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 antigentic expression of Reed–Sternberg (RS) cells is consistent in the same patient but not 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 serially or simultaneously from different sites or at different times. The panel of antibodies we used included CD3 (polyclonal antiserum), DAKO-M1 (CD15), L26 (CD20), B29 (CD30), MT1 (CD43), DAKO-LECA (CD38/LECA), UCCH1 (CD45RO), L22 (CD74), and L200-56. The phenotype of RS cells was identical in simultaneous biopsies in only 11 of 116 patients (28%), and remained consistent in consecutive biopsies in only 4 of 21 patients (19%). Major differences in expression of 13 cell surface antigens were observed in 10 of 20 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 single antigen) were observed in 22 of 56 patients with simultaneous biopsies and in 15 of 21 patients over time. These findings indicate the inherent variability of the immunophenotype of RS cells in the same patient may be due to inherent marker expression, as well as to the neoplastic cells and to modulation of antigentic expression in relation to the host environment. This inconsistency suggests caution when interpreting the relationship between HD and NHL by immunophenotyping alone. (Amer J Pathol 1992; 141: 11–17)
present in studies reporting the "co-existence" of HD with 19-20 or its "transformation" into 21-25 NIs.

Most of the conclusions drawn on cell lineage of HD, immunophenotypic overlap with NHL, or transition 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 that fulfilled the following criteria: 1) a confirmed diagnosis of classic HD 21-25 on multiple surgical specimens; and 2) availability of paraffin blocks on each specimen. The tissues were recovered fixed in formalin; only two specimens (obtained 1 week apart from the same patient) had been fixed in B5. A total of 118 specimens were available, including 11 lymph nodes, 5 spleens, and 1 tonsil. Sixty-eight specimens (57.1%) were classified as nodular sclerosis (NS); 21 (25.0%) were classified as mixed cellularity (MC); 22 specimens (18.5%) were classified as lymphocyte predominant (LP), all of the nodular type, and 4 (3.2%) 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 "progressive," if obtained 3 months apart or more (range 3 to 87 months; median 27) in a given patient. Thirty-five patients had consecutive biopsies; 13 had consecutive biopsies, and in four, both simultaneous and consecutive specimens were available. In the internet biotube consecutive biopsies, 11 patients received radiation therapy only; 6 received therapy; 1 received radiation therapy and chemotherapy (MOPP); and 1 received chemotherapy only (ADT). We found no patient with different morphologic classification in simultaneous or consecutive specimens.

Immunoperoxidase stains were carried out with the avidin-biotin complex technique. 20 Sections were incubated at room temperature for 30 minutes with the following antibodies: polyclonal CD5 (1:100), DAKO-M1 (C0195; 1:100), L26 (CD20) (1:200), BerH2 (CD20; 1:40), UCHL-1 (CD45RO) (1:500), DAKO-LCA (CD45R0) (1:200), DAKO-EMA (1:100) (DAKO, Cambridge, CA) and M1 (CD43) (1:40) and U2 (CD7) (1:5) (Becton, Dickinson, NJ). Staining with CD20 and ReHs1 were preceded by protein digestion using protease type VII-A (Sigma Chemical Co., St. Louis, MO) (0.05%) in 0.1 mol phosphate buffer (pH 7.8) at 37°C for 3 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 22 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 118 specimens, each considered as an independent case. Positive reactions were obtained in the RS cells with the following frequencies for CD20, 70%; CD15, 40%; CD10, 63%; CD3, 27%; CD20, 18%; EMA, 7.5%; CD43, 3%; CD45RO, 3%; CD16, 2%. Although reactivity with most antibodies was observed in the majority (80-100%) of RS cells, CD20, M11, 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 (67.5%) the RS cells did not react for both CD20 or CD3. In 30 cases (25%), a portion of the RS cells expressed CD20, but none expressed CD3. In the two of these 30 cases, some RS cells expressed CD43 in one, some expressed CD45RO, and in one, some expressed both CD20 and CD45RO. Reactivity for CD20, but not CD3, characterized 20 specimens (17%). In two cases, both CD20+ and CD20- RS cells were found; in one of those two, CD3+ cells were also present. The CD20+/CD20- phenotypes of RS cells were observed in 4 of 25 DLBCL cases (16%), 5 of 68 NS cases (6%), and 14 of 26 MC cases (54%), but in only 4 of 22 LP cases (18%). Specimens with CD20+, but no CD20+ RS cells represented 34%, 29%, and 14% of the cases classified, respectively, as MC, NS, and LP, and specimens that showed CD20+ and CD20- RS cells constituted 26%, 20%, 8%, and 6% of the cases, 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 antibodies (CD3, CD20, CD45RO, or CD5); or "minor," when it involved other antibodies.
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 (19.3%) and major (10.5%) in a quarter of 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 of minor (14%) of the HLA markers, CD15 in 29% of patients (Figure 1) and CD74 in 29%.

