Virchows Archiv A Pathol Anat Histopathol (1988) 412:241–245

Virchows Archiv A
Pathological Anatomy and Histopathology

Lymph node involvement in chronic neutrophilic leukemia

An immunohistochemical study

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Summary. An immunohistochemical study was performed on autopsy material from a patient with chronic neutrophilic leukaemia (CNL) using antibodies against various cell lineage-related antigens. Proliferation of immature neutrophils with occasional clusters of erythroblasts and megakaryocytes were noted in the retroperiotoneal lymph nodes, spleen, and kidneys as well as in the bone marrow. Predominance of immature neutrophils in the lymph nodes suggested the emergence of a blast crisis, although there was no increase of blasts in the peripheral blood. Since immature myeloid cells are difficult to distinguish from malignant lymphoid cells on tissue sections, we suggest that immunohistochemical identification of cell lineage-related molecules on these cells is necessary for the more accurate interpretation of lymph node lesions in myeloid neoplasms.

Key words: Chronic neutrophilic leukemia – Lymph node – Immunohistochemistry – Immature neutrophil

Introduction

Chronic neutrophilic leukemia (CNL) is a rare haematological disorder characterized by distinct clinical and laboratory features (Shindo et al. 1977; You and Weisbrot 1979; Dotten et al. 1982; Feremans et al. 1983). Lymph node involvement has been reported in a few CNL patients (You and Weisbrot 1979; Dotten et al. 1982; Feremans et al. 1983), but its histopathological features have not been fully described.

We report here an autopsy case of CNL in which retroperitoneal lymphadenopathy was noted. An immunohistochemical study was performed on the lymph nodes, and the significance of the features is discussed.

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Case report

A 51-year-old Japanese man was seen for the evaluation of leukocytosis in January 1985. Body temperature was 36.5° C. The liver, spleen, and peripheral lymph nodes were not palpable. Haemoglobin was 10.1 g/dl and platelet count was 114×10^9/l. Leukocyte count was 53.3×10^9/l with 4% myelocytes, 4% metamyelocytes, 75% mature neutrophils, 12% monocytes, and 4% lymphocytes. Eosinophils and basophils were rarely seen. Neutrophil alkaline phosphatase (AlP) score was 472/100 with 98% positive cells. Bone marrow aspiration showed a nucleated cell count of 561×10^9/l with M/E ratio of 4.73. Differential counts of granulocytic series were; 0.5% myeloblasts, 3.9% promyelocytes, 7.8% myelocytes, 9.5% metamyelocytes, 58.3% mature neutrophils, 0.6% eosinophils, and 0.3% basophils. Serum levels of LDH, AlP, uric acid, and vitamin B_{12} were 910 U/l (referential value: 220–640), 1,010 U/l (100–280), 410 μ mol/l (220–410), and 9,033 ng/l (200–1,000), respectively. Colony-stimulating activity (CSA) in the serum and urine was examined by one of the authors (H.H.) according to Burgess et al. (1977) with some modifications. It was found that there was no increase of CSA when compared with that of normal individuals. Chromosomal analyses of bone marrow and peripheral blood cells were performed twice and they gave normal karyotype with no Philadelphia chromosome (Ph^1).

Hepatosplenomegaly developed during his clinical course, and leukocyte count was elevated to 176.0×10^9/l (71% mature neutrophils). The patient died of intraperitoneal bleeding due to splenic rupture in May 1985.

At autopsy, 3,200 ml of blood was obtained from the peritoneal cavity. The spleen (1,500 g) showed a broad area of necrosis and destruction. The liver weighed 2,060 g. There was multiple swelling of the retroperitoneal lymph nodes up to 3 cm in diameter.

Methods

Tissues obtained at autopsy were either routinely fixed in 10% formalin or snap-frozen at −70° C. Imprint preparations of the spleen and retroperitoneal lymph nodes were stained with May-Grünwald & Giemsa. Paraffin sections were stained with

