Tohoku J. Exp. Med., 1995, **177**, 193-211

Immunophenotypes of Reed-Sternberg Cells and Their Variants: A Study of 68 Cases of Hodgkin's Disease

Hiroshi Takahashi, Katsumi Hideshima*, Kuniaki Kawazoe, Nobuo Tsuda†, Shuichi Fujita, Yasuaki Shibata, Haruo Okabe and Shigeru Yamabe‡

*Department of Oral Pathology, Nagasaki University School of Dentistry, *Clinical Laboratory, Nagasaki University Hospital of Dentistry, †Department of Pathology, Nagasaki University Hospital, and ‡The First Department of Maxillofacial and Oral Surgery, Nagasaki University School of Dentistry, Nagasaki 852*

Takahashi, H., Hideshima, K., Kawazoe, K., Tsuda, N., Fujita, S., Shibata, Y., Okabe, H. and Yamabe, S. *Immunophenotypes of Reed-Sternberg Cells and Their Variants: A Study of 68 Cases of Hodgkin's Disease.* Tohoku J. Exp. Med., 1995, **177** (3), 193-211 —— Utilizing a panel of monoclonal and polyclonal antibodies, routine paraffin sections in 68 cases of Hodgkin's disease were examined for the presence of immunoreactivity in Reed-Sternberg (R-S) and related cells by the avidin-biotin-peroxidase complex (ABC) technique. In 14 cases of lymphocyte-predominant Hodgkin's disease (LPHD), R-S cells and the polyploid lymphocytic and histiocytic (L & H) variants of R-S cells were immunoreactive for L26 and α_1-antitrypsin (α_1-AT) in 9 (64%) and 6 (43%), respectively, whereas the remaining antibodies were negative or rarely positive against L & H variants of R-S cells. R-S cells in 24 cases of mixed cellularity Hodgkin's disease (MCHD) were positive with α_1-AT in 63% of cases, positive with LN3 in 71% of cases and positive for BerH2 in 92% of cases. The lacunar cell type of R-S cells in 19 cases of nodular sclerosing Hodgkin's disease (NSHD) were reactive for α_1-AT in all cases, BerH2 in 18 cases (95%), and LN3 in 17 cases (89%). Pleomorphic variant of R-S cells in 11 cases of lymphocyte depleted Hodgkin's disease (LDHD) showed reactivity with α_1-AT in 9 cases (82%), BerH2 in 6 cases (55%), and LN3 in 9 cases (82%). The incidence of L26 in R-S cells was higher in LPHD than in other three subtypes, whereas the immunohistochemical finding of α_1-AT had reverse relevance to the result of L26. The incidence of BerH2 in MCHD and NSHD was higher than that of this antibody in the whole of Hodgkin's disease. R-S cells in NSHD and LDHD were highly positive to LN3, and detection rate of these two types was higher than that in the whole of Hodgkin's disease. No cases showed immunoreactivity with anti-T-cell antibodies (CD3, UCHL1 and DFT1), a marker for natural killer cell (Leu7), and a marker for interdigitating reticulum cell (S-100 protein). These results suggest that correlation between predominant

Received February 4, 1995; revision accepted for publication September 12, 1995.

Address for reprints: Dr. H. Takahashi, Department of Oral Pathology, Nagasaki University School of Dentistry, 1-7-1 Sakamoto, Nagasaki 852, Japan.

staining pattern and R-S cells and variants thereof in each histological subtype of Hodgkin's disease are as follows: LPHD shows L26$^+$, α_1-AT$^-$, BerH2$^-$; MCHD and NSHD show L26$^-$, α_1-AT$^+$, BerH2$^+$; and LDHD shows L26$^-$, α_1-AT$^+$, BerH2$^+$ or L26$^+$, α_1-AT$^+$, BerH2$^-$. ——— Hodgkin's disease; Reed-Sternberg cells; immunophenotypes; paraffin section

Hodgkin's disease is a distinct clinical and pathologic entity that has long been the subject of intense investigation. Four histologic subtypes are recognized in Hodgkin's disease, namely, lymphocyte predominance (LPHD), mixed cellularity (MCHD), nodular sclerosis (NSHD), and lymphocyte depletion (LDHD) (Lukes and Butler 1966; Lukes 1971). Diagnostic Reed-Sternberg (R-S) cells are well recognized as the neoplastic cells of Hodgkin's disease (Kaplan 1980), with certain variants characteristic of specific histologic subtypes, e.g. the polyploid lymphocytic and histiocytic (L & H) variants in LPHD, lacunar cells in NSHD, and pleomorphic variants in LDHD (Lukes and Butler 1966; Lukes 1971).

Using a wide variety of approaches derivation of R-S cells has been suggested from B-cells (Weiss et al. 1986; Sundeen et al. 1987; Hall et al. 1988; Werner et al. 1990; Bishop et al. 1991; Hansmann et al. 1991; Lauritzen and Ralfkiaer 1991; Mason et al. 1994), T-cells (Griesser et al. 1986; Cibull et al. 1989; Werner et al. 1990; Lauritzen and Ralfkiaer 1991), activated lymphocytes (Stein et al. 1985; Abe et al. 1988; Chittal et al. 1988; Hall et al. 1988; Schwarting et al. 1989; Werner et al. 1990; Bishop et al. 1991; Hansmann et al. 1991; Carbone et al. 1992), immature lymphoid cells (Herbst et al. 1989), macrophages (Payne et al. 1982; Strauchen 1984), interdigitating reticulum cells (Kadin 1982), and dendritic reticulum cells (Delsol et al. 1993). Therefore, the histogenesis of Hodgkin's disease has not been clearly understood.

The present study reports the application of a combination of routinely used antibodies in the R-S cell and its variants of the different subtypes of Hodgkin's disease. The specific purpose of the study was to determine whether their reactivity with antibodies effective in paraffin sections is sufficiently distinctive to allow the reliable diagnosis of Hodgkin's disease.

Materials and Methods

Tissues studied

Cases were retrieved from the surgical pathology files of the Department of Pathology, Nagasaki University Hospital, Nagasaki, Japan. Formalin-fixed and paraffin-embedded lymph node biopsy material from 68 patients with Hodgkin's disease was utilized for this study. Three micrometer-thick sections were prepared for routine diagnosis. Tissue sections from all cases were stained with hematoxylin-eosin, Giemsa and periodic acid-Schiff.

In all cases, histopathologic diagnosis was made with the strict application of the Rye modification of the Lukes and Butler classification (Lukes and Butler

TABLE 1. *Cases of Hodgkin's disease according to the Rye classification*

Histologic subtype	Number of cases (%)	
Lymphocyte-predominant	14	(21)
Nodular	4	
Diffuse	10	
Mixed cellularity	24	(35)
Nodular sclerosing	19	(28)
Lymphocyte-depleted	11	(16)
Total	68	(100)

1966; Lukes 1971). Based on histopathologic examination, they were divided into (a) lymphocyte-predominant Hodgkin's disease (LPHD), $n=14$ with its subtypes: nodular, $n=4$ and diffuse, $n=10$; (b) mixed cellularity Hodgkin's disease (MCHD), $n=24$; (c) nodular sclerosing Hodgkin's disease (NSHD), $n=19$; and (d) lymphocyte-depleted Hodgkin's disease (LDHD), $n=11$ at first biopsy (Table 1).

Immunoperoxidase method

In all cases, serial sections were immunostained for a large panel of monoclonal and polyclonal antibodies in Table 2. Staining reactions were carried out by a previously described avidin-biotinylated-peroxidase complex (ABC) technique (Takahashi et al. 1992, 1993). Briefly, after dewaxing the sections were blocked for endogenous peroxidase activity with 0.03% hydrogen peroxide in methanol. The sections were then incubated with 1% normal horse serum and 1% normal goat serum, followed by primary antibody for 1 hr, biotinylated horse anti-mouse antibody against mouse monoclonal antibody and biotinylated goat anti-rabbit antibody against rabbit polyclonal antibody for 30 min, and avidin-biotinylated peroxidase complex (DAKO, Copenhagen, Denmark) for 45 min. Between the steps, the sections were thoroughly washed with phosphate buffered saline (pH 7.4). Antibody localization was effected by the use of a peroxidase reactions with 0.03% hydrogen peroxide and 0.6% diaminobenzidine. Sections were then counterstained with Harris' hematoxylin, dehydrated, and mounted. Trypsinization was performed prior to staining with anti-CD3 antibody and BerH2.

