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Short Communication

Inconsistency of the Immunophenotype of Reed–Sternberg Cells in Simultaneous and Consecutive Specimens from the Same Patients

A Paraffin Section Evaluation in 56 Patients

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Both immunophenotypic overlaps between Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL), and evolution of one into the other have been reported. However, the underlying assumption that the antigenic expression of Reed–Sternberg (RS) cells is consistent in the same patient has not been evaluated. Such an evaluation was undertaken by immunophenotyping paraffin-embedded lymphoid tissue biopsies with HD from 56 patients in whom multiple specimens were obtained, either simultaneously from different sites or at different times. The panel of antibodies we used included: CD3 polyclonal antiserum, DAKO-M1 (CD15), L26 (CD20), BerH2 (CD30), MT1 (CD43), DAKO-LCA (CD45RB), UCHL1 (CD45R0), LN2 (CD74), and DAKO-EMA. The phenotype of RS cells was identical in simultaneous ***biopsies in only 11 of 39 patients (28%) and remained constant in*** consecutive ***biopsies in only 4 of 21 patients (19%). Major differences (relative to cell lineage specific antigens) were observed in 10 of 39 patients with simultaneous biopsies and in 10 of 21 patients over time; they mainly involved expression of T-cell antigens. Minor differences (relative to any other antigen) were observed in 22 of 39 patients with simultaneous biopsies and in 15 of 21 patients over time; these mainly involved CD15 or CD74. This striking variability of the immunophenotype of RS cells in the same patient may be due to aberrant marker expression, as a result of the neoplastic state, and/or to modulation of antigenic expression in relation to the host environment. This inconsistency suggests caution when interpreting the relationship between HD and NHL by paraffin immunophenotyping alone. (Am J Pathol 1992, 141: 11–17)***

The cell lineage of the neoplastic cells of Hodgkin's disease (HD), the Reed–Sternberg (RS) cells, remains controversial; lymphoid cells of B-cell[1–3] or T-cell[4–6] type or histiocytes/reticulum cells[7–10] have been favored as the "cells of origin" in different studies. Although there seems to be a consensus that the lymphocyte predominance, nodular (LPN) type is a B-cell disease with the CD20+ CD45+CD15−CD30− phenotype,[11–14] distinct from the other types, characterized by a "null" CD45−CD15 +CD30+ phenotype,[15–19] inconsistency of immunophenotypic characteristics of RS cells is recognized within both of these subdivisions. In addition, immunophenotypic overlaps have been described between HD and non-Hodgkin's lymphomas (NHL), such as large anaplastic cell Ki-1–positive lymphoma[20–21] and peripheral T-cell lymphoma,[22] producing uncertainties as to the immunophenotypic definition of HD. Such uncertainty is

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present in studies reporting the "co-existence" of HD with,[23–26] or its "transformation" into,[27,28] NHLs.

Most of the conclusions drawn on cell lineage of HD, immunophenotypic overlaps with NHL or transitions from HD to NHL seem to have been based on the implicit assumption of the stability of the immunophenotype of RS cells in a given case. However, information to support or disprove such an assumption is not available. This study attempts to answer whether the immunophenotypic characteristics of RS cells in paraffin sections remain constant in the same patient, by comparing the findings in specimens obtained simultaneously from different sites or at different times in the same patients.

