Chronic myelomonocytic leukaemia associated with T cell receptor δ gene rearrangement

A B Provan, R V Majer, A G Smith, B Wilkins, E Hodges, J L Smith

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 immunohistochemistry performed on the pharyngeal extramedullary infiltrate showed the presence of large monocytioid 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. TCRδ 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 TCRδ gene. To date TCRδ rearrangement has not been described in CMML. The aberrant TCRδ 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. The TCRδ locus is the most recent of 4 TCR loci to be described. Rearrangement or deletion of TCRδ has been reported in most acute and chronic T cell lymphoid neoplasms and in most early B cell lymphoproliferative disorders. About 10% of cases of acute myeloid leukaemia (AML) have been found to have rearrangement or deletion of the TCRδ gene. 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 TCRδ 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 x 10⁹/l, monocytes 1.6 x 10⁹/l and a platelet count of 50 x 10⁹/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 JH probe (C76 R51A), an Igκ chain constant region probe (PUCR17), an Igλ constant region probe (CH22Sb), a TCRβ chain gene probe (Jurkat 2), a TCRγ gene probe and TCRδ gene probes for Jδ1, Jδ16, and Jδ2, R21XH. A full description of the probes is given in Hodges et al.

Results
Immunophenotypic analysis of bone marrow showed primitive granulocytes, monocyttes, and blasts (9%). The blasts were positive for class II antigens (57%) but were negative for B and T cell markers (x, 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 monocyctoid cells which stained for muramidase (fig 1).

Both peripheral blood and lymph node DNA at presentation showed germline configuration when probed with JH, Cx, Cl, TCRβ and γ probes. Novel rearranged bands, however, were identified when the same DNA was probed using the Jδ1 and Jδ2 probes, indicating
clonal rearrangement of the TCRδ locus (figs 2A and B).

**Discussion**

CMML is characterised by a peripheral blood mononuclear cell count exceeding \(10^5/\text{l}\), a marrow blast 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. 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.

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, \(\alpha\), \(\beta\), \(\gamma\) and \(\delta\), undergo somatic rearrangement during lymphocyte ontogeny. Characteristically Ig genes rearrange in developing B cells and TCR genes in T cells. TCR\(\beta\) and TCR\(\gamma\) loci, however, are rearranged in 25–50% of pre-B lymphoid cells, although in B-ALL, for example, one TCR\(\gamma\) allele remains in the germline configuration—whereas in T-ALL both TCR\(\gamma\) alleles are rearranged. TCRδ 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 TCRδ rearrangement in 10% of cases of acute myeloid leukaemia. Such neoplasms express myeloid antigens and inappropriate rearrangement of the TCRδ 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 TCRδ rearrangement. The mechanism underlying this inappropriate TCRδ 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. The recombination events required for gene rearrangement demand that the chromatin is “open” and accessible to recombinase enzymes 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 TCRδ gene.

AP is supported by a Leukaemia Research Fund Clinical Training Fellowship.


