Immunophenotypes of Reed-Sternberg Cells: A Study of 19 Cases of Hodgkin’s Disease in Plastic-Embedded Sections

By Terence T. Casey, Sandra J. Olson, John B. Cousar, and Robert D. Collins

The immunophenotype of Reed-Sternberg (RS) cells in Hodgkin’s disease (HD) has not been clearly defined, partly owing to difficulties in studying RS cells in cell suspensions or identifying them with certainty in frozen sections. We studied the immunophenotype of RS cells with a recently developed plastic section immunohistochemical technique on acetone-fixed tissues that affords superior morphologic detail while preserving a wide variety of lymphoid differentiation antigens. Nineteen cases of HD [16 nodular sclerosing (NS), 2 mixed cellularity (MC), and 1 lymphocyte-depleted (LD)] were embedded in plastic and stained for pan-B, pan-T, and various T-subset markers, as well as leukocyte common antigen (CD45), interleukin-2 (IL-2) receptor (CD25), and RS cell markers CD15 and CD30. RS cells were positive for CD45, CD15, CD30, and CD25, except for 3 cases (2 NS, 1 MC) that were CD15 negative and 2 cases (NS) that were CD45 negative. In 10 cases (NS), RS cells were positive for at least two pan-T-cell markers and CD4; pan-B cell markers were uniformly negative. RS cells in 6 cases (3 NS, 2 MC, 1 LD) were positive for at least one T-cell marker (CD2) and one B-cell marker (CD22). Two cases of NSHD showed no T- or B-cell marking. These data provide further evidence that RS cells in some cases of NSHD have T-cell phenotypes and that RS cells are not homogeneous in their immunoreactivity.

REED-STERNBERG (RS) cells are generally accepted as the neoplastic component of Hodgkin’s disease (HD). Identification of the origin of this cell remains controversial, as conflicting results have been obtained by ultrastructural, immunohistochemical, or molecular genetic techniques. Immunohistochemical studies have been inconclusive, chiefly owing to difficulty in identifying RS cells in frozen-section immunoperoxidase preparations and the questionable specificities of antibodies (eg, CD15, CD30, UCHL-1, LN1, and LN2) reactive to hematopoietic/lymphoid subpopulations in paraffin-embedded tissues.

Two recent studies using novel immunohistochemical techniques showed that RS cells in some cases of HD express certain T- and/or B-cell differentiation antigens. Falini et al, using immunoperoxidase staining on cytocentrifuge preparations, and Agnarsson and Kadin, using frozen sections of paraformaldehyde-lysine-periodate-fixed tissues, showed relatively congruent data in this regard.

We modified a plastic-embedding method for immunohistologic staining allowing reliable analysis of a wide variety of lymphoid antigens in a morphologically superior preparation. Our technique is especially suited to study of HD in

MATERIALS AND METHODS

Nineteen cases of HD diagnosed by established histopathological criteria were included in this study. The cases were typical cases of HD and were subclassified into nodular sclerosing (NS, 16 cases), mixed cellularity (MC, 2 cases) and lymphocyte-depleted (LD, 1 case) categories. RS cells were scored as positive only when adjacent small cells were positive, I+ = 10% positive; 2+ = 10% to 50% positive; and 3+ = greater than 50% positive. Because RS cells in HD may be surrounded by many reactive cells of various phenotypes, RS cells were scored as positive only when adjacent small lymphocytes were negative and unequivocal positivity was demonstrated in the RS cell membrane. Back-to-back RS cells and variants were also scored. RS cells and variants were judged negative if portions of their cell membrane failed to show reactivity.

RESULTS

Reactivity of RS cells for leukocyte common antigen (CD45), CD15, Ki-1 (CD30), and Interleukin-2 (IL-2) receptor (CD25). RS cells in all cases studied were positive for CD15, CD30, and CD25 except for two cases of NSHD and one case of MCHD that were negative for CD15. These data are summarized in Table 2. RS cells in all but two cases (cases 11 and 17) were positive for CD45 in plastic sections.

Reactivity of RS cells for B- and T-cell markers. The reactivities of RS cells in plastic sections with the various B-
and T-cell markers tested are summarized in Table 3. RS cells in cases 1 through 3 were positive for all four pan-T-cell markers (CD3, CD5, CD2, UCHL-1) as well as CD4; the pan-B-cell markers were negative (Fig 2); RS cells in cases 4 and 5 expressed three of the four pan-T-cell markers plus CD4. Although fewer pan-T-cell antibodies marked RS cells in cases 6 through 11, CD4 was positive in all of the cases,

and the pan-B-cell markers were negative. Cases 6 through 11 were all examples of NSHD, and the pan-T-cell markers positive in this group were CD2 and UCHL-1.

In contrast to the predominance of RS cells expressing T-cell antigens in cases 1 through 11, RS cells in cases 12 through 14 were often positive for the pan-B-cell markers CD19 and/or CD22. In the same cases, at least one of the pan-T-cell markers (usually CD2) was expressed in RS cells. The two cases of MCHD (cases 17 and 18) and the one case of LDHD (case 21) showed the same overlap of T- and B-cell marking in RS cells as in this subgroup of NSHD (cases 12 through 14) (Fig 3). In these cases (cases 12 through 14 and 17 through 19), CD2 and UCHL-1 were the most common T-cell markers expressed by RS cells.

Two cases of NSHD (cases 16 and 17) showed no marking of RS cells with either T- or B-cell antibodies. RS cells in all but three cases (cases 15 through 17) were positive for CD4. No case of HD showed RS cells positive with CD8 or CD1. Two cases (NSHD, cases 3 and 14) possessed RS cells positive for Leu-7.