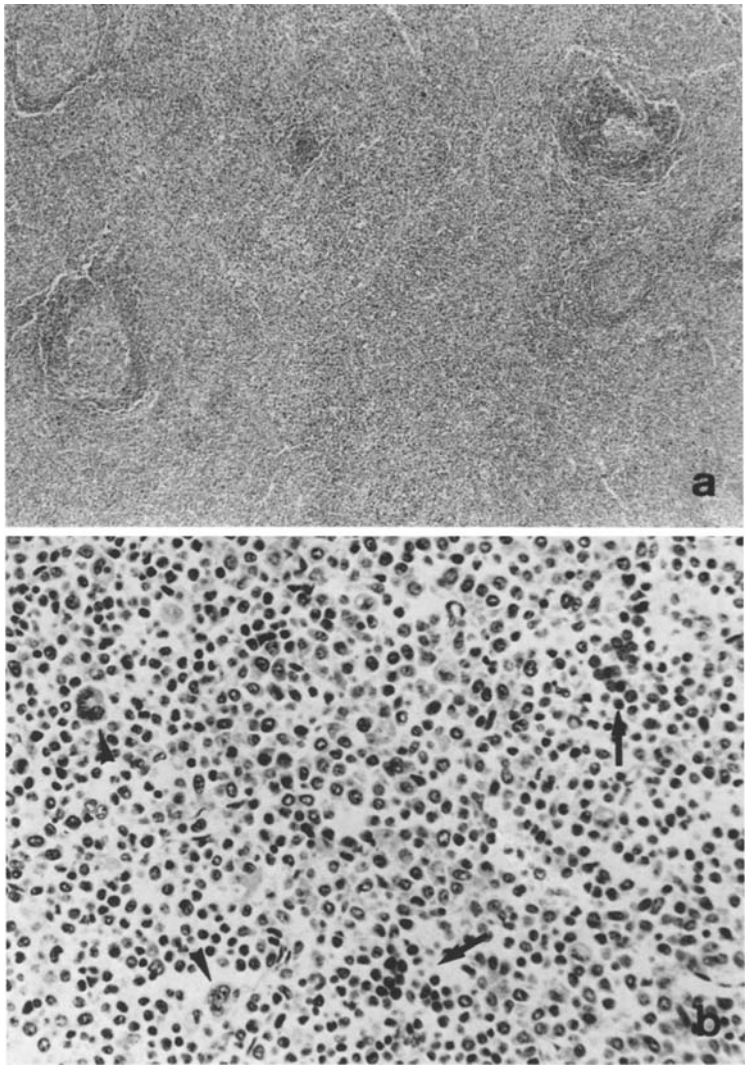

Fig. 1. Structure of the retroperitoneal lymph node. **a** The interfollicular areas are expanded and infiltrated by heterologous population of cells. Several residual lymph follicles with germinal centers are present. **b** At this higher magnification, infiltrates composed of numerous cells with oval or indented nuclei and moderate amount of cytoplasm (large mononuclear cells), round cells forming clusters (*arrows*), and giant cells with lobulated nuclei (*arrowheads*) can be seen. HE (**a** ×34, **b** ×255)

haematoxylin and eosin (HE), periodic acid-Schiff (PAS), silver reticulin, toluidine blue, and Biebrich scarlet (Luna 1968). The frozen sections of the spleen and lymph nodes were examined for peroxidase and naphthol AS-D chloroacetate esterase (NCE) activities.

Immunoperoxidase staining was performed on paraffin sections by the avidin-biotin-peroxidase complex (ABC) method (Hsu et al. 1981) using biotinylated secondary antibodies and ABC (Vector Laboratories, CA, USA). The primary reagents used were: antibodies to erythrocyte membrane antigen and haemoglobin (DAKOPATTS, Glostrup, Denmark) for erythocytic lineage, those to lysozyme (Behringwerke, Marburg, FRG), non-specific cross-reacting antigen (NCA) (DAKOPATTS, Glostrup, Denmark), and Leu M1 antigen (Becton-Dickinson, CA, USA) for granulomonocytic lineage, and an antibody to factor VIII-related antigen (Behringwerke, Marburg, FRG) for megakaryocytic lineage. Antibodies to Leu 7 antigen (Becton-Dickinson, CA, USA), leukocyte common antigen (LCA), S-100 protein, each heavy and light chain of immunoglobulin molecules (DAKOPATTS, Glostrup, Denmark), and J-chain of immunoglobulin molecules (Nordic Immunological Laboratories, Tilburg, The Netherlands) were also used.