Negative controls were used by omission of the primary antibody. Simultaneous staining of several different sections with the same antibody served as mutual controls. The following positive internal controls were used: parafollicular T-lymphocytes for CD3, UCHL1 and DFT1; follicular B-lymphocytes for L26 and KiB3; macrophages, monocytes and granulocytes for α_1-antitrypsin (α_1-AT), LN5, KP1 and cathepsin D; natural killer cells in germinal center for

TABLE 2. *Monoclonal and polyclonal antibodies used*

Antibodies	Predominant reactivity	Cluster designation	Source
T-cell markers			
CD3	T-cells	CD3	Dako Ltd., Copenhagen, Denmark
UCHL1	T-cells, epithelioid cells	CD45RO	Dako Ltd.
DFT1	T-cells, myeloid cells, macrophages, some B-cell lymphomas	CD43	Dako Ltd.
B-cell markers			
L26	Pan B-cells	CD20	Dako Ltd.
KiB3	Subtype of leukocyte common antigen predominantly expressed on B-cells		Pathol. Inst. Univ. Kiel, Germany
Macrophage/monocyte markers			
α_1-Antitrypsin	Macrophages, monocytes, granulocytes, some epithelial cells		Dako Ltd.
LN5	Macrophages, monocytes, mantle zone B-cells, gastric chief cells, interdigitating reticulum cells		Techniclone Int., CA, USA
KP1	Macrophages, monocytes, granulocytes, interdigitating reticulum cells	CD68	Dako Ltd.
Cathepsin D	Macrophages, histiocytes		Novocastra Lab. Ltd., Newcastle, UK
Miscellaneous			
LN3	Cells expressing HLA/DR		Techniclone Int.
PD7/26	Lymphocytes, myeloid cells, macrophages, monocytes	CD45RB	Dako Ltd.
BerH2	Hodgkin cells, Sternberg-Reed cells, activated lymphocytes	CD30	Dako Ltd.
Leu7	Natural killer cells, neuroendocrine cells	CD57	Becton-Dickinson, CA, USA
EMA	Epithelial cells, activated lymphocytes, plasma cells		Dako Ltd.
S-100 protein	Interdigitating reticulum cells, neuroendocrine cells		Dako Ltd.

Leu7; plasma cells for epithelial membrane antigen (EMA); peripheral nerves for S-100 protein; lymphoid cells for LN3 and leukocyte common antigen (LCA-CD45RB, PD7/26); and large cell anaplastic Ki-1 positive lymphoma for BerH2.

The staining reaction was estimated to be positive if only single R-S cell was labeled within one section, irrespective of intensity. When we investigated the immunostained paraffin sections for UCHL1, DFT1, L26, KiB3, LN3 and BerH2, the following point was noted. Namely, if R-S cells and variants show strong membranous staining pattern with circular and continuous fashion at the cell membrane, we regarded as positive cells for the above-mentioned six monoclonal antibodies.

In order to confirm the immunohistochemical phenotype of the same R-S cells, three serial sections were subjected to immunostaining for L26, α_1-AT and BerH2.

Results

The results of staining of Reed-Sternberg (R-S) cells and variants thereof are shown in Table 3. The detection rate of immunoreactive cases for R-S cells was the highest with BerH2 (75%), α_1-AT (72%) and LN3 (71%). The detection rate was much lower with L26 (32%). In 5 cases (7%) staining of some or the majority of R-S cells with other B-cell marker (KiB3) was seen. LN5, KP1 and cathepsin D, which were macrophage/monocyte markers other than α_1-AT, bound to R-S cells in 4 cases (6%), 9 cases (13%) and 10 cases (15%), respectively. In 12 cases, R-S cells and variants showed cytoplasmic positivity with PD7/26. EMA was detected in R-S cells of only 3 cases. In general, because R-S cells in Hodgkin's disease may be surrounded by many reactive T-cells, R-S cells were regarded as positive only when adjacent small lymphocytes were negative and unequivocal positivity was demonstrated at the surface membrane of R-S cells. We detected partial membranous positivity for UCHL1 and DFT1 in R-S cells and variants of only one case. However, R-S cells and variants in this case were judged negative because of lack of immunoreaction in portions of their cell membrane. T-cell markers including anti-CD3 antibody were evaluated negative in R-S cells of the present study. No positive staining of R-S cells with Leu7 and S-100 protein was observed.

Lymphocyte-predominant Hodgkin's disease (LPHD) (14 cases)

Six of 14 cases of LPHD showed reactivity of scattered L & H cells with α_1-AT, 2 cases stained with KP1, and one case stained with LN5 and cathepsin D. Of the 14 cases of LPHD, 9 showed definite staining of cell membrane in most of the L&H type of R-S cells with L26 (Fig. 1). KiB3 showed cytoplasmic reaction in a few R-S cells in 2 cases. Positive reaction with BerH2 was obtained in 5 of 14 cases of LPHD. The staining was membranous when present, commonly associated with a focal positivity restricted to the paranuclear area. HLA-DR

TABLE 3. *Results of immunohistochemical staining of Reed-Sternberg cells and thier variants*

Subtype	Number of cases	Number of positive cases of immunostaining														
		CD3	UCHL1	DFT1	L26	KiB3	α_1-AT	LN5	KP1	Cathepsin D	LN3	PD7/26	BerH2	Leu7	EMA	S-100 protein
Lymphocyte-predominant	14	0	0	0	9	2	6	1	2	1	5	3	5	0	1	0
Nodular	(4)	(0)	(0)	(0)	(2)	(0)	(2)	(0)	(0)	(0)	(2)	(0)	(1)	(0)	(1)	(0)
Diffuse	(10)	(0)	(0)	(0)	(7)	(2)	(4)	(1)	(2)	(1)	(3)	(3)	(4)	(0)	(0)	(0)
Mixed celluilarity	24	0	0	0	4	1	15	2	2	1	17	2	22	0	2	0
Nodular sclerosing	19	0	0	0	4	0	19	3	3	1	17	1	18	0	0	0
Lymphocyte-depleted	11	0	0	0	5	2	9	4	2	1	9	6	6	0	0	0
Total	68	0	0	0	22	5	49	10	9	4	48	12	51	0	3	0

TABLE 4. *Immunological phenotypes of Reed-Sternberg cells in histologic subtypes of Hodgkin's disease*

Immunological phenotype	Number of positive cases in histologic subtypes				Total
	LPHD	MCHD	NSHD	LDHD	
$L26^+/\alpha_1$-$AT^+/BerH2^+$	1	3	4	1	9
$L26^+/\alpha_1$-$AT^+/BerH2^-$	1	0	0	3	4
$L26^+/\alpha_1$-$AT^-/BerH2^+$	1	0	0	0	1
$L26^+/\alpha_1$-$AT^-/BerH2^-$	6	0	0	1	7
$L26^-/\alpha_1$-$AT^+/BerH2^+$	2	14	14	4	34
$L26^-/\alpha_1$-$AT^+/BerH2^-$	2	0	1	1	4
$L26^-/\alpha_1$-$AT^-/BerH2^+$	1	5	0	1	7
$L26^-/\alpha_1$-$AT^-/BerH2^-$	0	2	0	0	2
Total	14	24	19	11	68

α_1-AT, α_1-antitrypsin; LPHD, Lymphocyte-predominant Hodgkin's disease; MCHD, Mixed cellularity Hodgkin's disease; NSHD, Nodular sclerosing Hodgkin's disease; LDHD, Lymphocyte-depleted Hodgkin's disease.

antibody, detected by LN3, was identified in L & H cells in 5 cases of LPHD (Fig. 2). Staining with the antibody to PD7/26 showed a weak and focal cytoplasmic labeling of L & H cells in 3 cases of LPHD (Fig. 3). Only one case was reactive with anti-EMA antibody. In 7 cases of LPHD, R-S cells and their variants were $L26^+$, α_1-AT^-. Furthermore, 6 of these 7 cases were BerH2 negative (Table 4).

Mixed cellularity Hodgkin's disease (MCHD) (24 cases)

Diagnostic R-S cells and variants showed different staining patterns with LPHD. α_1-AT was expressed in the cytoplasm of R-S cells in 15 of 24 cases of MCHD (Fig. 4). Only 4 cases of MCHD showed reactivity of R-S cells with the pan-B marker L26, and one stained with KiB3. In the majority of cases, the staining pattern was uniform for BerH2 (Fig. 5). Expression of HLA-DR antigen stained by LN3 was strongly found in the diagnostic R-S cells of 17 cases. In 2 cases, R-S cells were positive for LN5, KP1, PD7/26 and anti-EMA (Fig. 6). Cathepsin D was positive in a single case out of 24 of MCHD. In over half of the cases of MCHD, diagnostic R-S cells displayed the combination of staining pattern with $L26^-$, α_1-AT^+ and $BerH2^+$ (Table 4).

Nodular sclerosing Hodgkin's disease (NSHD) (19 cases)

In the majority of cases of NSHD, the staining patterns for α_1-AT (19 of 19 cases; Fig. 7), BerH2 (18 of 19 cases) and LN3 (17 of 19 cases; Fig. 8) antibodies in RS cells and lacunar variants were remarkably parallel, with only minor variations in some instances. In a small proportion of cases of NSHD, a few R-S cells and lacunar variants exhibited a cytoplasmic staining for L26, LN5, KP1

(Fig. 9), cathepsin D and PD7/26. In the 14 cases of NSHD, R-S cells and lacunar variants displayed the staining pattern of L26$^-$, α_1-AT$^+$ and BerH2$^+$ (Table 4). This particular phenotype is similar to that of MCHD. In marked contrast, anti-EMA was negative in the cytoplasm of R-S cells and lacunar cells.

