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CASE REPORT

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Utility of immunophenotypic and immunogenotypic analysis in the study of necrotic lymph nodes

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Abstract We report a case of complete lymph node necrosis. No specific aetiology could be determined by morphology, but a B lymphoid population and clonal rearrangement of the immunoglobulin heavy chain gene were demonstrated in immunophenotypic and immunogenotypic studies performed using DNA extracted from paraffin embedded necrotic tissue. In the setting of lymph node necrosis, we suggest that immunohistochemical and gene rearrangement studies may provide additional diagnostic information.

Key words Immunohistochemistry · Gene rearrangement · Lymph node necrosis · Malignant lymphoma

Introduction

Total lymph node necrosis is unusual [2] and has been associated mainly with vasculitis, vascular thrombosis, mechanical vascular obstruction, systemic lupus erythematosis, nonreactive tuberculosis, and tumour [7]. Several reports have stressed the association of total lymph node necrosis with the presence of malignant lymphoma [1]. A similar histological appearance may occur following irradiation and/or chemotherapy for lymphomas and metastases. Thus the histopathologist has a duty to make a careful assessment of any infarcted lymph node, taking multiple blocks of tissue to exclude the possibility of lymphoma or metastases. In this setting there is not enough information about the role of additional techniques that might aid in obtaining a reliable diagnosis.

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Preservation of antigenicity in cells showing the histological features of coagulative necrosis has been reported [8, 10,11]. Immunohistochemistry can provide information complementary to that yielded by conventional histology; this is valuable in the evaluation of the infarcted lymph nodes and may be applied to necrotic tissue in general [8]. In such situations immunoperoxidase studies may yet be of value in establishing the basic histogenesis of the process. They may provide insight into the underlying lymphoid architecture and the cell size of any neoplastic proliferation. Almost all antibodies with a surface membrane pattern of binding have showed consistent reactivity in infarcted tissue (CD45, UCHL-1, MB1, MB2) [8]. However, antibodies to plasma constituents are worthless in the assessment of infarcted tissue, owing to nonspecific uptake by the cells via their damaged membranes [4, 8].

Immunoglobulin and T-cell receptor beta chain gene rearrangement studies have proven to be useful adjuvant procedures in the evaluation of lymphoid proliferations [6]. However, the value of these techniques when applied to DNA extracted from paraffin-embedded necrotic tissue has not been clearly addressed.

We report a case of a totally necrotic lymph node on which a diagnosis of malignant lymphoma was made on the basis of immunohistochemical and molecular methods. We discusss the utility and limitations of these techniques in the setting of necrotic samples.

Clinical history

A 62-year-old woman presented with a enlarged mass in the left side of her neck. The lesion had first been noticed approximately 3 months before, but had grown more rapidly during the last month. In another hospital, fine needle aspiration cytology of the enlarged lymph node was performed, which revealed a lymphoid population suggestive of lymphoma. On the basis of this, a chemotherapy course was proposed. However, after receiving only one cycle of chemotherapy the patient decided to seek a second opinion and came to our hospital. A CT scan showed several enlarged cervical nodes, the largest measuring 2 cm in diameter. A lymph node biopsy was performed to confirm a diagnosis on which oncologists

could base the treatment. No other enlarged nodes were found. The liver and spleen were normal. Physical examination was unremarkable.

Materials and methods

The biopsy specimen was routinely fixed in buffered formalin, sectioned at 5 μm, and stained with haematoxylin and eosin for morphological studies. For immunohistochemistry, deparaffinized, rehydrated sections were treated with 3% H_2O_2 in methanol for 30 min at room temperature to abolish endogenous peroxidase activity. Tissue sections were treated with trypsin (Sigma Chemical, St Louis, Mo.) for 10 min at 37° C and microwaved. After overnight incubation at 4° C with the primary antibody, slides were washed in phosphate-buffered saline (PBS) and then exposed to the biotinylated secondary antibody diluted at 1:100 in PBS for 1 h at room temperature. They were then washed in PBS for 10 min and incubated for 60 min with the streptavidin–biotin–peroxidase complex (1/100; Biogenex) at room temperature. Using these techniques sections were reacted with antibodies against CLA (CD45, Biogenex), UCHL-1 (CD45RO, Biogenex), CD20 (Dako), CD43 (Biogenex), CD3 (Zymed), MB2 (Biogenex), vimentin (Novocastra) and keratins (Biogenex). The slides were counterstained with Harris's haematoxylin, dehydrated, cleared and mounted.

