

# Molecular Evidence for a Single Clonal Origin in Biphenotypic Concomitant Chronic Lymphocytic Leukemia and Multiple Myeloma

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To establish the clonal origin of a case of concomitant B-cell chronic lymphocytic leukemia (IgM $\kappa$ ) and multiple myeloma (IgA $\lambda$ ), we analyzed the immunoglobulin (Ig) gene rearrangements in the patient's blood and bone marrow. Despite the different isotypes, pretreatment investigation of the heavy chain gene (J $\mu$ ) revealed a germline fragment and two identical rearrangements in the blood and marrow. Both  $\kappa$  and  $\lambda$  light-chain genes were rearranged in the

blood, suggesting peripheral blood lymphocyte involvement in the myeloma. Analysis of the Ig genes after chemotherapy demonstrated no change in the J $\mu$  or C $\kappa$  rearrangements; however, the  $\lambda$  genes were now in a germline configuration. Our results suggest that in this patient both chronic lymphocytic leukemia and myeloma originated from the same B-cell progenitor.  
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**C**HRONIC LYMPHOCYTIC leukemia (CLL) and multiple myeloma (MM) are chronic B-cell malignancies that represent different stages in B-cell ontogeny. The rare occurrence of CLL and MM, presenting in the same patient, provides a unique opportunity to study clonality in B-cell neoplasia.

The idea of separate clonal origins for most cases of concomitant CLL and MM has been based on the diseases having different isotypes, light-chain restrictions, idiotypes, and immunoglobulin gene (Ig) rearrangements in the few cases examined.<sup>1-3</sup> During normal B-cell differentiation, rearrangement of the variable (V) region gene segments occurs first for the heavy-chain genes, resulting in the appearance of  $\mu$  heavy chains in the cytoplasm of pre-B cells. After heavy-chain recombination, the light-chain genes undergo a similar rearrangement. The  $\kappa$  locus is rearranged initially, and if this results in a functional rearrangement, then  $\kappa$  light chains are produced. However, if the recombination results in a nonfunctional gene, then the  $\lambda$  locus will undergo rearrangement. Heavy-chain genes can subsequently switch classes by placing the rearranged V region in front of any one of the constant region genes downstream of C $\mu$ .<sup>4,5</sup> Therefore the normal developmental sequence of the Ig gene rearrangements provides an explanation why B cells with different isotypes and light chains could have originated from the same progenitor cell.

We have analyzed the Ig gene configurations in the blood and marrow from a patient with a biphenotypic concomitant CLL and MM. Identical heavy-chain gene rearrangements were demonstrated, suggesting that both malignancies had a common clonal origin.

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## CASE STUDY AND METHODS

**Patient.** A 52-year-old man was referred to the hematology department with a history of low back pain and fatigue. A physical examination at the time was within normal limits, with no palpable lymphadenopathy or hepatosplenomegaly. His initial investigations revealed a hemoglobin of 11.3 g/dL, white blood cell count (WBC)  $6.4 \times 10^9/L$  (26% neutrophils, 68% lymphocytes, 5% monocytes), and platelets  $274 \times 10^9/L$ . No plasma cells were seen in the peripheral blood. Serum immunoelectrophoresis demonstrated an IgA  $\lambda$  paraprotein, and urinary electrophoresis showed free  $\lambda$  light chains. A bone marrow aspirate revealed a hypercellular marrow with increased numbers of plasma cells (20%) with some abnormal forms (Fig 1). One year after diagnosis the patient developed an increase in his paraprotein and hypercalcemia and was started on chemotherapy.