Material and Methods

The study includes 56 patients from the Armed Forces Institute of Pathology files that fulfilled the following criteria: 1) a confirmed diagnosis of classic HD[29] on multiple surgical specimens, and 2) availability of paraffin blocks on each specimen. The tissues were received fixed in formalin; only two specimens (obtained 1 week apart from the same patient) had been fixed in B5. A total of 119 specimens were available, including 113 lymph nodes, 5 spleens, and 1 tonsil. Sixty-eight specimens (57.1%) were classified as nodular sclerosis (NS); 24 (20.2%) were classified as mixed cellularity (MC); 22 specimens (18.5%) were classified as lymphocyte predominance (LP), all of the nodular type; and five (4.2%) were classified as lymphocyte depletion (LD). The specimens were categorized as "simultaneous," if they were obtained at the same time or less than 3 months apart (a commonly used clinical cut-off) from separate sites in a given patient, or "consecutive," if obtained 3 months apart or more (range: 3.8–87 months; median: 27) in a given patient. Thirty-five patients had simultaneous biopsies, seventeen had consecutive biopsies, and in four, both simultaneous and consecutive specimens were available. In the interval between consecutive biopsies, 11 patients received radiation therapy only; 6 received no therapy; 3 received radiation therapy and chemotherapy (MOPP); and 1 received chemotherapy only (ABVD). We found no patient with different morphologic classification in simultaneous or consecutive specimens.

Immunoperoxidase stains were carried out with the avidin-biotin complex technique.[30] Sections were incubated at room temperature for 30 minutes with the following antibodies: polyclonal CD3 (1:100), DAKO-M1 (CD15) (1:100), L26 (CD20) (1:200), BerH2 (CD30) (1:40), UCHL1 (CD45RO) (1:200), DAKO-LCA (CD45RB) (1:200), DAKO-EMA (1:100) (DAKO, Carpinteria, CA) and MT1 (CD43) (1:40) and LN2 (CD74) (1:5) (Biotest, Denville, NJ). Staining with CD3 and BerH2 was preceded by protease digestion using protease type VIII-A (Sigma Chemical Co., St. Louis, MO) (0.05%) in 0.1 mol/l phosphate buffer (pH 7.8) at 37°C for 5 minutes. All reactions were developed with 3, 3-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO). Appropriate negative and positive controls were used with each reaction. The evaluation of the reactivity of RS cells[19] for each antibody was done on coded specimens.

Results

Cumulative Immunophenotypic Findings

To assess the comparability of our data with those available in the literature, we first overviewed the results obtained on the entire series of 119 specimens, each considered as an independent case. Positive reactions were obtained in the RS cells with the following frequencies: for CD30, 70%; CD15, 65%; CD74, 63%; CD3, 27%; CD20, 18%; EMA, 7.5%; CD43, 3%; CD45RB, 3%; CD45RO, 2%. Although reactivity with most antibodies was observed in the majority (60–100%) of RS cells, CD3, MT1, and DAKO-EMA decorated only a small percentage (10–20%) of RS cells. CD3 was detected in the cytoplasm and/or on the membrane of the RS cells.

When we analyzed specifically the expression of CD20 and CD3, in the majority of specimens (67; 56%) the RS cells did not mark for either CD3 or CD20. In 30 cases (25%), a portion of the RS cells expressed CD3, but none expressed CD20. In two of these 30 cases, some RS cells expressed CD43, in one, some expressed CD45RO, and in one, some expressed both CD43 and CD45RO. Reactivity for CD20, but not CD3, characterized 20 specimens (17%). In two cases, both CD3+ and CD20+ RS cells were found; in one of these two, CD43+ cells were also present. The CD3−CD20− phenotype of RS cells was observed in 4 of 5 LD cases (80%), 45 of 68 NS cases (66%), and 14 of 24 MC cases (58%), but in only 4 of 22 LP cases (18%). Specimens with CD3+, but no CD20+ RS cells represented 34%, 26%, and 14% of the cases classified, respectively, as MC, NS, and LP; and specimens that showed CD20+, but no CD3+ RS cells constituted 59%, 20%, 8%, 6% of the cases classified, respectively, as LP, LD, MC, and NS.

Immunophenotypic Comparison of Simultaneous and Consecutive Specimens

For evaluation, any difference in the results of our panel of nine reagents between specimens obtained at different sites or different times from the same patient was classified as "major," when it involved cell lineage specific antigens (CD3, CD20, CD45RO, or CD43), or "minor," when it involved any other antigens.

