Phenotypic Expression of Hodgkin's and Reed–Sternberg Cells in Hodgkin's Disease

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The phenotypic expression of Hodgkin's and Reed-Sternberg (H-RS) cells was determined by analysis with a panel of monoclonal antibodies and peanut agglutinin (PNA) by an immunohistochemical technique. Seven antibodies, including T200, anti-HLA-DR, anti-Leu 10, A1G3, anti-Tac, OKT9, and anti-Leu M1, were found to react with a great majority of H-RS cells. In some cases, H-RS cells also bound PNA. Other antibodies, including those highly specific for T cells (eg, Lyt 3) and B cells (eg, B1, anti-Leu 14) were consistently negative. The results argue against the derivation of H-RS cells from T or B lymphocytes. The H-RS cells were also negatively stained with antibodies which react with monocytes (OKM1, Mo-2, 63D-3), follicular dendritic cells (DRC-1), and natural killer/killer cells (Leu 7, Leu 11a, B73.1). The presence of Leu M1 and Tac in H-RS cells is of interest. Anti-Leu M1 positivity was seen in all 20 of Hodgkin's disease (HD) cases tested and should provide a very useful reagent for differential diagnosis of HD from other reactive and neoplastic conditions. Tac normally is present only on activated T cells. The presence of Tac in H-RS cells may reflect expression of T-cell growth factor receptor or a closely related protein during a stage of neoplastic transformation. Although the nature of the neoplastic cell of HD cannot be determined by these studies, they are consistent with an origin from interdigitating reticulum cells. Both H-RS cells and interdigitating reticulum cells have a similar antigenic phenotype (Leu M1$^+$, T200$^+$, HLA-DR$^+$, Leu 10$^+$, A1G3$^+$, and OKT9$^+$) and a similar pattern of lysosomal enzyme activity. (Am J Pathol 1985, 118:209–217)

ALTHOUGH Hodgkin's disease has been the subject of many studies, the origin of Hodgkin's (H) mononuclear and Reed–Sternberg (RS) cells is still a matter of debate. Recent studies indicate that these cells fail to express a phenotype characteristic of B or T lymphocytes.[1–3] In a previous report, we showed that H-RS cells react only with anti-Leu M1, among nine monocyte/granulocyte/histiocyte monoclonal antibodies tested.[4] Leu M1 normally is acquired during the later stages of myeloid differentiation.[5] Stein et al also reported a myeloid-associated antigen on H-RS cells and suggested that H-RS cells may be more related to granulocytic cells or granulocytic precursor cells than to any other group of cells in human hematopoiesis. However, H-RS cells, in general, lack morphologic and enzyme cytochemical properties of granulocytes. In an attempt to further clarify the nature and/or origin of H-RS cells, we studied 20 cases of Hodgkin's disease with a large panel (>50) of monoclonal antibodies. Because a possible origin of H-RS cells from histiocytes (macrophages) or interdigitating reticulum (IR) cells has been proposed, the results emphasized a comparison of the phenotypes of histiocytes/IR cells and H-RS cells.

Materials and Methods

Tissues

Twenty cases of Hodgkin's disease of various histologic subtypes (Table 1) were used for immunohistochemical study. Parallel frozen sections were stained with hematoxylin and eosin for identification of H-RS cells and other nonneoplastic cell types. Cases of lymphocyte-predominant Hodgkin's disease were not available for study. All tissues had been embedded in OCT (Miles Laboratories, Naperville, Ill), snap-frozen in a mixture of dry ice-2-methylbutane (−75 C), and stored at −150 C over liquid nitrogen. Five-micron sec-

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Table 1—Results of Phenotypic Expression of H-RS Cells

	Histologic subtype*	Anti-Leu M1	PNA	Anti-HLA-DR	Anti-Leu 10	Anti-Tac	A1G3	OKT9	T200
1	NS	+	−	+	+	−	+	+	+
2	NS	+	−	−	−	−	+	+	+
3	NS	+	−	+	−†	−	+	+	+
4	NS	+	+(20%)‡	+	+	+(20%)	−	+	+
5	NS	+	+	+	+	+	+	+	+
6	NS	+	+	+	+	+	+	+	+
7	NS	+	−	+	+	+	+	+	+
8	NS	+	+(40%)	+	+	+	+	+	+
9	NS	+	+(20%)	+	+	+(20%)	+	+	+
10	NS	+	−	+	+	−	+	+	+
11	NS	+	+(40%)	+	+	+	−	+	+
12	NS	+	+(40%)	+	−	−	+	+	+
13	NS	+	+	+	+	+	+	+	+
14	NS	+	−	+	+	−	+	+	+
15	NS	+	−	+	−	−	+	+	+
16	MC	+	+	+	+	−	+	+	+
17	MC	+	+(20%)	+	−†	−	+	+	+
18	LD	+	+	+	−	−	+	+	+
19	LD	+	+	+	+	+	−	+	+
20	LD	+	+	+	−	+	+	+	+

