The many faces of Hodgkin’s disease around the world: What have we learned from its pathology?

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Summary

In the past decade there have been many advances in our understanding of Hodgkin’s disease. Among the most important is the discovery that the Reed–Sternberg cell is a lymphoid cell, in most cases a B cell, and that it is clonal, and thus a true lymphoma, deserving of a name change, to ‘Hodgkin’s lymphoma’ (HL). Based on a combination of immunophenotype and morphology, the R.E.A.L. Classification recognizes two main types of HL: classical types (nodular sclerosis, mixed cellularity, lymphocyte-rich classical HL, and lymphocyte depletion) and nodular lymphocyte predominance type (NLPHL), which probably represent distinct biological entities. The immunophenotype and genetic features of both classical HL and NLPHL have been defined. These are useful in the subclassification of HL, and in distinguishing HL from two recently-described, aggressive lymphomas that were in the past often diagnosed as HL: anaplastic large-cell lymphoma, T-cell type (ALCL), and T-cell/histiocyte-rich large B-cell lymphoma (T/HRBCL). Epstein–Barr virus has been detected in approximately 40% of the cases of classical HL, and is clonal, suggesting that this virus may play a role in the pathogenesis of at least some types of HL. The frequency of HL varies in different populations, and the frequency of EBV-positive HL appears to be inversely related to the overall frequency of HL in a given population. Thus, it is possible that its presence may simply reflect the prevalence of EBV-infected B cells in the individual. Despite the advances of the past ten years, many questions remain to be answered, and these will provide the challenges of the next decade.

Key words: B cell, classification, EBV, epidemiology, Hodgkin’s disease, immunophenotype, lymphoma, molecular genetics, pathology

Introduction

This presentation represented the first Gianni Bonnadonna Lecture, at the IV International Symposium on Hodgkin’s Lymphoma, in Kolne. Gianni Bonnadonna is the only individual in our generation to make major contributions, which altered clinical practice, in two major neoplasms – lymphoma and breast cancer. I would like to take this opportunity to congratulate Gianni Bonnadonna on a wonderful and unique career in oncology.

Hodgkin’s lymphoma (HL) is one of the great clinical success stories of our generation: it has changed from a predominantly fatal disease to an almost uniformly curable one. Although the pathologic classification has remained fairly stable over the last 20 years (Table 1) [1–3], much has been learned during that time from the study of tissues involved by this disorder. In this presentation, I will address the following questions, to which pathology has provided some answers. What is the role of immunophenotyping? What is the normal counterpart of the Reed–Sternberg cell? Why does HL vary in different populations? What is the role of the Epstein–Barr virus in the pathogenesis of HL?

What is the role of immunophenotyping?

Immunophenotype and classification

Until recently, HL was a purely morphologic diagnosis [1, 2]. Recently, however, characteristic immunophenotypes were recognized that correlated with morphology and in some cases, refined it (Table 2). Most subtypes of HL (nodular sclerosis/NSHL, mixed cellularity/MCHL,
and lymphocyte depletion/LDHL) have neoplastic cells that are CD15+ CD30+ and lack pan-B and pan-T-cell antigens as well as leukocyte common antigen (CD45). In contrast, nodular lymphocyte predominance (NLPHL) is CD15– CD30– and uniformly expresses B-cell antigens and epithelial membrane antigen [4–7]. These differences in immunophenotype, as well as differences in clinical behavior, led the International Lymphoma Study Group (ILSG) to propose a modification to the classification of HL, recognizing that NLPHL is distinct from classical HL, and although it is quite distinct from typical non-Hodgkin's lymphomas, clearly deserves its own category (Table 3) [3]. With the application of immunohistochemistry to many cases of HL, it has become apparent that simply a predominance of lymphocytes in the background is not sufficient to classify a case as NLPHL; some cases with a lymphocyte-rich background may have the RS-cell morphology and immunophenotype of classic HL. These are now classified as lymphocyte-rich classical HL.

Lymphocyte-rich classical HL requires immunophenotyping for the diagnosis in most cases. Of 426 cases initially diagnosed as LPHL, review and immunophenotyping by a panel formed by the European Task Force on Lymphoma (ETFL) revealed that only half were confirmed as NLPHL, while one quarter were lymphocyte-rich classical HL [8]. In a study from the German Hodgkin’s Study Group (GHSG), a similar rate of misdiagnosis of NLPHL was found; only 44% of 208 cases considered by at least one pathologist as LPHL had the immunophenotype of LPHL, while 56% were CHL.

When the expert panel reclassified the cases by morphology alone, 75% of the cases classified as LPHL and 88% of the cases classified as CHL were confirmed by immunophenotyping [9].

Like NLPHL, LRCHL is often nodular, although diffuse areas and interfollicular involvement are more common, and RS cells and variants are located within the mantle zones and interfollicular regions. This has been termed ‘follicular’ Hodgkin’s lymphoma [10], or lymphocyte-rich classical HL, nodular [8]. The neoplastic cells may resemble the ‘L&H’ or ‘popcorn’ cells of NLPHL, but a variable number of classical or lacunar RS cells are typically present.