For immunogenotypic studies, DNA was extracted from paraffin blocks as follows: 10 μm sections were transferred to 1.5-ml microcentrifuge tubes. Paraffin was removed from each sample tissue by extraction twice with 1000 μl of xylene each time. The cell pellet was washed twice with 500 μl of absolute ethanol and dried. Samples were incubated overnight at 50° C in a buffer containing 10 mM Tris-HCL (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% (wt/vol) gelatin, 0.5% Tween-80, and 0.1 mg/ml of proteinase K (Sigma, St Louis, Mo.). After boiling for 10 min to destroy remaining proteinase activity, samples were centrifuged and the supernatants were used in the PCR reaction.

PCR was performed using a seminested procedure as previously described [6]. In the first PCR round, a "consensus" primer to the third framework (FR-III) region of the V_H genes was used with a "consensus" J_H primer. In the nested round, the same V_H primer was used with a consensus J_H primer, internal to the J_H used for the first round. Positive controls consisted of DNA extracted from the Namalwa B cell line diluted to 10^{-2} and 10^{-3} in DNA from the erythroleukaemia cell line K562. Negative controls consisted of sterile water. After PCR amplification ended 15 μl of the PCR products were electrophoresed in a 8% polyacrylamide gel and visualized with UV light after ethidium- bromide staining.

Pathological findings

Histological examination of the lymph node biopsy specimen showed massive necrosis of the lymph node and surrounding a thin zone of granulation tissue and fibroadipose tissue (Fig. 1). The node was represented by a confluent sheet of eosinophilic necrotic material with scattered pyknotic nuclei and areas showing the silhouettes of necrotic lymphocytes. The number of inflammatory cell infiltrates within the node was minimal. Granulomatous infiltrates, vascular thromboses, vasculitis and nuclear karyorrhexis were not seem.

The infarcted cells had a strong surface membrane reactivity with CLA (CD45), MB2, and CD20 (Fig. 2). The positive stain was stronger in the periphery than in the centre of the lymph node. Many of the more peripheral cells showed MT1 (CD43) expression (Fig. 2). CD3 and UCHTL-1 were uniformly negative in the necrotic cells,

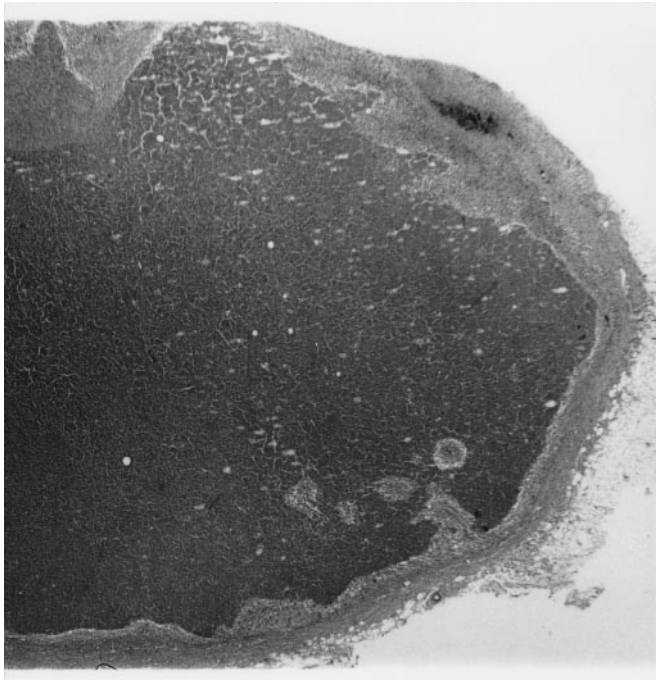

Fig. 1 Low-power view demonstrating total necrosis of lymph node. Only a narrow rim of fibrous granulation tissue survives. HE, ×10

and only small scattered viable reactive cells were stained by both antibodies. Vimentin and keratins were negative. No background staining was present in the infarcted tissue with any of these antibodies. These immunophenotypic data suggested a diagnosis of lymphoma.

Gene rearrangement analysis showed a unique band in the range of 90–120 bp indicating a B monoclonal population (Fig. 3). These findings show that, despite the necrosis,a sufficient amount of DNA was preserved for successful immunogenotypic analysis to be possible.

