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Expression of T-Cell Antigens on Reed–Sternberg Cells in a Subset of Patients With Nodular Sclerosing and Mixed Cellularity Hodgkin's Disease

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Expression of T-cell antigens by Reed–Sternberg (RS) cells has not been detected in most studies of Hodgkin's disease (HD). The authors employed an improved method of fixation (paraformaldehyde–lysine–periodate), which sharply defined cell borders and revealed T-cell antigens on RS cells in 8 of 30 (27%) cases of HD. Antigen-specific staining was confirmed by immunoelectron microscopy. RS cells expressed T11 (8/8 cases), Leu-3 or T4 (4/8 cases), Leu-4 or T3 (3/8 cases), but not other T-cell specific antigens (Leu-1, T8, T6, 3A1). RS cells were negative for leukocyte common antigen (LCA/T200), in contrast to positive LCA/T200 staining of RS-like cells in T-cell lymphomas. RS cells in all HD cases were positive for Ki-1 and/or Leu-M1 antigens. The percentage of RS cells expressing T-cell antigens was <20% (2 cases), 20–50% (3 cases), or >50% (3 cases). This percentage and the specific T-cell antigens expressed varied in tissues from different sites in each of 2 cases. Expression of T-cell antigens by RS cells was found in nodular sclerosis (6 of 20 cases) and mixed cellularity (2 of 5 cases) but not in lymphocyte predominance (2 cases), lymphocyte depletion (1 case), or unclassified types (2 cases). Two cases of nodular sclerosis contained areas of necrosis surrounded by sheets of lacunar cells (syncytial variant of NSHD). Two other cases were associated with cutaneous lymphoma. One of these cases was mixed cellularity HD, which appeared to be confined to the skin. In a second case, tumor cells of similar phenotype (T4$^+$, Ki-1$^+$) were found in skin and lymph nodes of a patient with coexistent mycosis fungoides and HD. These results are consistent with an origin of RS cells from T cells in some cases of nodular sclerosing and mixed cellularity HD. They also suggest that the same cell type, an activated helper T-cell, is involved in the pathogenesis of both skin lesions and lymphadenopathy of some patients with coexistent mycosis fungoides and HD. (Am J Pathol 1988, 130:345–353)

SEVERAL characteristics of Hodgkin's disease (HD) suggest that the malignant cell might be derived from a T cell. These T-cell characteristics include initial lesions of HD in thymic-dependent regions of lymph nodes and spleen,[1] frequent involvement of the thymus,[2] high concentrations of thymus-associated antigens in tissues affected by HD,[3] and depressed T-cell–mediated immunity proportional to the extent of disease.[4]

The Reed–Sternberg (RS) cell is generally accepted to represent the malignant cell in HD.[1] Numerous immunologic studies of RS cells for T-cell characteristics have given negative or ambiguous results.[5–11] Rosette studies have shown freshly isolated RS cells to be surrounded by E-rosette–forming T cells, but the RS cells themselves appeared to lack this T-cell characteristic.[9–11] Immunoperoxidase studies of RS cells with T-cell–specific antibodies in frozen tissues have most often been negative or inconclusive.[5–8] This may be due to low density of T-cell antigens on the surface of RS cells, with resultant weak or equivocal staining, or to difficulty in the distinction of staining of RS cells from the small T-lymphocytes surrounding them.

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In this study we have employed initial fixation of lymphoid tissues in periodate–lysine–paraformaldehyde (PLP) prior to freezing tissues for immunoperoxidase studies. This method has resulted in improved detection of lymphoid cell surface antigens, compared with unfixed frozen tissues.[12] Antigen-specific staining of RS cells was confirmed in electron micrographs and distinguished from nonspecific background staining or staining of adjacent lymphocytes. In this manner we have detected T-cell–specific antigens on RS-cells in 8 of 30 (27%) consecutive cases of HD. Correlations with histologic and clinical features are also made.