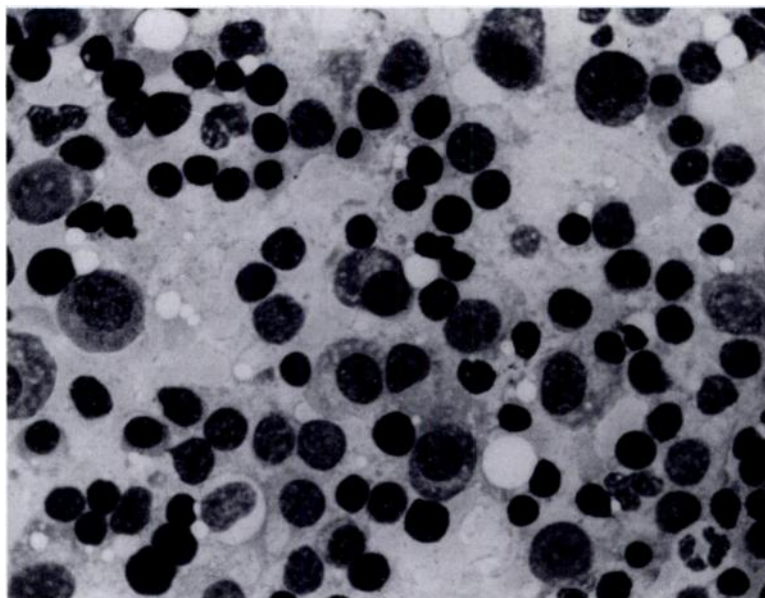
**Flow cytometry.** Mononuclear cells were separated from blood and marrow by centrifugation over Ficoll-Hypaque (FH, Pharmacia, AB, Uppsala, Sweden). Initial screening was carried out by incubating  $5 \times 10^5$  cells with combinations of fluoresoithiocyanate (FITC) and phycoerythrin (PE) directly conjugated antibodies (Becton Dickinson Ltd, Mountain View, CA, Dako Ltd, High Wycombe, UK, respectively).

For indirect immunofluorescence,  $5 \times 10^5$  cells were incubated with saturating concentrations of monoclonal antibodies (MoAbs) for 15 minutes at room temperature. This was followed by incubation in fluorescein-conjugated F(ab) sheep antimouse immunoglobulin (Sigma, UK Ltd, Poole) for 15 minutes at room temperature. Fluorescence was measured on a Facscan (Becton Dickinson, Sunnyvale, CA) flow cytometer. Cells were tested with MoAbs recognizing the cluster of differentiation (CD) antigens and are listed in Table 1.<sup>4</sup>

The antibodies used in this study were obtained from the following sources: DA6.31—MRC Human Genetic Unit; B1—Coulter, Hialeah, FL; RFT1—Scottish Antibody Production Unit; MHM6—A. McMichael, Oxford, England<sup>6</sup>; BU12, CLB.B1y1, HB8, 915, 758, HD28—Third International Leucocyte Differentiation Workshop. Intracytoplasmic immunoglobulins were detected using intracellular staining carried out on cytopins following storage at  $-10^\circ\text{C}$ , followed by thawing and fixation in 90% ethanol at room temperature. Washing in phosphate-buffered saline (PBS) was followed by incubation in 1:40 dilutions of FITC-conjugated goat anti-IgA, IgD, IgG, IgM, Kappa, Lambda, and rhodamine-conjugated antikappa and antilambda antisera (Kallestadt Ltd, Brill, Bucks, UK) for 1 hour at room temperature.

## SOUTHERN BLOT ANALYSIS

High molecular weight DNA was extracted from peripheral blood and marrow by standard techniques. Ten micrograms was digested with the restriction enzymes *Bam*HI and *Eco*RI, separated on 0.8% agarose gel by electrophoresis, and transferred to either Hybond-C or Hybond-N (Amer-



**Fig 1. Bone marrow aspirate demonstrating increased numbers of plasma cells and small lymphocytes (MGG).**

sham, UK), by the Southern technique.<sup>7</sup> Hybridization with hexanucleotide random-primed ( $\alpha$ -<sup>32</sup>P) dCTP (deoxycytidine triphosphate) probes was followed by washing with 0.1  $\times$  SSC (sodium chloride and sodium citrate) and autoradiography using Kodak X AR-5 film at  $-70^{\circ}\text{C}$  for 2 to 10 days.<sup>8</sup> The  $J_H$  and  $C_K$  probes were donated by T. Rabbitts (MRC, Cambridge).<sup>9</sup> The  $C_\lambda$  probe was donated by B. Blomberg (University of Miami).<sup>10</sup> The  $J_H$  probe is a 2.5-kb pair *Eco*RI to *Bgl*III human genomic fragment of the joining gene region. The  $C_K$  probe is a 2.5-kb *Eco*RI germ-line fragment of the constant region and the  $C_\lambda$  probe is a 0.8-kb *Eco*RI fragment of the constant region part of the  $\lambda$ -3 gene ( $C\lambda$ -3).

