Simultaneous Presentation of B- and T-Cell Malignant Lymphoma

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A. Introduction

Multiclonal NHL of the B-cell type has been described [1, 2], most often when two histological types of lymphoma have been noted in simultaneous or successive biopsies. This occurs predominantly when follicular centre cell (FCC) histology converts into a large cell diffuse histology [2]. Multiclonal disease of the B-cell type has been noted in heavily immunosuppressed patients [3]. Reports of composite lymphoma with diffuse T-cell histology arising from co-existant FCC have been described on morphological and immunological evidence [4–6]; however, no DNA analysis was reported. Recently, a multiclonal lymphoma, confirmed by DNA analysis, was reported with sequential B- and T-cell clones [7] in a patient with autoimmune disease who received chemotherapy for the initial B-cell lymphoma. Simultaneous presentation of B- and T-cell lymphomas, confirmed by DNA analysis, with no predisposing factors has not previously been reported.

B. Materials and Methods

I. Case Report

A fit 64-year-old man presented with lymphadenopathy in his left neck and right groin. He had no previous history of illness or exposure to carcinogens. Biopsy tissue from the neck lymph node was histologically a centroblastic/centrocytic follicular lymphoma. Staging investigations revealed no further sites of disease, and the patient was classified as having a stage-IIIA follicular lymphoma and treated with 6 weeks of oral chlorambucil at 10 mg per day. During the course of this treatment the swelling of neck glands disappeared, but the glands in the right groin continued to enlarge, leading to a biopsy of this second site of disease. The groin lymph node was histologically a diffuse T-cell lymphoma of the angioimmunoblastic lymphadenopathy type (AIL) with no evidence of a centroblastic/centrocytic lymphoma. Restaging revealed no new sites of disease. Treatment was commenced with weekly treatment of a regimen containing adriamycin, cyclophosphamide, vincristine, bleomycin and prednisolone, followed by a complete resolution of disease which has been sustained for 18 months.

II. Immunophenotype and Immunogenotype Studies

Fresh specimens were obtained from both biopsies and small portions were placed in air-tight plastic tubes, snap-frozen, and stored in liquid nitrogen. Immunostaining on paraffin sections (4 μm) and cryostat sections (6 μm), the latter air-dried and fixed in acetone, followed by periodate-lysine-paraformaldehyde as previously described [8], was performed using the indirect immunoperoxidase method with both B- and T-cell primary antibodies. Peroxidase-labelled second antisera were obtained from Dakopatts UK Ltd.

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III. Molecular Studies

DNA was extracted from the freshly frozen biopsy material by standard techniques [9]. Ten micrograms of DNA was digested with an appropriate restriction enzyme (Boehringer Mannheim, Federal Republic of Germany) and the fragments were studied by the Southern blot hybridization method [10] using Hybond-N filters (Amersham International PLC, Amersham, England). DNA probes were radiolabelled with the (32p)-dCTP random primer extension method [11]. Immunoglobulin heavy chain (JH) [12], pFL1 (kindly provided by Dr. M.L. Cleary) [13], and β-T-cell receptor gene (TCR) [14] probes were hybridized [15] and autoradiographed at $-70°$ C.

C. Results

I. Histology

The histological features of the first biopsy from the left neck were normal nodal architecture effaced by an infiltrate of malignant lymphoma with a nodular architecture composed of an admixture of centroblasts and centrocytes. The appearance was that of centroblastic/centrocytic follicular lymphoma. The second biopsy, taken from the right groin, had a completely different appearance. The nodal architecture was effaced by a diffuse infiltrate of small and medium-sized lymphoid cells. Plasma cells and occasional eosinophils were present, and arborizing high endothelial venules were