Lymphocyte-depleted Hodgkin's disease (LDHD) (11 cases)

α_1-AT and LN3 were found to be positive on a large number of R-S cells and pleomorphic variants in the majority of 11 cases of LDHD. L26 and PD7/26 were positive on R-S cell and pleomorphic variants in 5 and 6 cases, respectively. Although BerH2 reacted with R-S cells in 6 cases (Fig. 10), positive frequency of this antibody was lower than that in the whole of Hodgkin's disease. LN5 (Fig. 11), KP1 (Fig. 12) and cathepsin D in the group of macrophage/monocyte markers that showed any reactivity with R-S cells gave positive results in 4 cases, 2 cases and 1 case, respectively. The staining pattern of L26$^-$ and α_1-AT$^+$ was observed in 5 cases of LDHD, whereas 4 cases showed the staining pattern of L26$^+$ and α_1-AT$^+$ (Table 4).

Discussion

The origin and nature of R-S cells have long been a subject of intense investigation and discussion. Morphologic and immunologic evidence has suggested a lymphoid cell, macrophage, interdigitating reticulum cell, dendritic reticulum cell, or possibly a diverse origin for these cells (Kadin 1982; Stein et al. 1983, 1985; Hsu et al. 1985; Abe et al. 1988; Chittal et al. 1988; Hall et al. 1988; Cibull et al. 1989; Schwarting et al. 1989; Werner et al. 1990; Bishop et al. 1991; Hansmann et al. 1991; Lauritzen and Ralfkiaer 1991; Carbone et al. 1992; Delsol et al. 1993; Mason et al. 1994). The present study has indicated that the staining characteristics of R-S cells and variants may express markers generally regarded as B-cell, or macrophage/monocyte associated, with some relationship to histological subtype.

In the present study, immunoreactivity with L26 was observed in 22 cases of Hodgkin's disease, possibly suggesting a B-cell nature. Several authors have described cases of Hodgkin's disease in which the R-S cells show immunoreactivity with anti-B-cell antibodies (Hall et al. 1988; Werner et al. 1990; Bishop et al. 1991; Hansmann et al. 1991; Lauritzen and Ralfkiaer 1991; Mason et al. 1994). Although Knowles et al. (1986) have reported the absence of immunoglobulin gene rearrangements in cases of Hodgkin's disease, others have found such rearrangements in some but not all cases (Weiss et al. 1986; Sundeen et al. 1987). Immunoreactivity of occasional cases of Hodgkin's disease with antibodies that recognize antigens expressed on T-lymphocytes has been reported (Stein et al. 1985; Hall et al. 1988; Cibull et al. 1989; Werner et al. 1990; Lauritzen and Ralfkiaer 1991). However, our observation does not support a relationship between R-S cells and T-cells. In general, the staining reactions of

R-S cells with T-cell markers were difficult to assess, since in the majority of cases small T-lymphocytes were present in large numbers, tending to obscure the reactions of the R-S cell itself. Although a few R-S cells in single case of the present study were positive for UCHL1 and DFT1, we judged negative due to the lack of immunoreactions in portions of R-S cell membrane. Furthermore, positive staining of R-S cells for CD3 was not observed in the present study.

Several macrophage/monocyte markers seem to recognize determinants on R-S cells (Stein et al. 1982; Hsu et al. 1985; Norton and Isaacson 1985; Abe et al. 1988). Anti-LeuM1 (CD15) has in recent years been proposed as a useful and specific marker of Hodgkin's disease (Hsu et al. 1985; Norton and Isaacson 1985). Critical analysis, however, indicates that this is neither a sensitive nor specific marker of Hodgkin's disease (Hall and D'Ardenne 1987). Polyclonal antibody α_1-AT, as a marker generally observed for macrophages and monocytes (Cohen 1973; Isaacson et al. 1979), was used in the present study. Our results of α_1-AT staining were compared with those of α_1-AT in the work by Payne and co-workers (1982). In their investigation, although α_1-AT staining was positive in 19 (79%) of 24 cases of Hodgkin's disease, the number of cases positive for α_1-AT in each subtype of Hodgkin's disease was not clearly described. In our material, α_1-AT staining was positive in 49 (72%) of 68 cases and reacted with a larger number of R-S cells in these positive cases. Furthermore, our findings indicated that all cases of NSHD, and about 60% of MCHD and LDHD cases were marked with α_1-AT. With the exception of LP subtype α_1-AT among the macrophage/monocyte associated antibodies was of great value in diagnosing Hodgkin's disease.

Some studies of the LPHD have demonstrated its lack of homogeneity with the other histologic subtypes, clinically as well as immunologically, and have even proposed that it represents a distinct entity (Poppema et al. 1979; Abdulaziz et al. 1984; Werner et al. 1990; Bishop et al. 1991; Hansmann et al. 1991; Lauritzen and Ralfkiaer 1991; Mason et al. 1994). In contrast to the other subtypes of Hodgkin's disease, which initially involve the T zones of the lymph node (Lukes 1971), LPHD appears to originate in the B-cell areas (Poppema et al. 1979; Abdulaziz et al. 1984; Timens et al. 1986; Bishop et al. 1991; Hansmann et al. 1991; Mason et al. 1994). The results of our study further support the possibility that LPHD may represent an entity distinct from the other histologic subtypes. R-S cells and variants of NSHD and MCHD are similar in their expression of the macrophage/monocyte-related differentiation antigen detected by α_1-AT; whereas L & H variants of LP type are rarely reactive. Such a staining profile for α_1-AT in different types of Hodgkin's disease has not been reported. Another divergent staining pattern for L & H variants, as compared with other types of R-S cells, is the expression of B-cell antigens, further supporting a B-cell derivation for these cells, as suggested by earlier studies (Poppema et al. 1979; Coles et al. 1988; Bishop et al. 1991; Hansmann et al. 1991; Mason et al. 1994). In support of their

findings, our study demonstrates that immunohistochemical profiles of L & H cells have L26$^+$ and α_1-AT$^-$ in half cases of LPHD. Ki-1/BerH2 (CD30) antigen, an activation-associated lymphocyte antigen, was recognized on Hodgkin and R-S cells (Stein et al. 1985; Chittal et al. 1988; Hall et al. 1988; Schwarting et al. 1989; Werner et al. 1990; Bishop et al. 1991; Hansmann et al. 1991; Carbone et al. 1992). In the present study, BerH2 was expressed on the R-S cells in three-fourth of 68 cases, however, this protein was only rarely identified on L & H variants. The lower proportion of cases expressing BerH2 in LPHD reinforces the findings of previous studies (Chittal et al. 1988; Werner et al. 1990; Bishop et al. 1991; Hansmann et al. 1991). Monoclonal antibody LN3 that reacts with HLA-DR antigen (Marder et al. 1985) was observed on R-S cells in 48 of 68 Hodgkin's disease cases. Of considerable interest was the expression of LN3 on L & H cells in only 5 of our LPHD cases. This lower incidence of staining with LN3 in cases of LPHD was characteristic finding in the present study.

The clinical and epidemiologic differences between NSHD and the other histologic subtypes have suggested that these might be different diseases. In this study, R-S cells and lacunar cells in NSHD (19 cases) and MCHD (24 cases) were similar in their staining profiles, whereas the 14 cases of LPHD group tested failed to show α_1-AT and BerH2 expression in 8 and 9 cases, respectively. This difference might relate to a stage in evolution of the disease process or to a difference in the cytogenealogy of the R-S cells and variant thereof. Fourteen cases (51%) tested in the MCHD group showed L26$^-$, α_1-AT$^+$, BerH2$^+$ on R-S cells. Furthermore, the similar phenotype of L26$^-$, α_1-AT$^+$, BerH2$^+$ was observed in 14 cases (74%) of NSHD. There were some notable exceptions in the MCHD group. Five cases were both L26 and α_1-AT negative but positive for BerH2. From this finding, BerH2 positivity should be potentially useful for the diagnosis of MCHD and NSHD because of its high detection rate on R-S cells. In 13 cases of NSHD group (14 cases) with the combined data of L26$^-$, α_1-AT$^+$, BerH2$^+$, there was a strong expression of LN3 on R-S cells. Therefore, immunohistochemical panel of α_1-AT, BerH2 and LN3 showed diagnostically helpful reactivity in NSHD group.

The phenotypic position of LDHD is difficult to determine, because so complicated patterns were indicated. The immunophenotype was inconsistent in terms of the combination of L26 and α_1-AT expression. The phenotype of L26$^-$, α_1-AT$^+$ is observed in 5 cases (45%) of LDHD group, and this pattern predominated in MCHD and NSHD groups. On the other hand, 4 cases (36%) in the LDHD group were positive for L26 and α_1-AT on R-S cells and variants. Such a immunohistochemical profile was found in LPHD (2 cases), MCHD (3 cases) and NSHD (4 cases) groups. The present study may indicate that LDHD contains two subgroups with different immunophenotypes. The detection rate of BerH2 in LDHD indicates intermediate value between LPHD and MCHD or NSHD groups, and that of LN3 in LDHD group indicates intermediate value between

MCHD and NSHD groups.