Discussion

Total necrosis of superficial lymph nodes is an uncommon event. Initial reports of spontaneous lymph node infarction suggested vasculitis, vascular thrombosis, or mechanical vascular obstruction as possible aetiologies [2, 3, 7]. Later, a large series of infarcted lymph nodes was reported from the M.D. Anderson Hospital and Tumor Institute in Texas [1]. This series indicated an extremely ominous association between lymph node infarction and subsequent lymphoma (13/16; 80%). The majority of the lymph nodes were located in the head and neck region, and the most frequent histological finding was that of a diffuse large cell lymphoma (6/13 cases; 46%). In 1986, a multicentre study of 51 cases of lymph node infarction was conducted in order to assess the prognostic implications of the condition [7]. In this study, the overall incidence of lymphoma was approximately 40% (20/51 cases, and they were more evenly distributed between small and large cell lymphoid type.

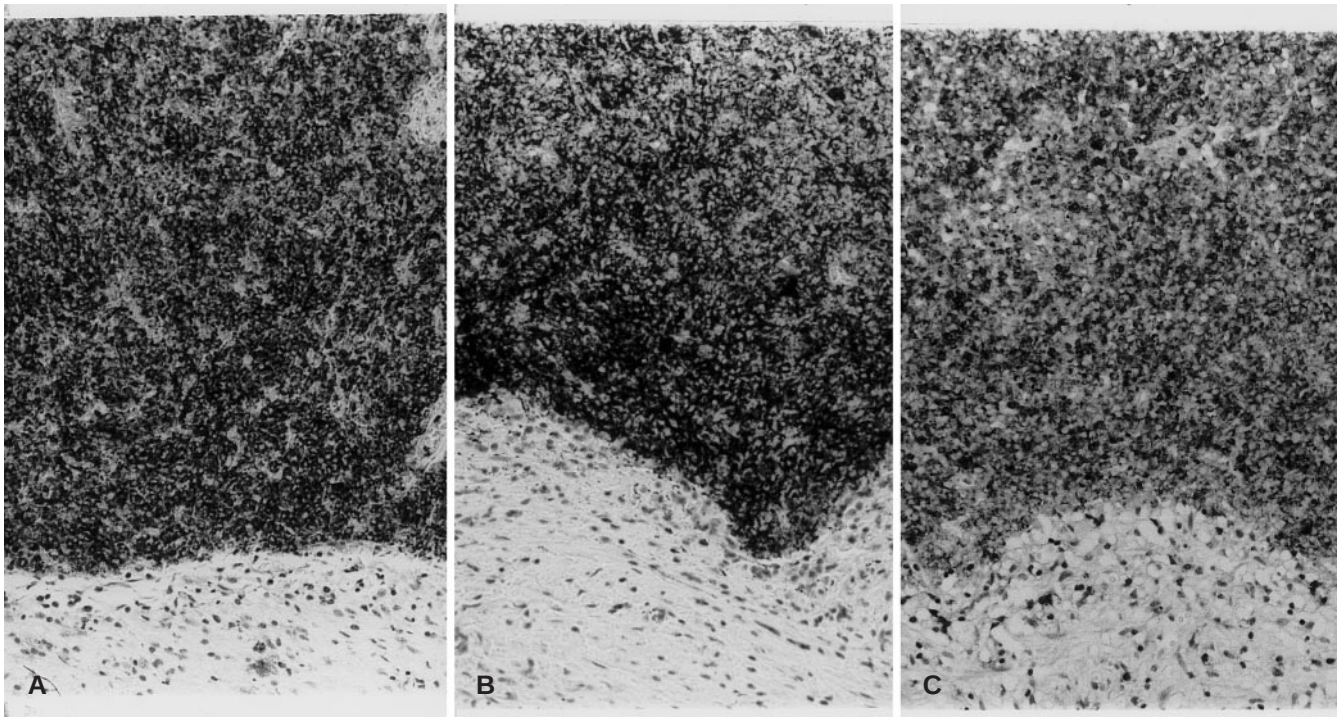

Fig. 2A–C Immunoperoxidase staining of necrotic tissue. A strong surface membrane reactivity with **A** CLA (CD45), and **B** CD 20. **C** Many of the more peripheral cells showed MT1 (CD43) expression. Immunoperoxidase, ×200