The neoplastic cells have a classical immunophenotype (CD20–/+ CD15+ CD30+ EMA– EBV+/-); however, the background may contain numerous B cells and a follicular dendritic cell (FDC) meshwork, similar to NLPHL. Staining for FDC often reveals a small, dense aggregate of FDC consistent with a regressed germinal center, associated with a broad mantle zone with more loosely spaced FDC processes. CD57+ T cells may also aggregate of FDC consistent with a regressed germinal center, associated with a broad mantle zone with more loosely spaced FDC processes. CD57+ T cells may also be present and may rim the RS cells; thus, it is really the immunophenotype of the RS cells that distinguishes this from NLPHL.

Overall, LRCHL appears to comprise about 5% of HL – a frequency similar to that of LPHL [9, 11]. In the ETFL and GHSG series, the clinical features at presentation of LRCHL seem to be intermediate between those of LPHL and classic HL: similarly to NLPHL, patients had early stage disease and lacked bulky disease or B symptoms; like both NLPHL and MCHL and in contrast to NLPHL they lacked mediastinal disease and had a predominance of males, and like MCHL they had an older median age than either NLPHL or NSHL (Table 4). In the ETFL series, the overall survival of both LPHL and LRCHL were excellent, but not significantly different from other types of HL. However, cases of NLPHL had an increased frequency of multiple relapses and better survival after relapse, compared with LRCHL, NSHL, and MCHL. In the GHSG study, the overall survival of LRCHL was significantly worse than that of NLPHL [8, 9, 11]. These data do not clearly define LRCHL as a distinct entity, but are consistent with either an early phase of MCHL or a novel subtype.

| Table 2. NLPHL and classic HL: morphologic and immunophenotypic features. |
|---------------------------------|-----------------|-----------------|
| Pattern                         | Classical HL    | NLPHL           |
| Tumor cells                     | Diffuse, interfollicular, nodular | Nodular, at least in part |
| Background                      | Lymphocytes, histiocytes, eosinophils, plasma cells | Lymphocytes, histiocytes |
| Fibrosis                        | Common          | Rare            |
| CD15                            | +               | –               |
| CD30                            | +               | –               |
| CD20                            | –/+             | +               |
| CD45                            | –               | +               |
| EMA                             | –               | +               |
| EBV (in RS cells)               | + (~ 50%)       | –               |
| Background lymphocytes          | T cells > B cells | B cells > T cells |
| CD57+ T cells                   | –               | –               |
| Ig genes (single-cell PCR)      | Rearranged, clonal, mutated, 'crippled' | Rearranged, clonal, mutated, productive, ongoing |
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reactive follicular lymphoid hyperplasia; however, on occasion, they may be numerous and associated with prominent lymph node enlargement, particularly in adolescents and young adults [19]. In several studies of de novo PTGC in children, there appears to be a small but increased risk of subsequent development of NLPHL; this progression is rare and may occur only after many years [20–23]. Nonetheless, young patients with lymphadenopathy and florid PTGC should be followed closely for development of NLPHL.

Architectural and cytologic features are most important in distinguishing LPHL from PTGC; however, immunophenotyping can be a useful adjunct. PTGC are typically round, well-circumscribed, and widely-spaced, with typical reactive follicles interspersed between them. In contrast, nodules of LPHL are typically back-to-back, with angulated borders and effacement of the architecture, with no intervening reactive follicles. At high magnification, PTGC contain small lymphocytes admixed with centrocytes and centroblasts, and popcorn cells are absent.

Both PTGC and LPHL consist of large nodules of B cells with FDC meshworks, and both may contain numerous CD57+ T cells. The nodules in LPHL typically contain more numerous T cells, which are often clustered, in contrast to the T cells in PTGC, which are evenly distributed as in normal germinal centers. In addition, the FDC meshwork often appears broken up as the follicles are invaded by T cells. These factors combine to give the nodules of NLPHL a ‘motheaten’ appearance on immunostained sections, rather than the smooth, rounded nodules of B cells that are seen in PTGC. Finally, in LPHL, there are large B cells surrounded by prominent rings of T cells; this phenomenon is usually absent in PTGC [24].

Immunophenotype and prognosis

The immunophenotype of classical HL varies from case to case: although most cases are CD15+ and CD30+, some lack one or the other antigen, and a variable number of cases in reported series express B-cell associated antigens [12–15]. The German Hodgkin’s Study Group reported 83% of 1751 cases to be positive for CD15, 96% positive for CD30, and 5% for CD20 [11]. The GHSG study found that cases that lacked CD15 but expressed CD30 had a significantly worse freedom from relapse and overall survival than CD15+ cases. Co-expression of CD20 with CD15 and/or 30 had no impact on outcome, but cases that expressed CD20 alone had poor survival [11]. This result is similar to that reported by McBride et al. [16], and raises the question whether these may represent cases of T-cell rich large B-cell lymphoma.