Most cases of large cell anaplastic lymphoma (LCAL) may be separated from LDHD on the basis of lack of typical R-S cells as well as the presence of large numbers or sheets of atypical cells with irregular nuclei. However, histological distinction between LCAL and LDHD may on occasion be difficult because of the frequent presence of R-S-like cells in a milieu of inflammatory cells including plasma cells and eosinophils in LCAL. The entity of LCAL (Lennert and Feller 1992), a few of which were classified as LDHD, was individualized by the presence of Ki-1 antigen on neoplastic cells. In the majority of cases with LCAL, the neoplastic cells express anti-EMA antibody. In contrast, R-S cells in Hodgkin's disease are rarely positive for anti-EMA antibody (Hall et al. 1988). In the present study, we used immunohistochemical result of anti-EMA antibody as an aid of distinction between LCAL and LDHD.

The following conclusions are drawn from the data presented here: (1) Our multiple marker analysis on R-S cells and variants thereof provided the immunohistochemical characters of B-cells and cells of the macrophage/monocyte system. (2) The staining profile for R-S cells of L & H type in our cases of LPHD suggests that these cells have high detection rate of L26 and most likely represent transformed lymphoid cells of B-cell derivation. By contrast, R-S cells and variants in MCHD, NSHD and LDHD subtypes, which showed high detection rate of cases with α_1-AT positivity, have immunohistochemical features most compatible with a derivation of macrophage/monocyte. (3) R-S cells and variants of all histological subtypes of Hodgkin's disease demonstrated a variety of antigens and confirmed the immunophenotypic heterogeneity. Therefore, Hodgkin's disease appears to be a heterogeneous disorder and the hypothesis of one specific cell line for the R-S cells of Hodgkin's disease must probably be revised.

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Fig. 1. Hodgkin's disease, lymphocyte predominant type, nodular; staining with L26 antibody. L&H variants in LP Hodgkin's disease reveals immunoreactivity for L26, characterized by membrane staining of these cells. (ABC, hematoxylin counterstain, ×200)

Fig. 2. Hodgkin's disease, lymphocyte predominant type, diffuse; staining with LN3 antibody. L&H cell shows cytoplasmic and membrane staining for LN3. (ABC, hematoxylin counterstain, ×200)

Fig. 3. Hodgkin's disease, lymphocyte-predominant type, diffuse; staining with PD7/26 antibody. Some L&H cells show membranous and contiguous cytoplasmic staining for PD7/26. Surrounding small and medium-sized lymphoid cells show strong reactivity. (ABC, hematoxylin counterstain, ×200)

Fig. 4. Hodgkin's disease, mixed cellularity type; staining with α_1-antitrypsin antibody. Typical R-S cell is strongly positive for α_1-antitrypsin antibody. The staining pattern is cytoplasmic positivity. (ABC, hematoxylin counterstain, ×200)


