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Paragranuloma is a variant of Hodgkin's disease with predominance of B-cells*

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Summary. Fifteen cases of Hodgkin's disease of nodular and diffuse paragranuloma subtype (nodular and diffuse subtype of lymphocyte predominant type of Hodgkin's disease) were studied by light and electron microscopy, using monoclonal antibodies recognizing T and B-lymphocytes and dendritic reticulum cells. The results were compared with findings on 10 cases of Hodgkin's disease of mixed type with lymphocyte predominance. The present study provides evidence that paragranuloma represents a special variant of Hodgkin's disease different from other subtypes. Paragranuloma is characterized by predominance of B-cells which were demonstrated with a new B cell reagent KiB3 on routinely processed paraffin sections.

Key words: Hodgkins disease – Paragranuloma, mixed type – Monoclonal antibody in paraffin sections

Introduction

Despite considerable progress in knowledge of different aspects of Hodgkin's disease (HD), the origin of the neoplastic cells is still obscure. Some findings support the view that in the majority of cases Hodgkin (H) cells represent transformed T-cells (Andresen et al. 1984; Stein et al. 1984). Others, however, indicate that at least in some cases H-cells may express B-cell properties (Poppema et al. 1979a; Pinkus and Said 1985).

Since the early observations on the paragranuloma variant of HD, evidence has accumulated indicating a preponderant role for B-cells in this lesion (Poppema et al. 1979b; Abdulaziz et al. 1984; Burns et al. 1984). However, it is difficult to detect cell surface antigens in formaldehyde fixed material in order to determine the corresponding phenotype. In the present paper we report the application of monoclonal antibodies to cases of HD

* This paper is dedicated to Professor Karl Lennert, Kiel, on the occasion of his 65th birthday

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in order to distinguish between paragranuloma and HD of mixed type with lymphocyte predominance (MLP), on the basis of paraffin sections.

Materials and methods

Cases of nodular ($n=10$) and diffuse paragranuloma ($n=5$), and HD of MLP ($n=10$) were investigated in paraffin and cryostat sections. Lymph nodes ($n=5$) and tonsils ($n=2$) with minor reactive changes were used as controls. Paraffin sections of lymph node biopsies were stained with H&E, Giemsa, Gomori silver impregnation and PAS for histopathological diagnosis.

The following monoclonal antibodies were used on cryostat sections: To15 (Dako pan-B, Copenhagen, Denmark) as pan-B, OKT11 (Ortho Diagnostic system, Heidelberg, FRG) for detecting sheep erythrocyte receptor, and anti-Leu-1 (Becton-Dickenson, Heidelberg, FRG) as pan-T reagent. In addition, the monoclonal antibody KiM4 (Behring, Marburg, FRG) enabled detection of Dendritic reticulum cells (DRC). For detection of B-cells in paraffin sections a new monoclonal antibody KiB3 was utilized. Details on its production and specificity are given elsewhere (Feller et al. in preparation).

Cryostat sections were immunostained as described by Stein et al. (1982a) and Feller et al. (1983). Paraffin sections for staining with KiB3 were deparaffinized in xylol for 10 min and processed as above.

Positively stained lymphocytes were counted with high magnification ($\times 40$ obj.) in 10 fields and the mean values were calculated.

For electronmicroscopic immunohistochemistry lymph node specimens (chronic nonspecific lymphadenitis ($n=2$); nodular paragranuloma ($n=2$)) were cut into $5 \times 5 \times 1$ mm pieces and fixed in freshly prepared 4% paraformaldehyde in 0.1 M PBS, pH 7.4 for 1 h. After washing in PBS supplemented with 2% sucrose (weight/volume) tissue blocks were snap frozen and 8 µm thick cryostat sections prepared. Sections were air dried for 2 h at room temperature. Immunoperoxidase staining was done as given above. Postfixation was done with 1% osmium tetroxide in Tris-HCl, pH 7.4, for 1 h. Sections were then dehydrated in graded alcohols and embedded in Araldit. Ultrathin sections were examined with Siemens Elmiskop 101 without contrasting (Hansmann et al. 1984a).

Results

Light microscopic immunohistochemistry

Reactivity pattern of KiB3. In normal lymphoid tissues such as lymph nodes and tonsils the reactivity of KiB3 was found to be restricted to small lymphocytes of the follicle mantle (Fig. 1a). A few B-lymphocytes in the interfollicular and pulp areas and many plasma cells reacted with KiB3. Centrocytes, centroblasts and immunoblasts of the germinal centers sometimes revealed a weak and more diffuse reactivity.