Immunophenotype in differential diagnosis

Immunophenotyping, as well as providing important insights into the classification of HL, has become increasingly useful in differential diagnosis with disorders that may mimic HL on routine histologic sections. These disorders include progressive transformation of germinal centers, T-cell/histiocyte-rich large B-cell lymphoma, and anaplastic large-cell lymphoma.

Progressive transformation of germinal centers (PTGC)

A distinctive type of follicular lymphoid hyperplasia, known as progressive transformation of germinal centers (PTGC), is seen focally in about 20% of lymph nodes involved by NLPHL, and may be seen in the absence of HL in other lymph nodes in the same patient [17, 18]. PTGC are enlarged follicles that contain numerous small B cells of mantle zone type; these follicles may closely resemble the nodules of NLPHL. This phenomenon has given rise to speculation that NLPHL may arise from PTGC. PTGC are usually seen as single or only a few enlarged follicles in a setting of nonspecific reactive follicular lymphoid hyperplasia; however, on occasion they may be numerous and associated with prominent lymph node enlargement, particularly in adolescents and young adults [19]. In several studies of de novo PTGC in children, there appears to be a small but increased risk of subsequent development of NLPHL; this progression is rare and may occur only after many years [20–23]. Nonetheless, young patients with lymphadenopathy and florid PTGC should be followed closely for development of NLPHL.

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T-cell/histiocyte-rich large B-cell lymphoma

In the last several years, several groups have reported an unusual type of lymphoma with morphologic features reminiscent of diffuse lymphocyte predominance or mixedcellularity Hodgkin’s lymphoma, with a predominance of small T lymphocytes, and scattered large neoplastic cells that express B-cell antigens. T-cell/histiocyte-rich large B-cell lymphoma (T/HRLBCL) is a diffuse lymphoma with a lymphocyte-rich background, with small clusters of epithelioid histiocytes and numerous scattered large mononuclear cells, suggesting either LP or classical Hodgkin’s lymphoma (CHL). The large cells may resemble popcorn cells, immunoblasts, or centroblasts, or all three. The neoplastic cells have an immunphenotype identical to NLPHL, expressing CD20 and other pan-B antigens, +/− cytoplasmic light chains, and may have detectable Ig gene rearrangement by Southern blot or whole-section PCR. Like LPHL, they are often EMA+, but are CD15− and CD30− and EBV−. The background lymphocytes are T cells, CD57−, and FDC aggregates are not seen.

The immunophenotype of the large cells is thus of limited value in the differential diagnosis with LPHL, since it is similar. However, evaluation of the cellular...
background can be helpful. Staining for CD20 reveals a nodular pattern and a B-cell-rich background in NLPHL, as well as follicular aggregates of FDC (anti-CD21) and large numbers of CD57+ cells. In T/HRLBCL, the background is predominantly T cells, even in nodular-appearing areas; FDC aggregates are absent, and CD57+ cells are rare. In distinction of T/HRLBCL from classical HL, immunophenotyping is essential and helpful; if the large cells express CD20 and lack CD15 and CD30, the diagnosis of T/HRLBCL is strongly favored, while expression of either CD15 or CD30 strongly favor a diagnosis of CHL.

This distinction is clinically important. Patients with T/HRLBCL typically present with advanced-stage disease involving lymph nodes, liver, spleen, and bone marrow, and have a poor prognosis [25, 26]. Furthermore, in cases diagnosed as CHL that express only CD20, the prognosis appears to be significantly worse than for cases expressing CD15 and/or CD30, with or without CD20 [11, 16]. Thus, although the relationship between this disorder and HL remains to be elucidated, it needs to be recognized because of its distinctive clinical behavior.

**HL vs. anaplastic large-cell lymphoma**

T/null anaplastic large-cell lymphoma is characterized by large malignant cells with prominent nucleoli and abundant cytoplasm, which may resemble mononuclear or multinucleated Reed–Sternberg cell variants. However, the tumor cells grow in cohesive sheets and frequently involve lymph node sinuses – a pattern that would be unusual in HL. In addition, the neoplastic cells are usually smaller than RS cells, have less conspicuous nucleoli, without perinucleolar halos, and often have bean-shaped or horseshoe-shaped nuclei, with a prominent paranuclear hof, in contrast to the round nuclei of mononuclear RS cells.

A subtype of ALCL has been described, called Hodgkin’s related [27–29] (modified to Hodgkin’s-like in the R.E.A.L. Classification – a provisional entity), which closely resembles NSHL. This variant has architectural features that resemble Hodgkin’s lymphoma of the nodular sclerosis type, with nodular growth of tumor cells and occasional fibrous bands, but with cytologic features similar to ALCL – confluent sheets of tumor cells, a cohesive growth pattern, and sinusoidal infiltration. The immunophenotype of ALCL–HL was reported to be similar to that of common ALCL, but some cases had CD15 expression and EBV infection. The patients were typically young adults with aggressive nodal disease, often with bulky mediastinal masses. Those who poorly responded to conventional therapy for HL, but had a good response to third generation chemotherapy regimens for high-grade non-Hodgkin lymphomas, with a better survival than patients with classic ALCL.