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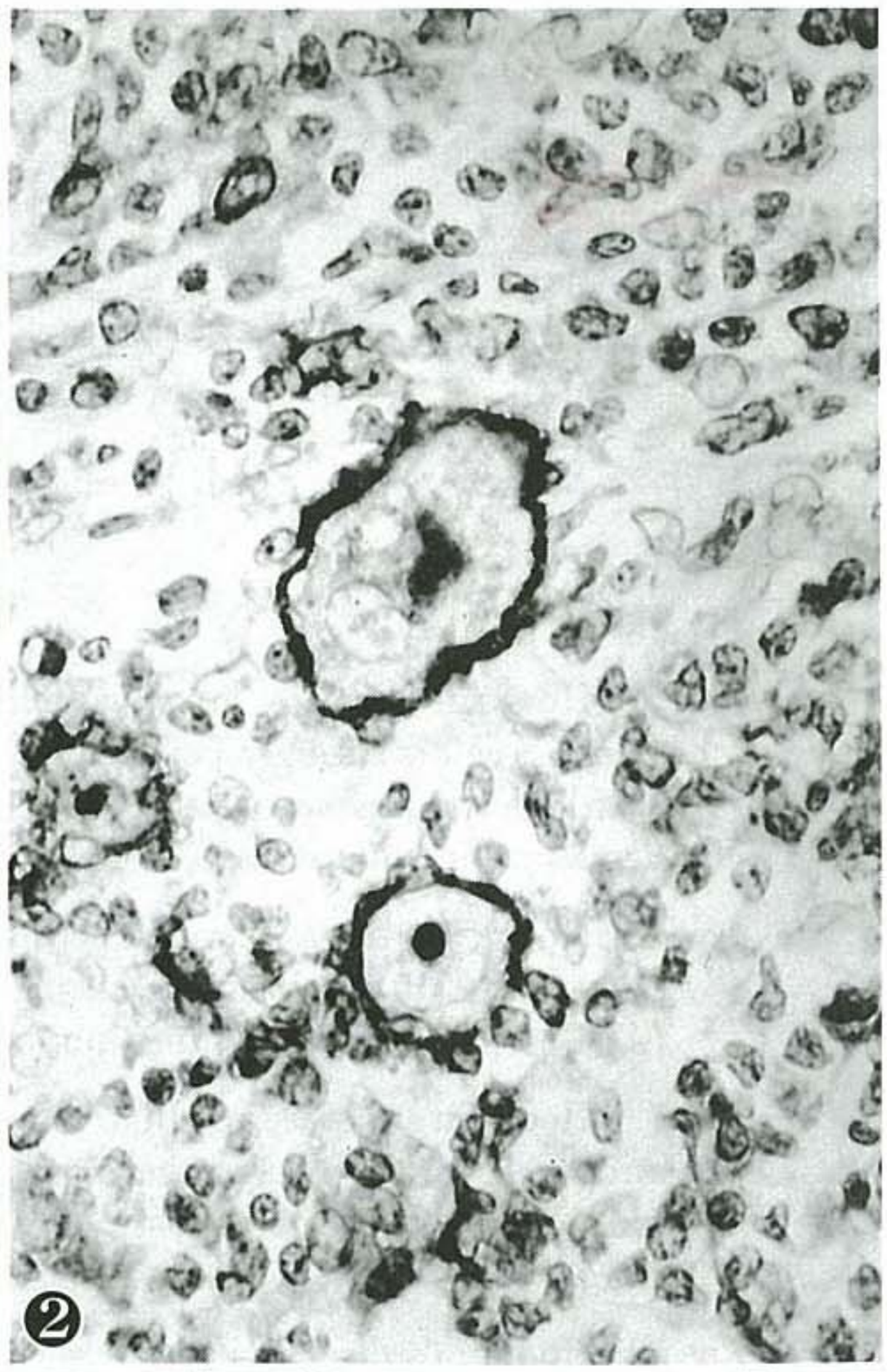
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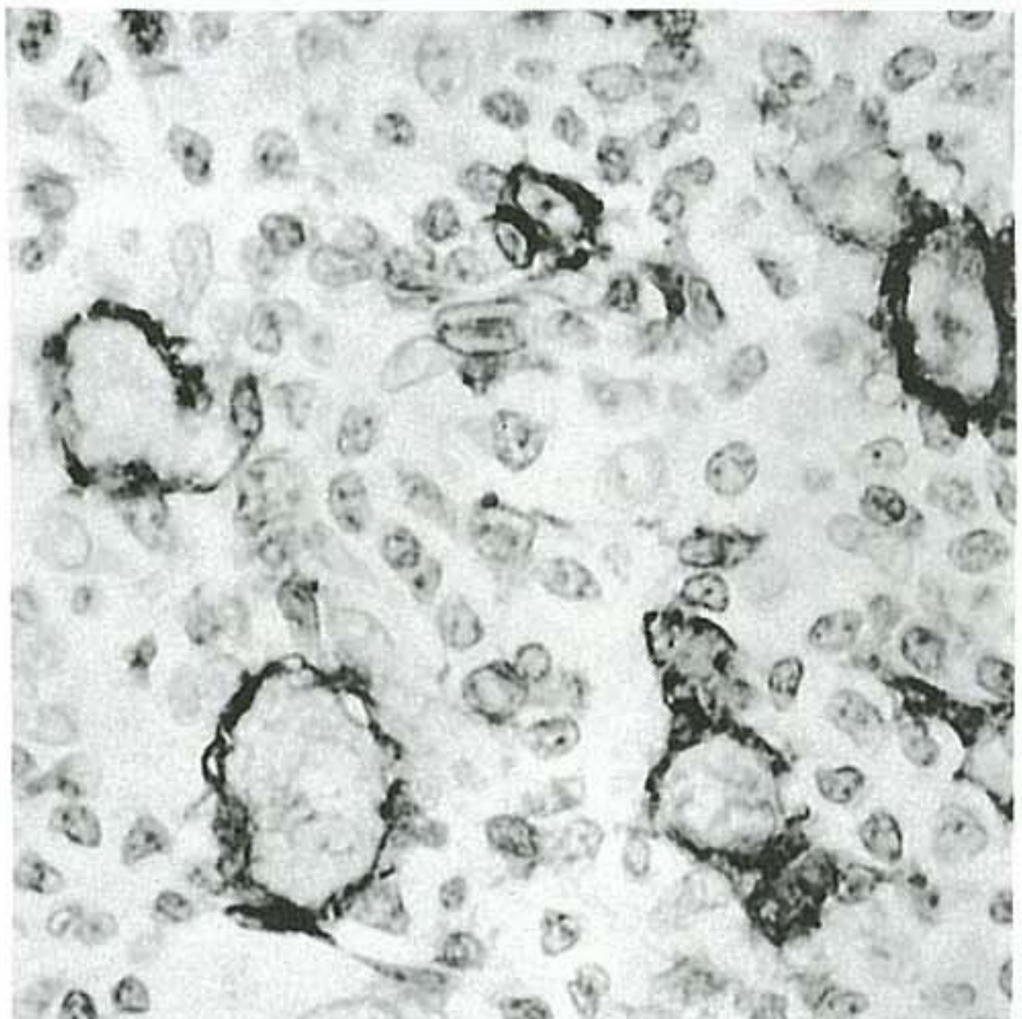
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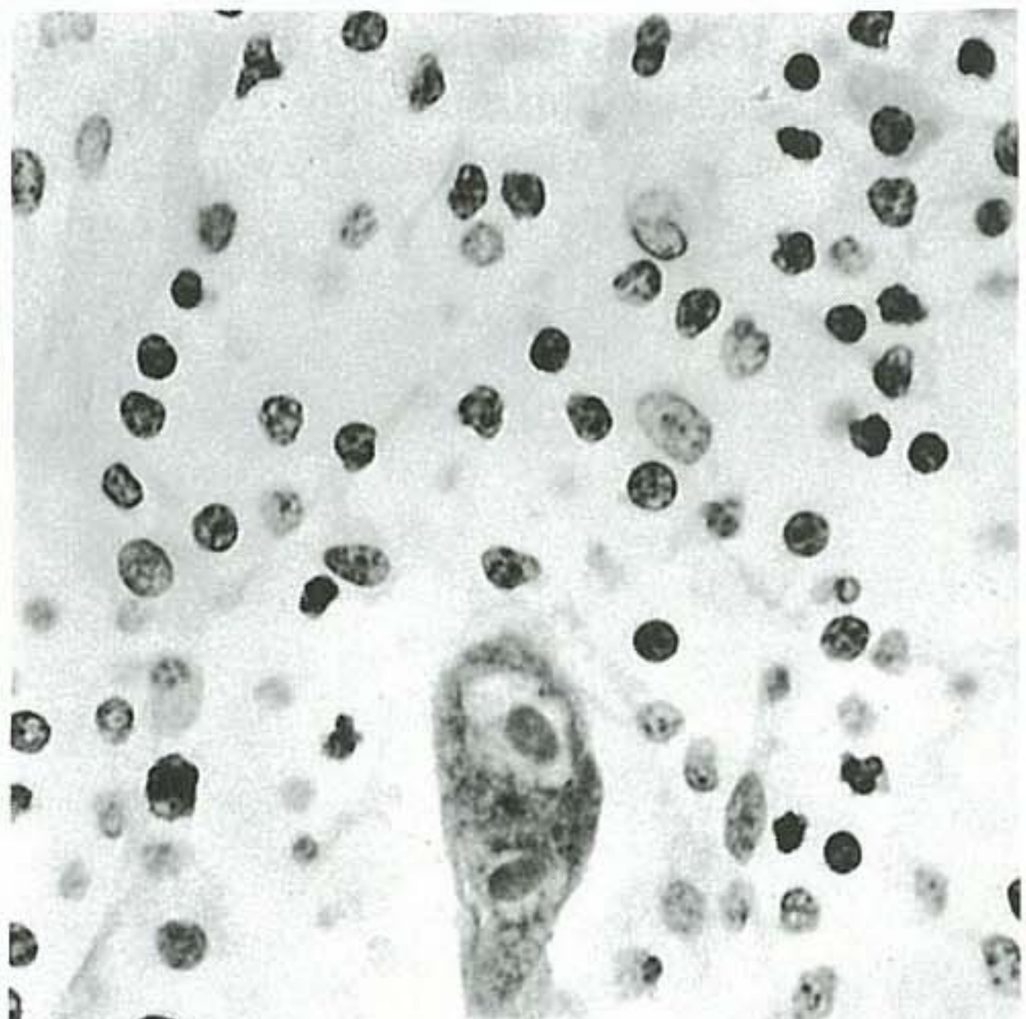
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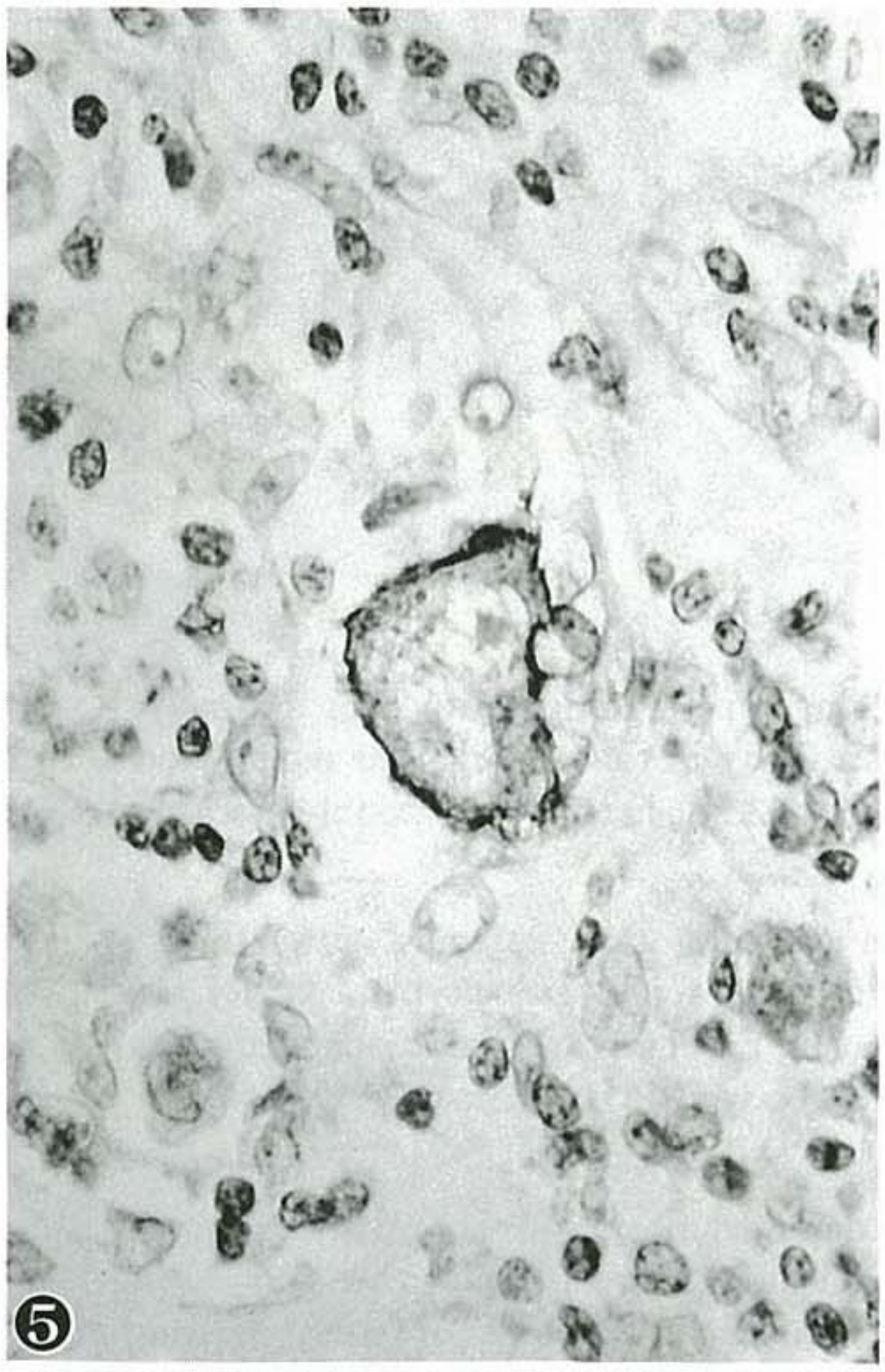
Fig. 5. Hodgkin's disease, mixed cellularity type; staining with BerH2 antibody. The R-S cell is strongly positive for BerH2 antibody. The staining pattern is clearly membrane-associated, with a dot-like paranuclear positivity. (ABC, hematoxylin counterstain, ×200)