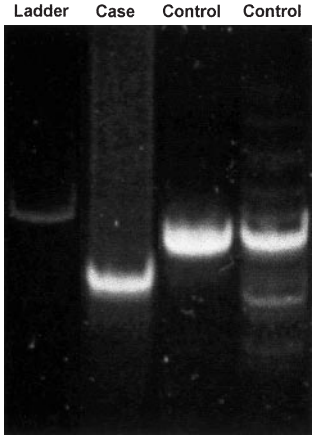

Fig. 3 IgH chain rearrangements in necrotic paraffin tisue. *Lane 1* 100-kb molecular weight marker, *lane 2* case, *lane 3* positive control (Namalwa 10^{-2}), *lane 4* positive control (Namalwa 10^{-3})

A similar histological appearance of lymph node infarction may occur following irradiation and/or chemotherapy for lymphomas or metastases. The differential diagnosis of lymph node infarction includes necrotizing lymphadenitis (Kikuchi's lymphadenitis, the mucocutaneous lymph node syndrome (Kawasaki's syndrome), and necrotic malignant tumours. The necrosis of necrotizing lymphadenitis is different: it is usually focal, and nuclear karyorrhexis, fibrin deposits and collections of large mononuclear cells are present. In the mucocutaneous lymph node syndrome the lymph nodes may show fibrin thrombi in the smaller vessels, accompanied by patchy infarcts [12]. Metastatic tumours to lymph nodes, particularly from melanoma, can undergo spontaneous necrosis in the absence of prior therapy. Therefore, thorough examination of the lymph node is mandatory, and multiple blocks of tissue must be taken to exclude the possibility of lymphoma or metastases.

In our case no viable tissue was found even though the whole lymph node was studied, and we believe the necrosis was caused by chemotherapy. There was definitive diagnosis, and we therefore applied a wide panel of antibodies to determine the nature of the necrotic cells. We also performed PCR to look for immunoglobulin gene rearrangement. On the basis of our results we made a diagnosis of diffuse B cell lymphoma, probably of small cell type.

Immunophenotypic and immunogenotype studies have a potential application in the evaluation of necrotic lymph nodes. Preservation of antigenicity in lymphoid cells showing the histological features of coagulative necrosis was demonstrated by Pallesen and Knudsen [10]. They have shown that in cryostat sections specific immunoreactivity with a wide range of leucocyte antigens is retained for up to 72 h post mortem [10]. Norton et al., in their study, have reported the contribution of immunostaining using monoclonal antibodies to fixation-resistant leucocyte antigens in making the diagnosis of lymphoma in the presence of coagulative necrosis of the entire lymph node [8]. The antibodies that showed the most consistent reactivity in infarcted tissue were CD45, CE45R, UCHL1, MB1, MT1, MT2 and LN1. The surface membrane pattern of binding of these antibodies provided an easy method of assessing the specificity of immunohistology in the infarcted tissue. CD30, L26, anti-alpha-1-antitrypsin, immunoglobulins and anti-factor VIII were of littel value in the infarcted material because

they led to diffuse, unstructured, and nonspecific staining of the infarcted tissue [8]. Thus, antibodies to plasma constituents are worthless in the assessment of infarcted tissue. However, the interpretation of immunophenotypic studies may be limited by nonspecific staining and a lack of definite cell surface light chain restriction.

Laszewski et al. have reported a necrotic lymph node in which a clonal rearrangement of the immunoglobulin gene was demonstrated by Southern blot hybridization of DNA extracted from the necrotic tissue [5]. There is only one report addressing the utility of PCR techniques as an adjuvent diagnostic aid in necrotic tissue [9]. In our case a clonal rearrangement of the immunoglobulin gene was demonstrated with the aid of a seminested PCR applied to DNA from paraffin-embedded tissue. We assume that DNA is partially degraded both by necrosis and by the fixation and paraffin-embedding procedures, but a unique band was observed in the range of 90–120 bp. In this case, it is quite likely that DNA fragmentation owing to necrosis is not enough as to make the tissue unusable for PCR analysis. It is possible that when lymph node infarction has happened longer before testing DNA fragmenation could be so severe as to make PCR studies useless.

This report illustrates the potential application and usefulness of immunohistochemistry and gene rearrangement analysis in the evaluation of necrotic lymph nodes.

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