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Correlation of content of B cells and Leu7-positive cells with subtype and stage in lymphocyte predominance type Hodgkin's disease

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Summary. Cases of lymphocyte predominance type Hodgkin's disease were investigated using immunohistochemical methods and compared for morphological subtype and clinical stage. Cases of nodular paragranuloma showed a high, diffuse paragranuloma a moderate, and the mixed type a low, content of B cells. There was no significant correlation between B cell content and clinical stage. The number of Leu7+ cells was significantly increased in stage I of nodular paragranuloma. Hodgkin cells did not react with the CD15 antibody in most cases of paragranuloma but showed reactivity in the mixed type.

Key words: B cells – Leu7 cells – Hodgkin's disease – Subtype – stage.

Introduction

Hodgkin's disease (HD) has been subdivided into several morphological subtypes (Lukes et al. 1966a). One criterion for this subdivision is the number and morphological features of Hodgkin (H) cells and whether they show differentiation into typical Sternberg-Reed (SR) cells, lacunar cells, or L&H cells (Lukes et al. 1966b). Another important criterion is the content of lymphocytes, hence the distinction between the lymphocyte depleted and the lymphocyte predominance subtypes (Lukes 1971). The subdivision into at least four subtypes has broad application because of its clinical relevance: the prognosis is good in cases with a high content of lymphocytes, whereas prognosis is poor in cases with a low content (Lukes et al. 1966b). In recent years, however, advances in therapy have improved prognosis irrespective of histological type.

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Recent immunohistological investigations have provided evidence for a B or T cell origin of the H cells and for a predominance of B cells in paragranuloma in contrast to all other subtypes of HD (Poppema et al. 1979; Hansmann and Lennert 1985a, b). In the present study, cases of HD with a high content of small lymphocytes were investigated to determine whether the content of B or T cells and the immunohistochemical properties show a correlation with the aggressiveness of disease.

Materials and Methods

Patients. A total of 142 cases of HD, lymphocyte predominance type (HDLP) were investigated. They were subclassified according to the criteria of Lennert and Mohri (1974) into three groups: nodular paragranuloma (NP; $n=104$), diffuse paragranuloma (DP; $n=16$), and mixed type with lymphocyte predominance (MTLP; $n=22$). Information on the clinical stage of the disease was obtained from questionaires and in some cases from clinical reports provided by numerous hospitals and physicians. Clinical results concerning NP, including 41 additional cases, have been published elsewhere (Hansmann et al. 1984).

Histological diagnoses were made on paraffin sections stained with Giemsa, H&E, PAS, and silver impregnation. All samples investigated in this study were taken from untreated patients.

Immunohistochemistry. Paraffin sections were deparaffinized and immunostained according to the method of Stein et al. (1982). The following primary antibodies were used: for B cell marker Ki-B3 (Hansmann et al. 1986; Feller et al. 1987) at a dilution of 1:32000, for H cell marker 3C4 [C3D-1; Dakopatts, Copenhagen, Denmark, Schienle et al. (1982); Stein et al. (1982)] concentrated, CD15, and for natural killer cells Leu7 (Becton Dickinson, Heidelberg/FRG, 1:100).

As secondary antibody peroxidase conjugated rabbit anti mouse (Dakopatts; 1:15), and as tertiary antibody peroxidase conjugated goat anti rabbit (Ortho Diagnostic, Neckargemünd/FRG, 1:100) were used. The reaction product was visualized according to Graham and Karnovsky (1966).

Morphometrical analysis. The B cell areas were measured using a MOP-AM02 (Contron Messgeräte GmbH, FRG) at a magnification of 25× (objective). The Leu7+ cells were counted in four areas per case at a magnification of 400×.

Results

In HDLP, Ki-B3 detected mainly small B lymphocytes with round to slightly irregular muclei and a sparse cytoplasm. Some centrocytes and a few centroblasts, plasma cells, and histiocytes were also detected. The reaction product appeared to be localized on the outer cell membrane.