In all 10 cases of nodular paragranuloma the nodules showed large amounts of densely packed small KiB3 positive lymphocytes (Fig. 1b, Table 1). In cases of diffuse paragranuloma the infiltrates showed many small B-cells which were poorly demarcated against the adjacent T-cell regions. However, in two cases of diffuse paragranuloma the B-cell areas detectable with the monoclonal antibody KiB3 were small.

In contrast to paragranuloma the cases of HD of MLP showed only a few B-cells, which probably represented residual B-cell areas (Fig. 2, Table 1). Three of 10 cases of nodular and one of five cases of diffuse paragranuloma showed a variable number positively stained H, SR, and L&H-cells (Table 2). The reaction product was localized on the cell membrane and/or

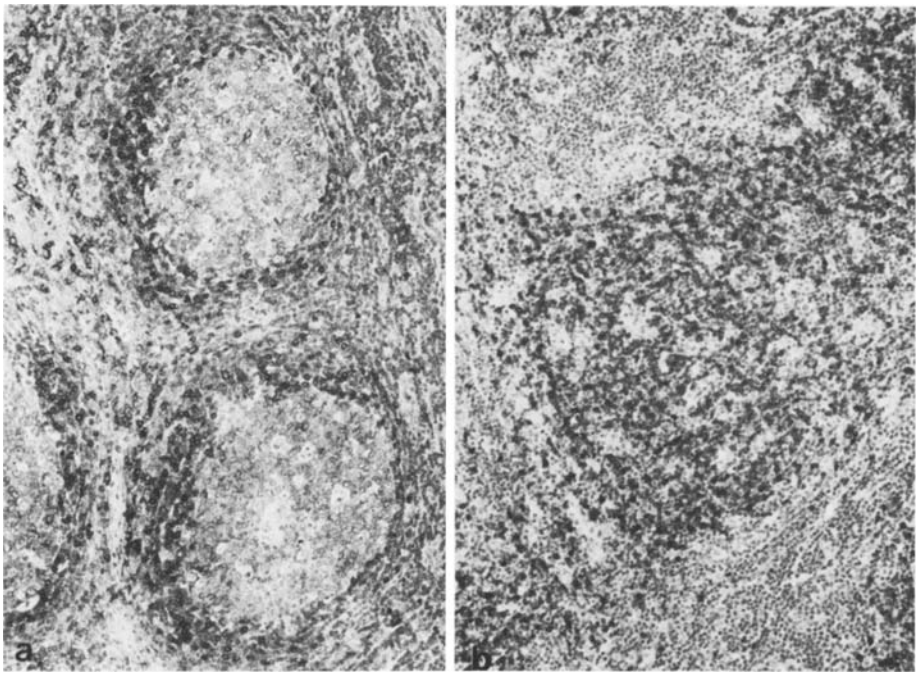

Fig. 1. a Chronic nonspecific lymphadenitis with 3 florid germinal centers. Small lymphocytes of the follicle mantel show a strong, centroblasts and centrocytes a weak reaction for KiB3. Paraffin section, Immunoalkaline phosphatase, × 105. **b** Nodular paragranuloma. Nodule composed of small lymphocytes with a strong reactivity for KiB3. Paraffin section, Immunoperoxidase, × 105

Table 1. Distribution of B and T-lymphocytes recognized by the monoclonal antibodies KiB3, To15, OKT11 in cases of paragranuloma as compared with Hodgkin's disease of mixed type with lymphocyte predominance. All numbers represent median values of up to 4927 cells per case counted

	n	KiB3	PAN-B To15	PAN-T OKT11 LEU1
Nodular paragranuloma	10 (3)[a]	132.5	194	47
Diffuse paragranuloma	5	140		
Mixed type with lymphocyte predominance (other than paragranuloma subtype)	10 (5)[a]	30	34	230

[a] Number of cases investigated in frozen sections

in the Golgi region (Fig. 3). In all cases of HD of MLP the H and SR-cells remained unstained with KiB3 (Table 2). All other lymph node structures, including T-lymphocytes, dendritic cells, histiocytes, epitheloid cells, tissue mast cells, endothelial cells and different types of granulocytes, remained negative.