There has been an ongoing debate about whether ALCL–Hodgkin’s-like is a variant of Hodgkin’s lymphoma, a variant of ALCL, a heterogeneous mixture of the two, or a distinct disease. Grogan and associates (T. Grogan, unpublished data) have reviewed cases of large-cell lymphoma and cases of NSHL from two Southwest Oncology Group (SWOG) studies, and found respectively 13% and 11% cases that appeared morphologically to be ALCL – approximately 50% common type and 50% Hodgkin’s-like. Cases were studied immunophenotypically, and reclassified as either ALCL (CD15–CD30–) (75%) or NSHL (CD15+CD30+) (25%). There were no differences in clinical presentation between the two types of ALCL, and both differed from HL in having fewer young patients and less bulky disease. The survival of ALCL Hodgkin’s like was better than that for common ALCL when treated as large-cell lymphoma, but slightly worse than that for HL in the cohort treated as HL (63% vs. 77%, five-year OAS).

Analysis of the t(2;5) associated with ALCL reveals no overlap between ALCL and HL. Studies of the translocation have shown that it is absent in cases of typical HL [30–32], and in immunophenotyping studies using antibodies to either the ALK protein or the p80 fusion product of the t(2;5) [33–35], several groups have found the protein to be present in a subset of ALCL, but not in HL. Occasional cases diagnosed as ALCL–HL express the protein.

It appears likely that cases reported in the literature as ALCL–Hodgkin’s-related or Hodgkin’s-like are heterogeneous. Some represent lymphocyte-depleted variants of Hodgkin’s lymphoma – either NSHL (syncytial, lymphocyte depleted, or NS1H) type or lymphocyte depletion (LDHL), reticular type (‘Hodgkin’s sarcoma’), while others are cases of ALCL with a nodular growth pattern. The studies cited above suggest that most cases can be resolved as either HL (CD45–, CD15+, T-cell antigen–, CD20–/+ , t(2;5)–, ALK1–, EMA–) or ALCL (CD45+, CD15–, T-cell antigen+, CD20–, t(2;5)+, ALK1+, EMA+) [30–35].

In summary, the data currently available suggest that there is no true biological borderline between HL and ALCL of T/null type as defined in the R.E.A.L. Classification: HL is in most cases a B-cell neoplasm, while ALCL is a T-cell neoplasm. HL may resemble ALCL by having areas of lymphocyte depletion – i.e., it may be ‘ALCL-like’ – but this is a morphologic resemblance only, not a true biological borderline. Although these cases are rare – approximately 6% of NSHL in the SWOG study, they may have a more aggressive course than more typical NSHL. Similarly, cases of ALCL may resemble HL by having areas of nodularity, sclerosis, or granulocyte infiltration – i.e., ‘Hodgkin’s-like’ – but these are in fact ALCL, not true biologically borderline cases.

In cases that are histologically borderline between HL and T-ALCL, immunophenotyping on paraffin sections with CD45, CD15, CD30, CD20, EMA, pan-T antigens, ALK1, and, if necessary, genetic studies should be undertaken to resolve the differential diagnosis. Expression of CD15 or CD20 tend to exclude a diagnosis of T-ALCL (CD20+ cases may be either HL or diffuse large B-cell lymphoma), while expression of
CD45, T-cell antigens, ALK1 or EMA tend to exclude HL. Southern blot or whole section PCR analysis showing T-cell antigen receptor gene rearrangement would tend to exclude HL and confirm the diagnosis of ALCL. Ig gene rearrangement by the above techniques would not usually be detectable in HL and would favor diffuse large B-cell lymphoma, but a weak band would not exclude HL. Cases that cannot be resolved by immunophenotype or genetic studies should be considered unclassifiable; clinical judgment should be used in deciding whether to re-biopsy, or to treat for either HL or ALCL.

Hodgkin's lymphoma: When to immunophenotype?

In histologically and clinically typical cases (NLP, NS, MC) immunophenotyping may not be necessary, but it is certainly justified if the pathologist is uncertain about the diagnosis. In clinically atypical cases, immunophenotyping is suggested, even if the histologic features are not atypical. This would include HL involving unusual sites – for example, extranodal presentations, or NLPHL with disseminated disease, or a case that seems otherwise unusually aggressive. In histologically borderline cases immunophenotyping is essential, including cases that are borderline between NLPHL and classical HL, LPHL or MCHL and T-cell/histiocyte-rich B-cell lymphoma, and lymphocyte depleted variants (NSHL grade 2 or LDHL) vs. T/null-ALCL or DLBCL 'anaplastic' type.