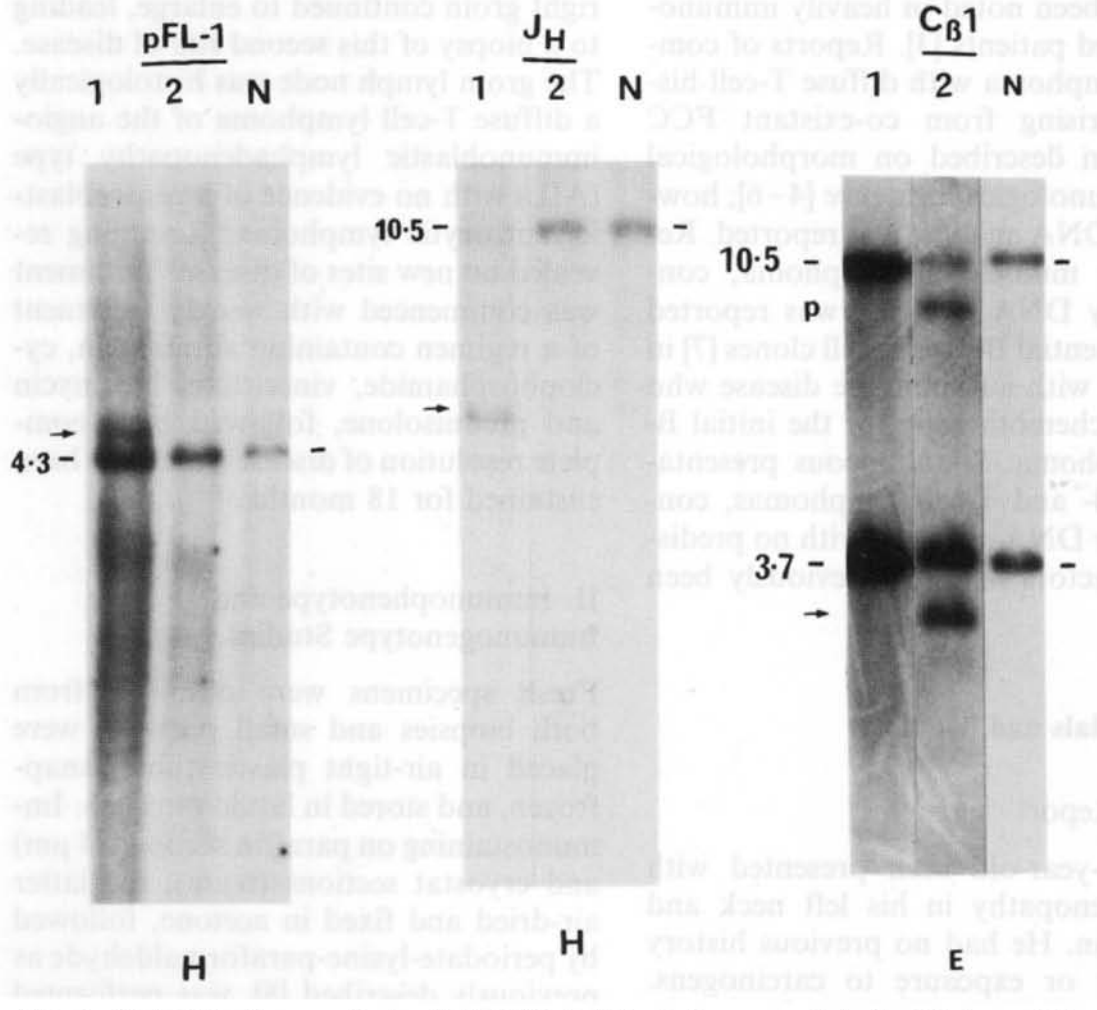

Fig. 1. Hybridization analysis of t(14;18) (*pFL · 1*), immunoglobulin (J_H), and T-cell receptor gene (C_β *1*) DNA. The probes used are indicated above each panel and the appropriate restriction enzyme used is shown below each panel. (*H*, *Hind*III, *E*, *Eco*RI). *Dashes* indicate germ line and *arrow* indicates clonal rearranged bands. Size makers are indicated in kilobases. (*P* represents in *Eco*RI site partly resistant to normal digestion; however, this is not a generalised partial digestion)

prominent. There was no evidence of centroblastic/centrocytic lymphoma, and a diagnosis of probable T-cell lymphoma of the AIL type was made.

II. Immunohistochemistry

The neoplastic cells seen in biopsy one (left neck) showed strong immunoreactivity with CD45, L26, MB1, MB2 and LN2 in paraffin section. Scattered cells with MT1 and UCHL1 immunoreactivity were present in the interfollicular areas, with occasional cells in the B-cell nodules. Cryostat section immunostaining showed an identical pattern using a large panel of antibodies. Kappa light-chain restriction was demonstrated. Staining with the antibody R4/23 (which recognises an antigen expressed on dendritic reticulum cells) accentuated the nodular pattern. In contrast, in biopsy two (right groin) occasional small clusters of lymphocytes with immunoreactivity with B-cell markers (L26, MB1, MB2) were seen, but the predominant population was T-lymphocytes (MT1, UCHL1-positive). Again, this pattern was repeated in the cryostat section immunostaining. The plasma cells and B-lymphocytes present were polyclonal. The pattern of staining with R4/23 seen in the first biopsy was not present, with only a few scattered cells staining.

III. Molecular Studies

Biopsy one showed immunoglobulin gene rearrangement with a clonal rearranged band, with the JH and pFL1 probes (Fig. 1) confirming the presence of a clonal B-cell neoplasm and the t(14:18) rearrangement found in the majority of follicular lymphomas (16,6). No gene rearrangement was seen with the TCR probe. Biopsy two confirmed the T-cell clonality by revealing a rearranged band with the TCR probe (Fig. 1). However, there was no rearrangement seen with the JH and pFL1 probes.

D. Discussion

This case revealed histological and immunophenotypical non-Hodgkin's lymphoma (NHL) with simultaneously presenting lymph nodes (LN) with both B- and T-cell clones. DNA analyses confirmed a clonal B-cell population in one LN together with a t(14:18) translocation (involving the BCL-2 gene) and a clonal T-cell population in the other LN. This has not previously been reported in a patient with no predisposing factors. This could be ascribed to independent development of two types of lymphoma. However, in view of previous evidence for multiclonal disease arising from FCC [1, 2, 4, 5, 7] this may not be the only possibility. The t(14:18) translocation was present in the FCC lymphoma but not in the T-cell lymphoma, suggesting that this translocation, present in nearly all FCC [16], may be a causative factor in the development of this histological type of disease. Subsequent or simultaneous development of diffuse histological types of lymphoma is reported to be associated with increasing absence of this translocation [17] and has been thought to have been lost with transformation of the lymphoma, and in addition is often associated with development of a second B-cell clone [1, 2]. A possible explanation for multiclonal lymphoma is the emergence from a malignant clone of pre-B-lymphocytes prior to B- or T-cell lineage commitment with the ability to develop into clonal B- or T-cell lymphoma. The presence of the BCL-2 gene rearrangement may be an indicator of a defective pre-B-lymphocyte and could have a bearing on the resistance of FCC to curative treatment [18, 19].

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