As shown in Table 1, the phenotype of RS cells was completely identical in *simultaneous* specimens in only 11 of 39 patients (28%). Differences at different sites were mostly minor (18/39, 46%). However, in a quarter of the patients, major differences were found, by themselves (six patients) or associated with minor differences (four patients). The major changes in phenotype (Table 2) involved mostly the expression, or lack thereof, of T-cell markers (15% of patients). The minor differences in phenotype (Table 3) involved mostly the expression, or lack thereof, of two markers, CD15 in 33% of patients (Figure 1) and CD74 in 20.5%.

As shown in Table 1, the phenotype of RS cells in *consecutive* specimens remained completely identical over time in only 4 of 21 patients (19%). In the other patients, minor changes only (seven patients), major changes only (two patients), or both (eight patients) were observed. As with simultaneous specimens, the major changes between successive biopsies involved either the acquisition or the loss of T-cell markers (Table 2) (Figure 2) and the minor changes, the expression of CD15 or CD74 (Table 3). CD15 was most often acquired with time than lost, whereas CD74 was most often lost than acquired.

Discussion

The question of the cell lineage(s) of HD remains open. With only minor support having being obtained from molecular genetic studies of antigen receptor gene rearrangements and *bcl-2* oncogene involvement,[31,32] most conclusions have been based on the immunophenotypic characteristics of the RS cells. However, the patterns of antigenic expression of RS cells are notoriously variable, even within the two main subdivisions accepted, i.e., the LPN and non-LPN (NS, MC, LD) types.

Some of these variations may be accounted for by differences in the fixation and/or staining methods used. For example, expression of CD45 by RS cells in non-LPN cases, which is uncommon (10%) in paraffin sections,[19] was observed in 89% of plastic-embedded cases[22] and was found to vary between 3% in NS and 50% in MC in a series of cases immunophenotyped in paraformaldehyde-lysine-periodate (PLP)-fixed frozen tissue.[33] Similarly, expression of T-cell antigens on RS cells of non-LPN HD, which is found exceptionally (around 4%) in paraffin sections,[19] was observed in 40% of lymph-node cytospin preparations,[34] 44% of PLP-fixed frozen cases,[33] and 58% of plastic-embedded cases.[22] Expression of B-cell markers in these same studies was detected in 15%, 10%, 11%, and 26% of cases, respectively.

However, since variations of antigenic expression are also found within cases of HD similarly fixed and immunostained, explanations other than purely technical are needed for such variability, especially as it relates to markers of cell lineage. One commonly offered explanation is that HD is a heterogeneous group of disorders of diverse cell origin[31,33,34]; the LPN type is accepted as a B-cell disease, but other histologic types may be B-cell, T-cell, or histiocytic disorders. Other workers seem to favor a "unitarian" view of HD and read their data and most of the evidence available as strongly supporting a B-cell[35,36] or histiocytic[37,38] origin for HD. However, relevant to support either view are several questions that remain unresolved: the cell lineage specificity of some commonly used markers, such as cytoplasmic and/or membranous CD3 or CD20; how to explain cases with markers of different lineages; why such variations in the expression of non-lineage restricted markers.

Table 1. *Comparison of Phenotypes in Multiple Specimens (bx) from the Same Patients*

	Simultaneous bx	Consecutive bx
	No. (%) of 39 patients	No. (%) of 21 patients
No differences	11 (28)	4 (19)
Differences	28 (72)	17 (81)
Major	6 (15.4)	2 (9.5)
Minor	18 (46.1)	7 (33.3)
Both	4 (10.3)	8 (38.1)

Table 2. *Major Phenotypic Differences in Multiple Specimens (bx) from the Same Patients*

	Simultaneous bx	Consecutive bx
	No. (%) of 39 patients	No. (%) of 21 patients
T ↔ null	6 (15)	
T → null		4 (19)
null → T		3 (14)
null → T → null		1 (5)
B ↔ null	2 (5)	
null → B → null		1 (5)
T ↔ B	1 (2.5)	
B & T ↔ B	1 (2.5)	
B & T → B		1 (5)

