American Journal of Pathology, Vol. 150, No. 2, February 1997

Fascin, a Sensitive New Marker for Reed-Sternberg Cells of Hodgkin's Disease

Evidence for a Dendritic or B Cell Derivation?

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Immunohistochemical localization of human fascin, a distinct 55-kd actin-bundling protein, was determined for a wide variety of lymphoid tissues (364 specimens total). In non-neoplastic tissues, reactivity was highly selective and localized predominantly in dendritic cells. In the thymus, this protein was distinctly localized to medullary dendritic cells. In reactive nodes, interdigitating reticulum cells of T zones, cells in subcapsular areas, and cells of the reticular network were reactive, with variable reactivity observed for follicular dendritic cells. Splenic dendritic cells of the white pulp and sinus-lining cells of the red pulp were reactive. Endothelial cells of all tissues exhibited variable reactivity. Lymphoid cells, myeloid cells, and plasma cells were uniformly nonreactive. In the peripheral blood, only dendritic (veiled) cells were reactive for fascin. A striking finding was observed for cases of Hodgkin's disease (total 187 cases). In all cases of nodular sclerosis (132), mixed cellularity (34), lymphocyte depletion (2), and unclassified types (5), all or nearly all Reed-Sternberg cells and variants were immunoreactive for fascin. Neoplastic cells exhibited strong diffuse cytoplasmic staining and frequently assumed dendritic shapes, particularly in the nodular sclerosis type, producing an interdigitating meshwork or syncytial network of cells. In cases of mixed cellularity type, neoplastic cells generally appeared more discrete. In all 14 cases of nodular lymphocyte predominance type, L&H variants were nonreactive. By contrast, neoplastic lymphoid cells of only 24 of 156 (15%) other lymphoid neoplasms (127 B cell, 27 T cell, and two null cell evaluated) were reactive for fascin. Fascin represents a highly effective marker for detection of certain dendritic cells in normal and neoplastic tissues, is an extremely consistent marker for Reed-Sternberg cells and variants of Hodgkin's disease (except L&H types), and may be helpful to distinguish between Hodgkin's disease and non-Hodgkin's lymphoma in difficult cases. The staining profile for fascin raises the possibility of a dendritic cell derivation, particularly an interdigitating reticulum cell, for the neoplastic cells of Hodgkin's disease, notably in nodular sclerosis type. However, as fascin expression may be induced by Epstein-Barr virus infection of B cells, the possibility that viral induction of fascin in lymphoid or other cell types must also be considered in Epstein-Barr virus-positive cases. (Am J Pathol 1997, 150:543–562)

More than 150 years have passed since the initial description of Hodgkin's disease, yet the origin of the Reed-Sternberg cell, the hallmark of this disorder, remains controversial. Possible etiologies for this cell have included lymphoid cells of T cell or B cell

Accepted for publication September 18, 1996.

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type,[1–13] histiocytes,[14–16] and dendritic cells of either follicular (B zone) or interdigitating (T zone) type[17–32] or the fusion of more than one cell type, eg, interdigitating reticulum cell and B or T lymphocytes.[33] Molecular biological studies have demonstrated immunoglobulin gene rearrangements or T cell receptor gene rearrangements for some cases of Hodgkin's disease,[34] with the former relatively more commonly observed. However, despite the high level of sensitivity achieved by this methodology, these results have been noted for only a small proportion of cases. Interestingly, B or T cell rearrangements[35] have also been documented in studies of established histiocytic cell lines, raising an element of uncertainty in accepting molecular evidence alone as a definitive basis for establishing the neoplastic cell type in this disorder.

Recent investigations of human peripheral blood cells utilizing a monoclonal antibody to human fascin, a 55-kd actin-bundling protein initially isolated from HeLa cells, demonstrated a localization pattern that appeared highly restricted to dendritic cells.[36–39] Analysis of peripheral blood by flow cytometry, immunofluorescence, and Western blots revealed that fascin was constitutively identified only in the population of dendritic cells (veiled cells) that typically constitute less than 0.1% of the mononuclear cells.[38,40] Granulocytes, lymphocytes, and monocytes were nonreactive. In cryostat section studies of reactive lymph nodes, reactivity appeared selective for dendritic cells in subcapsular areas, which would correspond to afferent lymphatics, and in cells consistent with interdigitating reticulum cells in T zones. Although previous studies of human dendritic cells have been hampered by the lack of specific or highly selective markers, antibodies to human fascin that are currently available potentially provide a means for identifying dendritic cells in reactive and neoplastic human tissues.

The dendritic cell system, as currently defined, includes a population of cells with unusual dendritic morphology with high levels of major histocompatibility complex (MHC) class II products and an accessory function for stimulation of T cells.[40] The distribution of these cells includes a trace population of cells in peripheral blood, veiled cells in afferent lymph, interdigitating reticulum cells (T zone) and other dendritic cells in lymphoid tissues, Langerhans cells, and interstitial dendritic cells. Dendritic cells are typically antigen-presenting cells capable of migrating to T cell zones of lymphoid tissues and clustering and activating T cells and are also potent stimulators of the mixed lymphocyte reaction. The presence of fascin in dendritic cells is not surprising in view of the cellular localization of this protein, which is similar to actin, and has been demonstrated in both microspikes and stress fibers[37] where it is apparently involved in the formation of microfilament bundles, structures that change dynamically during cell activities. Dendritic cells are motile antigen-presenting cells containing lamellipodia that extend and retract at various sites on the cell surface. This actin-bundling protein is undoubtedly involved in the ability of these cells to perform their biological functions.

Our initial studies of hematolymphoid tissues using the monoclonal antibody to fascin suggested a highly selective immunohistochemical staining pattern with reactivity observed for dendritic cells, particularly interdigitating reticulum cells, and an absence of reactivity for T and B lymphocytes.[38] mRNA for fascin, however, has been identified at various levels in a variety of human tissues, with particularly high levels noted in the brain and spleen.[41] Although normal B and T cells appear devoid of this protein, viral induction of fascin expression has been reported for Epstein-Barr virus (EBV)-infected B cells or B cell lines that express the full repertoire of EBV antigens.[41]

The goal of this study is to define a comprehensive staining profile for fascin in normal and neoplastic lymphoid tissues. A large series of Hodgkin's disease of various histological types and a variety of non-Hodgkin's lymphomas were evaluated to determine whether reactivity for fascin may provide insights into the pathogenesis of Hodgkin's disease and/or aid in its distinction from non-Hodgkin's lymphoma in difficult cases.

Materials and Methods

Cases were retrieved from the surgical pathology files of the Brigham and Women's Hospital (Boston, MA) and Cedars-Sinai Medical Center (Los Angeles, CA) and included 187 cases of Hodgkin's disease with 132 cases of nodular sclerosis, 34 cases of mixed cellularity, 14 cases of lymphocyte predominance (nodular), 2 cases of lymphocyte depletion, and 5 unclassified types. Tissues were fixed in B5 solution (57 specimens), 10% neutral buffered formalin (110 specimens), both B5 and formalin (8 specimens), Zenker's acetic acid solution (1 specimen), or Bayley's solution (11 specimens). Studies were also performed on non-neoplastic tissues of 14 reactive lymph nodes (8 formalin fixed, 2 B5 fixed, and 4 both B5 and formalin fixed), 4 thymuses (all fixed in both B5 and formalin), and 3 spleens (2 formalin fixed; and 1 formalin and B5 fixed). For comparison with cases of Hodgkin's disease, a total

of 156 specimens (79 formalin fixed, 75 B5 fixed, and 2 both formalin and B5 fixed) involved by B or T cell neoplasms, previously phenotyped by flow cytometric and/or immunocytochemical studies, were also analyzed.

Although preliminary studies were performed on cryostat sections,[38] further evaluation demonstrated that the epitope identified by the monoclonal antibody to human fascin was preserved after fixation and paraffin embedding. As optimal cytological detail may be achieved with paraffin sections, all studies in this report were performed on fixed tissues. For tissues fixed in B5 solution, mercury salts were not removed, as diminished reactivity was observed in some cases after this procedure. (Recent studies demonstrate that brief treatment with alcoholic iodine to remove mercury salts is not deleterious to antigen preservation.) After deparaffinization, slides were placed in 0.05 mol/L Tris buffer, pH 7.6. Slides were processed using a five-step alkaline phosphatase anti-alkaline phosphatase technique.[42] Monoclonal antibody to human fascin was prepared as previously described[37] and represents an IgG_1 immunoglobulin. Slides were incubated with primary antibody (1:10,000 dilution) for 1 hour, followed by sequential incubations for 40 minutes each with rabbit antibodies to mouse immunoglobulins (1:40 dilution; Dako Corp., Carpinteria, CA) and alkaline phosphatase anti-alkaline phosphatase immune complexes (APAAP; 1:50 dilution, Dako) with repeat sequential 20-minute incubations with the latter two reagents. Antibody localization was determined by an alkaline phosphatase reaction using naphthol AS-MX phosphate as substrate and Fast Red TR or New Fuchsin (Sigma Chemical Co., St. Louis, MO) as chromogen. Slides were counterstained with hematoxylin or methyl green. For studies with Fast Red TR, slides were mounted with glycergel (Dako); for studies with New Fuchsin, slides were processed through organic solvents and, for optimal preservation of chromogen, after ethanol, were processed through Pro-Par (Anatech, Battle Creek, MI) and mounted with permount. In some cases, additional studies were also performed using an immunoperoxidase technique as previously described[43] or with an automated immunostainer (Ventana Medical Systems, Tucson, AZ). Immunoperoxidase studies for EBV latent membrane protein were performed as previously described.[43]

Studies were also performed on cytocentrifuge preparations of enriched cell fractions (dendritic or veiled cells, T cells, B cells, and monocytes) isolated from peripheral blood (by E. Langhoff) as previously described[38] after fixation in cold methanol or methanol/acetone (1:1, v/v).