* NS, nodular sclerosis; MC, mixed cellularity; LD, lymphocyte depletion.
† The normal B lymphocytes in these two cases were also Leu 10^-.
‡ Parentheses indicate the percentage of tumor cells positive for that antibody. If not stated, the great majority (>80%) were positive.

tions were cut and placed on gelatinized slides prior to immunostaining. For analysis of antigenic distribution in normal and/or reactive lymphoid tissues, five tonsils and five lymph nodes were prepared in an identical manner. In addition, sections of lymph nodes showing dermatopathic lymphadenitis were used for study of the reactivity of interdigitating reticulum cells. Because anti-Leu M1 and another two reagents, PNA and *Bandeiraea simplicifolia* (BSA), can be applied to paraffin reactions, B-5-fixed tissues were also obtained.

Reagents

The mouse monoclonal antibodies used in this study are listed in Table 2. Biotin-labeled peanut agglutinin (PNA) and BSA, biotin-conjugated horse anti-mouse IgG, and avidin-biotin-peroxidase complex (Vectastain ABC Kit, PK 4002) were obtained from Vector Laboratories (Burlingame, Calif).

Staining Procedures

The staining procedure has been described elsewhere in detail.[7,8] Briefly, the sections were fixed in acetone at room temperature for 5 minutes. The primary antibodies were used at 2 μg/ml, followed by biotin-labeled secondary antibody (1:400) and avidin-biotin-peroxidase complex. The sections were developed in a DAB-Ni-H_2O_2 solution[7] and were counterstained with methyl green, dehydrated, and cleared as in routine processing. The PNA and BSA reactions were performed as previously described.[9,10]

Parallel sections were predigested with neuraminidase as previously described[4,11]; BSA and anti-Leu M1 were studied on both untreated and predigested sections.

Controls

Controls for method specificity were performed by omission of primary antibody or replacement of primary antibody with BALB/c mouse serum or ascites fluid. Controls for PNA and BSA reactions included sugar absorption, dissociation, and inhibition.[10]

Histochemical Localization of Histiocytes and IR Cells

The acid phosphatase reaction (hexazotized pararosanilin method) was used for staining histiocytes and IR cells. Histiocytes show strong cytoplasmic positivity, whereas IR cells show weak focal positivity.[12] Histiocytes are also diffusely positive with the acid α-naphthyl acetate esterase (ANAE) reaction, whereas IR cells are ANAE-negative or weakly positive.[12]

Double Immunohistochemical and Enzyme Histochemical Staining

In order to confirm antibody-histiocyte/IR cell reactions, a double immunohistochemical and enzyme histochemical method was performed. The sections

Table 2—Monoclonal Antibodies and Their Sources Used in This Study

I. T-cell monoclonal antibodies
Lyt 3 (NEN), anti-Leu 5 (BD), T11A (Coulter), anti-Leu 1 (BD)
Lyt 2 (NEN), T101 (Hybritech), OKT3 (Ortho), anti-Leu 4 (BD)
OKT 4 (Ortho), T4A (Coulter), OKT8 (Ortho), anti-Leu 2a (BD)
T8A (Coulter), 3A1[39] (Dr. B. Haynes), anti-Leu 9 (BD), OKT6 (Ortho), anti-Leu 6 (BD), T6A (Ortho), TA1 (Hybritech), anti-Tac (receptor for T-cell growth factor)[40] (Dr. T. Waldmann, NCI)

II. B-cell monoclonal antibodies
BI (Coulter), B2 (Coulter), BA-1 (Hybritech), IgM (μ) (BRL), IgG(γ) (BRL), IgD(γ) (BD), IgA_{12} (BD), κ(BD), λ(BD), anti-Leu 12 (BD), anti-Leu 14 (BD)

III. Monocyte/histiocyte/granulocyte antibodies
OKM1 (Ortho), Mo-2 (Coulter), Mo-1 (BRL), Mo-2 (BRL), anti-Leu M1 (BD), anti-Leu M2 (BD), anti-Leu M3 (BD), anti-Leu M4 (BD), Mac 1 (Hybritech)

IV. Natural killer/killer-cell antibody
anti-Leu 7 (BD), anti-Leu 11 (BD), B73.1 (Dr. Perussia, Wistar Institute)

V. "Leukemia lymphoma-associated antigens"
J5 (Coulter), anti-CALLA (BD), BA-2 (Hybritech), BA-3 (Hybritech)

VI. Miscellaneous
OKT9 (transferrin receptor[17]) (Ortho), OKT10 (Ortho), HLA-DR (BD), anti-Leu 10 (BD), anti-Leu 8 (BD), A1G3[41] (Dr. B. Haynes, Duke University), T200 (Hybritech), DRC-1[42] (follicular dentritic cell) (Accurate, New York)

NEN, New England Nuclear, Boston, Mass; Coulter, Coulter Immunology, Hialeah, Fla; BD, Becton-Dickinson, Sunnyvale, Calif; Hybritech, Hybritech, Burlingame, Calif.