Materials and Methods

We studied 30 consecutive cases of HD selected only on the basis of fresh tissues being available for fixation in PLP. A corresponding mirror-image piece of tissue was fixed in B-5 and embedded in paraffin for routine histologic study and classification of HD. Tissues selected for processing were cut into blocks 1–2 mm in thickness and fixed for approximately 4 hours at 4 C in freshly prepared PLP, which consisted of 0.037 M phosphate buffer, pH 7.4, containing 10 mM sodium m-periodate, 75 mM lysine, and 2% paraformaldehyde. PLP-fixed tissue was then rinsed and held for 1–3 days in 0.05 M sodium phosphate buffer, pH 7.4, containing 7% sucrose at 4 C, then changed into 0.05 M sodium phosphate buffer, pH 7.4, containing 15% sucrose for 4 hours at 4 C and 0.05 M sodium phosphate buffer, pH 7.4, containing 25% sucrose and 10% glycerol for 2 hours at 4 C. The PLP-fixed tissue was then embedded in 7.5% gelatin and snap-frozen in a mixture of acetone and dry ice. Frozen tissues were stored at −80 C and removed just before cryostat sectioning at −30 C. Six-micron-thick sections were applied to microscope glass slides coated with 0.05% poly-l-lysine (Sigma, St. Louis, Mo).

Immunoperoxidase Staining

Sections were fixed in cold acetone for 5 minutes and rinsed in phosphate-buffered saline (PBS). Nonspecific reactivity of antibodies was blocked with dilute normal horse serum. Sections were then allowed to react with various murine monoclonal antibodies, listed in Table 1. Appropriate dilutions of antibodies

Table 1—Monoclonal Antibodies and Their Reactivity

Antibody clone(s)	CD	Antigen recognized	Reference	Reactive cells
Ki-1	30	p125	13	Hodgkin (Reed–Sternberg) cells and activated lymphoid cells
Leu-M1	15	p180,110,68,50	14	Monocytes, granulocytes, Hodgkin (Reed–Sternberg) cells and activated T-cells
LCA/T200	45	p200	15	All lymphoid cells and hematopoietic progenitor cells
OKT11	2	E-rosette receptor p50	16	All E-rosette forming T-cells
Leu-1/10.2	5	p67	17	Thymocytes, all T cells except some cytotoxic/suppressor T cells
Leu-4/OKT3	3	p19-29	18	Mature thymocytes, peripheral T cells
Leu-3/OKT4	4	p55	19	Thymocyte subpopulations, helper T cells
Leu-2/OKT8	8	p32-33	20	Thymocyte subpopulations, cytotoxic/suppressor T cells
3A1	7	p41	21	Thymocytes, all T cells except some helper T cells
Tac	25	Interleukin-2 receptor	22	Activated and functionally mature T cells
HB10a		Framework region of Ia p29-34	23	B cells, dendritic cells, macrophages, activated T cells
OKT6	1a	p49	24	Cortical thymocytes, Langerhans cells

were applied to the sections and incubated at room temperature for 30 minutes. After rinsing with PBS, sections were incubated with biotinylated horse anti-mouse IgG (Vector Laboratories, Burlingame, Calif). Sections were incubated in 0.3% H_2O_2 in absolute methanol for 30 minutes for blocking endogenous peroxidase activity. After rinsing with PBS, the avidin–biotin peroxidase complex (ABC) (Vector Laboratories) was applied for 30 minutes. After rinsing with PBS, the reaction was developed with 0.1% (wt/vol) 3,3′-diaminobenzidine tetrahydrochloride and 0.01% (vol/vol) hydrogen peroxide. The reaction was darkened with 1% osmium tetroxide. Sections were counterstained in 2% methyl green, dehydrated through a series of alcohols and xylenes, and mounted with Permount.

Electron Microscopy

For electron microscopy, frozen sections prepared and antibody-labeled in the same manner as above were fixed in 2% glutaraldehyde before the diaminobenzidine reaction, dehydrated in a graded series of alcohols, and embedded in Epon (Ted Pella Inc., Tustin, Calif) by rapidly inverting gelatin embedding capsules filled with the epoxy resin. The Epon was allowed to harden overnight at 60 C. Detachment of the capsule from the glass slide was accomplished by gentle heating. Ultrathin sections were cut from the face of the Epon block with a diamond knife on an LKB ultramicrotome and examined unstained and stained with uranyl acetate and lead citrate under a JEOL electron microscope. Cell reactivity with monoclonal antibodies was detected as a continuous layer of granular electron-dense reaction product outlining the cell membrane and cell processes between adjacent cells. No reaction product was observed within the cytoplasm, overlying the nucleus, or in the interstitium. Control sections stained with an irrelevant antibody were examined ultrastructurally in all cases and revealed no staining.