Table 3. *Minor Phenotypic Differences in Multiple Specimens (bx) from the Same Patients**

	Simultaneous bx	Consecutive bx	
	No. (%) of 39 patients	No. (%) of 21 patients	
		w/gain	w/loss
CD15	13 (33)	8 (38)	4 (19)
CD74	8 (20.5)	2 (10)	5 (24)
CD30	3 (8)	3 (14)	
EMA	2 (5)	1 (5)	
CD45	1 (2.5)		1 (5)

* The sum of the differences in simultaneous or consecutive specimens is larger than the number listed in Table 1 because more than one marker may be different in any case.

present in studies reporting the "co-existence" of HD with,[23–26] or its "transformation" into,[27,28] NHLs.

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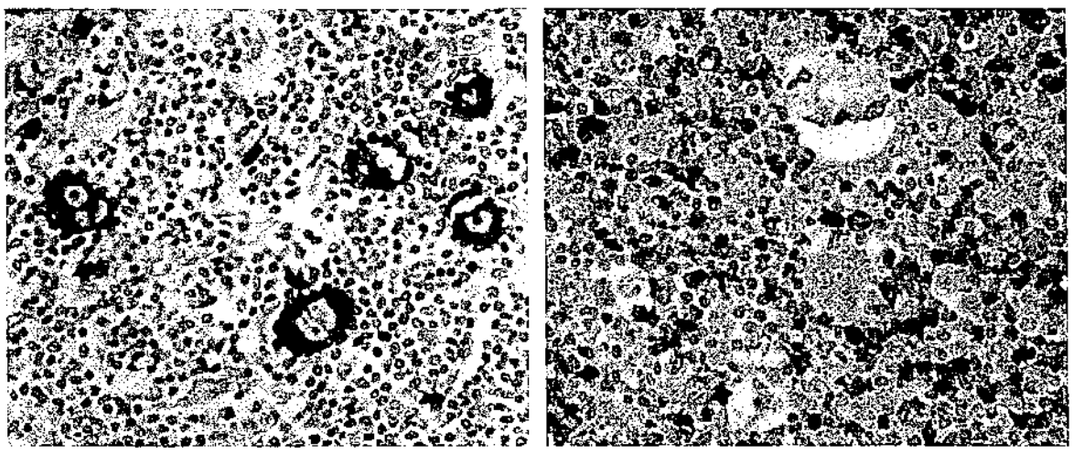

Figure 1. *Presence (left) and absence (right) of reactivity with Leu-M1 (CD15) in RS cells from two different lymph nodes obtained simultaneously from the same patient (immunoperoxidase, ×300).*

The question that prompted this study is even more basic: if the antigenic expression of RS cells is used for deciding the cell lineage(s) of HD, subclassifying the disease, and defining it in relation to NHLs, how stable is such phenotype in a given case? We tried to answer this question, as it pertains to paraffin sections immunophenotyping with a well-recognized panel of antibodies,[17,19,35] by comparing the reactivity of RS cells in multiple specimens obtained from the same patients, either simultaneously at different anatomic sites or at different times during their disease.

Our results over the entire series of 119 specimens we studied are closely comparable to those compiled from the literature.[19] As suggested by others, the most consistent findings in HD are the expression of CD15, CD30, and CD74 and the lack of expression of CD45RB, CD45RO, CD43, and EMA.[15–19,35] If a limited immunopanel may be suggested from these results for the diagnosis of HD, we favor one including CD15, CD20, CD30, CD45RB, and CD45RO. In most cases of the LP type, the RS cells had a "B-cell" (CD3−CD20+) phenotype, as expected; however, a "null cell" (CD3−CD20−) phenotype was observed in 18% of the lesions and CD3+ RS cells, alone or with CD20+ cells, were detected in 23%. Positivity for CD3 in RS cells was also found by Cibull et al[39] in 3 of 9 cases of LPN. As for the non-LP types of HD in our series, the RS cells had a "null cell" phenotype in the majority of cases. CD20 reactivity was seen in 7% of these specimens, a figure closer to the 15% obtained from the combined data of the literature[19] than to the 58% reported recently by Schmid et al.[35] Reactivity for CD3 in RS cells was seen in 28% of our non-LPN cases, a figure similar to that obtained by Cibull et al, 33%, using the same polyclonal antibody.[39]