For distribution of these antibodies and their specificities in normal human lymphoid tissues, see references 6, 15, 16, and 38.

were first developed for acid phosphatase or ANAE, which produced a bright red or brownish red reaction product. After a brief wash, the sections were stained by one of the seven monoclonal antibodies that reacted with H-RS cells with the use of DAB-Ni-H_2O_2, which produced a blue or grayish blue reaction product.

Results

Antigenic Phenotype of H-RS Cells

H-RS cells were stained by T200, anti-Leu M1, PNA, A1G3, anti-Tac, OKT9, HLA-DR, and anti-Leu 10 and consistently lacked markers specific for T or B cells or monocytes (Table 1) (Figures 1 and 2). Heterogeneity of marker expression was noted; for example, anti-Tac was positive in only 50% of cases tested. Because it was difficult to precisely quantitate neoplastic cells in frozen sections, the results for each antibody were evaluated in comparison with the antibody that reacted with most of the neoplastic cells, ie, anti-Leu M1.[4] Anti-Leu M1 stained H-RS cells in the absence of neuraminidase pretreatment, whereas histiocytes and IR cells were Leu M1-positive only after neuraminidase digestion. Both Hodgkin's mononuclear cells and Reed-Sternberg cells showed an identical phenotype in each case.

None of the H-RS cells were stained by anti-immunoglobulin antibodies. In 3 cases, very weak reactions with both anti-κ and anti-λ could be seen in <5% of H-RS cells. This staining was interpreted as nonspecific uptake.

Reactive Components in Hodgkin's Disease

In most cases, there was an abundant eosinophilic infiltrate. The eosinophils reacted intensely with diaminobenzidine-H_2O_2, which could not be blocked by methanoloic-H_2O_2 pretreatment. Plasma cells that were stained by either anti-κ or anti-λ antibodies were also randomly distributed in the tissues.

The small lymphocytes, B or T cells, stained by B1 or Leu 1/Lyt 3, were present in large numbers in the tissues. The T/B ratio was, in general, about 3–4:1. However, in 5 cases, B and T cells appeared in equal numbers. T lymphocytes tended to be scattered or mixed with neoplastic cells, whereas B cells were present in patches or aggregates. Among T cells, the helper/suppressor ratio was approximately 3–4:1 in all cases.

Leu 7^+ cells, presumptively natural killer cells, were present in small numbers and were randomly scattered in the tissues, as were histiocytes labeled by OKM1. The number of Leu $11a^+$ or $B73.1^+$ (natural killer) cells was less than that of Leu 7^+ cells.

Phenotypic Expression of Histiocytes and IR Cells in Normal Lymphoid Tissues

Table 3 summarizes the reactivities in normal peripheral lymphoid tissues of the reagents that reacted with H-RS cells. All seven antibodies, except anti-Tac, showed wide distribution with B and T lymphocytes, histiocytes, and IR cells.

The confirmation of an antibody reaction with histiocytes or IR cells was based on double labeling with both immunohistochemical and enzyme histochemical staining. The $ANAE^+$/acid $phosphatase^+$ histiocytes were scattered in germinal centers and sinuses as well as in the T-cell zone; and they were $T200^+$, $HLA\text{-}DR^+$, Leu 10^+, $A1G3^+$, $OKT9^+$, Leu $M1^+$, and BSA^+ (Figure 3). The IR cells showing weak focal intracytoplasmic acid phosphatase positivity were abundant in dermatopathic nodes and were confined to the T-cell zone in normal lymphoid tissues (Figure 4). IR cells and histiocytes had similar patterns of reactivity, with the exception of reactivity with BSA. Both BSA and anti-Leu M1 reactions in normal lymphoid tissues required predigestion with neuraminidase for positivity to be seen.

Anti-Leu M1 showed weak reactivity with follicular dendritic cells in germinal centers (Figure 3A). After neuraminidase treatment, anti-Leu M1 stained histiocytes, demonstrating an intracytoplasmic granular