Control slides substituting IgG_1 (isotype-specific) mouse immunoglobulin for the primary antibody in sequential paraffin sections were processed in all cases. As positive controls, cytocentrifuge preparations of peripheral blood mononuclear cells enriched for dendritic cells[38] and/or sections of reactive lymph node were employed.

Results

Non-Neoplastic Tissues

A total of fourteen lymph nodes were evaluated. In four lymph nodes with nonspecific reactive changes, reactivity for fascin was localized to the cytoplasm of dendritic-appearing cells consistent with interdigitating reticulum cells in the T zones of the node, in dendritic-appearing cells in subcapsular areas, and in cells of the reticular network (Figure 1). A strong diffuse cytoplasmic staining pattern was observed, although cells of the reticular network appeared relatively less intensely stained. Follicular dendritic cells exhibited variable reactivity and were frequently nonreactive or weakly reactive as were spindle cells (interstitial dendritic cells) in perinodal tissue. In all tissues examined, endothelial cells were reactive although intensity of staining was variable. Lymphoid cells were uniformly nonreactive. In five nodes with toxoplasmic lymphadenitis, a relatively similar pattern was observed. Reactivity of endothelial cells was slightly more prominent. Epithelioid histiocytes and monocytoid B cells were nonreactive. Five nodes with dermatopathic lymphadenitis exhibited a similar staining pattern; however, interdigitating reticulum cells in T cell zones were clearly more numerous as compared with other reactive processes.

Four thymuses were evaluated and revealed similar staining patterns. Reactivity for fascin was distinctly localized to dendritic cells of the medulla (Figure 2). Occasional single cells within the cortex, some of which appeared to be endothelial cells, revealed weak staining. Cortical and medullary lymphoid cells, Hassall's corpuscles, thymic epithelial cells, and other cell types were nonreactive.

In the spleen (three cases evaluated), reactivity for fascin was observed for dendritic cells in the white pulp, particularly in periarteriolar and marginal zones, for lining cells of splenic sinuses, and blood vessel endothelial cells. The staining pattern in the splenic red pulp appeared to accentuate the basement membrane of sinus-lining cells. Sheathed cap-

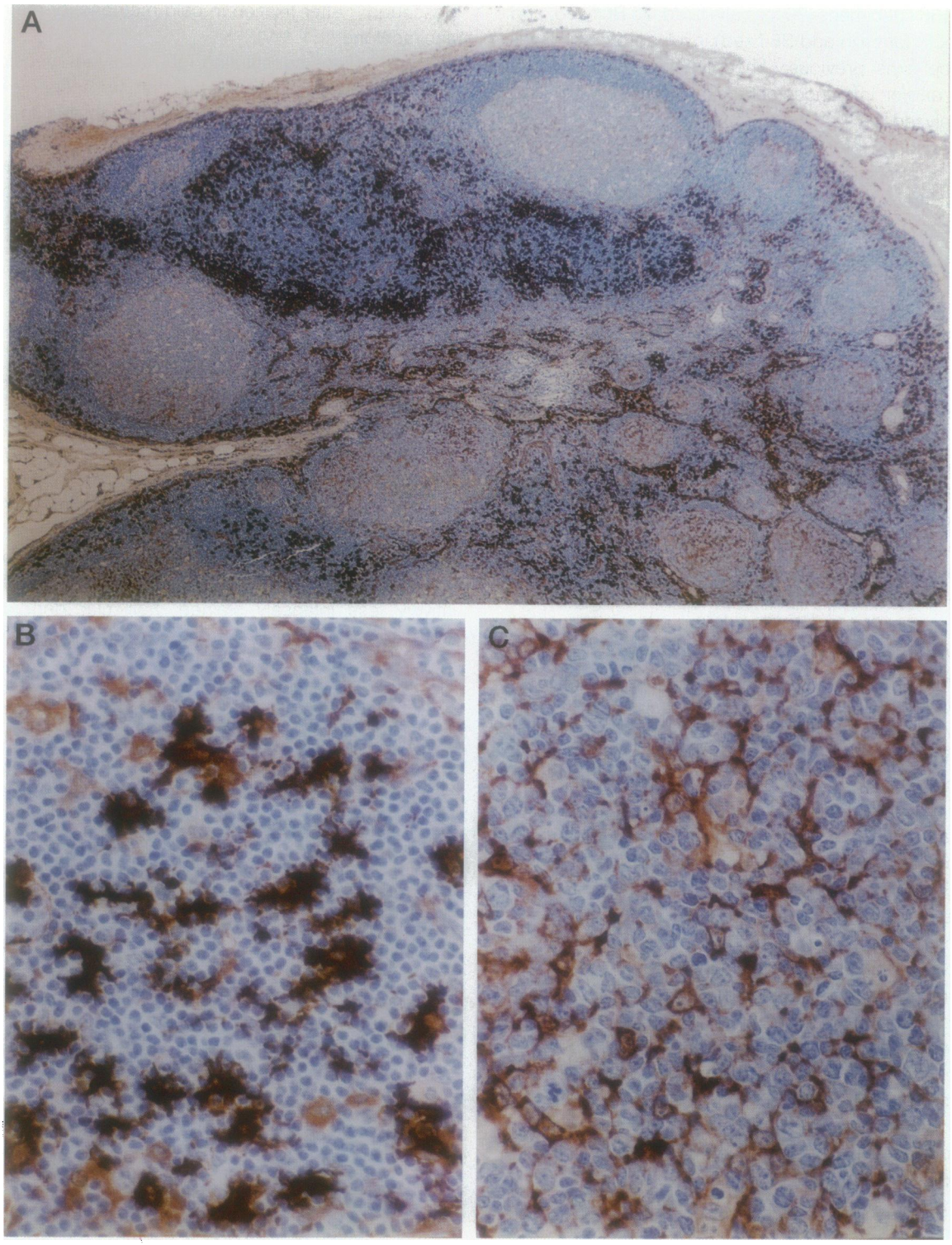

Figure 1. *Reactive lymph node, B5 fixation.* A: *At low power, strong immunoreactivity (dark brown) for human fascin is observed for interdigitating reticulum cells in the T cell zones, with reactivity also noted for cells of the reticular network and cells in subcapsular areas, with variable reactivity for follicular dendritic cells.* B: *Higher magnification of interdigitating reticulum cells in T zones reveals strongly immunoreactive cells with irregular cell margins and cytoplasmic extensions. Endothelial cells are also reactive, with a weaker staining pattern* (upper left). *Lymphoid cells are nonreactive.* C: *Higher magnification of a strongly reactive follicular area demonstrates immunoreactivity restricted to follicular dendritic cells with long slender cytoplasmic processes. Lymphoid cells are nonreactive. Immunoperoxidase technique; hematoxylin counterstain; magnification,* ×*50* (A) *and* ×*400* (B *and* C).