As shown in Table 1, the phenotype of RS cells in consecutive specimens was completely identical (100%) in only 4 of 39 patients (10%). 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 consecutive specimens involved either the accumulation or loss of T-cell markers (Table 2). Figure 2) and the minor changes, the expression of CD15 and CD74 (Table 3). CD15 was most often acquired with time, whereas CD74 was most often lost.

Discussion

The question of the cell lineage of HD remains open. With only minor support having been obtained from in vitro molecular genetics, studies of antigen receptor gene rearrangements and demonstration of rare clones by flow cytometry and Southern blotting have been less conclusive than those obtained by in situ hybridization procedures. In vivo studies of HD, however, have shown that the antigens expressed by RS cells are not related to these two cell subsets. Indeed, the lack of lymphocytes and non-lymphoid cells in HD tissue has been thought to be related to the lack of the expression of antigens related to these two cell subsets.

Some of these variations may be accounted for by differences in the location and/or sampling method of the lesions. For example, expression of CD138 in some cases of non-lymphoid tissues, which is uncommon in skeletal muscle, was observed in 14% of patients in 10 of 14 patients, while in the remaining 25 patients, 11 of 25 patients. In the present study, the expression of CD15 and CD74 was found to be related to the expression of T-cell antigens, while the expression of CD138 was found in non-lymphoid tissues.
present in studies reporting the "re-existence" of HD
with 26-28 ala transformation 29-31 NH-LH.

of the conclusions drawn on cell lines of HD,
immunocytochemical evidence for HD-LH, or the HD
in NH-LH, seem to have been based on the implicit
assumption of the stability of the immunocytochemical
characteristics of RS cells in non-paraffin sections
constant in the same patient, by comparing the findings in
sections obtained simultaneously from different sites or
from different times in the same patient.

Material and Methods

The study includes 56 patients from the Armed Forces
Institute of Pathology files that fulfilled the following crite-
ria: 1) a confirmed diagnosis of classic HD as
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 10%.
A total of 1.19 specimens were available, including 113
lymph nodes, 5 spleens, and 1 tonsil. Eighty-eight specimens
(57.5%) were classified as nodular sclerosis (NS); 24
(20.2%) were classified as mixed cellularity (MC); 22
specimens (15.5%) were classified as lymphocyte
predomination (LP), all of the nodular type, and five
(4.0%) were classified as lymphocyte depletion (LD).
The specimens were categorized as "similarities," if they were
obtained at the same time or less than 3 months apart (a
commonly used clinical cutoff) from separate sites,
given patient, or "consecutive," if obtained 3 months
apart or more (range, 2.8-67 months; median, 37) in a
given patient. Thirty-five patients had simultaneous
biopsies, seven had two consecutive biopsies, and in four,
both simultaneous and consecutive specimens were
available. If the biopsy was consecutive biopsies,
11 patients received radiation therapy only, 6 received no
therapy 3 received combination therapy and chemotherapy
(WCP), and 1 received chemotherapy only (WCP).
We found no patient with different morphologic classification in
simultaneous or consecutive specimens.