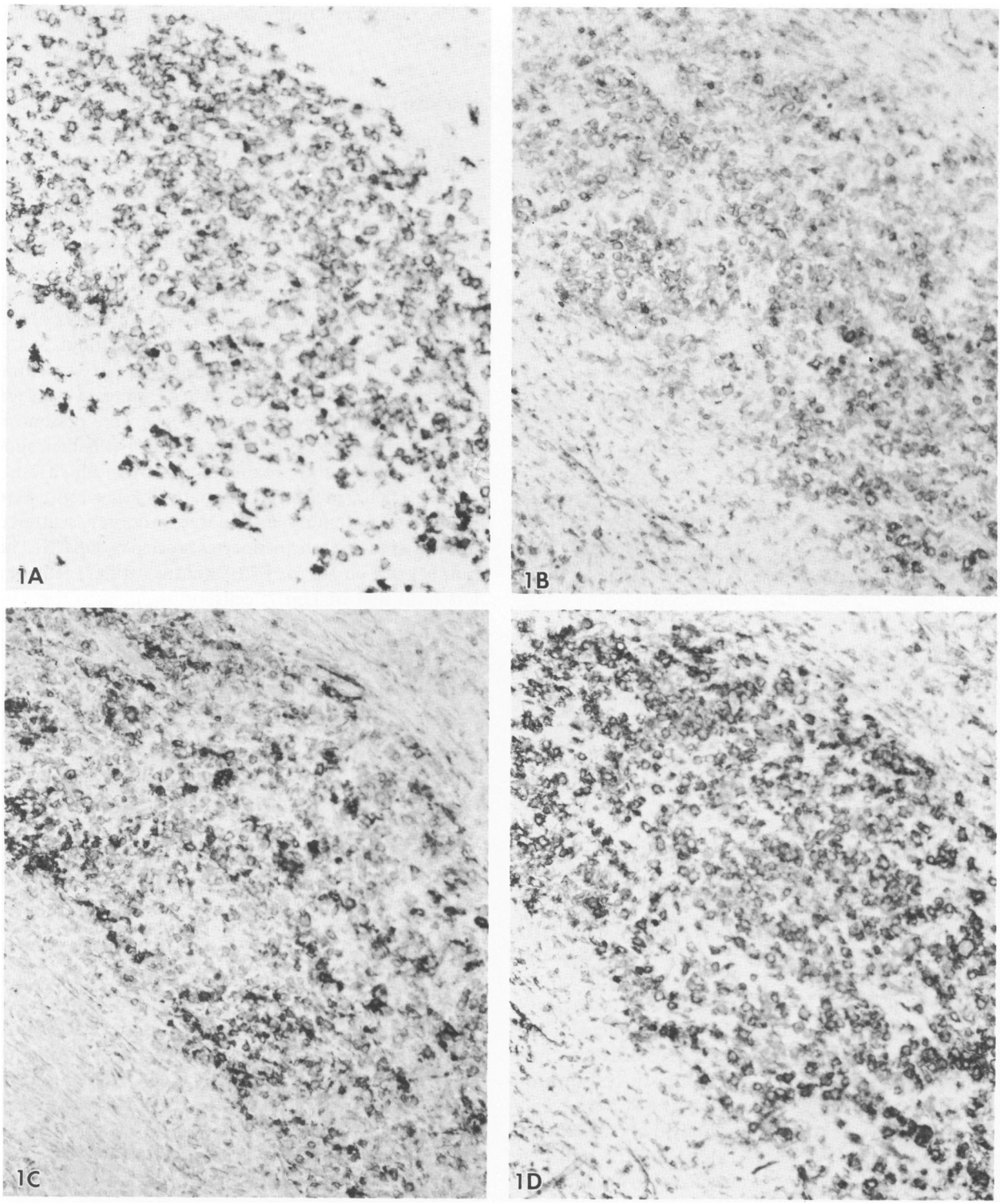

Figure 1—Frozen sections of tissue affected by Hodgkin's disease, stained with (**A**) anti-Leu M1, (**B**) anti-Tac, (**C**) OKT9, and (**D**) anti-HLA-DR. Hodgkin's tumor cells are intensely stained. A1G3 is negative in this case (not shown). Anti-Leu 10 and PNA show a staining pattern similar to that of anti-HLA-DR and anti-Leu M1, respectively (not shown). (×160)