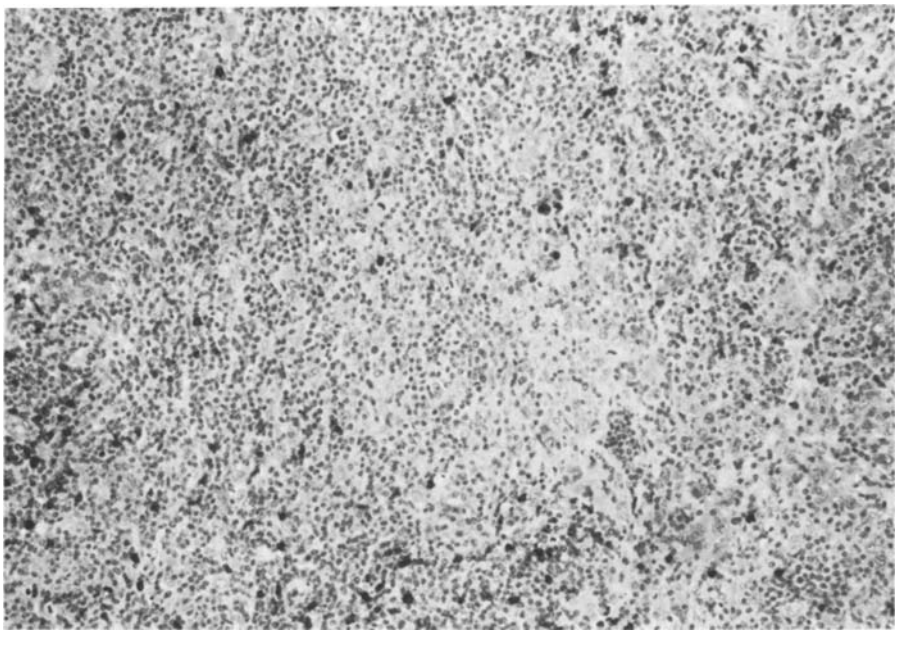

Fig. 2. Hodgkin's disease mixed type with lymphocyte predominance. Only a few B-cells are present. KiB3, Paraffin section, Immunoperoxidase, × 56

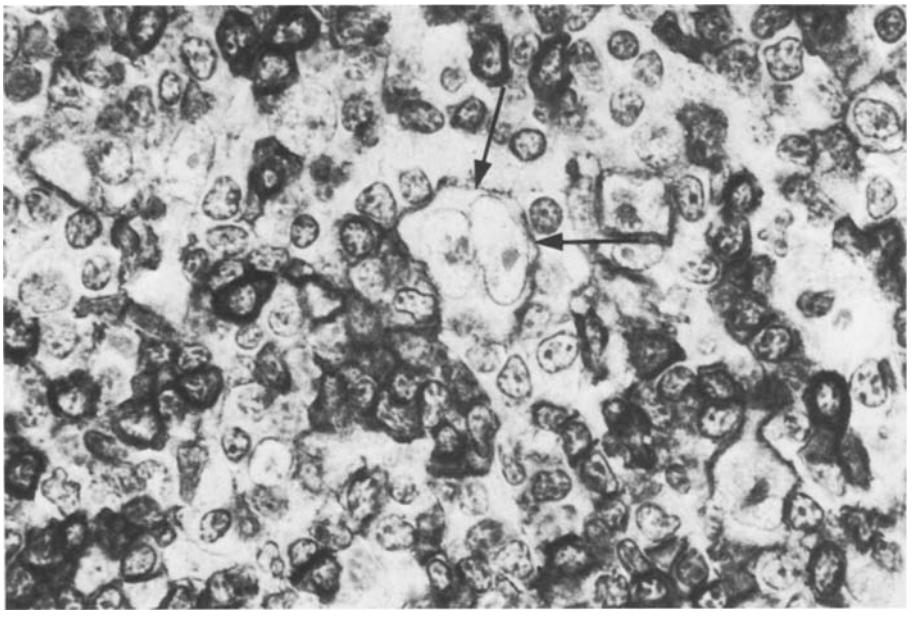

Fig. 3. Nodular paragranuloma. A Sternberg-Reed cell shows membrane bound reactivity (*arrows*) with the monoclonal antibody KiB3. Paraffin section, Immunoperoxidase, × 880

Table 2. Incidence of KiB3 positive L&H, H or SR-cells in cases of paragranuloma as compared with Hodgkin's disease of mixed type with lymphocyte predominance

	n	Cases with KiB3 positive L&H, H or SR-cells *n*
Nodular paragranuloma	10	3
Diffuse paragranuloma	5	1
Mixed type with lymphocyte predominance (other than paragranuloma subtype)	10	0