Immunoperoxidase stains were carried out with
the avidin-biotin complex technique. 32 Sections were
incubated at room temperature for 30 minutes with the follow-
ing antibodies: polyclonal CD3 (1:100), DAKO-M-1
(CD8) (1:100), LS-D (CD20) (1:200), RF4 (CD30) (1:
40), UCH-L1 (CD56) (1:200), DAKO-CA (CD68) (1:
200), DAKO-DA (1:100), DAKO, Carpinteria, CA
and Mo (CD45) (1:40) and L26 (CD2) (1:200, BioLife,
Denver, CO). Staining with CD20 and RF4-2H3 was
precluded by protease digestion using protease type IV-A
(Sigma Chemical Co., St. Louis, MO). 0.05% in 0.1 mol
phosphate buffer (pH 7.5) at 37°C for 5 minutes. All re-
actions 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 33 for each antibody was done on coated speci-
 mens.

Results

Cumulative Immunophenotypic Findings

To assess the comparability of our data with those avail-
able in the literature, we first overviewed the results ob-
tained on the entire series of 119 specimens, each con-
ceived as an independent case. Positive reactions were
detected in the RS-cells with the following frequencies:
for CD30, 70%; CD3, 56%; CD20, 63%; CD8, 27%; CD56, 12%;
EMA, 75%; CD4, 3%; CD8, 6%; CD56, 3%; CD56, 2%.
Although reactivity with most antibodies was ob-
served in the majority (65-100%) of RS cells, CD3, CD4,7,8
and DAKO-EMA decorated in 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 (67-96%)
the RS cells did not mark for either CD20 or CD30, in 30
cases (25%), a portion of the RS cells expressed CD3, but
not CD20, and conversely in 10 cases (9%), some RS cells
were expressed CD20, in one case (8%), some expressed
CD56, and in one, some expressed both CD3 and
CD56. Reactivity for CD20, but not CD3, character-
ized 20 specimens (17%); in two cases, both CD20 and
CD56 RS cells were found. In one of those two,
CD3+ cells were also present. The CD3+- CD30-
phenotype of RS cells was observed in 4 of 5 L2 cases
(80%), 45 of 69 NS cases (64%), and 14 of 24 MC cases
(60%); but in only 4 of 22 LP cases (18%). Specimens
with CD30+, but no CD20 + RS cells represented 5%
and 4% of the cases characterized, respectively, as
MC, NS, and LP, and specimens that showed CD20+, but
no CD3 + RS cells constituted 50%, 20%, 8%, and 6% of
the cases classified, respectively, as LP, MC, NS,
and MC.

Immunophenotypic Comparison of
Simultaneous and Consecutive Specimens

For evaluation, any difference in the results of our panel
of nine reagents between biopsies obtained at different
sites or different times from the same patient was clas-
sified as "major;" when it involved cell lineage specific an-
gerants (CD3, CD20, CD56, or CD4), or "minor;" when it
involved any other antigens.
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 parallel clinical immunophenotyping with a well-recognized panel of antibodies, by comparing the reactivity of RS cells in multiple specimens obtained from the same patients, either simultaneously or at different anatomic sites or at different times during their disease.