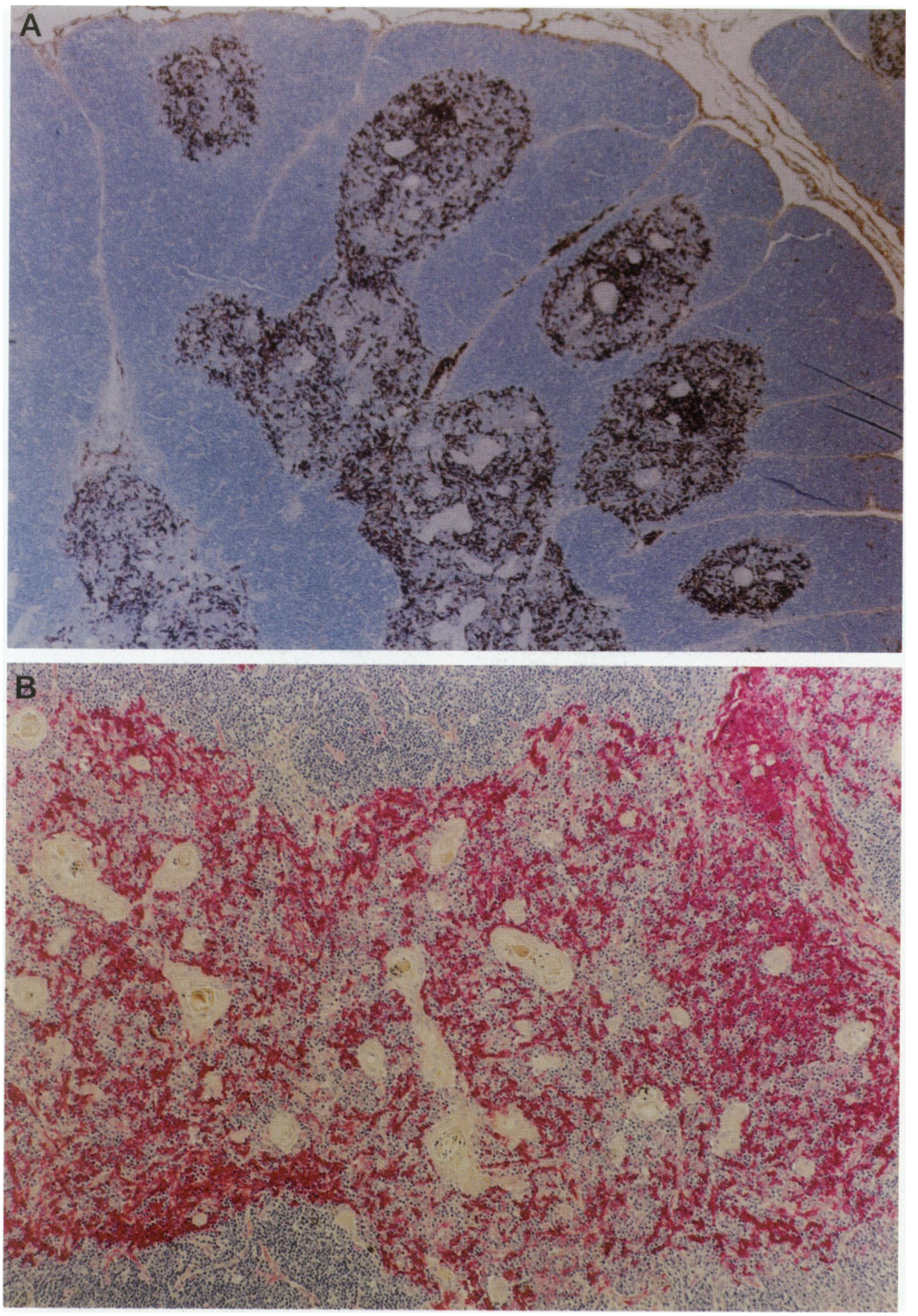

Figure 2. *Thymus, B5 fixation.* A: *Immunoreactivity for fascin is restricted mainly to dendritic cells of the medulla (dark brown). Immunoperoxidase technique; hematoxylin counterstain; magnification, ×50.* B: *Higher magnification of medullary dendritic cells with elongated cell processes, using another technique. Other cellular elements, ie, lymphoid cells, Hassall's corpuscles, and thymic epithelial cells, are nonreactive. APAAP technique; hematoxylin counterstain; magnification, ×115*

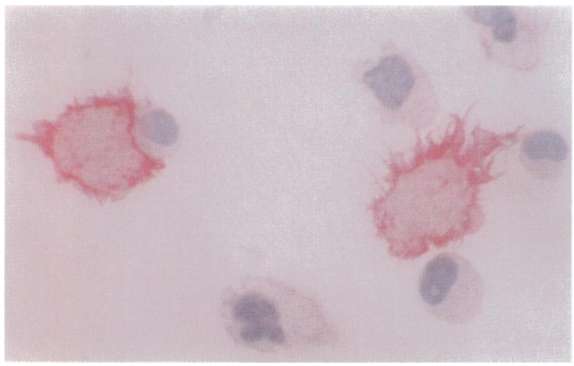

Figure 3. *Peripheral blood dendritic (veiled) cells, cytocentrifuge preparation (enriched cell fraction). In the peripheral blood, immunoreactivity for fascin (red) is observed only for this cell population, which is characterized by extremely long cell processes or lamellipodia. APAAP technique; methyl green counterstain; original magnification, ×1900.*

illaries in the marginal zone area were also reactive. Lymphoid cells and other splenic elements were nonreactive.

Studies of cytocentrifuge preparations of enriched cell fractions isolated from peripheral blood revealed cytoplasmic reactivity of varying intensity only for cells of dendritic type (Figure 3), as previously reported for immunofluorescent studies.[38] T cells, B cells, and monocytes, as well as occasional myeloid cells present in these preparations, were uniformly nonreactive.

The staining pattern observed for these tissues appears highly selective, although not absolutely specific, for cells of the dendritic system as previously described for human and various animal tissues.[40]

Control sections processed with IgG_1 mouse immunoglobulin as a substitute for primary antibody were nonreactive. For tissues processed in both formalin and B5 solution, staining intensity was stronger in B5-fixed tissues in some cases. Recent investigations in our laboratory (methods not employed in this study) have demonstrated enhanced staining intensity for formalin-fixed tissue after pretreatment of deparaffinized slides using a steamer (Black & Decker steamer, model HS80) with slides immersed in citrate buffer (30 minutes in steamer, followed by 20 minutes at room temperature, washing slides in water, placing in buffer, and proceeding with immunohistochemical detection method). For occasional B5-fixed tissues in which staining is not optimal, pretreatment using a steamer (as described) for slides immersed in target unmasking fluid (Signet Laboratories, Dedham, MA) may be employed to enhance reactivity.

Table 1. *Immunoreactivity for Fascin in Reed-Sternberg Cells and Variants of Hodgkin's Disease*

Histological type	Number of cases reactive/number of cases evaluated
Nodular sclerosis	132/132
Mixed cellularity	34/34
Lymphocyte depletion	2/2
Unclassified	5/5
Lymphocyte predominance, nodular	0/14

In the majority of reactive cases, essentially all Reed-Sternberg cells and variants were stained.

Hodgkin's Disease

A total of 187 cases were evaluated including 132 of nodular sclerosis, 34 mixed cellularity, 14 lymphocyte predominance (nodular), 2 lymphocyte depleted, and 5 unclassified types (Table 1).

In all cases of Hodgkin's disease, except for those of nodular lymphocyte predominance type, Reed-Sternberg cells and variants were uniformly reactive for fascin. In most cases, all or nearly all Reed-Sternberg cells and variants exhibited strong diffuse cytoplasmic staining. In some cases, the intensity of staining varied. In cases of nodular sclerosis type, reactive cells frequently occurred in aggregates, sheets, or syncytial masses, blending with identifiable interdigitating reticulum cells. Even in nodular areas of the infiltrate, frequently cytoplasmic processes of neoplastic cells were noted to contact each other (Figure 4). In more sheet-like areas, often a spectrum of cytological forms was apparent, ranging from interdigitating reticulum cells to Reed-Sternberg cells, with intermediate forms, which created a complicated interdigitating meshwork of cells (Figure 5). In nodes or spleens containing areas with early involvement, neoplastic cells were readily identified in T cell zones. In occasional cases, neoplastic cells were observed infiltrating intact follicular areas or were identified in vascular channels. In areas with recognizable follicles, neoplastic cells appeared to invade follicles; a pattern suggesting initial proliferation within follicles with extension to perifollicular regions was not observed. In cases with large areas of necrosis, immunoreactive neoplastic cells were observed in large numbers at the periphery of the necrotic lesions. Immunoreactivity for fascin was also observed for endothelial cells of many vessels present in the specimen. Follicular dendritic cells typically were nonreactive or exhibited weak reactivity, although occasionally stronger staining was observed. In some cases, weak to moderate staining was noted for dendritic-type spindle cells in fibrous

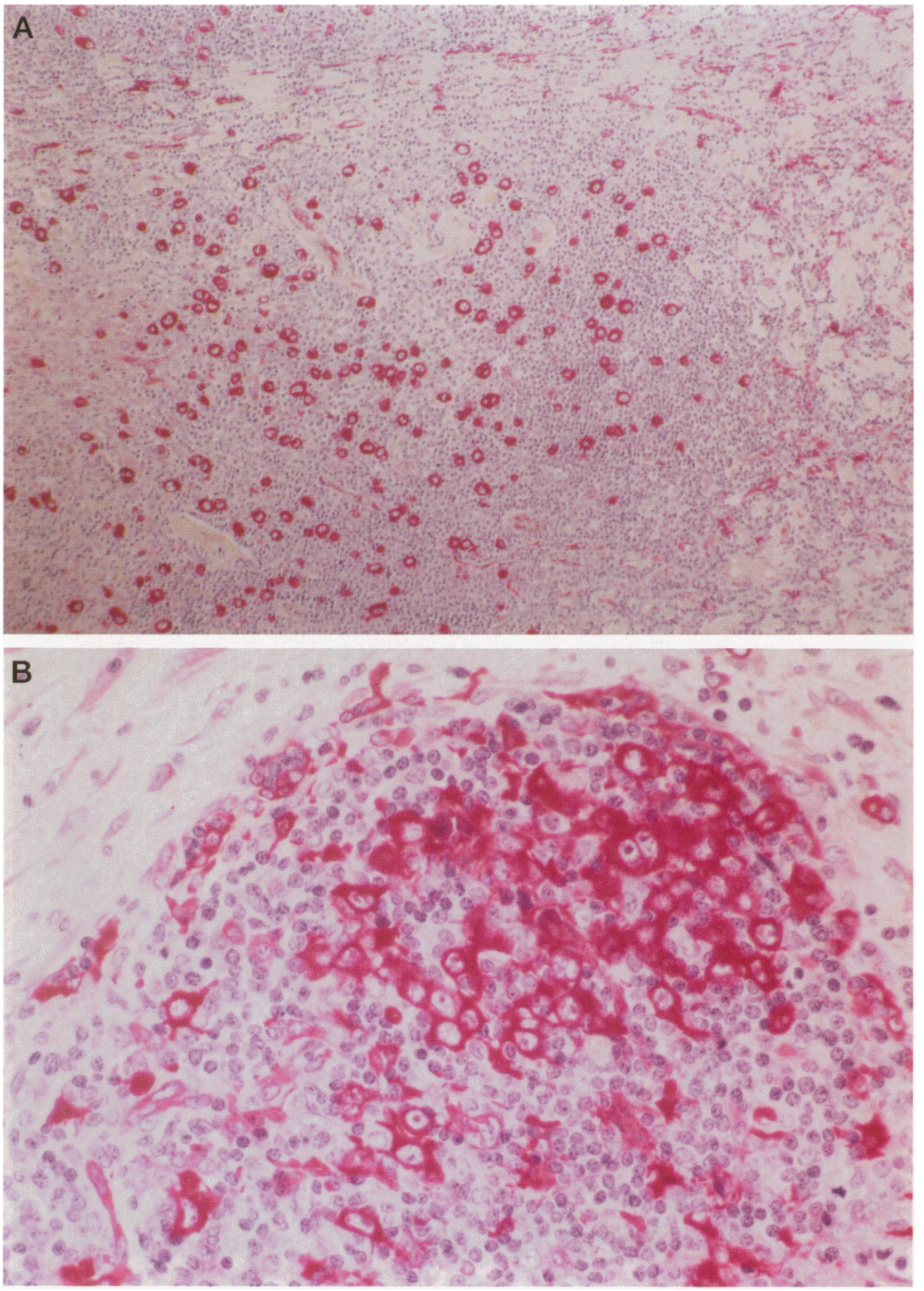

Figure 4. *Hodgkin's disease, nodular sclerosis type, B5 fixation.* **A:** *Strong staining for fascin (red) is observed for Reed-Sternberg cells and variants in a nodular area, with reactivity also noted for endothelial cells of blood vessels.* **B:** *In nodular areas, neoplastic cells frequently exhibited elongated dendritic shapes and tended to blend with each other. APAAP technique; hematoxylin counterstain; magnification, ×200 (A) and ×800 (B).*