Results

T-cell–specific antigens were detected on RS cells and their morphologic variants (lacunar cells) in 6 of 20 cases of nodular sclerosis and 2 of 5 cases of mixed cellularity types of HD. No T-cell antigens were detected on RS cells in 2 cases of lymphocyte predominance, 1 case of lymphocyte depletion, or 2 unclassified types of HD. Staining of RS cells in representative T-antigen–positive cases is shown in Figure 1. Surface antigen staining of RS cells was confirmed by the deposit of a dark linear reaction product along the cell membrane in electron micrographs (Figure 2). In some areas, staining was discretely localized to RS cells, leaving adjacent small lymphocytes and macrophages unstained.

As shown in Table 2, the frequency of T-cell antigens detected in the 8 positive cases was as follows: T-11 (8/8), Leu-3 or T4 (4/8), and Leu-4 or T3 (3/8). At least 2 separate T-cell antigens were detected on RS cells in 5 of the 8 positive cases. Leu-1, T8, T6, and 3A1 were all negative. The percentage of RS cells expressing T-cell antigens was <20% (2 cases), 20–50% (3 cases), and >50% (3 cases). In Cases 1 and 2, the percentage of T-antigen–positive RS-cells varied in tissues from two different sites.

In each case the diagnosis of HD was supported by the staining of RS cells with antibody Ki-1 and in 5 cases antibody Leu-M1. RS cells were unstained with antibodies against LCA/T200, in contrast to positive staining for LCA/T200 in T-cell lymphomas with RS-like cells.[25]

Two T-antigen positive cases of nodular sclerosis contained focal areas of necrosis surrounded by sheets of lacunar cells, corresponding to the syncytial variant of nodular sclerosing HD.[26]

Two remarkable cases were associated with cutaneous lymphoma. One case of mixed cellularity HD appeared to be confined to the skin. HD arose in lymph nodes of another patient who had a 7-year history of mycosis fungoides. This patient developed an anterior superior mediastinal mass characteristic of nodular sclerosing HD. His skin lesions contained occasional large transformed cells (Ki-1$^+$, Leu-M1$^-$,T11$^+$, T4$^+$). A right cervical lymph node had a pattern of nodular sclerosis with numerous lacunar variants of RS cells. These lacunar cells were Ki-1$^+$, T4$^+$ but T11$^-$, Leu-M1$^-$. A left inguinal lymph appeared dermatopathic but contained focal evidence of HD. RS cells were found focally in subcapsular sinuses and paracortical regions of the lymph node. They were Ki-1$^+$, Leu-M1$^+$, T11$^+$, T4$^+$ (Figure 3).

Discussion

This study reveals the expression of T-cell antigens on RS cells in a subset of patients with nodular sclerosing and mixed cellularity types of HD. Only one previous study, that of Stein et al,[27] demonstrated frequent T-cell antigen expression by RS cells in a large series of patients with HD. Their results were obtained with the sensitive APAAP technique. They did not confirm their results in electron micrographs, which help to distinguish staining of RS cells from that of surrounding T lymphocytes. Like us, they