What is the malignant cell? The answer from immunophenotyping and molecular genetic analysis

The malignant cell of NLPHL is a germinal center B cell

Immunophenotype

The immunophenotype is an important part of the definition of NLPHL. In contrast to classical HL, the atypical cells are CD45+, express B-cell associated antigens (CD19, 20, 22, 79a), and EMA but lack CD15 and CD30. In contrast to typical B-cell lymphomas, however, they are usually Ig- by routine techniques. Immunoglobulin light chain restriction has been reported by one group in the majority of the cases, but this has not been reproduced by others [36]. J-chain has been demonstrated in many cases [7, 37]. More recently, studies using in situ hybridization for light chain mRNA have shown clonal expression in the atypical cells [38]. Popcorn cells also express the nuclear protein encoded by the bcl-6 gene, which is associated with normal germinal center B-cell development [39], and the activation-associated molecules CD40 and CD86 (B7/BB1), which are involved in B-cell interaction with T cells [40, 41].

The nodules of LPHL are actually altered follicles or germinal centers. The small lymphocytes in the nodules are a mixture of polyclonal B cells with a mantle zone phenotype (IgM and IgD+), and numerous T cells, many of which are CD57+, similar to the T-cell population in normal and progressively transformed germinal centers [42]. T cells in NLPHL may have significant nuclear enlargement and irregularity, resembling centrocytes. In contrast to the T cells in reactive or progressively transformed follicles, which are scattered singly and often concentrated in the light zone or at the junction with the mantle zone, the T cells in NLPHL form small aggregates, often giving the follicle a broken up, moth-eaten, or irregular contour. They typically surround the neoplastic B cells, forming rings, rosettes or collarettes. Although several reports suggest that the T cells surrounding popcorn cells are mostly CD57+ [43, 44], this can be difficult to demonstrate in many cases, and absence of CD57+ cells in the rosettes does not argue against the diagnosis. A prominent concentric meshwork of FDC is present within the nodules. The interfollicular region contains predominantly T cells; when there are diffuse areas, the background lymphocytes are also predominantly T cells, and the FDC meshwork is lost [45].

Genetic features

Ig and TCR genes are germline when studied by Southern blot, and bcl-2 rearrangement has not been reported; the large cells are EBV- [46, 47]. When more sensitive PCR techniques are used to evaluate Ig gene rearrangement, using either whole tissue sections or single cells, the majority of cases in some series have been reported to show clonal Ig gene rearrangements [48, 49], while in other series only polyclonal rearrangements have been found [50]. Three recent studies report clonal Ig gene rearrangements in the majority of cases, with productively rearranged and hypermutated Ig genes and ongoing mutations, consistent with germinal center cells [51–53]. Specific cytogenetic abnormalities have not been reported in NLPHL.

The morphology, immunophenotype and genetic features all suggest a close relationship of the 'popcorn' cell to a centroblast (proliferating germinal center cell) [49, 51, 52]. Lack of CD15 and CD30 and absence of EBV suggest that NLPHL is not related to other types of HL, and lack of bcl-2 rearrangement suggest that it is not related to usual follicle center lymphoma. The morphologic continuum with progressively transformed germinal centers suggests that NLPHL is a neoplasm of centroblasts, possibly arising from an abnormal germinal center reaction.

The malignant cell of classical HL is an abnormal germinal center B cell

Immunophenotype

In classical HL the neoplastic cells typically express CD15 and CD30, and lack pan-B and pan-T antigens. Expression of B-cell associated antigens in a proportion of the cases has given rise to speculation that at least some cases are of B lineage. Expression of T-cell antigens is distinctly unusual, but some series report a rather high frequency of T-cell antigen-positive HL. In addition to CD15 and CD30, RS cells express CD25,
with faint bands. Using the more sensitive PCR technique on whole tissue sections to detect clonal VDJ rearrangements in cases of HL, several groups have documented Ig gene rearrangements 28%-50% of cases of classical HL, and in 58%-71% of cases with B-cell antigen expression [48, 63, 64]. In some of the cases, there were somatic mutations in the VH segments, suggestive of a germinal center or post germinal center stage of differentiation, while others lacked them, suggesting a pre-germinal center stage [48].

Recently, several groups have used single cell assays of RS cells obtained by either micromanipulation of frozen sections or cell suspension, and subjecting them to PCR to detect VDJ rearrangements; these studies have yielded conflicting results. One group [49, 65] has shown clonal rearrangements of Ig genes in all cells from all cases studied, while others have found either no rearrangements [66], polyclonal rearrangements [67], or a mixture of polyclonal and monoclonal cases [68]. Differences between the results of the different groups could be due to the small number of cases studied, differences in the subtypes of HL included, differences in isolation methods and differences in primers and PCR techniques. Nonetheless, taken together, these data provide suggestive evidence for the B-cell derivation and clonal nature of many cases of classical HL.

Despite their B-cell lineage, RS cells of classical HL, unlike those of LPHL, do not make Ig mRNA or protein [69, 70]. This may be explained by the findings of one group [65] that although rearranged and mutated, consistent with exposure to the germinal center environment, the Ig genes in classical HL had mutations that were out of frame or introduced stop codons, such that the gene could not be transcribed or translated into protein.