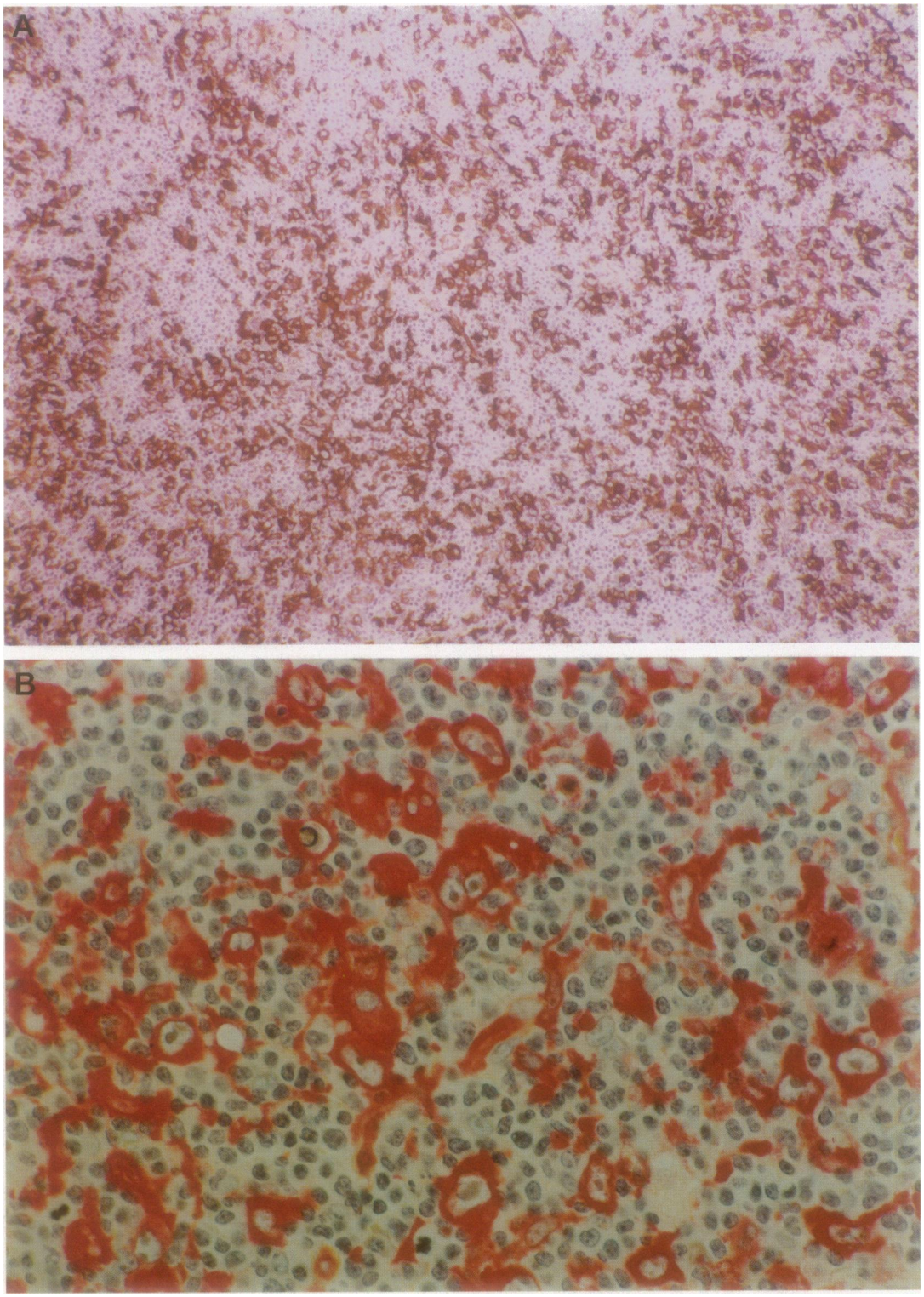

Figure 5. *Hodgkin's disease, nodular sclerosis type, B5 fixation.* A: *In areas with sheet-like proliferations, complicated networks or syncytia are formed by interdigitating cells that are strongly reactive for fascin (brown) and include Reed-Sternberg cells and variants, interdigitating reticulum cells, and forms that appear intermediate between the latter cell types. Immunoperoxidase technique; hematoxylin counterstain; magnification, ×200.* B: *High magnification of the spectrum of dendritic-type cells reactive for fascin (red), including mononuclear and diagnostic Reed-Sternberg cells, using another technique. APAAP technique; hematoxylin counterstain; magnification, ×1000.*

areas, interstitial tissue, or perinodal tissue. Lymphoid cells, plasma cells, neutrophils, eosinophils, and histiocytes were uniformly nonreactive.

In cases of mixed cellularity type, Reed-Sternberg cells and variants were also strongly immunoreactive for fascin, with nearly all neoplastic cells positive in these specimens (Figure 6). Neoplastic cells were scattered throughout the infiltrate, and although occasional cytoplasmic extensions were observed, imparting a limited dendritic appearance to the cells, they tended to appear more discrete, rather than as large syncytial masses as observed in nodular sclerosis type. Interdigitating reticulum cells were not a conspicuous feature in these proliferations. In specimens with interfollicular involvement, neoplastic cells were readily detected on the basis of immunoreactivity for fascin. Eosinophils, neutrophils, and lymphoid cells present within the proliferations were nonreactive. Epithelioid histiocytes were nonreactive or revealed weak ill defined staining.

In two cases of lymphocyte depletion type and five specimens with Hodgkin's disease, type unclassified, most Reed-Sternberg cells and variants were reactive. One specimen represented a Zenker's fixed bone marrow biopsy, which revealed weaker staining than that typically observed for formalin- or B5-fixed tissues.

The staining pattern of specimens involved by nodular lymphocyte predominance Hodgkin's disease was clearly divergent from that observed for other histological subtypes. In all cases, L&H variants of Reed-Sternberg cells were nonreactive (Figure 7), as were the rare large binucleated forms. A rare large cell of indeterminate type was reactive. However, immunoreactive dendritic cells were identified between and within nodules in the proliferation. In a number of cases, processes of dendritic cells were observed surrounding or in association with the L&H variants. Lymphoid cells and histiocytes were nonreactive. In all cases, endothelial cells were reactive for fascin and exhibited variable staining intensity.

As experimental studies have demonstrated fascin production in B cells or B cell lines after EBV infection,[41] immunoperoxidase studies for EBV latent membrane protein were performed for 170 cases of Hodgkin's disease. Of 121 cases of nodular sclerosis type, 27 (22%) contained reactive neoplastic cells, with only rare or few cells reactive in 7 of those cases. In cases of mixed cellularity type, 24 of 33 cases (73%) were reactive. The 2 cases of lymphocyte depletion type were both reactive. None of 14 cases of lymphocyte predominance type contained latent membrane protein-positive cells.

Non-Hodgkin's Lymphoma

For comparison with the striking consistent results observed for cases of Hodgkin's disease (except for lymphocyte predominance type), immunoreactivity for fascin was determined for a variety of immunologically phenotyped non-Hodgkin's lymphomas and other lymphoid neoplasms (156 total: 127 B cell, 27 T cell, and 2 null; Table 2). Overall, 24 of 156 specimens (15%) revealed reactivity for fascin, although staining patterns typically differed from those observed for Hodgkin's disease.