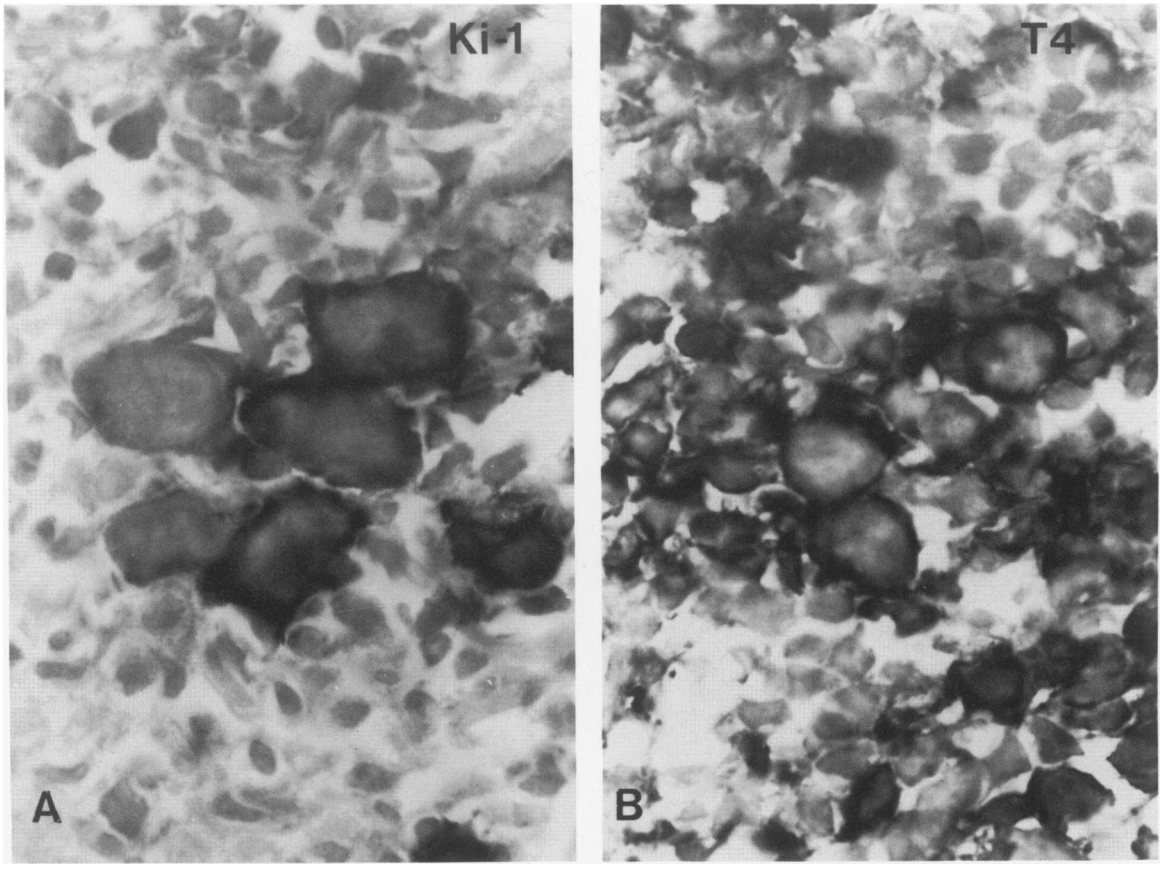

Figure 1—Immunoperoxidase staining of RS cells for Ki-1 antigen (A) and T4 antigen (B) in frozen sections of PLP-fixed spleen in mixed cellularity HD. (×1000)

found T3, T11, and T4 (as well as T1) to be the T-cell antigens most commonly expressed by RS cells. This T-cell antigen profile, together with the expression of several lymphoid activation antigens, Ia, Tac, and Ki-1, implies that RS cells are often derived from activated helper T cells.

We previously proposed a common activated helper T-cell origin for lymphomatoid papulosis, mycosis fungoides, and some types of HD.[28] This hypothesis was based on the unexpectedly common clinical associations between these entities, as well as morphologic and immunologic similarities, most noteworthy of these the expression of both T-cell antigens and Ki-1 antigen by RS or RS-like cells in each of the three disorders. Case 2 in the current study provides further support for this hypothesis. In this case of a patient with coexistent mycosis fungoides and HD, variable T-cell antigen expression was found for RS cells at different sites consistent with the concept of site-dependent phenotypic heterogeneity previously described in a peripheral T-cell lymphoma.[29] The patient had a 7-year history of mycosis fungoides in which occasional large Ki-1$^+$ cells were found. The diagnosis of HD was made on the basis of a superior mediastinal mass, a cervical lymph node with the pattern of nodular sclerosis, and diagnostic RS cells in an inguinal lymph node otherwise showing dermatopathic lymphadenopathy. Sinus infiltration and focal involvement of the inguinal node suggested the possibility that HD arose there as a metastatic process from the skin. To further investigate this possibility, it will be necessary to perform comparative cytogenetic and/or gene rearrangement studies of tissues obtained from different sites in patients with tumors of different histologic features and phenotypic heterogeneity.