Table 1. B cell areas (%) differentiated in subtypes of lymphocyte predominance type of Hodgkin's disease

	NP	DP	MTLP
n	104	16	22
Medium	37.8	23.3	15.9
SD	(15.4)	(13.9)	(11.4)

NP = nodular paragranuloma; DP = diffuse paragranuloma; MTLP = mixed type with lymphocyte predominance

Table 2. B cell areas (%) differentiated in stages of the subtypes of lymphocyte predominance type of Hodgkin's disease

	NP ($n=104$)	DP ($n=16$)	MTLP ($n=22$)
Stage I	41.4% ($n=47$)	26.7% ($n=7$)	20.3% ($n=9$)
Stage II	35.4% ($n=27$)	22.5% ($n=6$)	10.5% ($n=4$)
Stage III	35.4% ($n=25$)	24.5% ($n=2$)	18.7% ($n=4$)
Stage IV	31.8% ($n=4$)	2.4% ($n=1$)	10.2% ($n=5$)

Table 1 shows the proportion of B cells (B cell areas) in the various subtypes pf HDLP. The median incidence of Ki-B3+ areas was 37.8% in NP (Fig. 1), 23.3% in DP, and 15.9% in MTLP (Fig. 2).

Table 2 shows the B cell areas in the three subtypes and in stages I to IV for each subtype. The B cell content decreased from NP to DP and MTLP. However, a statistically significant difference ($P<0.05$) was only found between NP and MTLP in stage I (Table 2).

Evaluation of Leu7+ cells

The anti-Leu7 antibody detected some macrophages and variable numbers of small lymphocytes with round nuclei and scanty cytoplasm. The reaction product seemed to be localized on the outer cell membrane. Table 3 shows the number of Leu7+ cells (natural killer cells) in NP, DP, and MTLP. NP showed significantly higher numbers of Leu7+ cells (Fig. 3) than both other subtypes. In NP, large accumulations of Leu7+ cells were characteristic of stage I, whereas all other stages showed no differences in the number of these cells compared with the other subtypes of HDLP (Fig. 4). Excluding stage I, no statistically significant differences could be demonstrated between any of the other stages ($P<0.01$).

The monoclonal antibody 3C4 (CD15) was applied to all caes of lymphocyte predominance type. In some cases this antibody showed a clear granular reaction product which appeared to be localized in the paranuclear area (Golgi field) and/or a membrane bound reaction product on the surface of H, SR, and