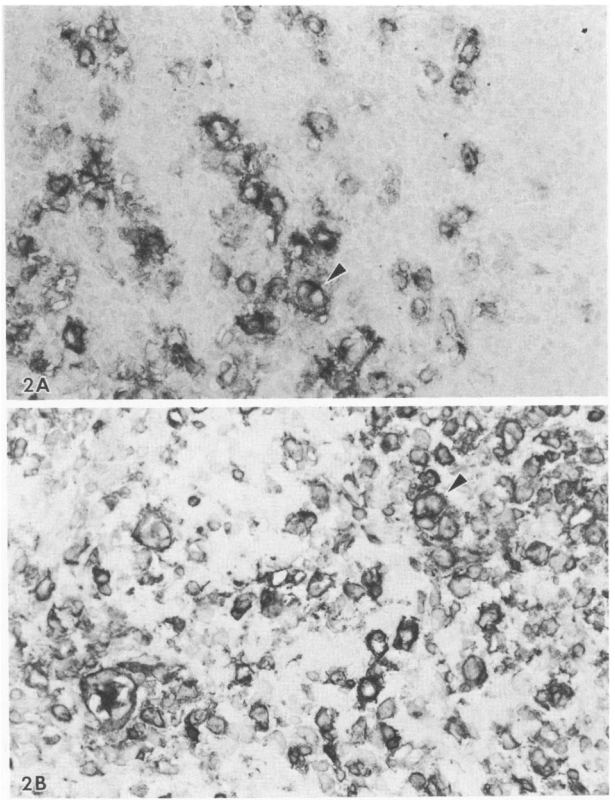

Figure 2—High magnification of Figure 1. **A**—Anti-Leu M1. **B**—Anti-Tac. Note that mononuclear Hodgkin's cells and Reed–Sternberg cells (*arrows*) are positively stained.

reactivity in the paranuclear region (Figure 3B). In contrast, IRCs exhibited intense membranous and cytoplasmic granular reactivity with anti-Leu M1 (Figures 3C and 4C). B or T lymphocytes remained Leu M1-negative even after neuraminidase treatment. BSA reacted mainly with histiocytes, exhibiting diffuse cytoplasmic staining (Figure 3D). IR cells were BSA negative.

PNA reacted strongly with a portion (25–50%) of IRCs and histiocytes. The reaction differed from that reported previously in formalin-fixed tissues.[9] The discrepancy is probably due to the difference in fixation and antigenic preservation.[13] Histiocytes and IR cells were generally negative for other antibodies (ie, OKM1) tested in this study.

Discussion

The data presented in this paper indicate that H-RS cells have a characteristic antigenic phenotype and are positive with T200, anti-HLA-DR, anti-Leu 10, OKT9, anti-Tac, A1G3, anti-Leu M1, and PNA. Other antibodies, including those specific for T or B cells, consistently failed to stain H-RS cells.

The expression of OKT9 and Tac in H-RS cells may reflect transformation and/or active proliferation of H-RS cells. OKT9 reacts with transferrin receptors, a characteristic of actively proliferating cells, including germinal center cells, gastrointestinal epithelium, endometrium, etc.[6,14–16] In addition, OKT9 has been identified on the proliferating cells of most neoplasms,[17] and thus was likely to be present on the proliferating cells of Hodgkin's disease. Alternatively, the OKT9 antigen may be an intrinsic component of histiocytes and IR cells, because the great majority of these cells are positive with OKT9 and yet show no signs of proliferation. Thus, the expression of OKT9 in H-RS cells may be indicative of a possible relationship between H-RS cells and histiocytes/IR cells.

Anti-Tac, the antibody against the T-cell growth fac-

Table 3—Distribution and Reactivities of PNA, BSA, and Monoclonal Antibodies in Normal Lymphoid Tissues

	PNA	BSA*	T200*	Anti-HLA-DR	Anti-Leu 10	Anti-Tac	OKT9	Anti-Leu M1*	A1G3
Germinal center and mantle zone lymphocytes (B cells)	+ (germinal center cells)	–	+	+	+†	–	+ (germinal center cells)	–	+ (mantle zone lymphocytes)
Paracortical lymphocytes (T cells)	–	–	+	–§	NR‡	–§	–§	–	+
Histiocytes (sinus histiocytes, macrophages)	+ (portion)	+	+	+	+	–	+	+ (Golgi)	+
Interdigitating reticulum cells	+ (portion)	–	+	+	+	–	+	+ (membranous and Golgi)	+

* The reactions of BSA and anti-Leu M1 require a predigestion of tissues with neuraminidase.
† Positive in 75–80% of normal individuals.
‡ NR, not reported; cannot be determined on the basis of tissue study.
§ Less than 10% of cells are positive. The small number of positive cells probably represent activated T cells.