In this preliminary study of 30 patients, only limited correlations between surface phenotype, histologic features, and clinical features could be made. As expected, no T-cell antigens were found on RS cells in cases of lymphocyte predominance, nodular type, which appears to be of B-cell derivation.[30] Two T-antigen–positive cases of nodular sclerosis had sheets of RS cell variants surrounding zones of necrosis appearing to meet the criteria for the syncytial variant of nodular sclerosing HD in which 1 patient with coexistent mycosis fungoides had been described.[26] RS cell

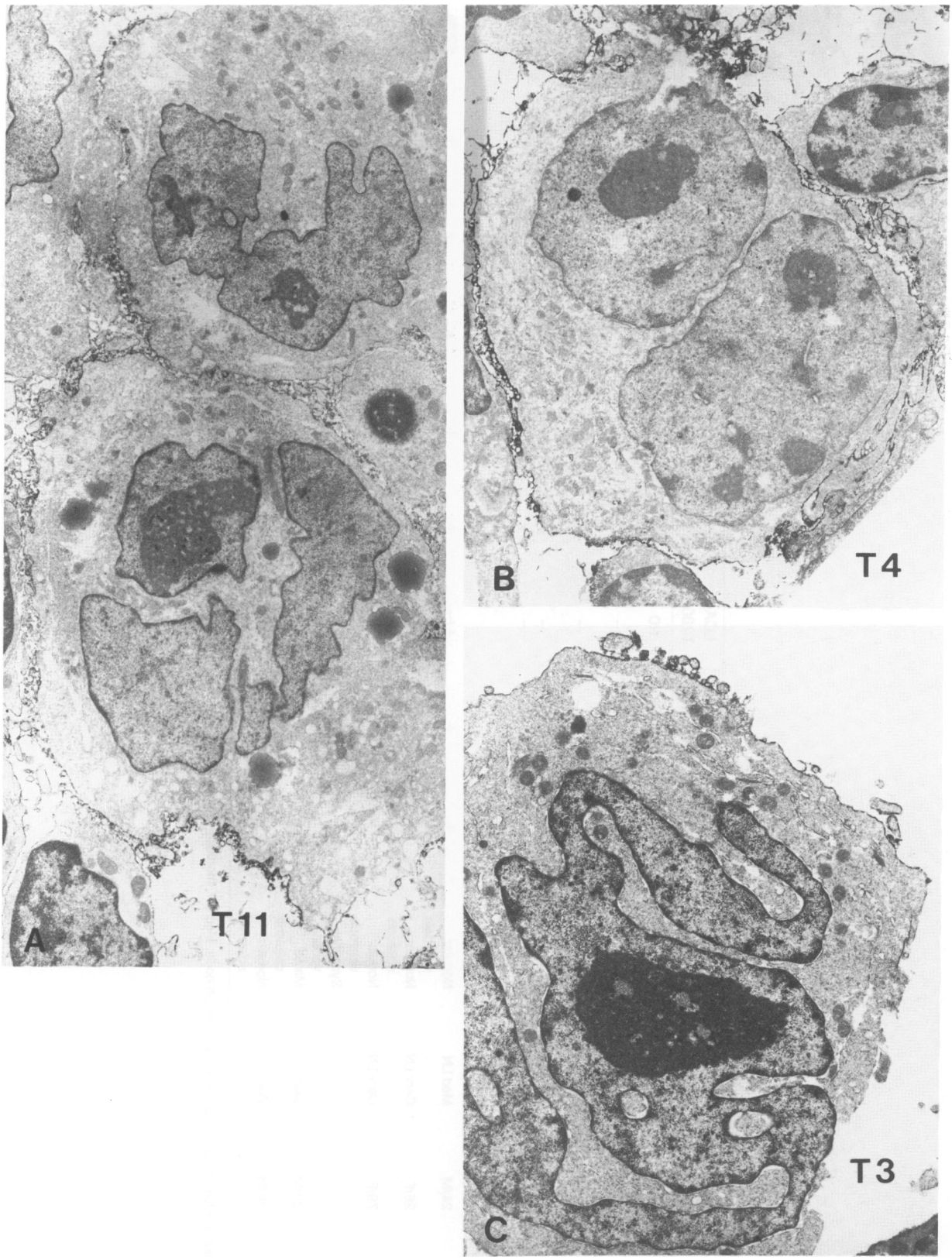

Figure 2—Electron micrographs showing dark surface staining of T-cell antigens on surface of RS cells in mixed cellularity (**A** and **B**) and nodular sclerosing (**C**) types of HD (**A**, ×4600; **B**, ×5530; **C**, ×6933)