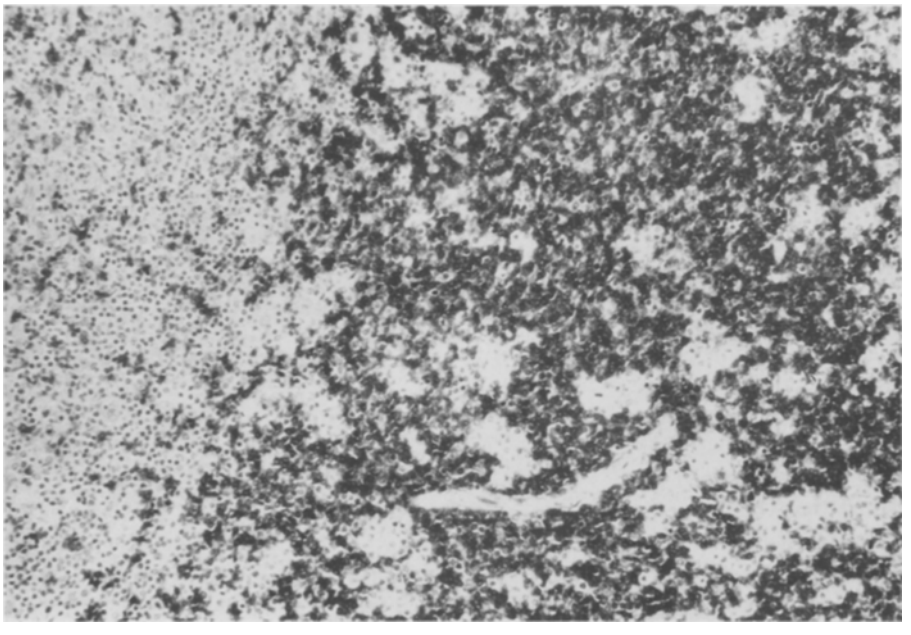

Fig. 1. Large nodule composed of B cells, NP. Immunoperoxidase staining, Ki-B3, ×140

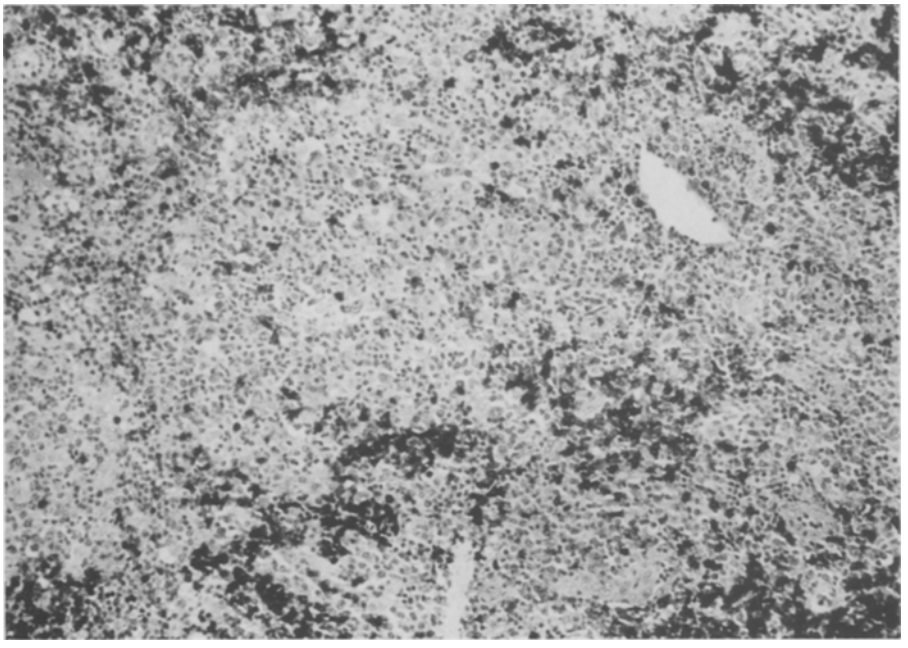

Fig. 2. Only a few B cells are detectable in a case of MTLP. Immunoperoxidase staining Ki-B3, ×140