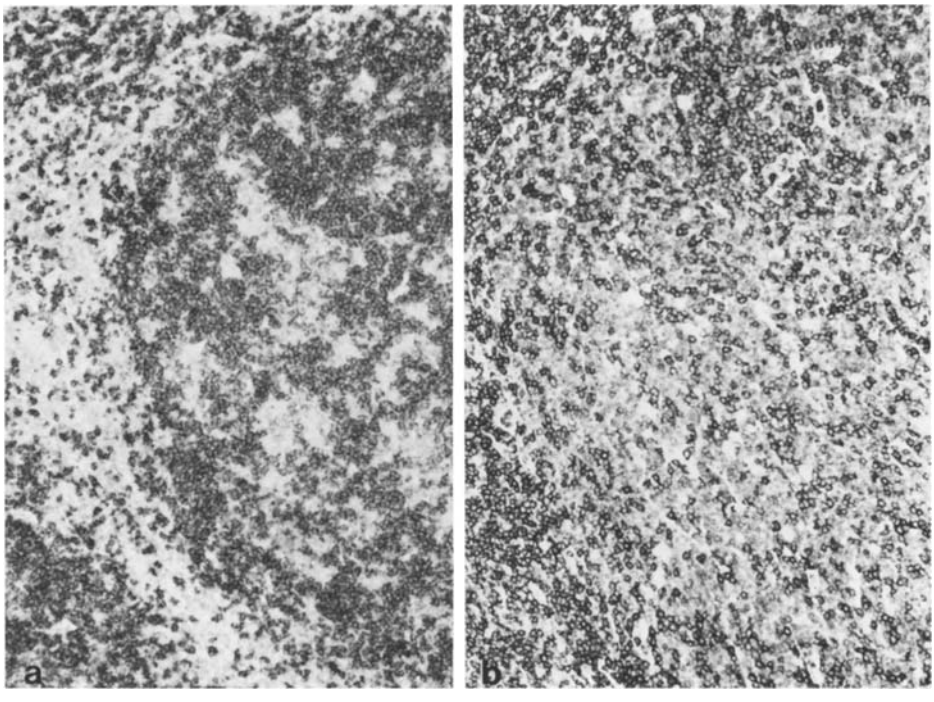

Fig. 4. a Nodular paragranuloma. Nodule with abundant B-lymphocytes. Pan-B-reagent; cryostat section, Immunoperoxidase, × 105. **b** In the middle of the picture a nodule of paragranuloma with some T-cells. Around the nodule in the T-zone T-lymphocytes densely packed. Pan-T-cell reagent; cryostat sections, Immunoperoxidase, × 105

Pan B-cell reagent To15

Large numbers of lymphocytes reacted positively to the pan-B reagent To15 in all cases of paragranuloma (Table 1, Fig. 4a). It was difficult to identify Hodgkin cells in frozen sections and to determine whether they were reactive to the monoclonal antibodies. Plasma cells and epithelioid cells remained