## RESULTS

**Immunophenotyping.** Initial screening for a suspected myeloma demonstrated an Ig $\lambda$  paraprotein on immunoelectrophoresis. Staining of the bone marrow for intracytoplasmic immunoglobulins demonstrated not only a population of Ig $\lambda$  plasma cells but also a population of IgM $\kappa$  small lymphocytes. Subsequent immunophenotyping by dual-fluo-

rescence flow cytometry indicated the presence of a population of small CD19- and CD5-positive lymphocytes, which were surface IgM and  $\kappa$  restricted, consistent with a diagnosis of CLL. The B cells present were further investigated using indirect immunofluorescence with a panel of antibodies (Table 1).

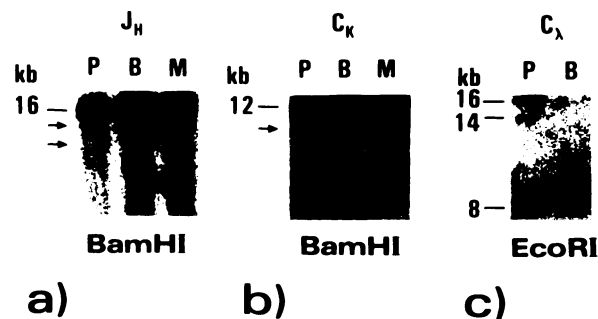
**Southern blot analysis.** Figure 2 shows the results of the initial investigations of peripheral blood and bone marrow DNA using the immunoglobulin gene probes: Analysis with the  $J_H$  probe showed a single 16-kb germline band was present in the placental control. In addition to the germ-line, two identical bands were present in the blood and marrow samples (Fig 2a). The kappa-chain gene was rearranged in the blood and marrow (Fig 2b), while there was a rearrangement of the lambda-chain gene when compared with the placental control (Fig 2c).

One year after diagnosis the patient was started on chemotherapy because of an increase in his paraprotein and hypercalcemia. Reanalysis of the immunoglobulin genes

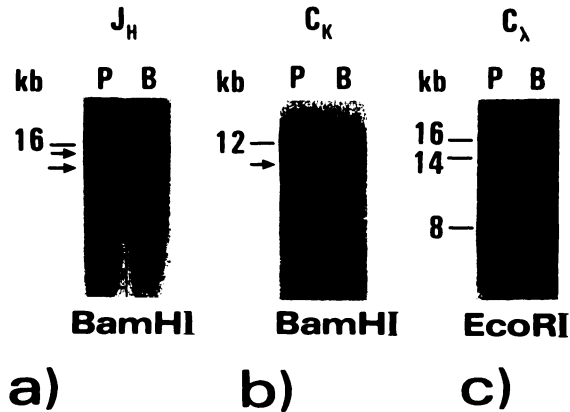
**Table 1. Expression of Cell Surface Antigens on Patient's Peripheral Blood Mononuclear Cells**

Specificity	Antibody	% +	Mfi
Control	AFP	02	193
CD45	PD7/26	99	650
CD5	RFT1	72	431
Class II (framework)	DA6.231	56	575
CD19	BU12	45	407
CD20	B1	27	346
CD22	CLB.Bly1	07	326
CD23	MHM6	38	362
CD24	HB8	37	504
CD37	HD28	45	422
CD38	915	34	397
CD40	758	46	398

The cells were stained by indirect immunofluorescence, and results are given as percentage and median fluorescence intensity (Mfi).