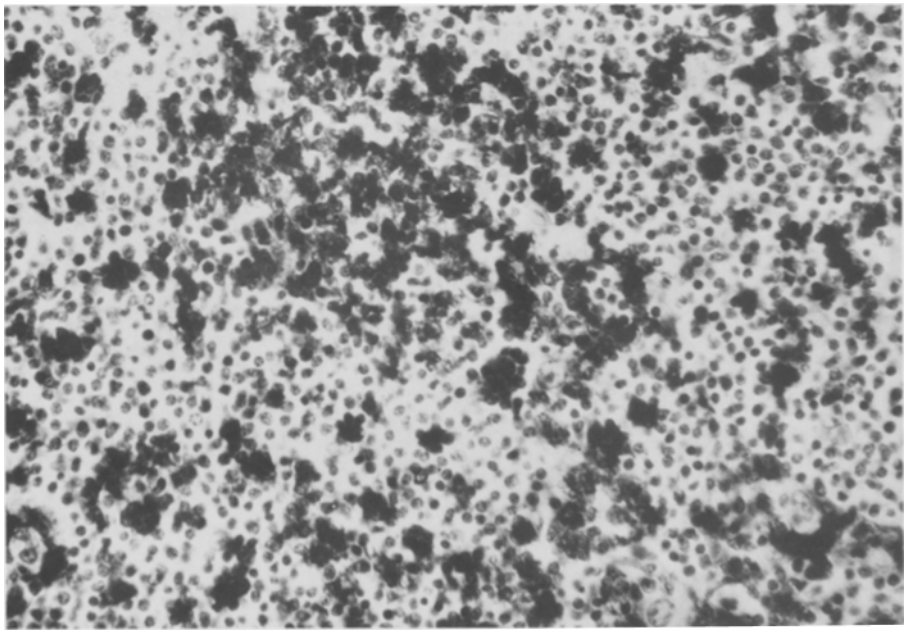

Fig. 3. High amount of Leu7+ cells in a nodule of NP stage I. Immunoperoxidase staining, ×350

Table 3. Content of Leu7+ cells in Hodgkin's disease lymphocyte predominance type

	NP	DP	MTLP
n	97	15	18
Medium	84	12	7
SD	(110.7)	(13.4)	(8.7)

Table 4. Cases with CD15+ or Ki-B3+ Hodgkin cells

	NP	DP	MTLP
CD15	14/96	2/15	16/18
Ki-B3	17/109	0/17	0/21

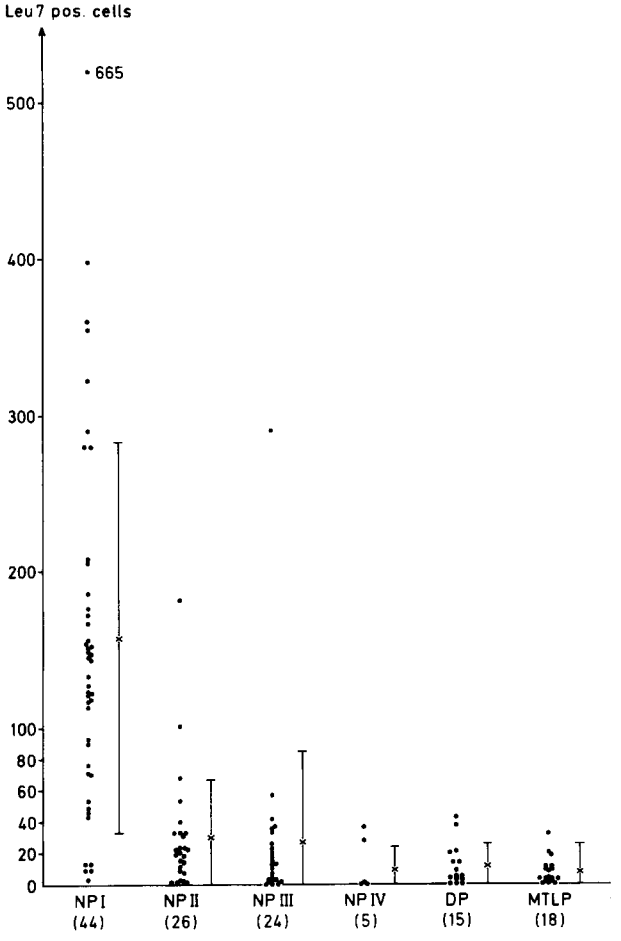

Fig. 4. Amount of Leu7+ cells in NP, DP, and MTLP (number of cases)

L&H cells (Fig. 5). The positive cases always showed a variable number of reactive H cells and their variants. CD15+ H cells were found in 14 out of 96 cases of NP (Table 4), in 2 out of 15 cases of DP, and in 16 out of 18 cases of MTLP.

H or L&H cells reacting with the monoclonal antibody Ki-B3 (Fig. 6) were found in 17 out of 109 cases of NP, but in none of the cases of DP or MTLP. In the positive cases the outer cell membrane of these cells was clearly stained.