Results

Retroperitoneal lymph nodes, bone marrow, spleen, and kidneys were infiltrated by heterologous cells, composed of numerous cells with oval or indented nuclei and moderate amount of cytoplasm (large mononuclear cells), occasional clusters of round cells with hyperchromatic nuclei and scant amount of cytoplasm, and giant cells with lobulated nuclei and abundant cytoplasm (Fig. 1). Significant numbers of mature neutrophils were present in the latter three organs but they were rarely seen in the lymph nodes. Bone marrow was totally replaced by the infiltrates, while there were some residual lymph follicles with germinal centers in the lymph nodes (Fig. 1a) and white pulp in the spleen. Large mononuclear cells and mature neutrophils also infiltrated the liver, lungs, adrenals, and cerebrum.

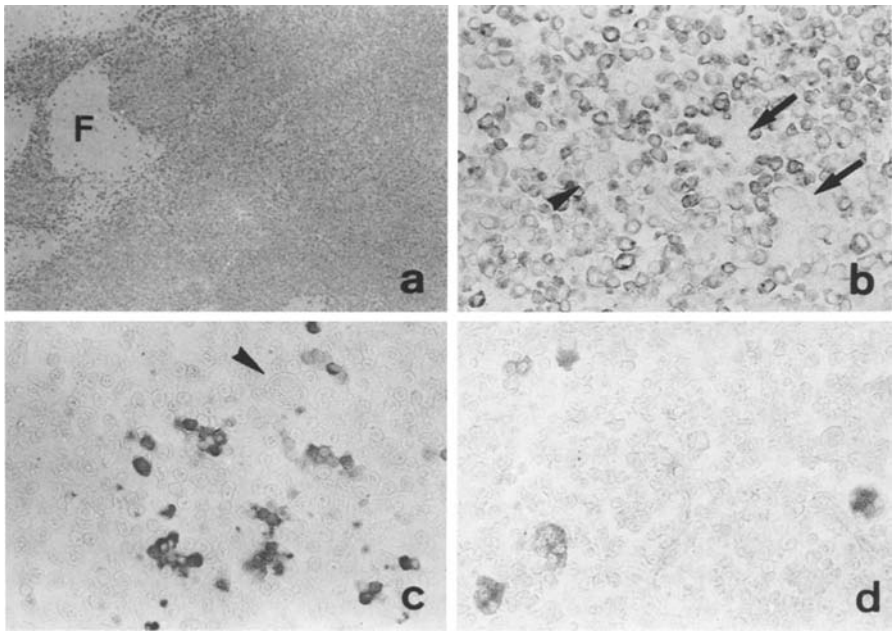

Fig. 2. Immunohistochemistry of the retroperitoneal lymph node. **a** Numerous Leu M1-positive cells are present in the interfollicular areas. Several lymph follicles (*F*) resisted staining with anti-Leu M1, resulting in a moth-eaten appearance (see Fig. 2a). **b** High-power view of Fig. 1a. Leu M1-positive cells are found to correspond to the large mononuclear cells seen in the HE-stained sections (see Fig. 1b). Clusters of blastoid cells (*arrows*) and a giant cell (*arrowhead*) resisted staining with this antibody. **c** Some haemoglobin-positive cells are nucleated and arranged in clusters. Numerous large mononuclear cells and a giant cell (*arrowhead*) resisted staining with anti-haemoglobin. **d** Giant cells with lobulated nuclei positive for anti-factor VIII-related antigen are present among the unstained large mononuclear cells. Immunoperoxidase stain with methyl green counterstain. (**a** × 25.5, **b–d** × 255)

Mature neutrophils and giant cells were stained positively with PAS. Silver impregnation showed scant reticulin fibers in the bone marrow. Eosinophils and basophils were rarely seen in imprint preparations and Luna- or toluidine blue-stained sections. On frozen sections, the large mononuclear cells showed both peroxidase and NCE activities.

Sections stained with antibodies against lysozyme, NCA, and Leu M1 antigen showed similar patterns of staining. These antibodies reacted positively with the large mononuclear cells and mature neutrophils. In the lymph nodes and spleen sections, residual lymphoid elements clearly appeared as non-stained areas giving a moth-eaten appearance (Fig. 2a). Clusters of blastoid cells and giant cells resisted staining with these antibodies (Fig. 2b). Antibodies against haemoglobin and erythrocyte membrane antigen stained erythrocytes and clusters of nucleated round cells (Fig. 2c). Factor VIII-related antigen was present in the giant cells and vascular endothelial cells (Fig. 2d). These cells resisted staining with antibodies against LCA, Leu 7 antigen, S-100 protein, and immunoglobulin molecules. LCA was present on the residual lymphoid cells in the spleen and lymph nodes. Cells having S-100 protein or Leu 7 antigen were found in the residual lymph follicles and paracortical areas.