Fig. 6. Hodgkin's disease, mixed cellularity type; staining with anti-EMA antibody. The R-S cell shows strong cytoplasmic reaction for this antibody. (ABC, hematoxylin counterstain, ×200)

Fig. 7. Hodgkin's disease, nodular sclerosing type; staining with α_1-antitrypsin antibody. R-S cells and their variants show diffuse cytoplasmic staining and some histiocytes are also strongly positive. (ABC, hematoxylin counterstain, ×200)

Fig. 8. Hodgkin's disease, nodular sclerosing type; staining with LN3 antibody. R-S cells and lacunar variants show strong expression of LN3 with membrane and cytoplasmic staining. A cluster of lymphoid cells is also positive for LN3. (ABC, hematoxylin counterstain, ×200)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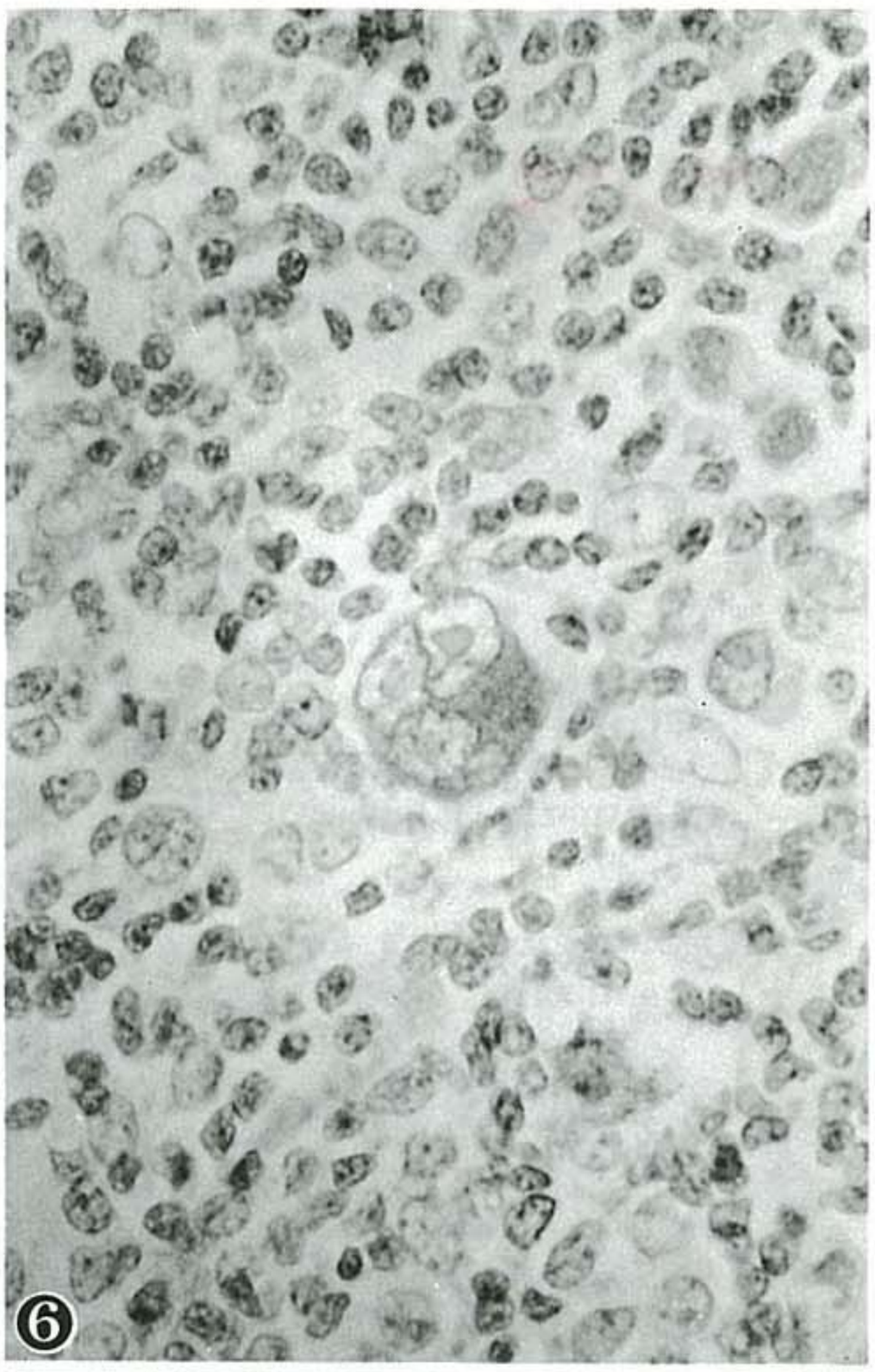
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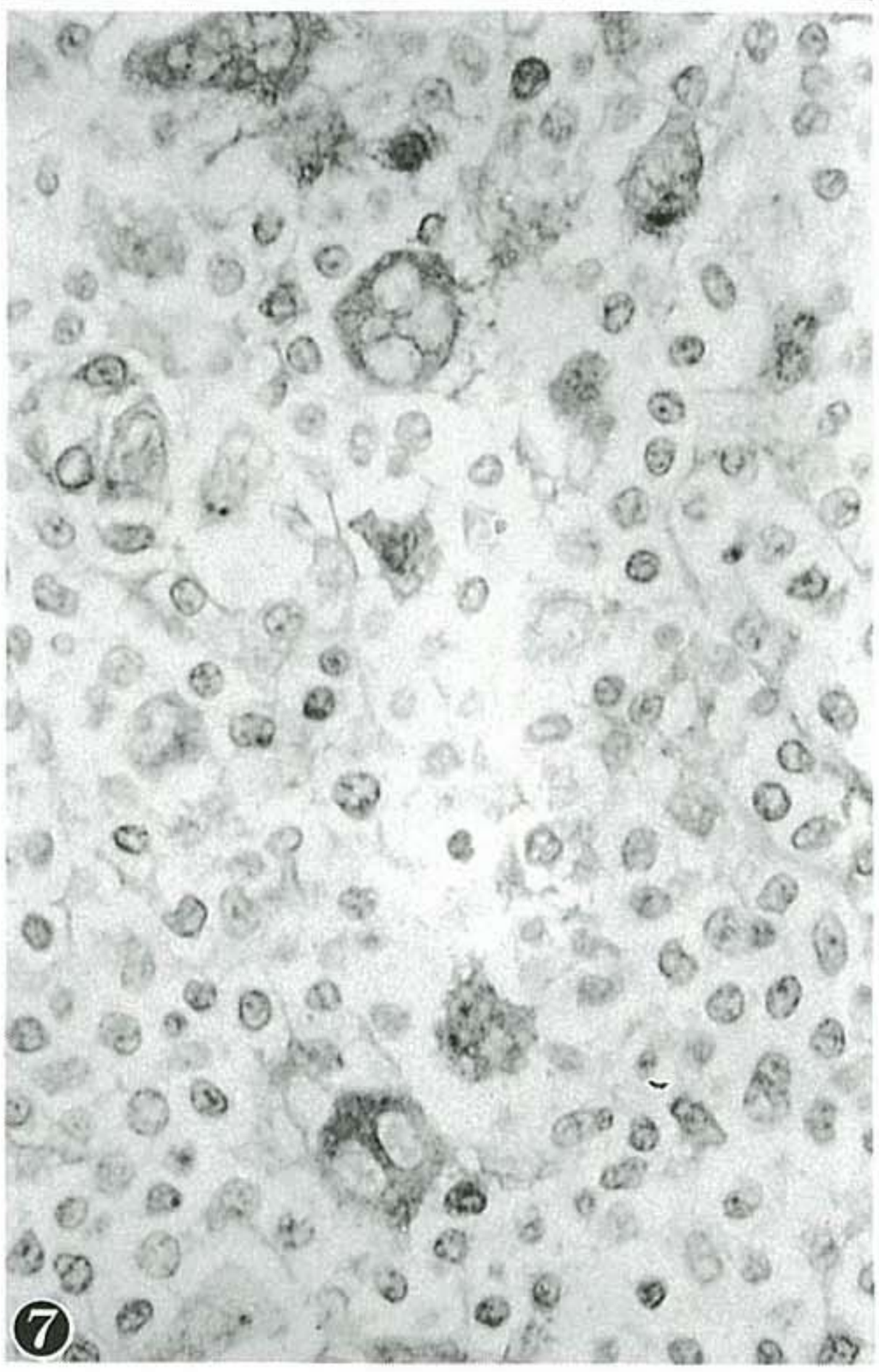
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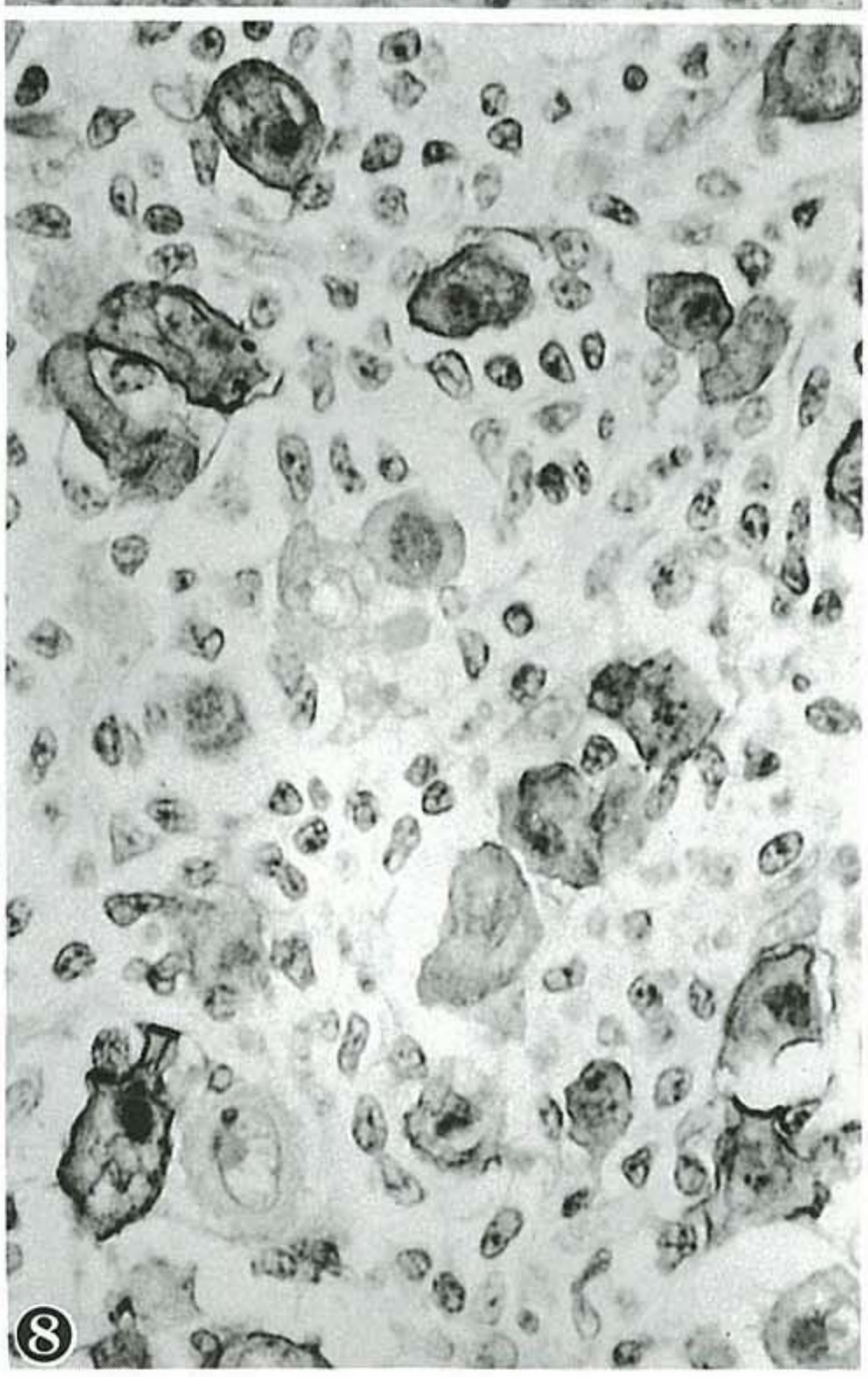
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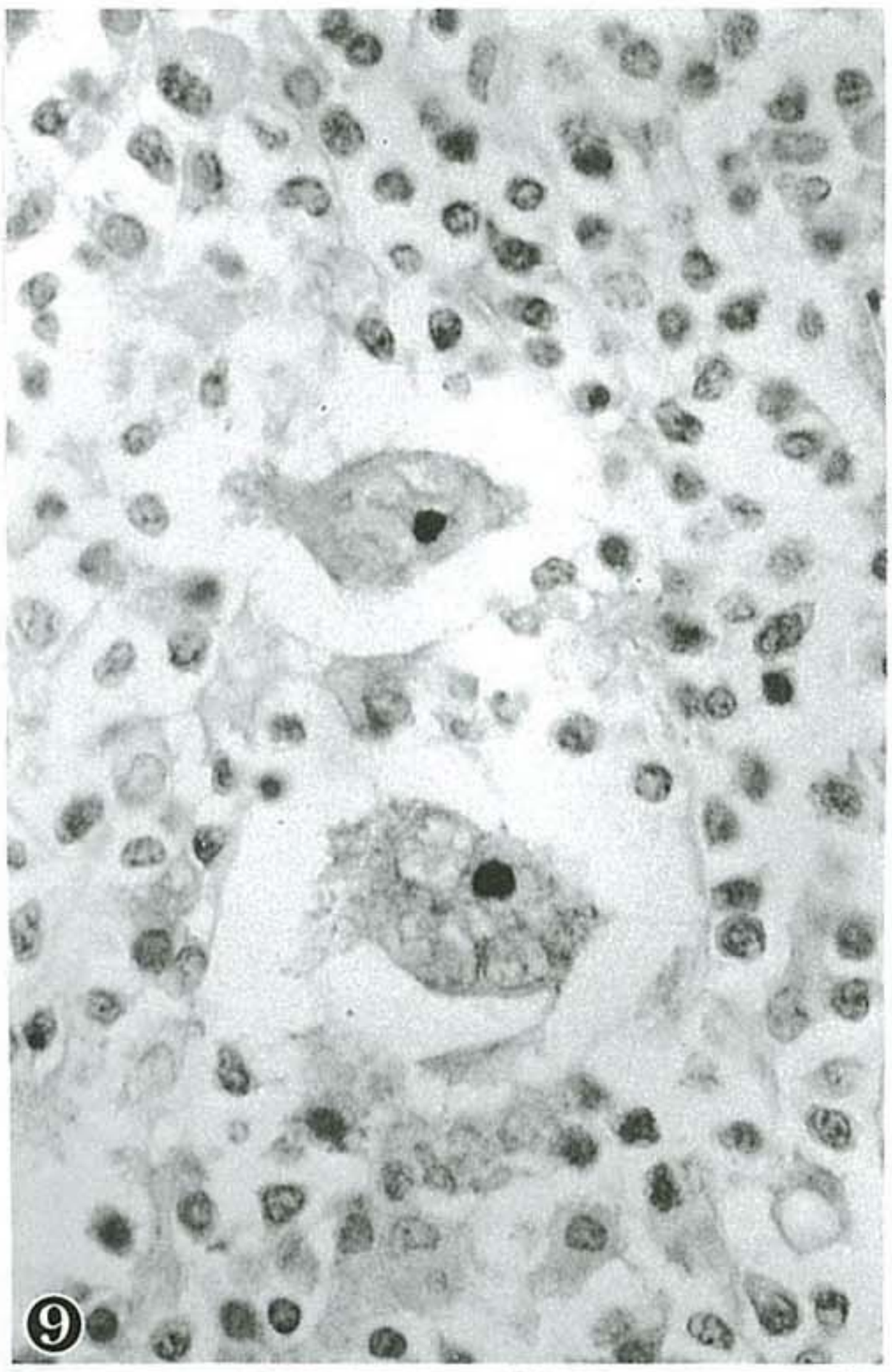
Fig. 9. Hodgkin's disease, nodular sclerosing type; staining with KP1 antibody. Lacunar cells strongly express KP1 antibody in the juxtanuclear region, and surrounding histiocytes are also stained. (ABC, hematoxylin counterstain, × 200)