Of 127 B cell neoplasms, cytoplasmic reactivity was observed in only 12 cases, many of which exhibited weak cytoplasmic (sometimes granular) staining, a pattern distinct from the uniform strong diffuse reactivity observed for immunoreactive Reed-Sternberg cells and variants. In 5 cases, less than 5% of the neoplastic cell population was reactive and included occasional polyploid forms. Of 8 EBV-associated post-transplant B cell lymphoproliferative disorders, 5 were nonreactive. In the 3 positive cases, approximately 5, 30, or 50%, respectively, of the neoplastic cell population was reactive. Neoplastic cells in 11 cases of T-cell-rich and/or histiocyte-rich B cell lymphoma (B cell neoplasms of large cell type in which 75% or more of the background cells consisted of T lymphocytes and/or histiocytes), 2 spleens involved by hairy cell leukemia, and 2 extramedullary plasmacytomas were nonreactive. A total of 23 peripheral T cell lymphomas were evaluated, including 10 anaplastic large cell lymphomas; 7 of the latter neoplasms and 1 other T cell lymphoma exhibited weak reactivity for fascin (Figure 8). In addition, 2 large cell anaplastic lymphomas of null-cell type also revealed weak granular cytoplasmic staining, apparent in only 5% of the neoplastic cells in 1 case. Of 5 lymphoblastic lymphomas, 3 (1 pre-B cell and 2 of 4 T cell) were reactive for fascin, generally with weak staining. Within these proliferations, non-neoplastic dendritic cells were reactive for fascin and were observed in varying numbers in the specimens. In some cases, these cells occurred as exuberant proliferations. In B cell lymphomas with nodular areas, immunoreactive dendritic cells were noted mainly between nodules, with occasional reactive cells in nodules. Vascular endothelial cells revealed reactivity that varied in extent and in intensity. Dendritic cells were more prominent in T cell proliferations, with reactivity observed for large cohesive clusters of these cells in a case of angioimmunoblastic lymphadenopathy with dysproteinemia-like T cell lymphoma. An exuberant dendritic cell proliferation was also apparent in a lymph node in-

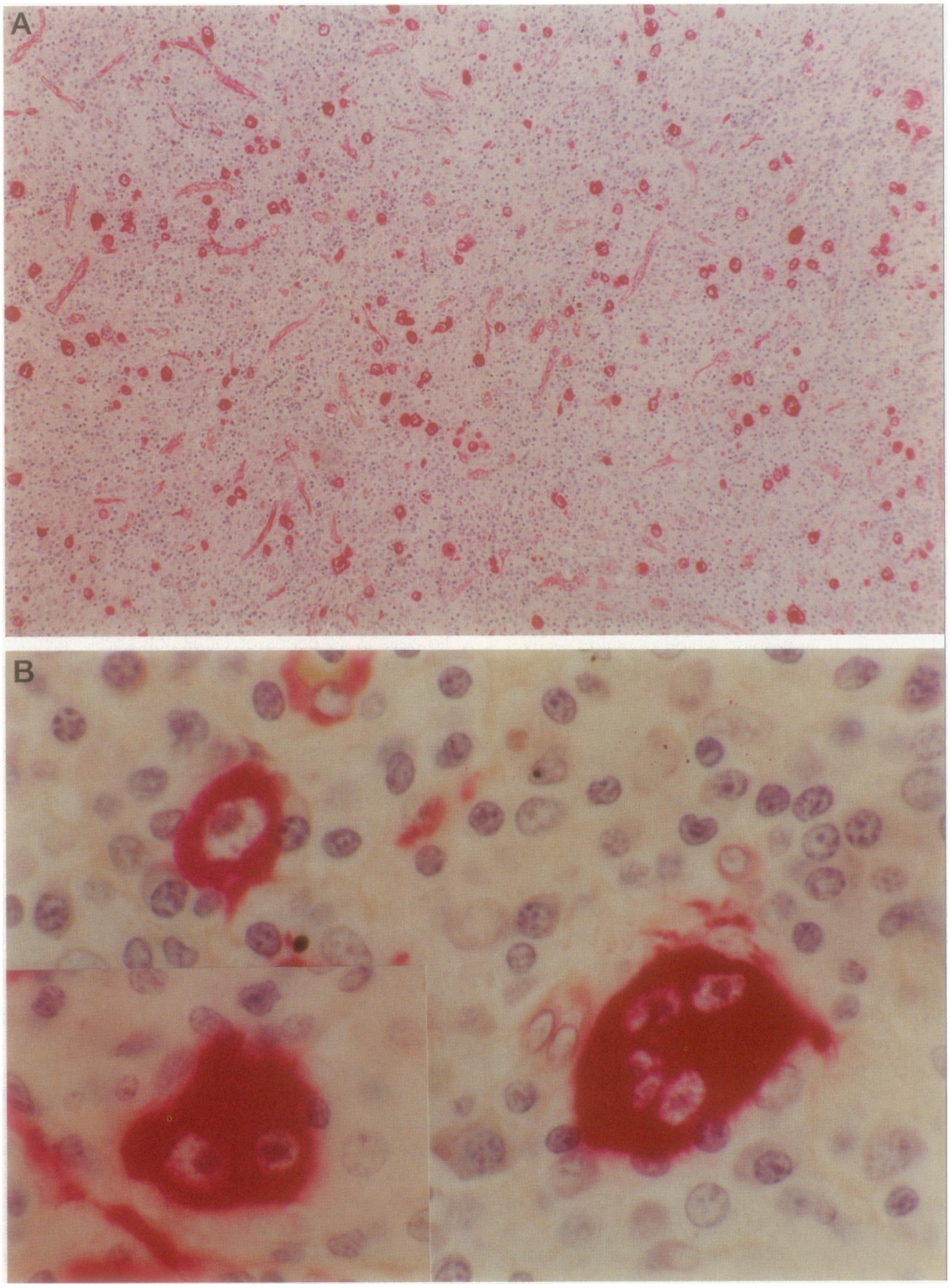

Figure 6. *Hodgkin's disease, mixed cellularity type, B5 fixation.* A: *At low power, strong immunoreactivity (red) for fascin is selectively observed for Reed-Sternberg cells and variants, with staining of varying intensity also apparent in blood vessel endothelial cells.* B: *Higher magnification of neoplastic cells including mononuclear, binucleated* (lower left), *and multinucleated forms of Reed-Sternberg cells. Background cell population (mainly lymphocytes and plasma cells) is nonreactive. Endothelial cell reactivity* (upper left) *is also noted. APAAP technique; hematoxylin counterstain; magnification,* ×*200* (A) *and* ×*1500* (B).

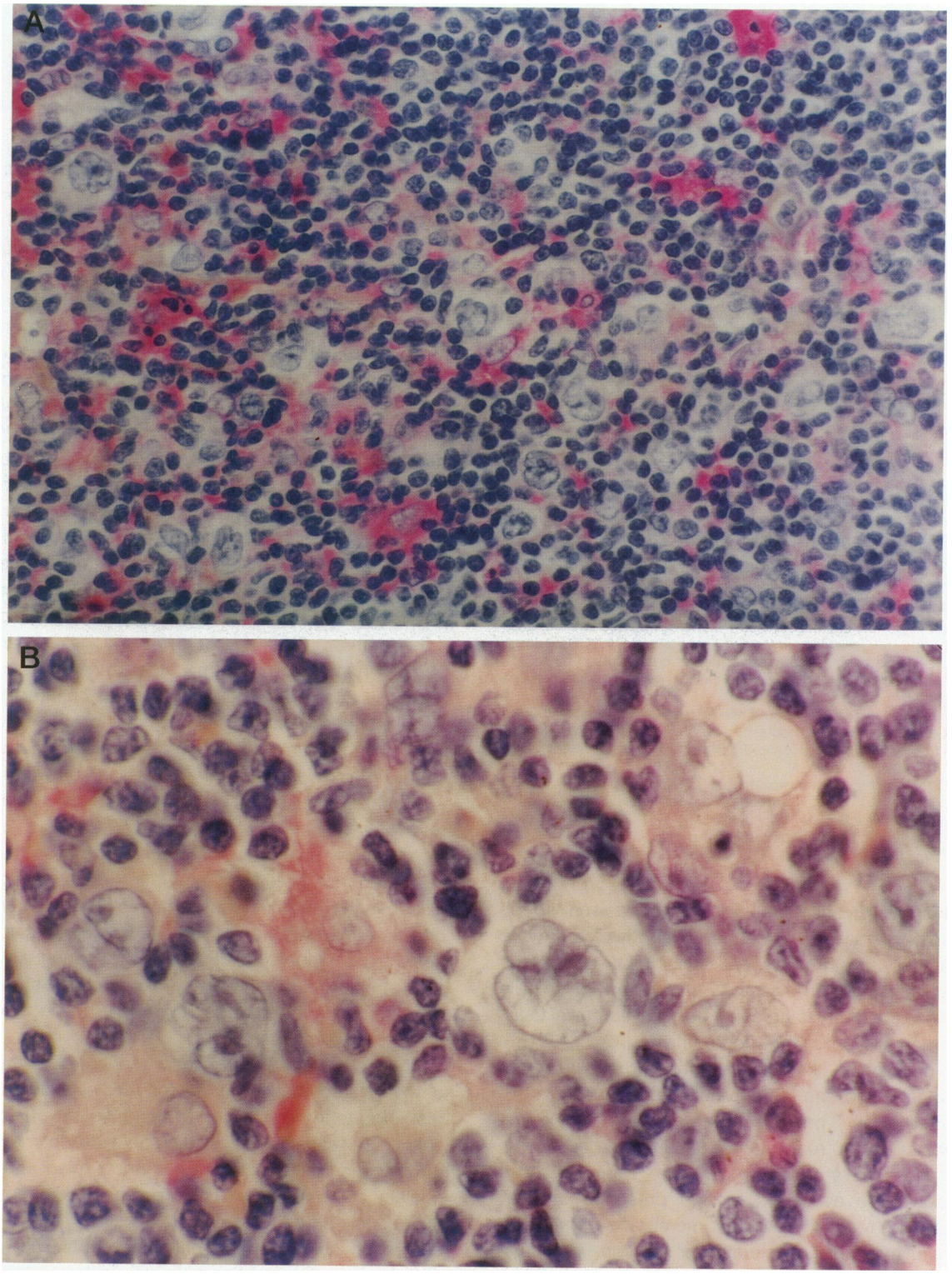

Figure 7. *Hodgkin's disease, nodular, lymphocyte predominance type, B5 fixation.* **A:** *L&H variants and background lymphocytes are nonreactive for fascin. Occasional dendritic cells exhibit staining (red).* **B:** *L&H variants at higher magnification. APAAP technique; hematoxylin counterstain; magnification, ×600* (**A**) *and ×1600* (**B**).