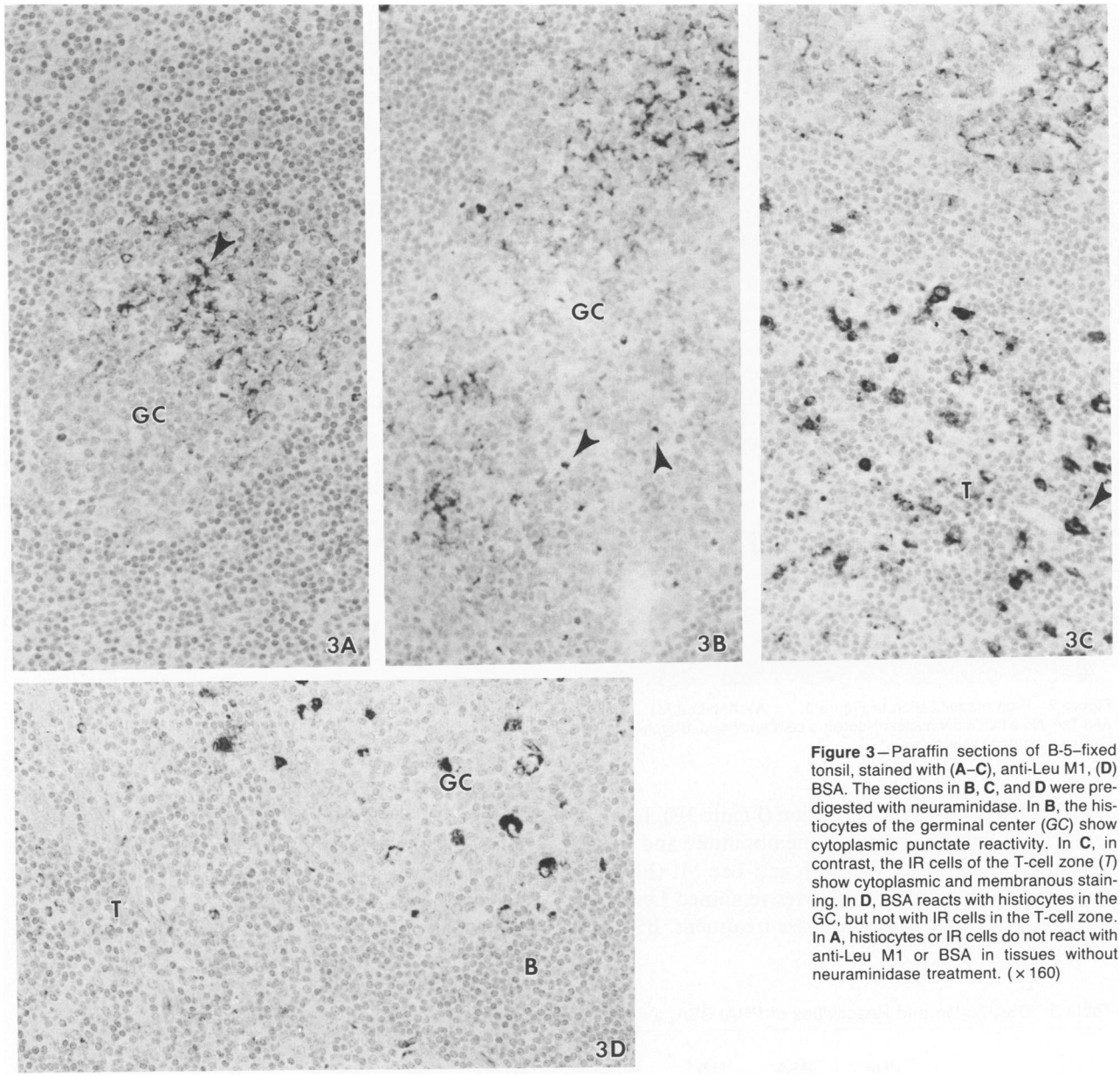

Figure 3—Paraffin sections of B-5–fixed tonsil, stained with (**A–C**), anti-Leu M1, (**D**) BSA. The sections in **B**, **C**, and **D** were predigested with neuraminidase. In **B**, the histiocytes of the germinal center (*GC*) show cytoplasmic punctate reactivity. In **C**, in contrast, the IR cells of the T-cell zone (*T*) show cytoplasmic and membranous staining. In **D**, BSA reacts with histiocytes in the GC, but not with IR cells in the T-cell zone. In **A**, histiocytes or IR cells do not react with anti-Leu M1 or BSA in tissues without neuraminidase treatment. (×160)

tor (TCGF) receptor, in general, is restricted to activated normal T cells and certain T-cell leukemias/lymphomas; whereas resting T cells, B cells, monocytes, and thymocytes are unreactive.[18] Recently, we also found Tac expression in hairy-cell (a B-cell) leukemia,[19] some low grade B-cell lymphomas, and a true histiocytic lymphoma cell line (SU-DHL-1) (unpublished data). Thus, the expression of the Tac antigen cannot be used to indicate a T-cell lineage. However, the significance of the expression of the TCGF receptor or a like substance in H-RS cells is not known.