Discussion

In the present case, clinical diagnosis of CNL was established on the basis of the following fingings; hepatosplenomegaly, absence of fever, severe sustained mature neutrophilic leukocytosis with elevated AlP activity, absence of Ph^1, increased serum vitamin B_{12}, and no increase of serum or urinary CSA. Autopsy provided anatomical evidence of multiple organ infiltration, which must be added to the clinical criteria for the diagnosis of CNL to rule out a leukaemoid reaction (You and Weisbrot 1979). In addition to these findings, cytological and histochemical examinations showed rare eosinophils and basophils in the involved organs and peripheral blood. Since these are not features in chronic myeloid leukemia (CML) including a Ph^1-negative form (Canellos et al. 1976), clinical diagnosis of CNL was histopathologically confirmed.

Infiltration of the neoplastic cells into the spleen and/or liver is a common finding in CNL, but lymph node involvement has been reported in only four patients (You and Weisbrot 1979; Dotten et al. 1982; Feremans et al. 1983) and its histopathological features have not been fully described. In the lymph nodes of our case, large mononuclear cells, clusters of round cells, and giant cells infiltrated among the residual lymph follicles. Large mononuclear cells, which could not be characterized by conventional morphology, were confirmed to be immature neutrophils because of the presence of peroxidase, NCE (Yam et al. 1971), lysozyme (Pinkus and Said 1977), NCA (Burtin et al. 1975), Leu M1 antigen (Hanjan et al. 1982), and the absence of eosinophilic or basophilic granules. LCA was negative in these cells, and this is a feature of granulocytic cells, but not lymphocytes, in formalin-fixed sections (Kurtin and Pinkus 1985). Round cells forming clusters were identified as erythroblasts by the presence of erythrocyte membrane antigen and haemoglobin (Pinkus and Said 1981). Giant cells with lobulated nuclei, which were positive for PAS and factor VIII-related antigen, were identified as megakaryocytes (Innes et al. 1982). Though there was no fibrosis in the bone marrow, these features are characterized as 'myeloid metaplasia' irrespective of reactive or clonal process. 'Myeloid metaplasia' has already been reported in some patients with CNL, but the studies relied solely on conventional morphology (You and Weisbrot 1979). To the best of our knowledge, this is the first CNL case in which detailed histopathological features and immunohistochemical identification of 'myeloid metaplasia' are described.

Because of the difficulty in distinction between immature granulocytic cells and neoplastic lymphoid cells on tissue sections, lymph node lesions in CML have been misinterpreted as second malignancies (usually lymphomas) in the past. Although this confusion can be resolved by either imprint preparations or identification of eosinophil precursors on the tissue sections (Garfinkel and Bennett 1969; Pascoe 1970), examination of cell lineage-related molecules on these cells seems to provide more accurate information. This was especially valuable in our CNL case, because eosinophil or basophil precursors were rarely present. Therefore, it is stressed that immunohistochemical examinations using antibodies to various cells lineage-related antigens should be performed on the lymph node lesions occurring in the myeloid neoplasms. The procedure can be done retrospectively, since all of the antibodies used in the present study are applicable to formalin-fixed, paraffin-embedded materials.

Another interesting finding in our patient is the lymph node infiltration predominantly by **immature** neutrophils in the presence of a sustained **mature** neutrophilic leukocytosis. In most reported cases, mature neutrophils are the predominant cell type in both the peripheral blood and tissues (You and Weisbrot 1979; Dotten et al. 1982; Feremans et al. 1983). The difference in maturity of neutrophils in our case may be explained by blast crisis, which has been reported in a CNL case (Shindo et al. 1977). Although there was no increase of blasts in the peripheral blood of our case, lymph node enlargement preceding the blast crisis is well-known to occur and lymph nodes have been suggested to be the most common extramedullary sites of blast crisis in CML (Garfinkel and Bennett 1969; Boggs 1976). Another less feasible explanation is that the immature neoplastic neutrophils underwent differentiation as well as proliferation and only the mature forms appeared in the peripheral blood.

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Accepted October 2, 1987