Chronic myelomonocytic leukaemia associated with T cell receptor δ gene rearrangement

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Abstract

Morphological, immunophenotypic, and genetic analyses were carried out on peripheral blood, bone marrow, and pharyngeal biopsy material from a patient with chronic myelomonocytic leukaemia (CMML). Morphological analysis of bone marrow was diagnostic of CMML; immunophenotypic analysis of peripheral blood and bone marrow were negative for B and T cell antigens, and immunochemistry performed on the pharyngeal extramedullary infiltrate showed the presence of large monocytoid cells which stained positively for muramidase. Genotypic analysis, however, showed clonal rearrangement of the T cell receptor (TCR) δ chain gene, a marker of T cell or, less commonly, B cell lymphoid neoplasms. Other TCR genes, β and γ , were germline in all tissues examined. TCR δ is rearranged in precursor B cell and most T lymphoid neoplasms. A small proportion of cases (10%) of acute myeloid leukaemia (AML) also show rearrangement of the TCR δ gene. To date TCR δ rearrangement has not been described in CMML. The aberrant TCR δ rearrangement shown in this patient with CMML provides further evidence of the clonal nature of this disorder.

Analysis of immunoglobulin and T cell receptor gene rearrangements has been shown to be of value in the determination of clonality and, in certain circumstances, lineage of a variety of lymphoproliferative disorders as well as aiding in the differentiation between neoplastic and benign populations.¹ The TCR δ locus is the most recent of 4 TCR loci to be described.² Rearrangement or deletion of TCR δ has been reported in most acute and chronic T cell lymphoid neoplasms and in most early B cell lymphoproliferative disorders.³ About 10% of cases of acute myeloid leukaemia (AML) have been found to have rearrangement or deletion of the TCR δ gene.⁴ We report a case of CMML with extensive extramedullary lymph node, oropharyngeal, and anal infiltration by tumour in whom genotypic analysis showed clonal rearrangement of the TCR δ gene.

Case report

A 61 year old Caucasian woman presented with a two year history of recurrent oral ulceration.

Peripheral blood indices were: haemoglobin, 10.1 g/dl, white blood count $10.4 \times 10^9/l$, monocytosis of $1.6 \times 10^9/l$ and a platelet count of $50 \times 10^9/l$. Bone marrow examination showed trilineage dysplasia with increased blast (9%) and monocyte (9%) counts. On this basis CMML was diagnosed. Cytogenetic analysis of the bone marrow showed a normal karyotype (46,XX). Because of the atypical extramedullary dissemination, inguinal lymph node and pharyngeal biopsies were performed.

The oral ulceration was refractory to treatment. Intensive antileukaemic chemotherapy was used in the later stages of her illness but she died in the hypoplastic phase.

Genotypic analysis

High molecular weight DNA was extracted from frozen lymph node and blood and digested to completion with *Bam* HI, *Eco* RI, and *Hind* III restriction endonucleases before size separation of the fragments on a 0.7% agarose gel. After Southern blotting, hybridisation of ³²P-oligolabelled gene probes was visualised by autoradiography. The probes used were Ig heavy chain joining region J_H probe (C76 R51A), an Ig κ chain constant region probe (PUCR17), an Ig λ constant region probe (CHR22 λ S8c), a TCR β chain gene probe (Jurkat 2), a TCR γ gene probe and TCR δ gene probes for J δ 1, J δ 16, and J δ 2, R21XH. A full description of the probes is given in Hodges *et al.*⁵

Results

Immunophenotypic analysis of bone marrow showed primitive granulocytes, monocytes, and blasts (9%). The blasts were positive for class II antigens (57%) but were negative for B and T cell markers (κ , λ , CD19, CD3, CD7 and Tdt). Examination of the paraffin wax embedded lymph node biopsy specimen showed extensive infiltration by blast cells in keeping with myelomonocytic leukaemia. A pharyngeal biopsy specimen showed submucosal infiltration by an abnormal population of large cells and small reactive lymphocytes. Immunohistochemical analysis confirmed the presence of an atypical infiltrate of large monocytoid cells which stained for muramidase (fig 1).

Both peripheral blood and lymph node DNA at presentation showed germline configuration when probed with J_H, C κ , C λ , TCR β and γ probes. Novel rearranged bands, however, were identified when the same DNA was probed using the J δ 1 and J δ 2 probes, indicating

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Figure 1 Atypical monocytoïd cells showing granular cytoplasmic staining for muramidase (streptavidin-biotin complex; polyclonal anti-muramidase).

clonal rearrangement of the TCR δ locus (figs 2A and B).

Discussion

CMML is characterised by a peripheral blood monocytoïsis exceeding $1.0 \times 10^9/l$, a marrow blast cell population of less than 20%, and a peripheral blood blast population of less than 5%. Auer rods are absent from blast cells. In contrast to acute myeloid leukaemia, extramedullary infiltration is rare but has been reported.⁶ Patients with CMML in whom extramedullary infiltration is a feature often have a high peripheral monocyte count which was not a feature of this case. Serous effusions and skin infiltration have both been reported previously.⁷

Genotypic analyses are not routinely performed on peripheral blood or marrow from patients with CMML as this investigative technique is reserved for the determination of lineage and clonality of lymphoproliferative disorders such as lymphoid leukaemia and non-Hodgkin's lymphoma. The four T cell receptor loci that have been identified, α , β , γ and δ , undergo somatic rearrangement during

lymphocyte ontogeny. Characteristically Ig genes rearrange in developing B cells and TCR genes in T cells.⁸ TCR β and TCR γ loci, however, are rearranged in 25–50% of pre-B lymphoid cells, although in B-ALL,—for example, one TCR γ allele remains in the germline configuration—whereas in T-ALL both TCR γ alleles are rearranged. TCR δ is the first of the four genes to undergo rearrangement and is rearranged or deleted in most acute and chronic T cell malignancies as well as in immature B cell precursor neoplasms. A recent study of TCR gene rearrangements in a variety of haematological neoplasms has shown TCR δ rearrangement in 10% of cases of acute myeloid leukaemia.⁴ Such neoplasms express myeloid antigens and inappropriate rearrangement of the TCR δ gene within the same clone. This may represent “lineage infidelity” or a deviation from a primitive stem cell in the form of “lineage promiscuity” as a normal event.

To our knowledge this is the first reported case of CMML with documented evidence of clonal TCR δ rearrangement. The mechanism underlying this inappropriate TCR δ rearrangement is unclear but this report suggests that CMML, like AML, can have disordered growth and differentiation. The coincident occurrence of myelodysplasia (MDS) with lymphoid or plasmacytic neoplasms has been described previously and suggests that in MDS progenitor cells are capable of clonal differentiation along a variety of pathways.⁹ The recombination events required for gene rearrangement demand that the chromatin is “open” and accessible to recombinase enzymes¹⁰ at the appropriate phase of the developmental cycle. It seems, therefore, that some cases of myeloid leukaemia, including CMML, have disordered recombination resulting in inappropriate rearrangement of the TCR δ gene.

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Figure 2 Autoradiographs of peripheral blood DNA probed with J δ 1 (A) and J δ 2 (B) oligonucleotide probes. C = control; MF = patient DNA.

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