Table 2. *Immunoreactivity for Fascin in Neoplastic Cells of Various Lymphoid Malignancies*

Diagnosis	Number of cases reactive/number of cases evaluated
SL/CLL	0/1
SC/mixed FCC	0/4
LC/LNCFCC	1/53* (weak)
Small non-cleaved	0/10
T cell-rich/histiocyte-rich B cell	0/11
Immunoblastic	
B cell	6/32†
T cell	1/4‡
Anaplastic large cell	
T cell	7/10 (weak)
B cell	1/3§
Null	2/2 (weak)
Other peripheral T cell lymphomas	0/9
Lymphoblastic	3/5‖
Post-transplant EBV-associated	3/8¶
Hairy cell leukemia (spleen)	0/2
Plasmacytomas	0/2
Total	24/156 (15%)

All lymphomas were diffuse, except for five cases (two SC and three LC/LNC cell types) that revealed a follicular growth pattern. The series includes 13 lymphomas (12 B immunoblastic, 1 LNC) in HIV^+ patients, with 3/8 EBV^+ cases (all B immunoblastic) reactive for fascin. SL/CLL, small lymphocytic lymphoma/chronic lymphocytic leukemia; SC/mixed FCC, small cleaved/mixed small and large cell follicular center cell lymphoma; LC/LNCFCC, large cleaved/large noncleaved follicular center cell lymphoma.

*Approximately 50% of neoplastic cells exhibited weak cytoplasmic staining.

†In most cases, <5% cells were reactive. A variable pattern was seen, from weak cytoplasmic staining to occasional strongly reactive large polyploid cells. Included are three of eight EBV^+ cases in HIV^+ patients.

‡Weak reactivity in approximately 50% of cells.

§Less than 5% of neoplastic cells exhibited variable cytoplasmic reactivity.

‖Two of four T cell (weak); one pre-B cell.

¶Reactivity in 5%, 30%, or 50% of cells, respectively.

volved by mycosis fungoides. In occasional specimens, interstitial or perinodal dendritic cells were reactive, usually with weak staining. In specimens that included skin, reactivity was noted for spindle cells in the dermis and some adnexal epithelial cells. Langerhans cells were nonreactive.

Thirteen non-Hodgkin's lymphomas (twelve immunoblastic and one large noncleaved type) were observed in HIV^+ patients. In three cases, all of immunoblastic type, reactivity for fascin was detected in small numbers of neoplastic cells. Twelve of these cases were evaluated for the presence of EBV, based on reactivity for latent membrane protein and *in situ* hybridization studies for Epstein-Barr virus-encoded ribonucleic acid. Three of eight EBV^+ cases were focally reactive for fascin. A comparison of fascin reactivity and detection of EBV in post-transplant EBV-associated lymphoproliferative disorders, B cell lymphomas in HIV^+ patients, and Hodgkin's disease of various histological types is presented in Table 3 and emphasizes the discordance observed for most neoplasms.

Discussion

This study comprehensively defines the staining profile for human fascin in lymphoid tissues. This protein, initially described in 1985,[36] was isolated from HeLa cells and characterized as a monomeric globular protein that made F-actin aggregate side by side into bundles, an effect similar to that observed for other actin-bundling proteins such as fimbrin. Based on recent cloning studies, this protein is likely encoded by a single copy gene termed *hsn*, which is localized to chromosome 7p22 and exhibits strong homology with the sea urchin fascin and the *Drosophila sn* genes.[39] The group of globular actin-bundling proteins, as represented by fascin, is responsible for the formation of microfilament bundles in microspikes, in contrast to the linear rod-like gelation proteins (eg, filamin, α-actinin, and spectrin) that are involved in the formation of meshworks at the basal portions of microspikes, ultimately producing rigid gel structures. Studies employing monoclonal antibody to human fascin demonstrated localization in microspikes and stress fibers of various cultured cell lines, including HeLa cells,[37] implying its involvement in the dynamic formation of microfilament bundles. Certain microspike structures are highly motile, eg, lamellipodia of cultured cells, and extend and retract on the cell surface. This property is also a feature of peripheral blood dendritic cells, as well as other dendritic cells, and is unique to this cell population in peripheral blood. Dynamic changes in microfilament bundles are also associated with cytokinesis and changes in cell shapes and overall would play a major role in the biological functions of dendritic cells, which include migration, homing, and antigen presentation.

Studies of peripheral blood mononuclear cells demonstrated that reactivity for fascin is uniquely restricted to the population of dendritic cells (veiled cells[38]; current study), providing an excellent marker for detection of this distinct extremely minor mononuclear cell population. Fascin also is an effective marker to characterize dendritic cells in a distinct and highly selective manner in non-neoplastic lymphoid tissues. Sites of dendritic cells in these tissues, based on fascin immunoreactivity, conform to sites of localization for these cells, which have been described for human and animal tissues.[40,44] Although Langerhans cells of skin represent dendritic cells, they were not immunoreactive for fascin in the few

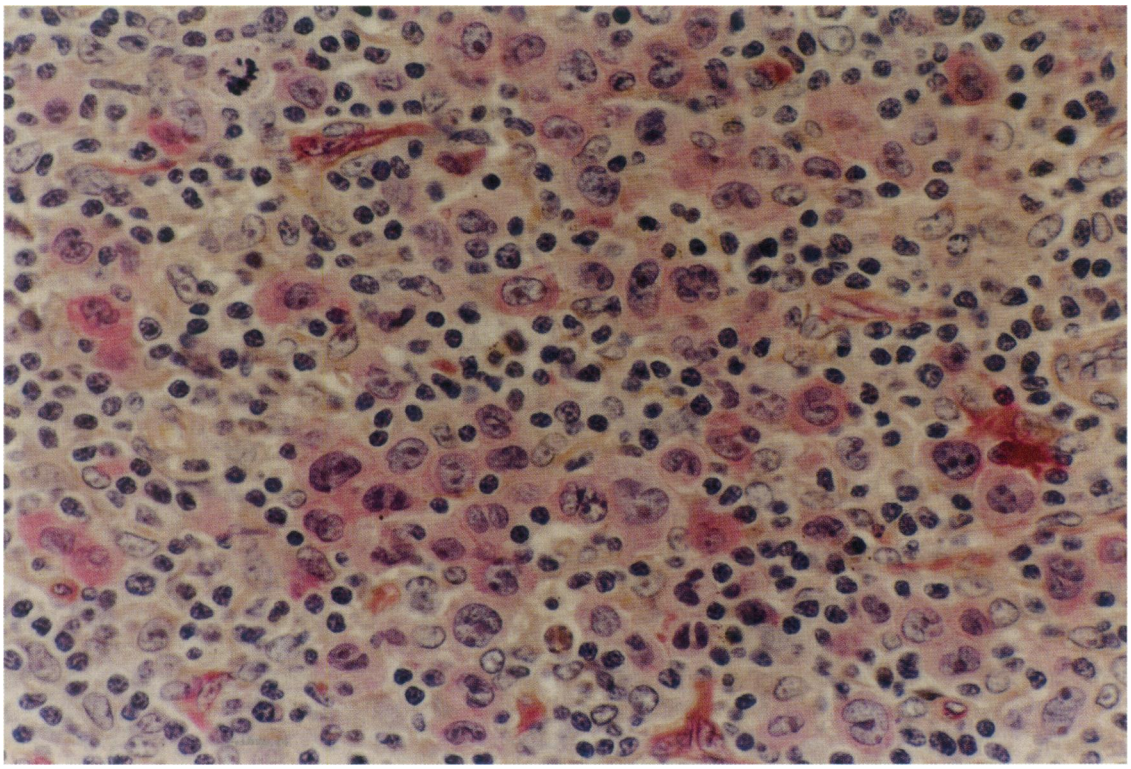

Figure 8. *Anaplastic large cell lymphoma, T cell phenotype, B5 fixation. Neoplastic cells are weakly reactive (pink) for fascin. Although most non-Hodgkin's lymphomas were nonreactive for fascin, this tumor type frequently exhibited weak reactivity. A dendritic cell* (right) *exhibits strong staining (red) for this protein, as do occasional blood vessel endothelial cells. APAAP technique; hematoxylin counterstain; magnification, ×800.*

specimens that included skin. However, after initial isolation, these cells reportedly are not active antigen-presenting cells for the mixed lymphocyte culture, despite their association with high levels of MHC class II products and Fc receptors. Interestingly, in culture, Langerhans cells are known to undergo structural and phenotypic changes and ultimately resemble peripheral blood and lymphoid tissue dendritic cells. Perhaps the Langerhans cells of skin represent a stage of differentiation of dendritic cells that lacks fascin *in situ* yet has the potential for induction of this protein under other conditions.[40] In our studies of non-neoplastic lymphoid tissues and in most neoplastic lymphoid tissues, lymphoid cells were nonreactive or exhibited weak or limited reactivity, even in proliferations that were EBV positive (Tables 2 and 3). Previous studies of B cells or B cell lines have demonstrated the ability of EBV to induce fascin production.[41]

A striking and consistent finding in this study is the uniform reactivity of Reed-Sternberg cells and variants for fascin, except in nodular lymphocyte predominance type. This protein was observed for most or nearly all Reed-Sternberg cells and variants in all

Table 3. *Detection of Fascin and Epstein-Barr Virus in Hodgkin's Disease and Selected Non-Hodgkin's Lymphomas*

Diagnosis	Number of cases positive/number of cases evaluated	
	EBV positive*	Fascin positive
Hodgkin's disease		
Nodular sclerosis	27/121 (22%)	121/121 (100%)
Mixed cellularity	24/33 (73%)	33/33 (100%)
Lymphocyte depletion	2/2	2/2
Lymphocyte predominance	0/14	0/14
Post-transplant B cell lymphoproliferative disorders	8/8 (100%)	3/8 (38%)
B cell lymphomas in HIV^+ patients	8/12 (67%)	3/12† (25%)

*Only cases evaluated for both EBV and fascin were included. Studies for EBV latent membrane protein were performed for Hodgkin's cases; for other lymphomas, EBV detection included studies for EBNA-2, LMP, Southern blot analysis, and/or *in situ* hybridization for EBV RNA (EBER-1).