Cytogenetic abnormalities. Clonal cytogenetic abnormalities are found in the majority of the cases of classical HL, when studied with karyotyping techniques; however the abnormalities vary from case to case, and there is often intraclonal variability, indicating chromosome instability [71]. Many cases show 14q abnormalities, like bcl-6 protein associated with follicle center B cells [39]. In EBV+ cases, the tumor cells express EBV latent membrane protein (LMP) but not EBNA2.

The relationship of antigen expression to the functional properties of the RS cells can be speculated upon. Cultured and primary RS cells produce a number of cytokines that may be responsible for some of the systemic symptoms and laboratory abnormalities associated with HL, as well as with the tissue fibrosis and eosinophilia. Many of the surface antigens that characterize the tumor are either cytokine receptors, adhesion molecules, or antigens involved in interaction with T cells. CD30, CD40, CD70, and CD95 are members of the tumor necrosis factor receptor/nerve growth factor (TNF/NGF) superfamily of surface receptor molecules (reviewed in [57, 58]). These receptors and their ligands are involved in regulation of cell proliferation, activation, differentiation, and apoptosis; studies on cell lines have shown that engaging these receptors results in increased proliferation, cytokine production, and upregulation of costimulatory and adhesion molecules by RS cells [57].

### Table 5. Differential diagnosis of Hodgkin's lymphoma.

<table>
<thead>
<tr>
<th>Morphology (large cells)</th>
<th>Immuno-phenotype (large cells)</th>
<th>T-cell rings</th>
<th>Genetics (Southern blot)</th>
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<tr>
<td>LPHL</td>
<td>Popcorn cells</td>
<td>CD20+</td>
<td>Ig polyclonal</td>
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<td>EMA+ 15-30-</td>
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<td>LRCHL</td>
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<td>T/HRLBCL</td>
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EMA may be difficult to detect in formalin-fixed tissues. Classical HL may be CD20+ (15%) or CD15- (15%).

HLA-Dr, ICAM-1, CD95 (apo-1/fas), and both CD40 and CD86 (B7), molecules associated with B-cell activation and interaction with T cells [40, 41, 54-56]; T cells surrounding the RS cells express both CD40 ligand and CD28, the ligand for CD86 [41, 55]. In contrast to NLPHL, the RS cells of classical HL lack the nuclear bcl-6 protein associated with follicle center B cells [39]. In EBV+ cases, the tumor cells express EBV latent membrane protein (LMP) but not EBNA2.

The relationship of antigen expression to the functional properties of the RS cells can be speculated upon. Cultured and primary RS cells produce a number of cytokines that may be responsible for some of the systemic symptoms and laboratory abnormalities associated with HL, as well as with the tissue fibrosis and eosinophilia. Many of the surface antigens that characterize the tumor are either cytokine receptors, adhesion molecules, or antigens involved in interaction with T cells. CD30, CD40, CD70, and CD95 are members of the tumor necrosis factor receptor/nerve growth factor (TNF/NGF) superfamily of surface receptor molecules (reviewed in [57, 58]). These receptors and their ligands are involved in regulation of cell proliferation, activation, differentiation, and apoptosis; studies on cell lines have shown that engaging these receptors results in increased proliferation, cytokine production, and upregulation of costimulatory and adhesion molecules by RS cells [57].

### Genetic features

**Immunoglobulin genes.** In classical HL, immunoglobulin (Ig) and T-cell receptor (TCR) genes are usually germline when studied by Southern blot, but rearrangements of Ig genes are reported in some cases, usually with faint bands [59-62]. Using the more sensitive PCR technique on whole tissue sections to detect clonal VDJ rearrangements in cases of HL, several groups have documented Ig gene rearrangements 28%-50% of cases of classical HL, and in 58%-71% of cases with B-cell antigen expression [48, 63, 64]. In some of the cases, there were somatic mutations in the VH segments, suggestive of a germinal center or post germinal center stage of differentiation, while others lacked them, suggesting a pre-germinal center stage [48].

Recently, several groups have used single cell assays of RS cells obtained by either micromanipulation of frozen sections or cell suspension, and subjecting them to PCR to detect VDJ rearrangements; these studies have yielded conflicting results. One group [49, 65] has shown clonal rearrangements of Ig genes in all cells from all cases studied, while others have found either no rearrangements [66], polyclonal rearrangements [67], or a mixture of polyclonal and monoclonal cases [68]. Differences between the results of the different groups could be due to the small number of cases studied, differences in the subtypes of HL included, differences in isolation methods and differences in primers and PCR techniques. Nonetheless, taken together, these data provide suggestive evidence for the B-cell derivation and clonal nature of many cases of classical HL.

Despite their B-cell lineage, RS cells of classical HL, unlike those of LPHL, do not make Ig mRNA or protein [69, 70]. This may be explained by the findings of one group [65] that although rearranged and mutated, consistent with exposure to the germinal center environment, the Ig genes in classical HL had mutations that were out of frame or introduced stop codons, such that the gene could not be transcribed or translated into protein.