Discussion

Cases of HDLP were investigated with pan-B (Ki-B3), cytotoxic T cell (Leu7), and H cell markers (CD15). The cases were subdivided into three morphological subtypes (Lennert and Mohri 1974): NP, DP, and MTLP. The NP showed typical features such as large aggregations of B cells. The DP differed from NP only in its diffuse infiltration pattern, although often a few areas resembling remnants of nodules could be seen. Unlike paragranuloma, MTLP showed the typical features of mixed cellularity subtype with classic H and SR cells but, like paragranuloma, it had a high lymphocyte content.

These lymphocytes often reacted with a monoclonal pan-B cell antibody in NP. In contrast a low B cell content was found in MTLP. A high B cell content in the nodules of paragranuloma has been found in frozen sections (Abdulaziz et al. 1984; Hansmann et al. 1986). A variable, often low B cell content and a

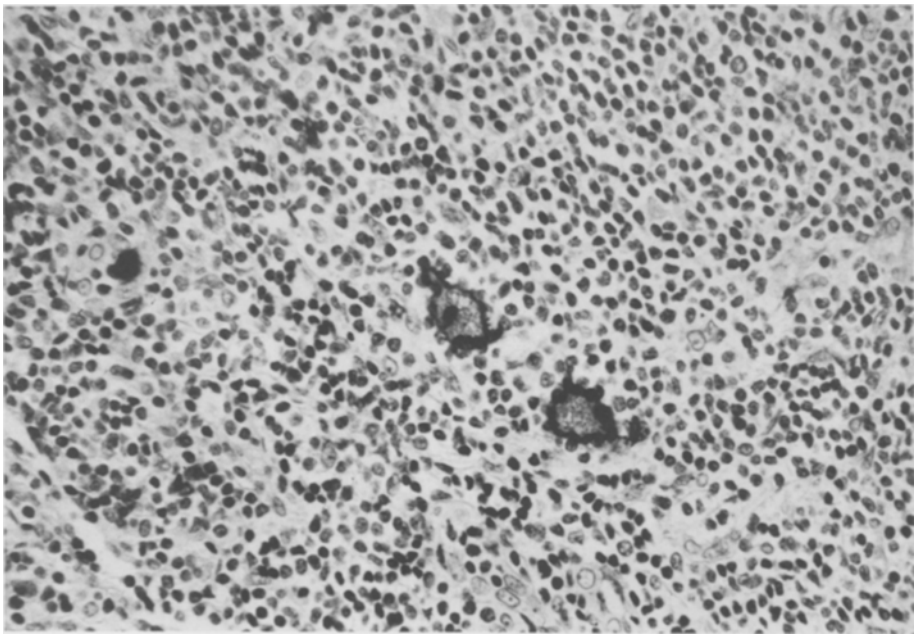

Fig. 5. Positively stained L&H cells of NP with the CD15 monoclonal antibody. The reaction product is confined to the outer cell membrane and in one cell to the perinuclear area. Immunoperoxidase staining, × 560