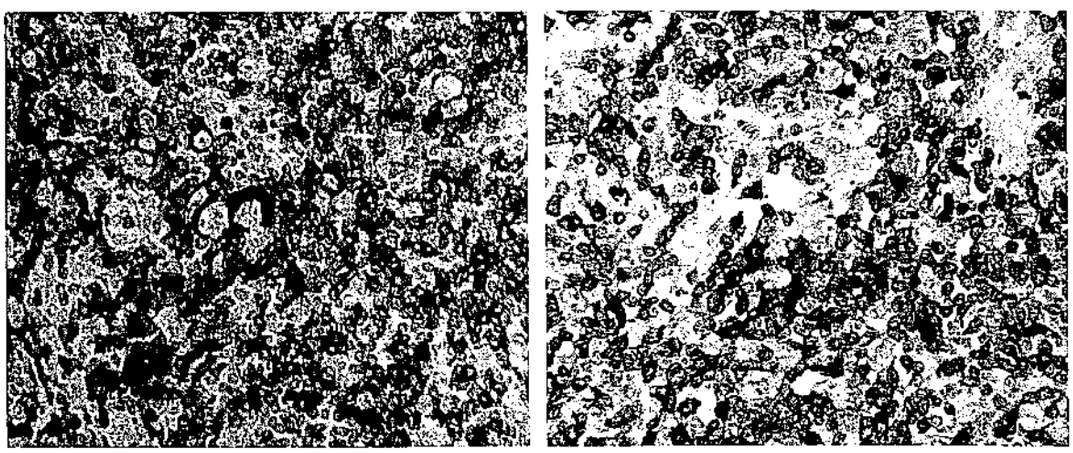

Figure 2. *Cytoplasmic reactivity for CD3 (left) and lack of it (right) in RS cells from lymph nodes obtained 65 months apart from the same patient (immunoperoxidase, ×300).*

Whatever the pattern of antigenic expression of RS cells, our study demonstrates that a given immunophenotype was identical at two different sites of the same patient in only 28% of cases and remained unchanged in consecutive biopsies in only 19% of patients. Major differences, i.e., involving the expression of cell lineage markers, were observed at different sites in 26% of patients and over time in 48% of patients. The great majority of these differences involved the expression of CD3. The significance of this finding is unclear. In most of the positive cells in our study and others,[34,35,39] the reactivity for CD3 is cytoplasmic. This has been interpreted as an "aberrant" expression by B cells.[35] This interpretation would be consistent with the expression of CD3 in three of our patients, in whom most of the RS cells in the same and/or other biopsies were CD20 positive (Table 2). Alternatively, since this and other T-cell markers[22,33,34,39] have been demonstrated on the surface membranes of RS cells, most convincingly by immunoelectronmicroscopy,[40,41] RS cells with cytoplasmic CD3 might be T cells, which during activation "have lost the ability ... to insert it into the surface membrane."[39] If this second interpretation is correct, one would need to postulate in several cases of HD a biphenotypic (B and T) pattern of differentiation that has been reported only exceptionally in NHLs.[42,43] Finally, reactivity of B cells for CD3 may be explained by crossreactive epitopes on unrelated molecules.[44]