Table 2—Antigens Expressed on Reed–Sternberg Cells

Patient	Age/sex	Tissue	Histology	Ki-1	Leu-M1	LCA T200	T11	Leu-3 T4	Leu-4 T3	Tac	Ia	% of RS cells T^+
1	26/M	Spleen	Mixed cellularity	+	−	ND	−	+	−	−	+	20–50%
		Cerv LN	Mixed cellularity	+	−	−	wk+	+	−	+	+	>50%
2	66/M	Cerv LN	Nodular sclerosis	+	−	−	−	+	−	+	+	20–50%
		Ing LN	Interfollicular	+	+	−	+	+	−	+	+	>50%
3	31/M	Cerv LN	Nodular sclerosis Syncytial variant	+	+	−	+	−	−	+	+	>50%
4	24/M	Med LN	Nodular sclerosis	+	+	+/−	+	+	+	+	+	<20%
5	28/F	Cerv LN	Nodular sclerosis	+	+	−	+	+	+	+/−	+	<20%
6	79/F	Cerv LN	Nodular sclerosis Syncytial variant	+	−	−	+	−	+	−	+	20–50%
7	20/M	Skin	Mixed cellularity	+	+	−	+	−	−	+	+	>50%
8	18/M	Cerv LN	Nodular sclerosis	+	+	−	+	−	−	+/−	+/−	20–50%

Cerv LN, cervical lymph node; Ing LN, inguinal lymph node; Med LN, mediastinal lymph node; ND, not done; +/−, equivocal.

