Papers

Clonality of chronic neutrophilic leukaemia associated with myeloma: Analysis using the X-linked probe M27 β

G R Standen, F J Steers, L Jones

Abstract

Aims—To determine whether myeloid proliferation was monoclonal or polyclonal in a woman with chronic neutrophilic leukaemia and myeloma.

Methods—The X-linked probe, M27 β was used to determine the clonality of the neutrophil population by analysis of restriction fragment length polymorphisms and X inactivation pattern.

Results—A polyclonal pattern of X inactivation was obtained for the neutrophil population in this patient.

Conclusion—The myeloid expansion in chronic neutrophilic leukaemia associated with myeloma represents a polyclonal reactive response to the plasma cell clone rather than a co-existent myeloproliferative disorder.

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Chronic neutrophilic leukaemia is a rare myeloproliferative disorder characterised by persistent neutrophilia, hepatosplenomegaly, increased serum vitamin B₁₂, hyperuricaemia, and raised neutrophil alkaline phosphatase activity.¹ Additional criteria for diagnosis include the absence of any underlying disease capable of provoking a reactive neutrophilia and exclusion of chronic myeloid leukaemia (CML) by appropriate cytogenetic and molecular studies.

Although rare, an association has been recognised between chronic neutrophilic leukaemia and plasma cell dyscrasia.^{2,3} Eleven cases of coexistent chronic neutrophilic leukaemia and multiple myeloma have

recently been reviewed and attention was drawn to the disproportionate excess of λ light chain restriction in this subset of patients.⁴ It remains unclear, however, whether the chronic neutrophilia and gross myeloid hyperplasia seen in these cases represents a distinct clonal haemopathy or a polyclonal reactive response to the plasma cell population.

We used the X-linked probe M27 β and Southern analysis⁵ to study the clonality of the neutrophil population in a woman presenting with IgG λ myeloma who subsequently developed chronic neutrophilic leukaemia.

Case report

The patient studied was a 67 year old woman whose clinical features have already been described in detail.⁴ She presented in 1986 with an orbital plasmacytoma and was found to have an IgG λ paraproteinaemia and excess plasma cells in the bone marrow. After surgical removal of the tumour and radiotherapy she developed severe neutrophilia rising to $27 \times 10^9/l$ over an 18 month period. This paralleled a progressive rise in paraprotein concentration from 5 g/l to 15 g/l. A further bone marrow aspirate showed gross myeloid hyperplasia with predominantly mature neutrophil expansion. The proportion of neutrophils in the marrow was 59% and the percentage of plasma cells 15% (as a function of non-neutrophil nucleated cells). Hyperuricaemia, increased serum B₁₂, and raised neutrophil alkaline phosphatase score were also documented. Karyotype analysis was normal.

Methods

Neutrophils were separated from the peripheral blood of the patient by Ficoll-hypaque density centrifugation and high molecular weight DNA was isolated by phenol extraction and ethanol precipitation.⁶ For controls, DNA was also isolated from the leucocytes of a healthy woman and a Ph⁺ patient with CML.

Aliquots (5 μ g) of DNA from each person were digested for 16 hours with either *Pst*I alone or in combination with *Msp*I or *Hpa*II (Northumbria Biochemicals Ltd, England). Agarose gel electrophoresis and Southern blotting were performed by standard methods, as described previously.⁷ The M27 β probe was radiolabelled using a Multiprime

Department of
Haematology Bristol
Royal Infirmary
Bristol BS2 8HW
G R Standen
F J Steers
L Jones

Correspondence to:
Dr G R Standen

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Southern blots of genomic DNA probed with M27 β : normal female (A), chronic neutrophilic leukaemia patient (B), and Ph⁺ CML patient (C). Results for *Pst*I digests (track 1, 4, 7), *Pst*I/*Msp*I (track 2, 5, 8), and *Pst*I/*Hpa*II double digests (tracks 3, 6, 9) are shown.

DNA labelling kit (Amersham International UK) and blots were hybridised for 24 hours at 65°C. The membranes were then washed twice in 2 × sodium chloride, sodium citrate (SSC)/0.1% sodium dodecyl sulphate (SDS) for 10 minutes at 65°C, followed by two washings in 0.1 × SSC/0.1% SDS for 5 minutes. Finally, the membranes were exposed to x-ray film for 2–7 days at –70°C.

Results

The patient with chronic neutrophilic leukaemia and myeloma was heterozygous for the multiallelic variable number tandem repeat (VNTR) detected by M27β following digestion of neutrophil DNA by *Pst*I (track 4) (figure). The *Pst*I/*Hpa*II double digest revealed a polyclonal pattern with partial digestion of both maternal and paternal derived alleles (track 6). Because the two alleles are similar in size, the middle band in track 6 is a composite of the partially digested larger fragment and the uncut component of the smaller band. *Msp*I, the isoschizomer of *Hpa*II, recognises an identical restriction site but cleavage is independent of methylation status (track 5). Similar patterns were seen following digestion of leucocyte DNA from a normal heterozygous woman (tracks 1, 2, 3). In this case the *Pst*I/*Hpa*II digest shows four bands. For comparison, the results for a female patient with Ph⁺ CML are shown (tracks 7–9). A typical monoclonal pattern is seen in track 9 with complete digestion of the larger fragment ("inactive" unmethylated allele) by *Hpa*II but with the smaller fragment remaining uncut.

Discussion

Many patients with multiple myeloma have been described who, at presentation, have severe neutrophilia and gross myeloid expansion in the bone marrow accompanying the plasma cell clone.⁴ Such patients commonly have other clinical, morphological, and metabolic abnormalities consistent with the diagnosis of chronic neutrophilic leukaemia. Consequently, most authors have concluded that the myeloid expansion represents a coexistent myeloproliferative disorder. In support of this thesis associations between multiple myeloma and other myeloproliferative diseases including primary polycythaemia and myelofibrosis have been well documented.^{8,9}

In our study the X-linked probe M27β was used to analyse the clonality of the myeloid proliferation in a patient with myeloma associated chronic neutrophilic leukaemia. The results clearly show that the neutrophil population in this patient was polyclonal and that the expansion therefore represents a reactive response to the plasma cell infiltrate rather than an associated myeloproliferative disorder.

In support of a polyclonal reactive state we monitored a second patient with myeloma associated chronic neutrophilic leukaemia after treatment with melphalan and observed that the neutrophil count and hepatosplenomegaly progressively declined in parallel with the paraprotein concentration. Furthermore, although karyotype abnormalities have been described in de novo chronic neutrophilic leukaemia,¹⁰ clonal chromosomal abnormalities characteristic of myeloproliferative disease have not been identified in patients with coexistent myeloma and chronic neutrophilic leukaemia.

We have previously failed to prove that the paraproteins in our patients have a stimulatory effect on the growth of myeloid progenitor cells in culture.⁴ It is conceivable, however, that the paraprotein or the plasma cells themselves might act on ancillary populations or marrow stromal cells at the microenvironmental level indirectly to upregulate myelopoiesis. Monoclonal B cell clones in myeloma can produce cytokines, including IL-1β and M-CSF, which are able to activate stromal cells to produce IL-6, IL-7, and IL-11. IL-7 may, in turn, stimulate T lymphocytes to produce IL-3 and GM-CSF. Alternatively, the paraprotein might interfere with the dynamics of neutrophil transit from the marrow and blood compartments—for example, via an effect on the expression of leucocyte adhesion molecules.

Additional studies are clearly required to delineate the nature of the myelopoietic stimulus in this unusual subgroup of myeloma patients.

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