†All fascin-positive cases were also EBV positive and represent 3/8 (38%) EBV-positive cases.

cases of nodular sclerosis, mixed cellularity, lymphocyte depletion, and unclassified types of Hodgkin's disease (173 total). The consistency of staining of these neoplastic cells for fascin distinguishes it as a more sensitive marker for these cells than others that have previously been described, eg, Leu-M1 (CD15) and Ki-1 (CD30).[5,7,13,23,24,45,46] Although immunoreactivity is not absolutely restricted to dendritic cells as defined in this and previous studies,[41] eg, endothelial cells, some epithelial cells, and neural cells exhibit reactivity, these staining patterns pose no problem in interpretation and the latter cells are not candidates for a cell of origin of Reed-Sternberg cells. More importantly, lymphoid cells of T and B cell type, which have been proposed as a cell of origin for Reed-Sternberg cells, are nonreactive for fascin, although limited staining was observed for some B or T cell neoplasms. Also, in view of the limited reactivity for fascin in non-Hodgkin's lymphomas, this protein may represent a useful marker for distinguishing between these neoplasms in difficult cases. Based on this staining profile, a possible dendritic cell derivation for the neoplastic cells of Hodgkin's disease, particularly an interdigitating reticulum cell, deserves serious consideration, notably in nodular sclerosis type, in which a prominent interdigitating pattern was observed. However, as fascin production may be induced after EBV infection of B cells, a possible role for this virus in EBV-positive cases must also be addressed (discussed subsequently).

A possible dendritic cell origin for neoplastic cells of Hodgkin's disease, in at least some of the cases, provides a unifying concept for various characteristics that have been described for Reed-Sternberg cells and for certain clinical manifestations of Hodgkin's disease. Cytologically, in this study, the spectrum of cells immunoreactive for fascin ranged from cells consistent with interdigitating reticulum cells to Reed-Sternberg cells and variants, with intermediate forms also apparent. The processes of these cells interdigitated with each other forming a meshwork of dendritic-appearing cells, a particularly prominent finding in the nodular sclerosis type of Hodgkin's disease (Figures 4 and 5). Previous studies also provide support for this proposal. A cell line (L428) derived from pleural fluid of a patient with Hodgkin's disease has also been shown to have extensive dendritic processes and to have other characteristics typical for dendritic cells, including potent stimulation of human mixed lymphocyte cultures.[22] Dendritic cells are 100 times more potent than lymphocytes or monocytes in stimulating mixed lymphocyte cultures.[47] T cells of helper/inducer type account for most of the T cell proliferation, a feature similar to the cellular proliferations observed in cases of Hodgkin's disease.[48] Another property of dendritic cells is their ability to cluster and activate T cells.[40] T cell clustering is a feature that has been described for Reed-Sternberg cells and variants of Hodgkin's disease, a disorder that is generally associated with a background population of T cells,[48,49] except in lymphocyte predominance type. The presence of high levels of adhesion molecules, eg, ICAM (CD54) and LFA-3 (CD58), on both dendritic cells and Reed-Sternberg cells provides a basis for the capacity of these cells to aggregate T cells for long periods of time.[40,50] In tissue sections, one obtains a perception of this clustering in Hodgkin's disease in that the cytoplasmic borders of the Reed-Sternberg cells and variants frequently exhibit a scalloped appearance seemingly as a consequence of impinging T cells.[45] Experimental injection studies using animal models have shown that dendritic cells will home to T-cell-dependent areas of the node where they are recognized as interdigitating reticulum cells. The proposal that Reed-Sternberg cells and variants represent neoplastic transformation of interdigitating reticulum cells, correlates well with the known occurrence of early nodal involvement in T cell zones (except lymphocyte predominance type[21,48,51]). Alteration and/or neoplastic transformation of interdigitating reticulum cells also would provide a basis for the impaired T cell immunity observed in patients with Hodgkin's disease[21,52,53] in that the roles of dendritic cells include capture of antigen and presentation to T cells, migration to T-cell-dependent regions of lymphoid tissues where binding of antigen-specific T cells occurs, and initiation of T cell growth and function.

Cytochemical studies observed for neoplastic cells of Hodgkin's disease also parallel those observed for interdigitating reticulum cells. Rather than the diffuse cytoplasmic staining observed for acid phosphatase or α-naphthyl acetate esterase in histiocytes or macrophages, both Reed-Sternberg cells and interdigitating reticulum cells exhibit localized reaction products.[20,21] Both cell types typically reveal high levels of MHC class I and class II products, are potent stimulators of mixed lymphocyte cultures, exhibit similar patterns of lysosomal activity, are not actively phagocytic, are generally nonreactive for B and T cell markers and for a variety of macrophage antigens, are reactive for CD40, and express adhesion molecules CD54 and CD58 and β2 integrins (CD11a and CD11c).[12,13,22,29,54] Dendritic cells are reportedly CD15 negative whereas Reed-Sternberg cells are generally reactive for CD15. However, CD15 reactivity has been observed for interdigitating

reticulum cells after neuraminidase treatment,[23] suggesting a potential for reactivity after neoplastic transformation. Using a monoclonal antibody (anti-IRac) raised against 12-*O*-tetradecanoylphorbol-13-acetate-treated Reed-Sternberg cells, reactivity was observed nearly exclusively for interdigitating reticulum cells in lymphoid tissues[31]; in Hodgkin's disease, Reed-Sternberg cells were reactive. In these studies, staining was rarely detected for normal or mitogen-treated lymphocytes but was observed in virus-transformed B cells (EBV) or T cells (HTLV-1). Studies of Hodgkin's cell lines have also provided evidence to support a histiocyte/dendritic cell derivation for Reed-Sternberg cells. Using a combination of phorbol esters, retinoic acid, and extracellular matrix to induce maximal differentiation of Hodgkin's cell lines, the differentiated cells exhibited cytological, immunohistochemical, biochemical, and other properties most consistent with cells of histiocytic/dendritic cell lineage.[30]

Some studies have raised the possibility of a follicular dendritic cell derivation for the neoplastic cells of Hodgkin's disease. Using a silver staining technique, Curran and Jones[17] demonstrated the dendritic nature of Reed-Sternberg cells and variants and implied an association or derivation from follicular dendritic cells by analysis of cytological features. Immunohistochemical studies have demonstrated reactivity for CD21, an antigen observed for follicular dendritic cells, for some Reed-Sternberg cells, and variants.[19] Most recently, immunoreactivity for acid cysteine proteinase inhibitor, a marker observed for follicular dendritic cells in nodes, was observed for Reed-Sternberg cells and variants, except in lymphocyte predominance type.[18] In the latter series, 20 to 60% of neoplastic cells were reactive in 15 of 20 cases (10 mixed cellularity, 2 nodular sclerosis, and 3 lymphocyte depletion). The remaining 5 cases (3 lymphocyte predominance, 1 nodular sclerosis, and 1 lymphocyte depletion) were nonreactive. This staining profile for non-lymphocyte predominance cases is clearly less consistent than that observed for fascin. It would be of interest to determine whether interdigitating reticulum cells exhibit any reactivity for this enzyme and/or whether alteration or neoplastic transformation of these cells would result in acid cysteine proteinase inhibitor production. Although the monoclonal antibody to human fascin employed in our study appeared more sensitive for detection of interdigitating reticulum cells of T cell zones of non-neoplastic tissue, as compared with follicular dendritic cells, one could raise the issue of whether this staining pattern could be altered in a neoplastic process. Although one cannot completely exclude this possibility, certain features render it less likely. The staining pattern in cases of Hodgkin's disease, particularly nodular sclerosis type, demonstrates a blending of cells that includes the neoplastic population and cells consistent with interdigitating reticulum cells. Early nodal or splenic involvement is observed in T cell zones[21,48,51] that are occupied by interdigitating reticulum cells, not in follicles. Also, growth characteristics of the neoplastic cells do not suggest a proliferation originating in follicular centers. Furthermore, alterations in follicular dendritic cell function would not provide a basis for the impaired T cell immunity observed for patients with Hodgkin's disease.

Detection of B or T cell markers for Reed-Sternberg cells and variants in some cases of Hodgkin's disease has been well documented.[1–13,34] Exclusive of lymphocyte predominance type Hodgkin's disease, these markers are not uniformly observed and may reflect either a shared epitope or possibly true evidence of a B or T cell origin for these neoplastic cells. The latter findings contrast sharply with the uniform consistent staining of Reed-Sternberg cells and variants observed for fascin. Underscoring the lack of absolute lineage fidelity for the B and T cell markers is the observation of aberrant reactivity for myeloid/monocyte/histiocyte-derived leukemia and lymphoma cell lines.[35] These studies further demonstrated that reactivity for CD30, a feature of Reed-Sternberg cells and activated lymphoid cells, may also be observed for granulocyte/monocyte/histiocyte-related cells. Recent immunohistochemical studies to detect CD79a, the mb-1 polypeptide that, together with the B29 polypeptide, constitute part of the B cell antigen receptor complex, demonstrated consistent reactivity only in the L&H cells of lymphocyte predominance Hodgkin's disease.[55] Of the non-lymphocyte predominance cases, only 20% (19 of 94) were reactive for CD79a, with staining observed in only a small proportion of neoplastic cells in most cases. Studies for this highly selective B cell marker do not support a B cell derivation for Reed-Sternberg cells in the majority of non-lymphocyte predominance cases.