Minor differences, i.e., involving the expression of nonlineage specific antigens, were observed with even higher frequency, i.e., in 56% of patients in simultaneous biopsies and in 71% of patients in consecutive biopsies. Most of the minor differences in both situations involved the expression of CD15 or CD74. Recently, Zukerberg et al[45] have noted that the RS cells express a CD20+ CD45+ phenotype in the absence of the classic inflammatory background of eosinophils and/or polymorphonuclears, but lose these antigens and become CD15+ CD30+ when this is present. Although the cause-effect relationship of the two phenomena remains unresolved, Zukerberg et al suggested that RS cells might be able to modulate antigen expression and that such modulation may be "related in some way to host-tumor cell interaction."[45] This provocative hypothesis fits well with the high degree of variability of antigenic expression of RS cells found in our study at different anatomic sites and over time in the same patients. Since this variability was higher among consecutive than among simultaneous specimens, the therapy administered in the interval could be a factor responsible for some of these variations, by producing changes in host-tumor interaction.

In conclusion, on one hand, our study confirms again the immunophenotypic heterogeneity of HD, even within the same histologic category. On the other hand, it demonstrates for the first time a striking variability in the patterns of antigenic expression of RS cells even in the same patient. Most likely reasons for these variations include the expression of inappropriate markers, inherent to the neoplastic transformation or clonal evolution, or the modulation of antigen expression in relation to the host environment. The poor consistency of the immunophenotype of RS cells suggests caution in drawing dogmatic conclusions about the cell lineage of HD or its relation with coexistent or subsequent NHLs by paraffin immunophenotyping alone.

References

1. Taylor CR: The nature of Reed–Sternberg cells and other malignant "reticulum" cells. Lancet 1974, 2:802–807
2. Boeker WR, Hossfeld DK, Gallmeier WM, Schmidt CG: Clonal growth of Hodgkin's cells. Nature 1975, 258:235–236
3. Linch DC, Jones HM, Berliner N, MacLennan K, O'Flynn K, Huehns ER, Kay LA, Goff K: Hodgkin-cell leukaemia of B-cell origin. Lancet 1985, 1:78–80
4. DeVita VT Jr: Lymphocyte reactivity in Hodgkin's disease: a lymphocyte civil war. N Engl J Med 1973, 289:801–802
5. Andreesen R, Osterholz J, Loehr GW, Bross KJ: A Hodgkin cell-specific antigen is expressed on a subset of auto- and alloactivated T (helper) lymphoblasts. Blood 1984, 63:1299–1302
6. Kadin ME: Common activated helper T-cell origin for lymphomatoid papulosis, mycosis fungoides, and some types of Hodgkin's disease. Lancet 1985, 2:864–865
7. Kadin ME, Stites DP, Levy R, Warnke R: Exogenous immunoglobulin and the macrophage origin of Reed–Sternberg cells in Hodgkin's disease. N Engl J Med 1978, 299:1208–1214
8. Strauchen JA: Lectin receptors as markers of lymphoid cells. II. Reed–Sternberg cells share lectin-binding properties of monocyte macrophages. Am J Pathol 1984, 116: 370–376
9. Kadin ME: Possible origin of the Reed–Sternberg cell from an interdigitating reticulum cell. Cancer Treat Rep 1982, 66: 601–608
10. Hsu S-M, Hsu P-L, Lo S-S, Wu KK: Expression of prostaglandin H synthase (cyclooxygenase) in Hodgkin's mononuclear and Reed–Sternberg cells. Functional resemblance between H-RS cells and histiocytes or interdigitating reticulum cells. Am J Pathol 1988, 233:5–12
11. Timens W, Visser L, Poppema S: Nodular lymphocyte predominance type of Hodgkin's disease is a germinal center lymphoma. Lab Invest 1986, 54:457–461
12. Pinkus GS, Said JW: Hodgkin's disease, lymphocyte predominance type, nodular. Further evidence for a B cell der-

ivation. L & H variants of Reed–Sternberg cells express L26, a pan B cell marker. Am J Pathol 1988, 133:211–217