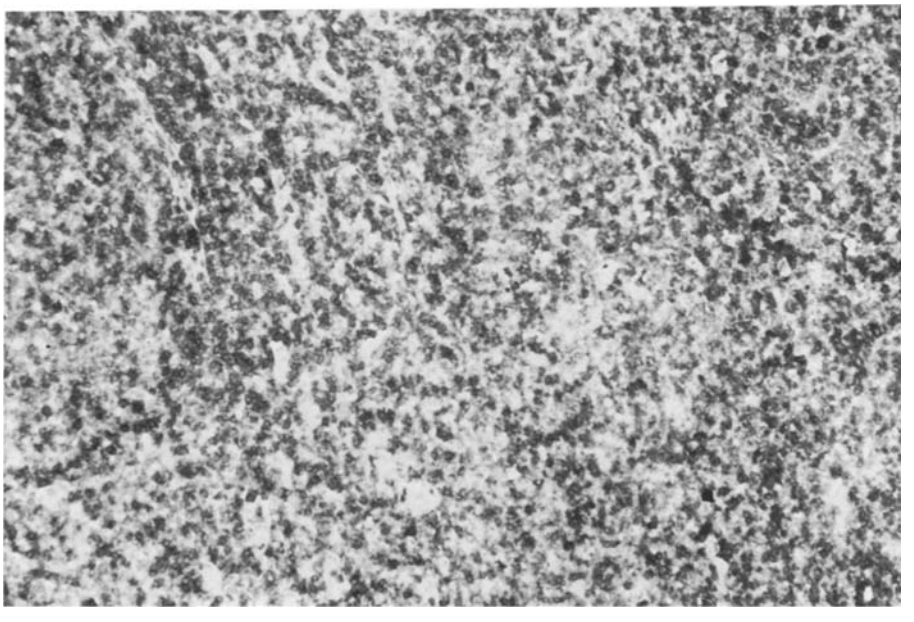

Fig. 5. Densely distributed T-lymphocytes are shown in Hodgkin's disease mixed type with lymphocyte predominance. Cryostat section, immunoperoxidase, Pan T reagent, ×140

nonreactive. In contrast, in infiltrated lymph nodes of HD of MLP most of the small lymphocytes were negative for pan-B To15 (Table 1).

Pan-T cell reagents OKT11 and Leu1

In cryostat sections of paragranuloma infiltrated lymph nodes pan-T reagents revealed an irregular or nodular distribution pattern containing a variable number of loosely distributed T-lymphocytes within the nodules (Fig. 4b, Table 1). The internodular spaces were densely packed with T-lymphocytes in contrast to the nodules, which were rich in B-lymphocytes. In diffuse variants of the paragranuloma the overwhelming majority of lymphocytes were recognized as B-cells with the exception of two cases, which showed large T-cell areas. The distribution pattern showed large confluent areas of B-cells poorly demarcated against the neighboring T-cell areas. Cases of HD of MLP lacked such large nodules and showed an even distribution of T-lymphocytes (Fig. 5). Residual B-cell areas were confined to minor follicles or small groups of lymphocytes.

Dendritic reticulum cells (DRC) recognized by KiM4

In all cases of paragranuloma, KiM4 positive DRCs were detectable in considerable numbers (Fig. 6). They were mostly concentrated within the dense areas of infiltrates. They showed an incomplete, loosely connected

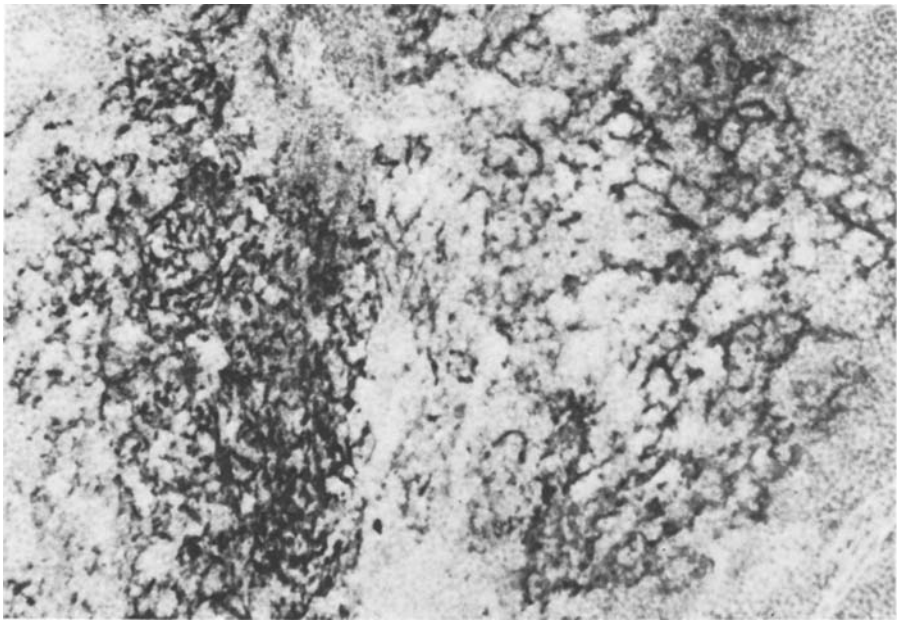

Fig. 6. Large numbers of DRCs occur in nodular paragranuloma. The dendritic reticulum cells are labelled with the monoclonal antibody KiM4. Cryostat section; immunoperoxidase, ×140