Our results on the entire series of 119 specimens we studied are closely comparable to those compiled from the literature. As suggested by others, the most consistent findings in HD are the expression of CD15, CD20, and CD74 and the lack of expression of CD45RB, CD45RO, CD43, and EMA. If a limited immunopanel may be suggested from these results for the diagnosis of HD, we favor one including CD15, CD20, CD74, and CD45RB. 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 10% of the lesions and CD3 - RS cells, alone or with CD20 + cells, were detected in 29%. Reactivity for CD3 in RS cells was also found by Cibull et al. in 3 of 8 cases of LNP. 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 79% of these specimens, a figure close to the 15% obtained from the combined data of the literature than to the 98% reported recently by Schnitz et al. Reactivity for CD3 in RS cells was seen in 36% of our non-LP cases, a figure similar to that obtained by Cibull et al., 33%, using the same polyclonal antibody.
Whether the pattern of antigenic expression of RS cells, our study demonstrates that a given immunohistological picture was identical in two different, if not the same patient in only 28% of cases and remained unchanged in consecutive biopsies in only 15% of patients. Major differences, i.e., involving the expression of cell-type 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 CD20. The significance of the finding is obvious: in most of the positive cases in our study, it is CD20 positivity that has been interpreted as an "absence" of B cells. This interpretation would be consistent with the expression of CD20 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 the other two CD20 markers have been demonstrated to be surface membrane of RS cells, most convincingly by immunoelectron microcopy, and RS cells with cytoplasmic CD20 might be T cells, which during activation lose this ability . . . To include it into the surface membrane, 15 it is important to note that in several cases of HD a lymphoid B pattern (B and T pattern) of differentiation has been reported, only exceptionally in HDs. 15,16 Finally, reactivity of B cells for CD25 may be explained by cross-reactive antibodies on viral membrane palisades. 15,16

Minor differences, i.e., involving the expression of antibodies specific against antigens, were observed with even higher frequency, i.e., in 52% of patients in simultaneous biopsies and in 77% of patients in consecutive biopsies. Most of the minor differences in both situations involved the expression of CD15 or CD57. Recently, Zulekberget al. 17 have noted that these RS cells express a CD20+/ CD45- phenotype in the absence of the classic immunohistological background of essential histiocytic polymorphonuclear cells. In these cases, the antibody expression is similar to HD, but these antibodies are also found in CD15+ CD68- when this is present. Although the cause-effect relationship of the two phenomena remains unresolved, Zulekberget al. 17 suggested that RS cells might be able to modulate antigen expression and that such modulation may be related to the way histiocytic cell functions. This hypothesis seems unlikely 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 patient. Since the variability was higher among consecutive than among simultaneous specimens, the therapy administered in this 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 immunohistological heterogeneity of HD, even within its same histologic category. On the other hand, it demonstrates for the first time a striking variability in the pattern of antigenic expression of RS cells even in the same patient. Most likely reasons for these variations include the expression of inappropriate malignants, inherent to the neoplastic transformation of normal cells, or the modulation of antigenic expression in relation to the host environment. The pool consistency of the immunohistological type of RS cells suggests caution in drawing conclusive conclusions about the cell lineage of HD or its relation with persistent or subsequent NHLs by paroxysmal immunohistochemistry alone.

References

12. Fisher GS, Small JD. Hodgkin's disease, lymphocytic proliferation type, nodular. Further evidence for a B-cell-de-
16 Chiu, Abdonato, and Frizzera
Appl. J. 1992 Vol. 141, No. 1


dilution, L.H. 


14. Belpah PV, Hank M, Smith AP, Essam KJ. Immunohisto-


16. Bollman HF, Gutter CK, Hruban KA, Mason DJ. 


19. Nadler AJ. Isolation of PG-1. Lymphoma phenotyping in bone-


22. Cawte TT, Chou SJ, Cawte JB, Coles DD. Immunohisto-


29. Lukes RJ. Criteria of involvement of lymph node, bone mar-

30. Chiu SM, Raines L, Fangar H. The use of anti-immunoh-

31. Arasanz J, Vellado G. Heterogeneity in Hodgkin's dis-


33. Agarwala BA, Walsh ME. The immunophenotypic analysis of Reed-


36. Chiu SS, Belin W, Alvarado expression of T-cell and B-cell 

37. Hsu SM, Xue SG, Hsu PC. Oudified Reed-Sternberg cells 