13. Coles FB, Cartun RW, Pastuszak WT: Hodgkin's disease, lymphocyte-predominant type: Immunoreactivity with B-cell antibodies. Mod Pathol 1988, 1:274–278
14. Bishop PW, Harris M, Smith AP, Elsam KJ: Immunophenotypic study of lymphocyte predominance Hodgkin's disease. Histopathology 1991, 18:19–24
15. Hsu SM, Jaffe ES: LeuM1 and peanut agglutinin stain the neoplastic cells of Hodgkin's disease. Am J Clin Pathol 1984, 82:29–32
16. Dorfman RF, Gatter KC, Pulford KA, Mason DY: An evaluation of the utility of anti-granulocyte and anti-leukocyte monoclonal antibodies in the diagnosis of Hodgkin's disease. Am J Pathol 1986, 123:508–519
17. Chittal SM, Caveriviere P, Schwarting R, Gerdes J, Al Saati T, Rigal-Huguet F, Stein H, Delsol G: Monoclonal antibodies in the diagnosis of Hodgkin's disease. The search for a rational panel. Am J Surg Pathol 1988, 12:9–21
18. Hall PA, D'Ardenne AJ, Stansfeld AG: Paraffin section immunohistochemistry II. Hodgkin's disease and large cell anaplastic (Ki-1) lymphoma. Histopathology 1988, 13:161–169
19. Norton AJ, Isaacson PG: Lymphoma phenotyping in formalin-fixed and paraffin wax—embedded tissues: II. Profiles of reactivity in the various tumour types. Histopathology 1989, 14:557–579
20. Leoncini L, Del Vecchio MT, Kraft R, Megha T, Barbini P, Cevenini G, Poggi S, Pileri S, Tosi P, Cottier H: Hodgkin's disease and CD30-positive anaplastic large cell lymphomas—a continuous spectrum of malignant disorders. A quantitative morphometric and immunohistologic study. Am J Pathol 1990, 137:1047–1057
21. Rosso R, Paulli M, Magrini U, Kindl S, Boveri E, Volpato G, Poggi S, Baglioni P, Pileri S: Anaplastic large cell lymphoma, CD30/Ki-1 positive, expressing the CD15/Leu-M1 antigen. Immunohistochemical and morphological relationships to Hodgkin's disease. Virchows Arch (A) 1990, 416:229–235
22. Casey TT, Olson SJ, Cousar JB, Collins RD: Immunophenotypes of Reed–Sternberg cells. A study of 19 cases of Hodgkin's disease in plastic-embedded sections. Blood 1989, 74:2624–2628
23. Sundeen JT, Cossman J, Jaffe ES: Lymphocyte predominant Hodgkin's disease nodular subtype with coexistent "large cell lymphoma." Histological progression or composite malignancy? Am J Surg Pathol 1988, 12:599–606
24. Gonzalez CL, Medeiros LJ, Jaffe ES: Composite lymphoma. A clinicopathologic analysis of nine patients with Hodgkin's disease and B-cell non-Hodgkin's lymphoma. Am J Clin Pathol 1991, 96:81–89
25. Grossman DM, Hanson CA, Schnitzer B: Simultaneous lymphocyte predominant Hodgkin's disease and large-cell lymphoma. Am J Surg Pathol 1991, 15:668–676
26. Williams J, Schned A, Cotelingam JD, Jaffe ES: Chronic lymphocytic leukemia with coexistent Hodgkin's disease. Implications for the origin of the Reed–Sternberg cell. Am J Surg Pathol 1991, 15:33–42
27. Hansmann ML, Stein H, Fellbaum C, Hui PK, Parwaresch MR, Lennert K: Nodular paragranuloma can transform into high-grade malignant lymphoma of B type. Hum Pathol 1989, 20:1169–1175
28. Casey TT, Cousar JB, Mangum M, Williams ME, Lee JT, Greer JP, Collins RD: Monomorphic lymphomas arising in patients with Hodgkin's disease. Correlation of morphologic, immunophenotypic, and molecular genetic findings in 12 cases. Am J Pathol 1990, 136:81–94