**Fig 2. Autoradiograph of Southern blot of immunoglobulin genes from the patient's PBLs (B) and the bone marrow (M). Placental (P) DNA digests were used as a control. (a) *Bam*HI digest probed with  $J_H$ , (b) *Bam*HI digest probed with  $C_K$ , (c) *Eco*RI digest probed with  $C_\lambda$ . The dash marks indicate the germ-line position, and the arrows denote the position of the rearranged alleles. Molecular weight markers are in kilobase (kb) pairs.**



**Fig 3.** Autoradiograph of the immunoglobulin gene analysis of the patient's peripheral blood after one course of chemotherapy. P, placental; B, peripheral blood (a) *Bam*H1 digest probed with  $J_H$ ; (b) *Bam*H1 digest probed with  $C_\kappa$ ; (c) *Eco*R1 digest probe with  $C_\lambda$ . Compared with Fig 2 (c) the peripheral blood shows only the germ-line configuration (dash marks).

after one course of chemotherapy revealed no changes in the heavy-chain or kappa light-chain gene configuration (Fig 3a,b). However, the previously noted rearrangement of the lambda light-chain gene (Fig 2c) was not detected at this time (Fig 3c).

#### DISCUSSION

We have investigated a case of concomitant CLL and MM using the Ig gene probes. DNA from the peripheral blood and bone marrow generated two identical Ig heavy-chain gene rearrangements, suggesting that despite the different isotypes and light-chain restrictions, the two diseases had a common clonal origin.

Our patient presented at diagnosis with both CLL and MM. Whether the MM represents an evolution of a pre-existing leukemia is unclear. The normal hierarchy of Ig gene rearrangements suggests that both malignancies evolved from a single B-cell progenitor.<sup>4,5</sup> During differentiation with the initiation of immunoglobulin gene activity both heavy-chain alleles were rearranged at the pre-B-cell stage. Cells that effectively rearranged  $\mu$  and  $\kappa$  alleles were capable of maturing into CLL expressing IgMK. Cells in which the  $\kappa$  gene recombination was ineffective could have rearranged

the  $\lambda$  genes. These cells with functional rearrangements of the  $\mu$  and  $\lambda$  alleles were capable of a class switch from  $\mu$  to  $\alpha$  with maturation to multiple myeloma expressing IgA $\lambda$ . Some of these cells may not have fully differentiated, as suggested by the detection of peripheral blood lymphocytes (PBLs) with a rearranged  $\lambda$  gene (Fig 2c), suggesting they were involved in the myeloma.<sup>11,12</sup>

The results of a previous investigation of coexisting CLL and MM using anti-idiotype antibodies suggested a single clonal origin.<sup>13</sup> The authors of this study found a shared idiotype between CLL (IgG $\kappa$ ) and MM (IgA $\kappa$ ). They also demonstrated the expression of  $\gamma$  and  $\alpha$  chains in the cytoplasm of cells that underwent a plasmacytoid differentiation in vitro after exposure to antigens and allogeneic T cells.

In a more recent investigation of concomitant CLL and MM expressing different isotypes, the Ig heavy-chain rearrangements suggested two distinct clones.<sup>3</sup> However, in cases of biphenotypic and bigenotypic follicular lymphoma that have been analyzed in more detail, somatic mutation has occurred in the Ig heavy-chain V region genes, producing new restriction enzyme recognition sites leading to different heavy-chain rearrangements.<sup>4,15</sup> In the cases described by Cleary et al,<sup>16</sup> a common clonal origin was established by demonstrating comigration of t(14;18) gene rearrangements on Southern blot analysis. In another case without comigration of t(14;18) gene rearrangements, cloning and sequencing of the t(14;18) breakpoint DNA confirmed the single clonal origin of the two subpopulations of lymphoma.<sup>16</sup> There is also evidence that myeloma may arise from a pluripotent stem cell. This is based on the finding of erythroid and megakaryocytic antigens on some myeloma cells and also on the demonstration of a rearrangement of the gamma T-cell receptor genes in myeloma cells with concomitant rearrangements of these heavy-chain genes.<sup>17,18</sup>

In summary, we have investigated a case of concomitant CLL and MM. Our data suggest that despite the different isotypes and light-chain restrictions, both malignancies have arisen from a single B-cell progenitor. This may be the case for most cases of concomitant CLL and MM, similar to biphenotypic follicular lymphomas.

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