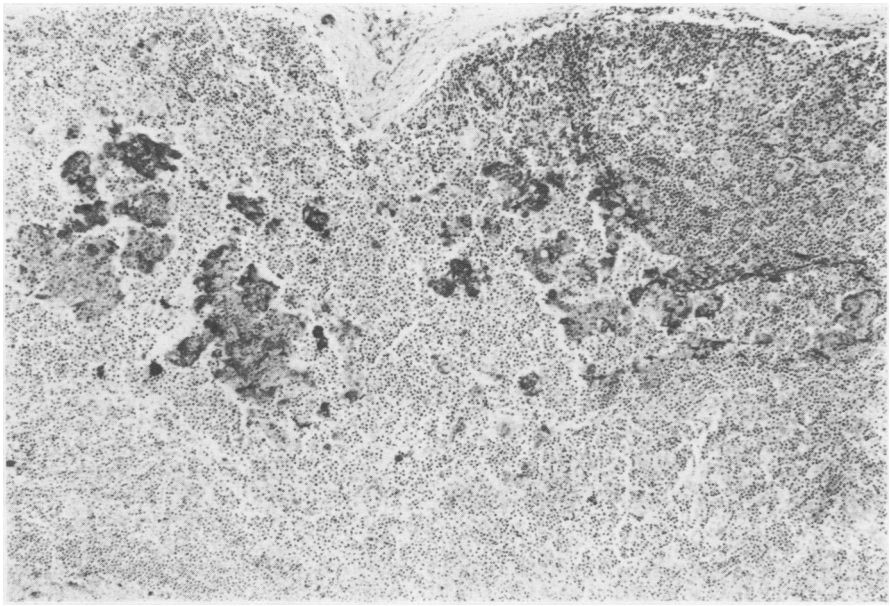

Fig 2 *Advanced stage of centrorrhexis.*

*Lymph node sections from patient with persistent generalised lymphadenopathy immunostained for acid cysteine proteinase inhibitor (peroxidase-antiperoxidase, haematoxylin.) × 120.*

describing the above events as it covers the essential features of this peculiar phenomenon.

Centrorrhexis is important as: DRC may represent a reservoir of HTLV-III (HIV)[6]; the extent of it may be of prognostic importance in patients infected with (HIV)[1]; and it is found in some other disorders, including *Toxoplasma gondii* infection,[4] the acute phase of which[10] shares with persistent generalised lymphadenopathy, AIDS related complex, and AIDS, the phenomenon of a reversed helper:suppressor cell ratio for peripheral blood lymphocytes. Appreciation of centrorrhexis may shed new light on the interplay between events taking place in organised lymphoid tissue and subsets of circulating lymphocytes.

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## Immunoreactivity of Reed Sternberg cells in paraffin and frozen sections

Recently much attention has been focused on the reactivity of the Reed Sternberg cell and its morphological variants with the monoclonal antibody Leu M1.[1–4] This can be detected both in paraffin and frozen sections using standard immunohistochemical techniques. In the series reported[1–4] Reed Sternberg cells reacted with Leu M1 in most cases of Hodgkin's disease in all histological subgroups with the exception of the nodular variant of lymphocyte predominant Hodgkin's disease. This was taken to support the

Table 1 *Reactivity of non-Hodgkin's lymphoma with Leu M1*

| | |
|---|---|
| **B cell lymphomas** | |
| Lymphocytic | 1/8 |
| Lymphoplasmacytic | 0/1 |
| Centrocytic-centroblastic follicular | 0/9 |
| Centrocytic-centroblastic diffuse | 0/3 |
| Centroblastic/immunoblastic | 0/18 |
| Lymphoblastic | 0/2 |
| Hairy cell leukaemia | 0/1 |
| **T cell lymphomas** | |
| Lymphocytic | 0/1 |
| Cutaneous | 1/2 |
| Pleomorphic | 0/2 |
| Immunoblastic | 2/3 |
| Lymphoblastic | 0/2 |
| **Histiocytic** | |
| Histiocytic sarcoma | 0/1 |
| Malignant histiocytosis of the intestine | 0/4 |
| **Unclassifiable** | |
| Large cell (non T non B) | 0/2 |
| Lymphoblastic | 0/1 |

hypothesis that this variant of Hodgkin's disease is histogenetically distinct, being associated with the B zone of the lymph node and, particularly, with progressively transformed germinal centres.[5 6] The reported expression of the leucocyte common antigen and epithelial membrane antigen by Reed Sternberg cells only in this group[3 7] further supports this hypothesis.