**Cytogenetic abnormalities.** Clonal cytogenetic abnormalities are found in the majority of the cases of classical HL, when studied with karyotyping techniques; however the abnormalities vary from case to case, and there is often intraclonal variability, indicating chromosome instability [71]. Many cases show 14q abnormalities, like B-cell lymphomas, but this only rarely involves a t(14;18). Using fluorescence in situ hybridization, with or without fluorescence immunophenotyping, two groups found that RS cells showed clonal numerical abnormalities in all cases of HL [72, 73].

**Oncogene rearrangements.** Bcl-2 rearrangement has been detected with the polymerase chain reaction in a variable proportion of the cases in some laboratories [74] but not in others [75]; these rearrangements have not been proven to be in the neoplastic cells, and in fact one study demonstrated convincingly that in cases with bcl-2 rearrangements, no t(14;18) was present in the neoplastic cells [75].

**Recent studies** have suggested that the t(2;5) is also associated with HL, as well as with the tissue fibrosis and eosinophilia. Many of the surface antigens that characterize the tumor are either cytokine receptors, adhesion molecules, or antigens involved in interaction with T cells. CD30, CD40, CD70, and CD95 are members of the tumor necrosis factor receptor/nerve growth factor (TNF/NGF) superfamily of surface receptor molecules (reviewed in [57, 58]). These receptors and their ligands are involved in regulation of cell proliferation, activation, differentiation, and apoptosis; studies on cell lines have shown that engaging these receptors results in increased proliferation, cytokine production, and upregulation of costimulatory and adhesion molecules by RS cells [57].

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In summary, the combination of immunophenotype and, in particular, single-cell PCR of the Ig genes leads to the conclusion that the RS cells of classical HL are in most cases abnormal, clonal B cells that have been exposed to antigen in the germinal center, but that are incapable of producing an immunoglobulin protein and are thus incapable of further differentiation or antigen-driven selection.

Is there a T-cell Hodgkin's lymphoma [12]? Most studies show no T-cell antigen expression or TCR rearrangement; however, the number of cases studied by molecular methods is small. Some studies in which a T-cell immunophenotype is reported show an association with lymphomatoid papulosis or mycosis fungoides [77]. Are these examples of T-ALCL with a nodular pattern (Hodgkin's like ALCL), or are they really T-cell HL? Further studies are required to resolve this question.

Why does Hodgkin's lymphoma vary in different populations?

It has been known for some time that there is variation in the incidence, age and sex distribution, and morphology of HL in different populations; more recently, it has become clear that the frequency of EBV positivity also varies. These parameters vary according to geographic location, socioeconomic status, and immunologic status of the population.

The earliest epidemiologic studies of HL found differences in the age-specific incidence of HL according to the level of industrial development, and three patterns were described. In pattern 1, seen in non-industrialized countries and in low socioeconomic groups and rural areas in industrialized countries, there was an overall low incidence of HL, with early childhood peak, no third decade peak, and a steady rise in incidence with advancing age. The histologic subtypes were predominantly MC, LD, and LP. Pattern 3 was seen in industrialized countries, in high socioeconomic groups, and urban areas; there was an overall increased incidence of HL with a third decade peak, an increased incidence in females, and a predominance of NS over MC or other types. Pattern 2 was intermediate, with both a childhood and a second decade peak, an equal frequency of MC and NS, and was seen in early industrialized or transitional economies [78–81].

Analysis of the incidence and pattern of HL over time has shown progression in some areas, such as Latin America, from a pattern 1 to a pattern 2 distribution, probably in reflection of increasing industrialization and standards of living [82]. Tables 6 and 7 compare the approximate pre- and post-1985 distribution of HL in selected countries for which data are available [78–98]. Diagnostic accuracy or changes in classification have also been proposed as a reason for the apparent changes in distribution of HL; one study from the US suggests that this may account for some of the decreases seen in frequency of the LP and LD subtypes, and also in the decrease in cases in elderly individuals; however, it does not appear to account for the increase in young adult NSHL [92, 93].

Patients in non-industrialized countries and lower socioeconomic groups, as well as children, who develop HL are more likely to have EBV+ HL than are patients from high socioeconomic groups in the young adult age group [89–91, 94–98]. In these groups, even NSHL has a higher incidence of EBV positivity than it does in the young adult cases (Table 8).