Molecular biological studies have demonstrated B cell and T cell receptor gene rearrangements in only a minority of cases of Hodgkin's disease,[34,56–58] although studies employing high sensitivity polymerase chain reaction (PCR) techniques have demonstrated clonal rearrangements of the IgH gene in over 50% of cases.[59,60] Although these results may reflect a T or B lymphoid derivation for the neoplastic cells, these findings must be viewed with some caution. As material examined in most studies includes

neoplastic cells of Hodgkin's disease as well as other cells, one cannot be certain that the clonal rearrangements are indeed derived from Reed-Sternberg cells or from the associated lymphoid populations. In an attempt to address this issue, micromanipulation techniques have been employed to isolate single Reed-Sternberg cells and variants.[56–58] Using PCR for DNA amplification, analysis of these cells revealed immunoglobulin gene rearrangements (V_H) in some cases, supporting a B cell derivation. In regard to T cell gene rearrangements, most have been described for the γ-T-cell receptor. As this gene has a limited number of variable regions, studies of polyclonal T cell populations may produce pseudoclonality. Studies of cultured true histiocytic cell lines have cast further skepticism on the true meaning of T or B cell gene rearrangements in tissues involved by Hodgkin's disease or cell lines derived from this disorder.[28] Other investigations using PCR to analyze chromosomal breakpoints also have failed to provide definitive results in Hodgkin's disease.[34,61–63] Most studies have evaluated the t(14;18) (q32;q21) translocation, which is associated with juxtaposition of the *bcl-2* proto-oncogene on chromosome 18 to the joining region of the immunoglobulin heavy chain gene. Most studies failed to detect either *bcl-2* major or minor cluster breakpoints. Even if *bcl-2* rearrangements were observed, again the question arises as to whether they reflect changes in neoplastic cells or in associated lymphoid populations. Using immunohistochemical techniques that allow cell identification, overexpression of *bcl-2* in Reed-Sternberg cells was not observed.[62]

Recent studies of cell cultures have demonstrated that production of fascin may be induced in B cells after EBV infection.[41] This finding raises the possibility that the presence of this protein in some cases of Hodgkin's disease, particularly those of mixed cellularity or lymphocyte depletion type, which are frequently EBV positive, may actually reflect viral induction of fascin in B cells or possibly other cell types. In our study, 22% of nodular sclerosis cases, 73% of mixed cellularity cases, and all of lymphocyte depletion type (two cases) exhibited reactivity for EBV latent membrane protein (Table 3). However, as previously noted, definitive evidence for a B cell derivation for neoplastic cells of Hodgkin's disease is lacking in most cases. If the EBV-positive neoplastic cells are indeed B cells, they have lost all or most characteristics permitting their identification in the majority of cases. It is also conceivable that the cell of origin may be diverse in Hodgkin's disease. In view of the ability of EBV to induce fascin production, Reed-Sternberg cells and variants may represent viral-infected cells of lymphoid or possibly even other cell types or even hybridoma tumor cells in EBV-positive cases. However, a role for EBV cannot be invoked in all cases of Hodgkin's disease. Using extremely sensitive *in situ* hybridization techniques for detection of Epstein-Barr virus-encoded ribonucleic acid transcripts,[34] EBV has been detected in only 50% of cases of this disorder. Although this finding supports a possible role for this virus in some cases of Hodgkin's disease, it precludes a role for EBV in all cases of this disorder. By contrast, fascin was detected in essentially all cells in all cases of Hodgkin's disease, exclusive of lymphocyte predominance type. Other sensitive techniques, such as PCR, suggest that EBV is present in up to 80% of cases of Hodgkin's disease, but it appears likely that EBV DNA in latently infected B cell lymphocytes, rather than Reed-Sternberg cells alone contributed to these high percentages. As EBV DNA may be detected in up to 43% of normal nodes using PCR techniques, this explanation is high plausible. An additional argument against EBV as the sole basis of production of fascin in neoplastic cells of Hodgkin's disease is its apparent lack of consistency in inducing production of this protein based on our tissue section studies of EBV-positive lymphomas (Tables 2 and 3). In eight cases of EBV-positive (based on Epstein-Barr virus nuclear antigen-2 and latent membrane protein studies and Southern blot analysis) post-transplant lymphoproliferative disorders, fascin was observed in some cells (5, 30, and 50%, respectively) in only three cases. Also, fascin was detected in only small numbers of neoplastic B cells in three of eight EBV-positive lymphomas in HIV^+ patients, underscoring the lack of consistency of fascin production in EBV-infected cells as evaluated in tissue sections. In studies of B cells or B cell lines,[41] only those exhibiting the full repertoire of EBV antigens (Epstein-Barr virus nuclear antigens and latent membrane proteins) were associated with fascin production. In all of our post-transplant cases, neoplastic cells were reactive for Epstein-Barr virus nuclear antigen-2 and latent membrane protein-1 (other EBV antigens were not evaluated). Neoplastic cells of Hodgkin's disease, although often latent membrane protein-1 positive, are not typically reactive for Epstein-Barr virus nuclear antigen-2,[43] a finding that perhaps would mitigate against fascin production by EBV, unless these neoplastic cells represent an exception to established conditions for inducing this protein. Perhaps the presence of EBV in the neoplastic cells of Hodgkin's disease may augment production of fascin in cells if they are of dendritic cell

derivation but induce its production if the neoplastic cell is of lymphoid origin. A possible origin for Reed-Sternberg cells from fusion of more than one cell type, eg, interdigitating reticlum cells and lymphoid cells, has also been proposed.[33]

Lymphocyte predominance type Hodgkin's disease revealed a divergent pattern of reactivity for fascin, representing yet an additional feature of this subtype that differs from other variants of this disorder. In contrast to Reed-Sternberg cells and variants of other subtypes that exhibited uniform reactivity for fascin, L&H variants of lymphocyte predominance type were nonreactive. Occasionally, elongated processes of dendritic cells surrounded or partially enveloped these variants. This type of Hodgkin's disease also differs from other types in a number of ways. Nodular lymphocyte predominance type of Hodgkin's disease arises in B zones, rather than T zones, of lymphoid tissues. L&H variants are typically reactive for leukocyte common antigen and one or more B cell antigens, particularly CD20 and CD79a, suggesting a B cell derivation or B cell association.[9,11,55] This disorder is frequently observed in nodes that also exhibit progressive transformation of germinal centers, a B cell proliferation. In occasional cases of lymphocyte predominance Hodgkin's disease, large cell lymphomas of B cell type have been documented, apparently representing evolution of the underlying disease. Despite these features that strongly support a neoplastic process of B cell type, B cell immunoglobulin gene rearrangements and *bcl-2* gene rearrangements are not a feature of this disorder, making a definitive origin elusive for this subtype.[34,61,63] The reactivity for B cell antigens may represent aberrant staining rather than true evidence of a B cell origin, as has been suggested for many cases of other subtypes of Hodgkin's disease, although this would appear less likely based on uniform reactivity for CD79a. Alternatively, these cells may represent a type of B cell that cannot be effectively characterized by most existing techniques. Based on single-cell analysis and PCR amplification, Reed-Sternberg cells and variants in one case demonstrated clonal heavy chain gene rearrangements and somatic mutations, consistent with a germinal center B cell derivation.[56] L&H cells are also noted to be nonreactive for Leu-M1 (CD15), a marker typically observed for other types of Reed-Sternberg cells and variants. However, those cases of lymphocyte predominance with a diffuse growth pattern were found to contain sialylated Leu-M1, detectable after neuraminidase treatment.[23] Generally, L&H cells do not exhibit strong reactivity for Ki-1 (CD30) as noted for other Reed-Sternberg cells and variants, although scattered large Ki-1-positive cells may be identified in these proliferations. These cells are also typically EBV negative[43] (Table 3). Also unique to lymphocyte predominance Hodgkin's disease is the presence of Leu-7 (CD57)-positive lymphoid cells that circumscribe L&H variants.[64] In other subtypes, CD4-positive T cells generally are observed around the neoplastic cells. Based on these composite findings, perhaps this process should be regarded as a unique B cell lymphoproliferative disorder rather than a variant of Hodgkin's disease.

In summary, fascin is highly restricted in its distribution in lymphoid tissues and is highly selective for cells of the dendritic system. This restricted staining profile, the uniformity of fascin reactivity for Reed-Sternberg cells and variants in all cases of Hodgkin's disease, except for lymphocyte predominance type, the striking parallels between properties of dendritic cells and those of Reed-Sternberg cells, the results of many other studies that provide evidence for a histiocytic/dendritic cell of origin for Hodgkin's disease, and the limited support for a lymphoid derivation for Hodgkin's disease in most cases all provide strong evidence for a dendritic cell origin (particularly an interdigitating reticulum cell) in many cases of Hodgkin's disease. Based on studies of B cells and B cell lines,[41] the role of viral induction of fascin in lymphoid or possibly other cell types, must also be considered in EBV-positive cases. In cases of Hodgkin's disease of lymphocyte predominance type, most studies favor a B cell derivation. Fascin is an extremely sensitive and consistent marker for Reed-Sternberg cells and variants (except L&H type) in paraffin sections, represents a valuable marker for dendritic cells in peripheral blood[38] and in other tissues, and may aid in distinguishing Hodgkin's disease from non-Hodgkin's lymphomas in difficult cases.

Acknowledgments

The authors gratefully acknowledge the skilled secretarial assistance of Ms. Janet Murphy.

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