29. Lukes RJ: Criteria for involvement of lymph node, bone marrow, spleen and liver in Hodgkin's disease. Cancer Res 1971, 31.1755–1767
30. Hsu SM, Raine L, Fanger H: The use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981, 29: 577–580
31. Anastasi J, Variakojis D: Heterogeneity in Hodgkin's disease: no simple answer for a complex disorder. Hum Pathol 1988, 19:1251–1254
32. Slivnik DJ, Nawrocki JF, Fisher RI: Immunology and cellular biology of Hodgkin's disease. Hematol/Oncol Clin North Am 1989, 3:205–220
33. Agnarsson BA, Kadin ME: The immunophenotype of Reed–Sternberg cells. A study of 50 cases of Hodgkin's disease using fixed frozen tissue. Cancer 1989, 63:2083–2087
34. Falini B, Stein H, Pileri S, Canino S, Farabbi R, Martelli MF, Grignani F, Fagioli M, Minelli O, Cianci C, Flenghi L: Expression of lymphoid associated antigens on Hodgkin's and Reed–Sternberg cells of Hodgkin's disease. An immunocytochemical study on lymph node cytospins using monoclonal antibodies. Histopathology 1987, 11:1229–1242
35. Schmid C, Pan L, Diss T, Isaacson PG: Expression of B-cell antigens by Hodgkin's and Reed–Sternberg cells. Am J Pathol 1991, 139:701–707
36. Jaffe ES: The elusive Reed–Sternberg cell. N Engl J Med 1989, 320:529–531
37. Hsu S-M, Hsu P-L: Aberrant expression of T cell and B cell markers in myelocyte/monocyte/histiocyte-derived lymphoma and leukemia cells. Is the infrequent expression of T/B cell markers sufficient to establish a lymphoid origin for Hodgkin's Reed–Sternberg cells? Am J Pathol 1989, 134: 203–212
38. Hsu S-M, Xie S-S, Hsu P-L: Cultured Reed–Sternberg cells HDLM-1 and KM-H2 can be induced to become histiocyte-like cells. H-RS cells are not derived from lymphocytes. Am J Pathol 1990, 137:353–367
39. Cibull ML, Stein H, Gatter KC, Mason DY: The expression of the CD3 antigen in Hodgkin's disease. Histopathology 1989, 15:597–605
40. Kadin ME, Muramoto L, Said J: Expression of T cell antigens on Reed–Sternberg cells in a subset of patients with nodular sclerosing and mixed cellularity Hodgkin's disease. Am J Pathol 1988, 130:345–353

41. Oka K, Mori N, Kojima M: Anti-Leu-3a antibody reactivity with Reed–Sternberg cells of Hodgkin's disease. Arch Pathol Lab Med 1988, 112:139–142
42. Lippman SM, Miller TP, Spier CM, Slymen DJ, Grogan TM: The prognostic significance of the immunotype in diffuse large cell lymphoma: a comparative study of the T-cell and B-cell phenotype. Blood 1988, 72:436–441
43. Herbst H, Tippelmann G, Anagnostopoulos I, Gerdes J, Schwarting R, Boehm T, Pileri S, Jones DB, Stein H: Immunoglobulin and T-cell receptor gene rearrangements in Hodgkin's disease and Ki-1-positive anaplastic large cell lymphoma: dissociation between phenotype and genotype. Leuk Res 1989, 13:103–116
44. Haegert DG, Smith JL: Unexpected MAb reactivities in lymphoid neoplasia. Immunol Today 1988, 9:193 (letter)
45. Zukerberg LR, Collins AB, Ferry JA, Harris NL: Coexpression of CD15 and CD20 by Reed–Sternberg cells in Hodgkin's disease. Am J Pathol 1991, 139:475–483