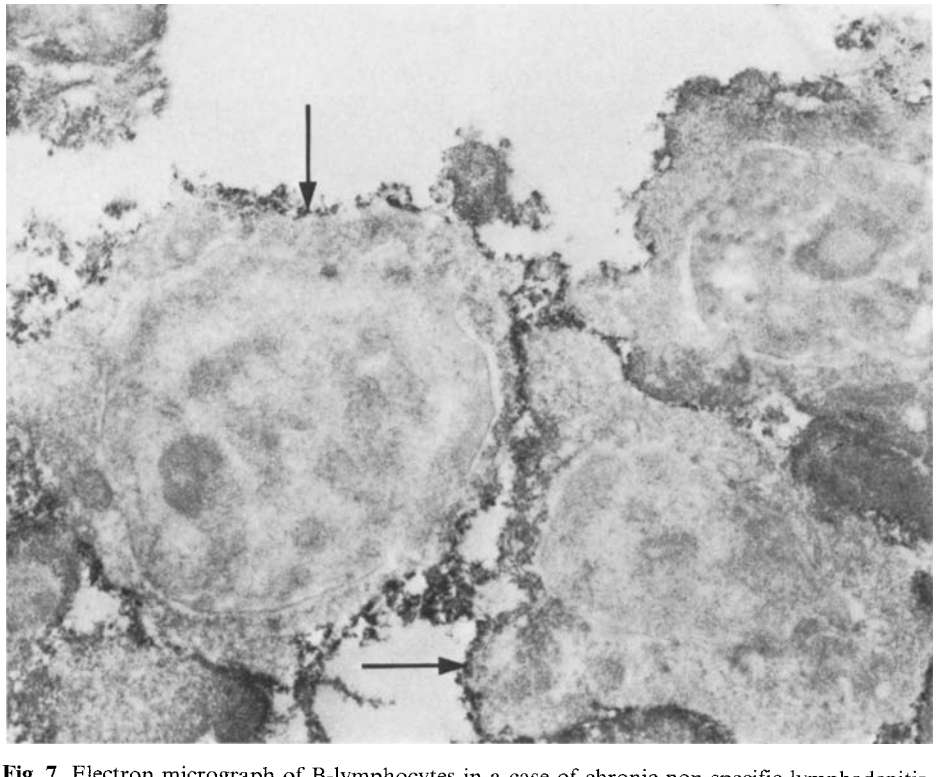

Fig. 7. Electron micrograph of B-lymphocytes in a case of chronic non-specific lymphadenitis exhibiting strong depositing of KiB3 on the outer membrane surface (*arrows*). Paraformaldehyde fixation, immunoperoxidase reaction, no contrasting, ×8,400

network of fusiform cells with elongated, arborized cytoplasmic projections. HD of MLP lesions, by contrast, had few of these cells, which were confined to the areas of the residual lymphatic follicles.

Electron microscopic results

At the electron microscopic level in normal lymphatic tissue KiB3 was found to be restricted to lymphoid cells and plasma cells. Peroxidase reaction product was mainly localized in a more or less continuous line of reactivity on the outer surface of the cytoplasmic membrane of B-lymphocytes (Fig. 7). Intracytoplasmic base material and cellular organelles showed almost no reactivity. In addition, almost no peroxidase activity could be observed in the ergastoplasm and perinuclear envelope. Results of electron microscopic investigation on the reactivity pattern of KiB3 accorded well with the findings at the light microscopic level.

In cases of HD, KiB3 recognized a large number of B-lymphocytes densely distributed within the infiltrates of paragranuloma subtype. The ultrastructural distribution pattern of KiB3 positive sites did not differ from that found in non-specific lymphadenitis. Macrophages, epithelioid cells and various types of granulocytes observed in Hodgkin infiltrates remained negative.

Discussion

Variants of HD rich in small lymphocytes have attracted attention due to their different prognostic behaviour. Lukes (1966a) distinguished a lymphocytic and/or histiocytic nodular and diffuse type from other types of HD. These cases were characterized by the presence of so called L&H cells. In the Rye classification, however, these variants were not considered as separate entities and subsummed under the term "lymphocyte predominant type" (Lukes et al. 1966b).