To evaluate the diagnostic potential of Leu M1 and to further examine the above hypothesis we performed a retrospective immunohistochemical study on 60 cases of Hodgkin's disease and 60 non-Hodgkin's lymphomas. All of the material was fixed in 4% buffered formaldehyde and embedded in paraffin. Immunostaining was carried out using Leu M1, antiepithelial membrane antigen, and antileucocyte common antibodies as primary antibodies in a two stage immunoperoxidase technique.[8] The 60 cases of non-Hodgkin's lymphoma comprised a wide variety of histological types, and each had been fully phenotyped on frozen section. Frozen material was also available from 28 of the cases of Hodgkin's disease, and this was also stained with an immunoperoxidase technique, using Ki1, pan B (B1), and pan T (Leu4) cell antibodies in addition to the antibodies used in paraffin section.

Table 1 summarises the reactivity of the non-Hodgkin's lymphomas. Four of the 60 cases reacted with Leu M1; one diffuse B cell lymphocytic lymphoma and three T cell lymphomas (one cutaneous and two immunoblastic).

Table 2 summarises the results for the cases of Hodgkin's disease in paraffin sections. Reed Sternberg cells and mononuclear Hodgkin's cells showed paranuclear (Golgi) and membrane binding of Leu M1 in most (52 of 60) of cases of Hodgkin's disease, including five of the nine cases of nodular lymphocyte predominant Hodgkin's disease. Three of these cases also expressed the leucocyte common antigen. Expression of the leucocyte common antigen was not restricted to the Reed Sternberg cells in nodular lymphocyte predominant Hodgkin's disease but was detected in 21 of the 51 cases in the remaining subgroups. Reed Sternberg cells reacted with antiepithelial membrane antigen in only five of the 60 cases, again this reaction was not restricted to nodular lymphocyte predominant Hodgkin's disease.

In frozen sections Leu M1 again reacted with Reed Sternberg cells in the most cases (table 3); Reed Sternberg cells in a smaller number of cases reacted with Ki1. There was no staining of Reed Sternberg cells using pan B or pan T markers, while in 11 cases from all subtypes staining occurred using antiepithelial membrane antigen, which had not been apparent in the paraffin embedded material.

Of the cases in which frozen material was available for comparison with the paraffin section results, four failed to react with Leu M1 in paraffin section (one lymphocyte predominant Hodgkin's disease diffuse, one lymphocyte predominant Hodgkin's disease nodular, and two nodular sclerosing). Each of these cases reacted with Leu M1 in frozen section. One case of nodular sclerosing Hodgkin's disease reacted with Leu M1 in paraffin section but not in frozen section, although there was reactivity with Ki1.

Our results support the view that Leu M1 is a useful agent for identifying Reed Sternberg cells and mononuclear Hodgkin's cells. In the small number of cases in which Leu M1 failed to react in formalin fixed paraffin embedded tissue positive results were obtained using frozen material when available, suggesting that the antigen is, to some extent, sensitive to these processing procedures.

Ki1 shows a similar spectrum of reactivity to Leu M1 in frozen tissue, although a small number of cases were Leu M1 positive Ki1 negative. One case was Leu M1 negative Ki1 positive. Ki1, however, is only reactive on frozen section material, and it has been shown to be reactive in various reactive

Table 2 *Immunoreactivity of Reed Sternberg cells in Hodgkin's disease in paraffin section*

| | *Leu M1* | *Antileucocyte common* | *Antiepithelial membrane antigen* |
|---|---|---|---|
| Mixed cellularity | 23/23 | 8/23 | 1/23 |
| Nodular sclerosing | 14/17 | 6/17 | 1/17 |
| Lymphocytic depleted | 5/6 | 2/6 | 1/6 |
| Lymphocyte predominant* (nodular) | 5/9 | 6/9 | 1/6 |
| Lymphocyte predominant (diffuse) | 5/5 | 5/5 | 1/5 |
| Total | 52/60 | 27/60 | 5/60 |

*Six of these cases showed progressively transformed germinal centres

Table 3 *Immunoreactivity of Reed Sternberg cells in Hodgkin's disease in frozen section*

| | *Leu M1* | *Ki1* | *Antiepithelial membrane antigen* |
|---|---|---|---|
| Mixed cellularity | 13/13 | 13/13 | 9/13 |
| Nodular sclerosing | 8/9 | 8/9 | 2/9 |
| Lymphocyte depleted | 1/1 | 0/1 | 0/1 |
| Lymphocyte predominant (nodular) | 2/2 | 1/2 | 1/2 |
| Lymphocyte predominant (diffuse) | 3/3 | 1/3 | 0/3 |
| Total | 27/28 | 24/28 | 12/28 |

lymphadenopathies and some non-Hodgkin's lymphomas.[9] Its use as a diagnostic reagent in Hodgkin's disease is therefore limited.

