomas and leukaemias has not so far been determined.

A subset of normal B-cells (Ly-1 B) that is considered to be a specific lineage with characteristic surface antigen expression, appearance during development, tissue distribution and responsiveness to antigens has been described [19]. Lymphoma cells derived from Ly-1 B cells and stimulated with bacterial lipopolysaccharide produce a low proportion of clones expressing both κ and λ light chains and exhibiting rearrangement of IGLκ and IGLλ genes [20]. Most of these cells express only λ light chains on the surface although κ message and protein can be found in the cytoplasm. Some Ly-1 B lymphocytes may exhibit a failure of the control of IGL gene rearrangements. It remains to be determined whether the patient we report represents malignant transformation of Ly-1 B cells. Moreover, further studies are needed to find out whether these malignancies represent a specific clinical subtype.

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