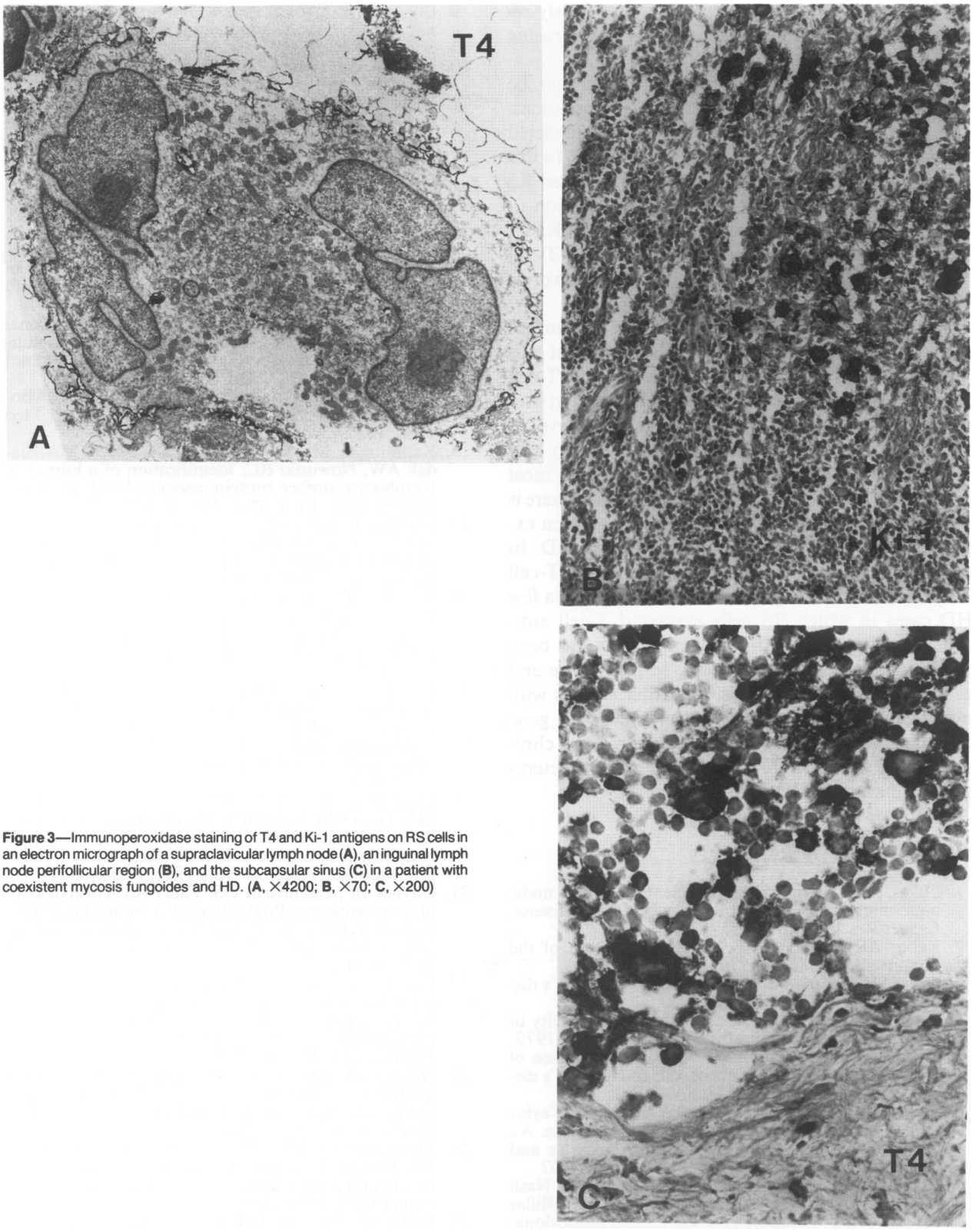

Figure 3—Immunoperoxidase staining of T4 and Ki-1 antigens on RS cells in an electron micrograph of a supraclavicular lymph node (**A**), an inguinal lymph node perifollicular region (**B**), and the subcapsular sinus (**C**) in a patient with coexistent mycosis fungoides and HD. (**A**, ×4200; **B**, ×70; **C**, ×200)

expression of T-cell antigens was not previously reported in the syncytial variant of nodular sclerosing HD.

A sharp histologic distinction cannot always be made between HD and non-Hodgkin's lymphomas. This is especially true for the lymphocyte depleted type and syncytial variant of nodular sclerosis. In this study, as previously reported,[25] we distinguished RS cells immunologically by their usual expression of Ki-1 and Leu-M1 and weak or absent LCA ($Ki\text{-}1^+$, $Leu\text{-}M1^+$, LCA^-), whereas RS-like cells in T-cell lymphomas were LCA^+ and usually did not express Ki-1 or Leu-M1 antigens.

The expression of T-cell antigens by RS cells in 8 of 30 cases suggests a T-cell origin for the malignant cells in some cases of HD. The variable frequency of T-cell antigen expression between cases and at different sites of HD within a given case suggests that the level of expression or antigen density may vary widely and often be below our threshold of detection, as in most previous studies. It is not readily apparent why there is sometimes a discrepancy between T-cell antigen expression and gene rearrangement results in HD. In two recent studies, immunoglobulin, but not T-cell receptor gene rearrangements were detected in a few HD cases in which RS cells expressed T-cell antigens.[31,32] The concept of lineage infidelity has been used in explain an inconsistency in phenotype and genotype of malignant cells.[33] Further studies with more sensitive markers and correlations with gene rearrangement analysis, histologic features, and clinical features should more fully characterize the heterogeneous nature of HD.

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