Both PNA and anti-Leu M1 react with H-RS cells. Under certain circumstances these reagents also react with histiocytes and IR cells. However, in contrast to H-RS cells, a positive anti-Leu M1 reaction in histiocytes and IRCs, requires predigestion of sections with neuraminidase. Neuraminidase removes sialic acid from sialoglycoconjugates. Thus, the results suggest that the Leu M1 antigen in histiocytes and IR cells is sialylated, whereas in H-RS cells it is not. Only lymphoid neoplasms have been reported to have a deficiency of sialylated glycoproteins.[20,21] Although both histiocytes and IR cells are positive for Leu M1, the staining patterns of these two types of cells are distinct. Histiocytes show an intracytoplasmic perinuclear punctate reaction, presumably in the Golgi region; whereas IR cells ex-

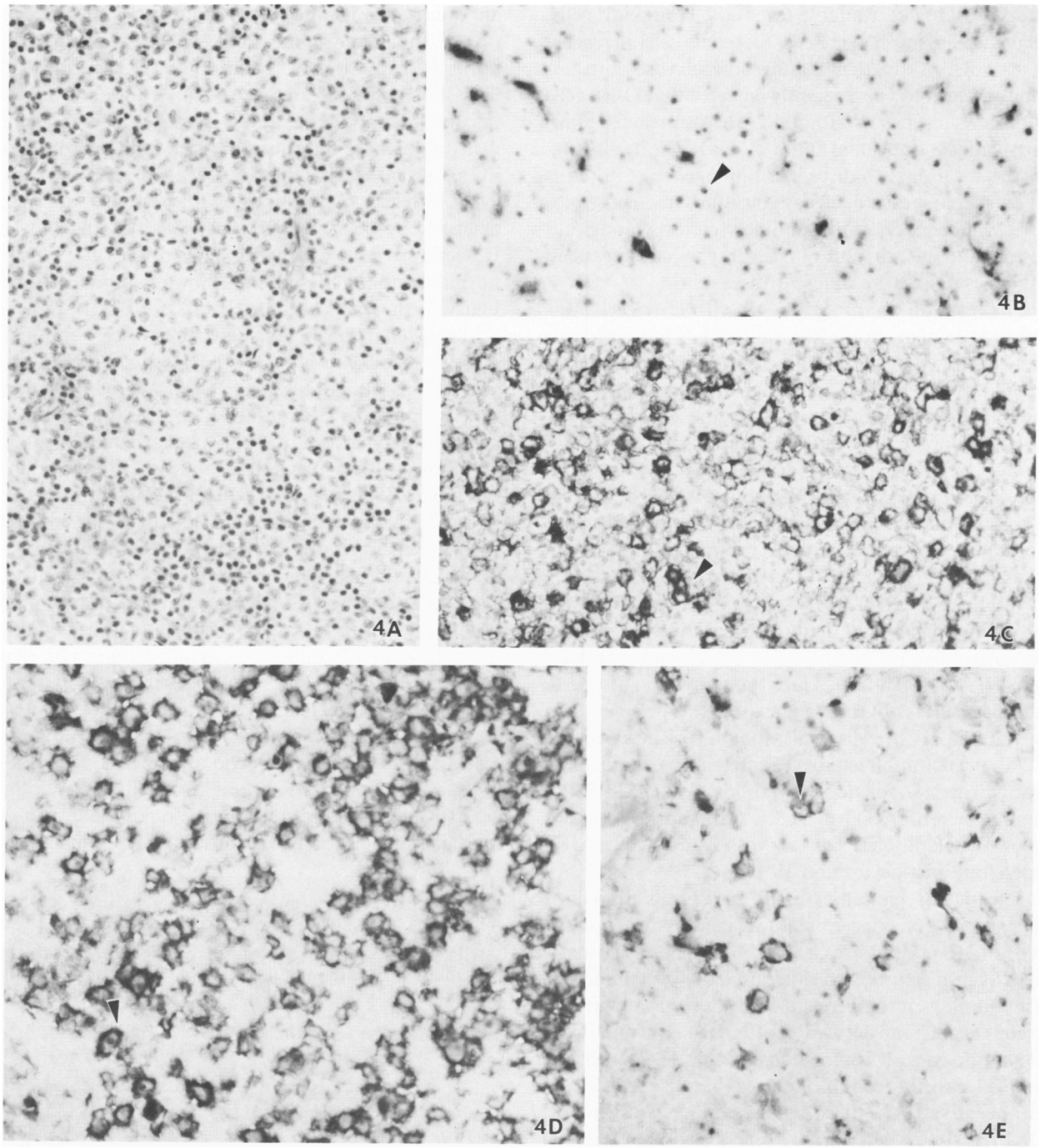

Figure 4A—Lymph node exhibiting dermatopathic lymphadenitis contains abundant IR cells. B-5–fixed paraffin section. **B**—Frozen section of the same lymph node stained for acid phosphatase. Note punctate reactivity of IR cells. **C**—The same lymph node as in **A**, stained with anti-Leu M1 after predigestion with neuraminidase. IR cells show intense reactivity. **D**—Frozen section double stained for acid phosphatase and with anti-Leu 10. The reaction confirms that IR cells are indeed Leu 10-positive. The same staining technique was also used to confirm HLA-DR/T200/A1G3/OKT9–IR cell reaction. **E**—Frozen section double-stained for acid phosphatase and with PNA. A small proportion of IR cells are PNA-positive. (**A–C**, ×160; **D** and **E**, ×250)

hibit both membranous and perinuclear staining resembling the pattern in H-RS cells.