For determining the prognostic relevance of tumour content in small lymphocytes various suggestions have been made for discriminating between the types of HD containing abundant lymphocytes. Lennert and Mori (1974) pointed out the heterogeneity of HDs with abundant small lymphocytes and subdivided them into nodular paragranuloma (Ia), diffuse paragranuloma (Ib), and subtype other than paragranuloma (Ic). The latter showed all the features of the mixed type but contained numerous lymphocytes (HD of mixed type with lymphocyte predominance). The three subtypes share the property of lymphocyte predominance but paragranuloma and HD of MLP differ considerably in prognosis (Hansmann et al. 1984b; Hansmann and Lennert 1985a, b). Paragranuloma and HD of MLP show further differences which justify making a distinction between these two lesions: histologically, both nodular and diffuse paragranuloma show a number of clearly discernable L&H-cells, which are usually lacking in cases of HD of MLP. In paragranuloma cases typical H and SR-cells are often absent or inconspicious, whereas HD of MLP lesions are characterized by some

H and SR-cells. Histochemically, SR-cells and L&H-cells are regularly Ki1 positive (Stein et al. 1982a). Immunoreactions for J-chain of immunoglobulins are often positive in L&H-cells but usually negative in SR-cells found in HD of MLP (Hansmann and Lennert 1985a, b; Stein et al. submitted for publication). The reverse behaviour is observed with the monoclonal antibody 3C4 (Stein et al. 1982b), which immunostains H and SR-cells in mixed type lesions but not L&H-cells in most cases of paragranuloma (Hansmann and Lennert 1985a, b; Stein et al. submitted for publication). H-cells reacted positively with the B-cell reagent KiB3 in 3 of 10 of the paragranuloma cases investigated. Similar observations have been reported by other authors. Pinkus and Said (1985) showed reactivity of SR-cells in nodular paragranuloma with B-cell markers. Okon et al. (1985) reported reactivity of large mononuclear cells in three cases of HD and of SR-cells in one case of HD of mixed and nodular sclerosis type. Thus, despite the prevailing view that the majority of HD cases are T-cell in origin, it seems probable that at least in a limited number of cases H-cells may express B-cell properties. Typical paragranuloma lesions are characterized by a dense distribution of DRCs, which can be selectively immunostained with the monoclonal antibodies KiM4 (Parwaresch et al. 1983a, b) and CR4/23 (Naiem et al. 1983). DRCs in nodules of paragranuloma were also demonstrated by Abdulaziz et al. (1984) and Burns et al. (1984). In contrast, these cells occur in only small numbers in HD of MLP lesions. Finally, the type of growth pattern seen in the lesions may be usefull in differential diagnosis. Paragranuloma may exhibit a nodular or diffuse histological pattern, whereas HD of MLP invariably show a diffuse growth pattern.

Another important feature distinguishing paragranuloma from HD of MLP is the phenotypic equipment of their small-lymphocyte populations. Various studies have dealt with this issue (Poppema et al. 1979a; Poppema et al. 1980; Abdulaziz et al. 1984). In this study all cases of nodular or diffuse paragranuloma were characterized by large foci of B-lymphocytes, with the exception of two cases of diffuse paragranuloma which showed only small B-cell areas. In these two cases, however, it was difficult to distinguish between residual B and T-cell areas. The high number of B-cells in cases of diffuse paragranuloma given in Table 1 does not reflect an overall median for the whole section but only for areas which consisted predominantly of B-cells. The number of KiB3 positive cells was in line with the pan-B positive lymphocyte counts in all cases investigated, as shown in Table 1. HD of MLP, however, is characterized by a large number of T-lymphocytes, occasionally intermingled with some probably residual B-lymphocytes. Similar results were reported by Poppema et al. (1980), who investigated HD, nodular paragranuloma, mixed and nodular sclerosis type in cryostat sections.

Lymphocyte phenotyping with cell sorter technique or other fluorescent methods in such cases does not reveal reliable information due to the distribution pattern of the various lymphocyte populations. For immunohistochemical studies with corresponding T or B-cell reagents fresh or deep frozen tissue specimens and cryostat sections are generally required. This represents

a major handicap to routine diagnosis. Immunohistochemical techniques applicable to paraffin sections, such as those using antisera with polyclonal antibodies against different Ig classes, are not appropriate for solving the problem at hand due to the impossibility of monitoring surface Ig on lymphocytes in paraffin sections.

A reliable method for distinguishing between paragranuloma and HD of MLP is essential considering the striking prognostic differences between these two types of HD. The monoclonal antibody KiB3 offers a convenient solution to this problem. It enables reliable recognition of B-lymphocytes in cryostat sections and routinely processed paraffin material. In this reaction B-lymphocytes populating paragranuloma lesions demonstrate a selective staining demarcating them against the intermingled T-cells or residual areas of the lymph node T-zone, thus distinguishing paragranuloma from other subtypes of HD.

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