Fig. 10. Hodgkin's disease, lymphocyte depleted type; staining with BerH2 antibody. Pleomorphic variant of R-S cells (cells with atypical nuclei not characteristic of R-S cells) demonstrating distinctive staining of membranes and juxtanuclear region by BerH2 antibody. (ABC, hematoxylin counterstain, ×200)

Fig. 11. Hodgkin's disease, lymphocyte depleted type; staining with LN5 antibody. Immunoreactivity with LN5 antibody is seen in a pleomorphic variant of R-S cell. Surrounding histiocytes show positive reaction. (ABC, hematoxylin counterstain, ×200)

Fig. 12. Hodgkin's disease, lymphocyte depleted type, staining with KP1 antibody. R-S cells and variants thereof exhibit cytoplasmic staining pattern for KP1. Histiocytes in the background stain with KP1. (ABC, hematoxylin counterstain, ×200)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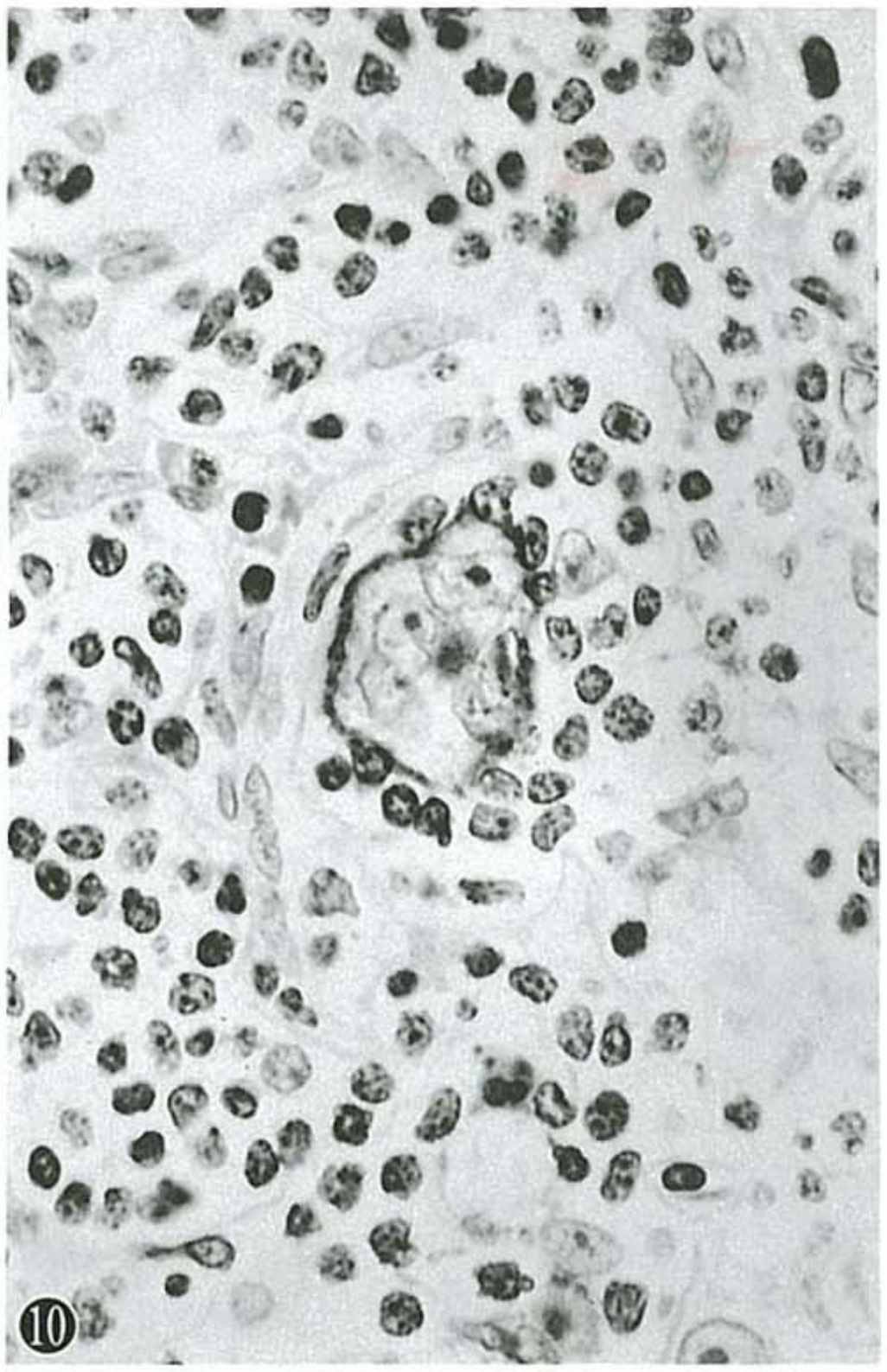
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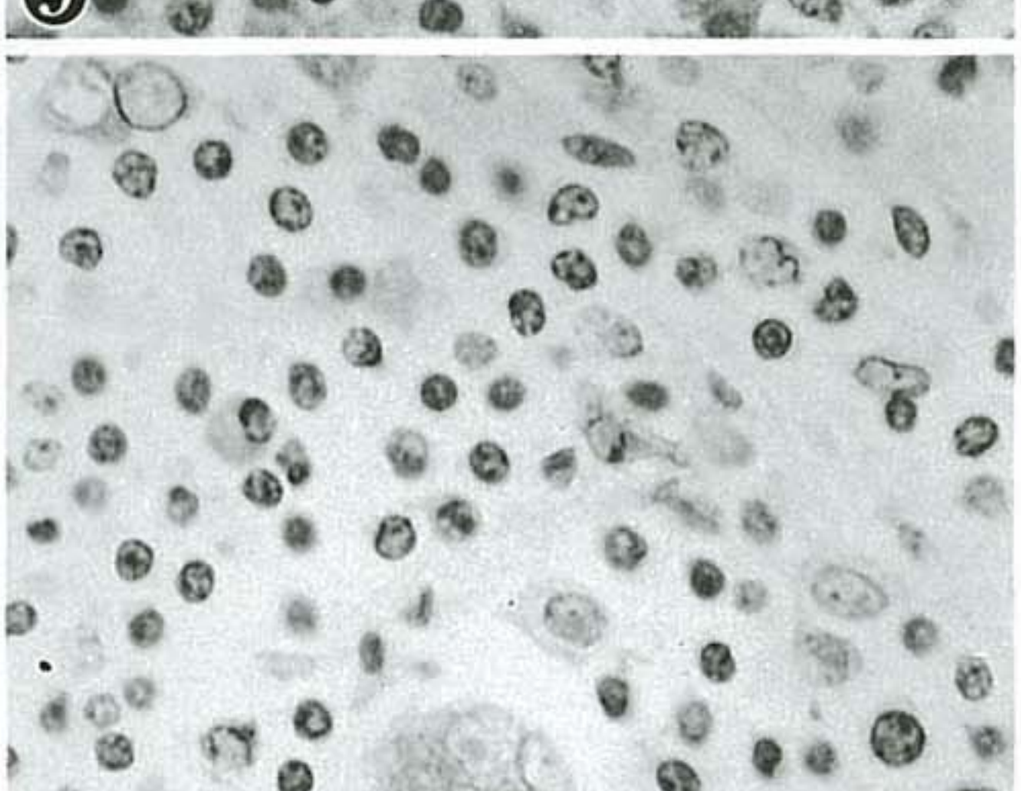
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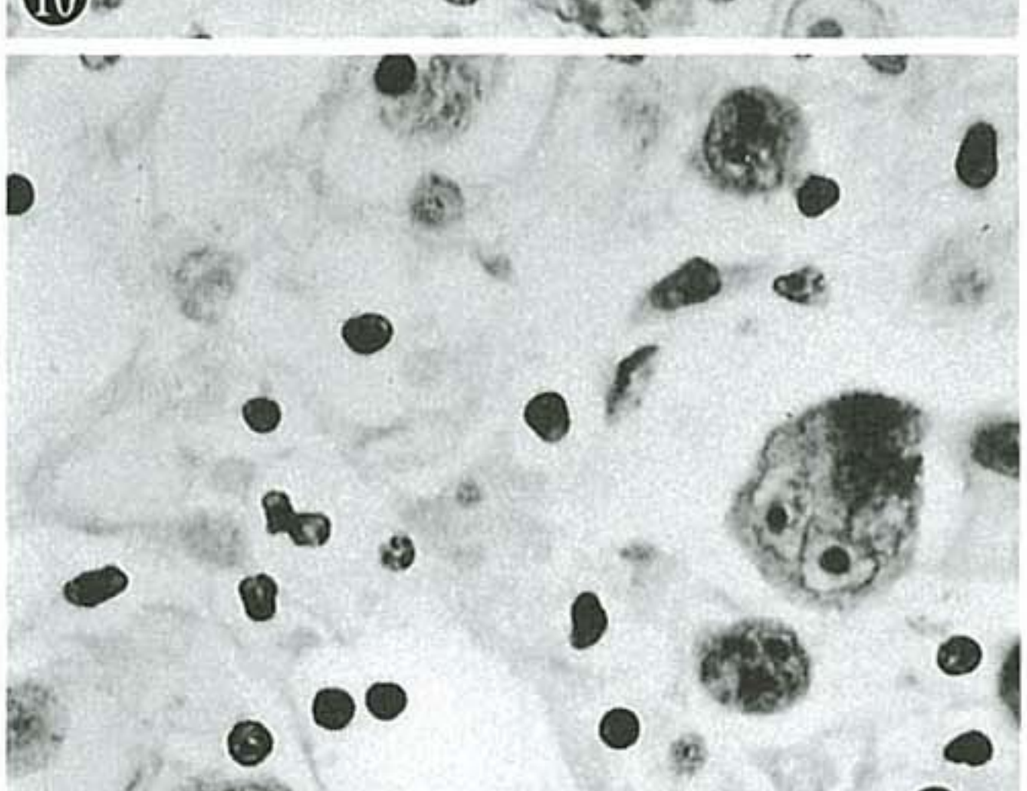
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