Anti-Leu M1 reacted with virtually all cases of Hodgkin's disease tested, whereas other reagents (anti-Tac, A1G3) showed minor variations from case to case. However, the reactivities of PNA, anti-Leu M1, and the other six antibodies were not different among the subtypes of Hodgkin's disease that were tested, namely, nodular sclerosis, mixed cellularity, and lymphocyte depletion. Fresh tissues from cases of the lymphocyte-predominant

subtype were not available for study. Hodgkin's cells, lacunar cells, and classic Reed–Sternberg cells all reacted in a similar or identical manner in each case. Furthermore, Stein et al[22] also recently reported that H-RS cells from all subtypes of Hodgkin's disease reacted similarly with a monoclonal antibody, Ki-1. The results suggest that Hodgkin's disease is homogeneous in terms of marker expression, although clinical and epidemiologic differences between nodular sclerosis Hodgkin's disease and the other histologic subtypes had suggested that these might be different diseases.

The reaction of anti-HLA-DR with H-RS cells was reported previously.[3,22] Both anti-HLA-DR and anti-Leu 10 show a similar staining pattern in normal lymphoid tissues.[16] Leu 10, a human D region-associated antigen equivalent to murine Ia, is present in association with most DR specificities but absent from DR-7 homozygous cell lines.[23] We have also noted that anti-Leu 10 staining is negative in about 10–15% of normal lymphoid tissues (unpublished data). In this study, the normal B lymphocytes in two of the patients with Hodgkin's disease (Cases 3 and 17) among 20 tested were negative for Leu 10. Both Leu 10 and HLA-DR were co-expressed in a great majority of non-Hodgkin's lymphomas tested, including B- and T-cell lymphomas (unpublished observation). Thus, the expression of Leu 10 in H-RS cells is not totally unexpected. The expression of T200 in H-RS cells is also not surprising, because T200, the common leukocyte antigen, is a characteristic of all hematopoietic and lymphoreticular cells.[16] The significance of A1G3 in H-RS cells, however, is not known. In addition to reacting with B and T cells, A1G3 does stain histiocytes and IR cells.[24]

The phenotypic expression of H-RS cells allows further speculation concerning the origin of the neoplastic cells of Hodgkin's disease. H-RS cells are consistently negative for markers specific for T or B lymphocytes. This finding, in agreement with previous reports,[3,22] argues against the derivation of H-RS cells from B and T lymphocytes. It is also unlikely that H-RS cells are derived directly from monocytes, because H-RS cells are negative for at least eight common antigens (ie, OKM1, Mo-2, Leu M3, . . . etc.) found on normal monocytes.[4]

It is more difficult to dismiss a histiocytic/IR cell origin for the cells of Hodgkin's disease. In studies with short- or long-term cultures of Hodgkin's disease-derived cell lines, Kaplan[24] and Diehl et al[25] showed that the tumor cells exhibited properties characteristic of monocytoid or macrophagelike cells. The antigenic phenotype described in this study is also consistent with an origin in histiocytes or IR cells. The presence of α_1-antitrypsin and/or lysozyme has been used to favor an origin from macrophages.[26,27] However, as in the present study, this has been a very inconsistent observation.[22] That the origin is in IR cells has been a more attractive hypothesis.[28–30] Kadin[28] and Beckstead et al[29] have shown that the presence of small amounts of acid phosphatase and nonspecific esterase with a punctate distribution in H-RS cells, a pattern similar to that seen in IR cells rather than phagocytic histiocytes or monocytes. Furthermore, histiocytes/macrophages are characterized by BSA reactivity, whereas IR cells and H-RS cells are not. Finally, IR cells are the only type in normal lymphoid tissues that show membranous anti-Leu M1 reactivity similar to that of H-RS cells. IR cells are localized in the thymus-dependent (T-cell) zone and appear to be involved in T-cell–mediated immunity as antigen-presenting cells.[12,31,32] The proposed malignant transformation of IR cells to H-RS cells thus could result in the impairment of T-cell–mediated immunity that commonly occurs in untreated patients with Hodgkin's disease.[28,33] This may also explain the observation that in partially involved lymphoid tissues, Hodgkin's disease selectively involves the thymic-dependent zones.[33]

Finally, the reactive, nonneoplastic components of Hodgkin's disease also have been the subject of many studies.[1,34–37] In general, the results had indicated that an increase of T lymphocytes, mainly helper cells, were present in involved sites.[1,34] The presence of T lymphocytes, especially helper T cells in the tumor-involved areas, is also evident in this study. However, the distribution of T lymphocytes, ratio of helper/suppressor T cells, and frequency of natural killer cells (Leu 7$^+$ cells) was variable and did not appear to correlate with either histologic subtype or number of malignant cells.

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