Three of the four cases of non-Hodgkin's lymphoma reacted with Leu M1 were T cell lymphomas. This is in keeping with the observation that Leu M1 reacts with some T cell lines and a proportion of mitogen activated T cells[10]; other authors have also noted reactivity of T cell lymphomas with Leu M1.[11 12] In cases such as this, where ambiguities in the immunohistochemical findings can arise, these should be interpreted in conjunction with careful histological examination of the tumour. Where possible, immunophenotyping with a further range of monoclonal antibodies should be undertaken, preferably on frozen material.

In contrast to the results of other workers we found that Reed Sternberg cells in all subgroups of Hodgkin's disease expressed the leucocyte common antigen and reacted with antiepithelial membrane antigen, the reaction to epithelial membrane antigen being stronger on frozen material. Furthermore, Reed Sternberg cells reacted with Leu M1 in a high proportion of the cases of nodular lymphocyte predominant Hodgkin's disease. Thus there was no consistent difference between the immunophenotype of the Reed Sternberg cells in this subgroup, and these results do not support the hypothesis that nodular lymphocyte predominant Hodgkin's disease is of a different histogenetic origin.

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## Bone marrow necrosis and treatment with interferon

Interferon was originally described as an antiviral agent and has recently been shown to have antithumoral activity in man.[1 2] Response to interferon in acute leukaemia has been reported in a few patients. (International Cancer Congress, Seattle, 1982).[3]

We treated a 15 year old boy with interferon, in whom acute lymphoblastic leukaemia had been diagnosed 18 months earlier. Immunological markers showed a cortical phenotype ($TdT^+$; $T_{10}^+$; $T_6^+$; $T_4^+$; $T_8^+$; $3A1^+$; $T_{11}^+$). Complete remission succeeded six months of treatment with $LSA_2$-$L_2$ but he then relapsed and became resistent to several chemotherapeutic regimens. When treatment with interferon was started he had generalised skin infiltration refractory to radiotherapy. His white cell count was $5 \times 10^9$/l with 2% blast cells; the haematocrit was 39%, and platelet count was $336 \times 10^9$/l. The bone marrow aspirated was hypercellular, and smears showed 10% immature blast cells.

He was treated with a daily intramuscular injection of recombinant leucocyte A clone $\alpha$ interferon (kindly provided by Boehringer Ingelheim, West Germany) at a dose of $2{\cdot}5 \times 10^6$ IU/m$^2$ during the first week and $5 \times 10^6$ IU/m$^2$ during the second week.

On day 10 of treatment he presented with bone pain in the extremities, fever, malaise, and anorexia. His white blood cell count decreased to $0{\cdot}4 \times 10^9$/l, the haematocrit to 20%, and his platelet count to $37 \times 10^9$/l. The reticulocyte count was 1%; the results of the coagulation study and other laboratory data yielded normal results. The bone marrow aspirated showed a gelatinous transformation compatible with bone marrow necrosis.[4] Treatment with interferon was stopped, and the patient died two days later.

Secondary effects of treatment with interferon include a rise in temperature, bone pain, malaise, fatigue, and anorexia. Leucopenia and thrombocytopenia have also been observed, though both are reversible when treatment is stopped. (International Cancer Congress, Seattle, 1982).[4]

Bone marrow necrosis has been recognised in patients with leukaemia, and a causal relation with cancer chemotherapy and radiotherapy has been postulated.[4] Although acute leukaemia infiltration could have been the most likely cause in our patient, the drop in the haematocrit (19%) in 10 days remains unexplained, and the possible role of treatment with interferon in the production of bone marrow necrosis is yet to be defined.

As treatment with interferon is still regarded as investigational any unusual change observed in patients thus treated warrants a mention in the literature. We think sequential bone marrow biopsy should be performed when changes in peripheral blood are observed.

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