Proliferation antigen (Ki-67), anti-DRC, and anti-macrophage (EBM11). RS cells in all but two cases had nuclear positivity for the proliferation antigen Ki-67. The numbers of Ki-67-positive RS cells varied from case to case, as shown in Table 2, but both cases of MCHD tested possessed more than 50% RS cells positive for Ki-67. RS cells in all cases were negative for anti-DRC (R4/23) and anti-macrophage (EBM11) markers.

DISCUSSION

The RS cells in this study were positive for CD15 (Leu-M1), CD30 (Ki-1), and CD25 [interleukin-2 (IL-2) receptor], apparently the typical phenotype of RS cells in NS, MC, and LDHD.13,14,19 RS cells have generally been reported

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<th>Table 1. MoAbs Used in This Study</th>
<th>Table 3. Reactivity or RS Cells With Markers of T and B Cells</th>
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*Obtained from Dako, Carpinteria, CA. †Obtained from Becton Dickinson, Mountain View, CA.
to be negative for CD45 (leukocyte common antigen), with use of aldehyde-fixed paraffin-embedded tissues. Falini et al and Agnarsson and Kadin, using more sensitive immunocytochemical techniques, showed, however, that some RS cells may express CD45. RS cells clearly expressed CD45 in plastic sections in 17 of 19 cases regardless of the histologic subtype of HD. This finding strongly indicates that the RS cell is of hematopoietic/lymphoid origin.

Various T- and B-cell markers have been reported to be positive in RS cells, in frozen sections or cytologic preparations. One of these studies concluded that frozen-section immunoperoxidase preparations were difficult to interpret in HD and that there was no characteristic T- or B-cell phenotype of RS cells. With use of immunoperoxidase technique staining procedure on cytospin preparations, T-cell antigens were demonstrated on RS cells in 8 of 20 cases of HD, with weak expression of B-cell antigens in 2 of 20 cases. Similar results were obtained in frozen sections of aldehyde-fixed tissues. In this frozen-section immunohistochemical study, RS cells expressed one or more T-cell markers in 20 of 50 cases of HD and B-cell markers in 9 of 50 cases; RS cells in one case of MCHD expressed both T- and B-cell markers. These latter two studies apparently used more sensitive immunological techniques; their study design facilitated precise recognition of RS cells, and large panels of antibodies were evaluated.

Our study showed that RS cells express T- and/or B-cell markers in most cases of HD. Most of our cases of HD had T-cell phenotypes with negative B-cell markers (cases 1 through 11, 58%); some had both T- and B-cell marking (cases 12 through 14 and 17 through 19, 32%); a minority showed no T- or B-cell marking (cases 15 and 16, 10%).

All cases with RS cells having T-cell phenotypes were in the NSHD category, but only a few cases of MCHD and LDHD were tested. The five cases (cases 1 through 5) with RS cells expressing three or more T-cell markers were believed to show the most convincing evidence of T-cell differentiation. In cases 6 through 11, the only pan-T-cell markers expressed on RS cells were CD2 and UCHL-1, admittedly weaker evidence of T-cell differentiation.

RS cells in six cases (cases 12 through 14 and 17 through 19), including cases of NS, MC, and LDHD, expressed both T- and B-cell antigens. These cases clearly bring into question the specificity of certain B- and T-cell antibodies. These results also suggest a degree of phenotypic overlap between the subtypes of HD, paralleling the overlap in morphologic features noted for some time. Two cases (cases 15 and 16) showed no T- or B-cell marking; one of these cases reacted strongly with CD45, and one was negative. Thus, a major conclusion of this study is that HD probably is as phenotypically diverse as it appears morphologically diverse, perhaps reflecting an intrinsic heterogeneity in histogenesis.

In summary, this study of HD using an improved technique for fixation and plastic embedding showed that RS cells in approximately two-thirds of the cases of NSHD possessed T-cell phenotypes; approximately one third of these cases exhibited strong evidence of T-cell differentiation with several positive T-cell markers. In addition, RS cells in almost all cases showed the expected positivity with CD15, CD30, and CD25. In contrast to previous reports, RS cells in the majority of cases (17 of 19) reacted with CD45, and the reaction in 15 of these cases was very distinct and present on most RS cells.

Several important conclusions may be drawn from these results. The distinction between HD and peripheral T-cell lymphomas is blurred even further; using sensitive techniques, typical cases of HD may show T-cell marking. We are currently studying a larger group of HD/peripheral T-cell lymphoma cases in an effort to establish diagnostic criteria for histologically borderline cases. Gene rearrangement assays in this group, as well in cases of HD with T-cell phenotypes, should be useful in this regard.

Second, this study shows the immunophenotypic heterogeneity within recognized histopathologic categories of HD. Such diversity may reflect antigenic variation in neoplastic cells rather than histogenetic differences within a category such as NSHD. Finally, our studies are additional evidence for the proposed diversity of HD. B-cell types have been recognized in nodular L H LPHD, and we now have evidence of a T-cell subset of HD as well.

REFERENCES

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Fig 1. (Top) RS cell in a case of NSHD (case 14) is surrounded by numerous small T lymphocytes staining for CD3, but the RS cell itself is negative for CD3 as no membrane staining is evident at the point of contact with an adjacent negative cell (arrow). (Original magnification ×500.) Fig 2. (Middle) Lacunar RS cells in a case of NSHD (case 1) show cytoplasmic membrane staining for the T-cell marker CD3. (Original magnification ×500.) Staining is apparent between adjacent lacunar variants (arrow). Fig 3. Mononuclear RS cells (A) (original magnification ×500) and binucleate RS cells (B) (original magnification ×750) are positive for B-cell marker CD22 in a case of MCHD (case 17).
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