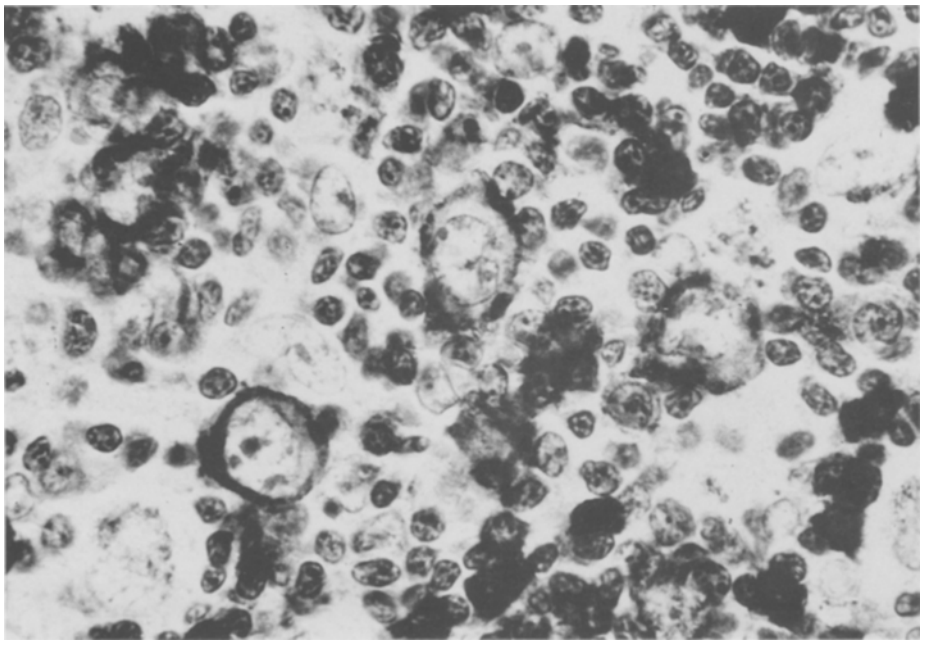

Fig. 6. L&H cells reactive with the pan-B cell antibody Ki-B3. The reaction product is confined to the outer cell membrane. Immunoperoxidase staining, × 880

high T cell content in MTLP has been reported previously (Hansmann et al. 1986). With regard to B cell content, DP is intermediated between NP and MTLP. Obviously in these cases T cells invade the nodular B cell regions. Considering subtype, we found a significant difference only between NP and MTLP (stage I). We also looked for differences in B cell content between various stages of NP, DP, and MTLP and found no significant correlation between B cell content and the stage of disease. However, the groups in DP and MTLP were rather small.

A high content of Leu7+ cells has been reported in progressively transformed germinal centers (Hansmann and Lennert 1985c; Lennert and Hansmann 1987), which seemed to be prestages of NP. We found a significant increase in the number of Leu7+ cells in stage I of NP in comparison to the other stages of the same and both other subtypes of HD. On the significance of large numbers of Leu7+ cells in stage I of NP one can only speculate. It is possibly a sign of a marked immunological reaction against the tumor or of a good control of this low grade malignant tumor with a favorable prognosis at this early stage (Hansmann et al. 1984).

Immunohistochemical differences in the H cells in the three subtypes were also seen. In most cases of NP and DP, H, SR, and L&H cell variants did not express the typical H cell properties defined by the H cell CD15 antibody (3C4). Similar findings have been published by other authors (Pinkus et al. 1985; Dorfman et al. 1986), and were taken as an argument for paragranuloma as a special entity. However, our results conflict with those of Pinkus and Said (1985) in that we also found CD15+ H cells in some cases of typical paragranuloma. This may be due to the larger number of cases in our study.

Several authors have proposed a B cell origin of the H cells in paragranuloma (Poppema 1980; Hansmann and Lennert 1985a, b; Stein et al. 1986; Timens et al. 1986). In favor of this theory is development of the disease in B areas (Lennert et al. 1978), the ultrastructural features of the H and B cells (Poppema et al. 1979), and the expression of J chains (Poppema 1980; Hansmann and Lennert 1985a, b; Stein et al. 1986). However, the pan-B cell monoclonal antibody Ki-B3 only reacted with H, SR, and L&H cells in paragranuloma in a few cases. Ki-B3 positivity of H cells was not detected in cases of MTLP. The low number of paragranuloma cases with Ki-B3+ H and L&H cells accords with a previous study on a small number of cases (Hansmann et al. 1986).

The present results could provide evidence on B cell content and H cell properties useful in differentiating between subtypes of HD and evidence on the high content of Leu7+ cells characteristic of stage I of NP. Possibly new monoclonal antibodies working in paraffin sections will provide further information on prognosis.

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