Assessment of the frequency of HL in individuals with altered immune status may shed some light on the differences of HL incidence and histology in different geographic populations. Differences are seen among immunosuppressed populations such as congenital immune deficiency, iatrogenic immune deficiency for organ transplantation or autoimmune disease, and HIV infection. In HIV infection, it is still controversial whether HL in increased in frequency [99]; if it is, the increase is markedly less striking than the increase in incidence of non-Hodgkin's lymphoma. Interestingly, the type of lymphoma that HIV+ patients develop depends on the degree of immunosuppression: patients with near-normal CD4 counts may develop HL; those with moderately reduced CD4 counts develop Burkitt's lymphoma, and the most severely immunosuppressed patients develop immunoblastic lymphoma [100]. In contrast to HIV+ patients, those with congenital immune deficiency and iatrogenic immune deficiency for solid organ transplants rarely develop HL, although the incidence of EBV+ B-cell lymphomas is markedly increased. Interestingly, patients with only mild immunosuppression, such as those with ataxia-telangiectasia or occasional bone marrow allograft recipients may occasionally develop HL [101]. Finally, patients with autoimmune disease who are treated with

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**Table 6. Hodgkin's lymphoma – international differences in morphology: Pre-1985.**

<table>
<thead>
<tr>
<th>Country</th>
<th>NS</th>
<th>MC</th>
<th>LD</th>
<th>LP</th>
</tr>
</thead>
<tbody>
<tr>
<td>US/Canada</td>
<td>50%</td>
<td>40%</td>
<td>5%</td>
<td>5%</td>
</tr>
<tr>
<td>North Europe</td>
<td>55%</td>
<td>25%</td>
<td>10%</td>
<td>10%</td>
</tr>
<tr>
<td>South Europe</td>
<td>20%</td>
<td>60%</td>
<td>10%</td>
<td>10%</td>
</tr>
<tr>
<td>India</td>
<td>10%</td>
<td>55%</td>
<td>15%</td>
<td>20%</td>
</tr>
<tr>
<td>Latin America</td>
<td>15%</td>
<td>55%</td>
<td>15%</td>
<td>15%</td>
</tr>
<tr>
<td>Japan</td>
<td>20%</td>
<td>50%</td>
<td>10%</td>
<td>20%</td>
</tr>
<tr>
<td>Africa</td>
<td>10%</td>
<td>20%</td>
<td>45%</td>
<td>25%</td>
</tr>
</tbody>
</table>

**Table 7. Hodgkin's lymphoma – international differences in morphology: Post-1985.**

<table>
<thead>
<tr>
<th>Country</th>
<th>NS</th>
<th>MS</th>
<th>LD</th>
<th>LP</th>
</tr>
</thead>
<tbody>
<tr>
<td>US/Canada</td>
<td>70%</td>
<td>25%</td>
<td>&lt;1%</td>
<td>5%</td>
</tr>
<tr>
<td>North Europe</td>
<td>70%</td>
<td>25%</td>
<td>&lt;1%</td>
<td>5%</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>70%</td>
<td>20%</td>
<td>5%</td>
<td>5%</td>
</tr>
<tr>
<td>Africa</td>
<td>65%</td>
<td>20%</td>
<td>10%</td>
<td>5%</td>
</tr>
<tr>
<td>China</td>
<td>50%</td>
<td>40%</td>
<td>5%</td>
<td>5%</td>
</tr>
<tr>
<td>Mexico</td>
<td>40%</td>
<td>45%</td>
<td>15%</td>
<td>2%</td>
</tr>
<tr>
<td>Korea</td>
<td>20%</td>
<td>60%</td>
<td>10%</td>
<td>10%</td>
</tr>
</tbody>
</table>
rather mild immune suppression with methotrexate occasionally develop an EBV+ HL-like lesion [102, 103].

Patients with HIV infection who do develop HL tend to have MC or LD histology, and the majority of the cases are EBV+. Taken together, these observations suggest that immunosuppression, per se, does not predispose to the development of HL, but that when it does develop, it is likely to be of MC or LD type and EBV+ [99, 104].

Could the variation in incidence, histologic features, and EBV positivity among geographic populations be related to differences in the status of the immune systems in these populations? HL is most prevalent in young adult females of high socioeconomic status in developed countries, where it is usually of NS type. These patients often have a history of infectious mononucleosis and unusually high titers of antibodies to EBV, suggesting a normal to hyperactive immune system [80, 105]. In contrast, patients who are severely immunosuppressed rarely if ever develop HL. Thus, one could argue that an intact immune system is required for the development of HL, particularly NS type, and perhaps a hyperactive immune system predisposes to it. It is possible that young children, older adults, and individuals living in relatively poor socioeconomic conditions may have relatively less 'active' immune systems for a variety of reasons, and this may result in a lower likelihood of developing HL. When HL does develop in one of these individuals, it is likely to be of MC type and EBV+. Patients with severely impaired immunity, such as allograft recipients and most HIV+ patients, cannot develop HL and instead develop NHL.

Table 8. Hodgkin's lymphoma: differences in EBV association (approximate).

<table>
<thead>
<tr>
<th>Percentage EBV+ cases</th>
<th>Total</th>
<th>NS</th>
<th>MC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>25%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>US/Europe</td>
<td>40%</td>
<td>35%</td>
<td>65%</td>
</tr>
<tr>
<td>Mexico</td>
<td>70%</td>
<td>50%</td>
<td>80%</td>
</tr>
<tr>
<td>Other Latin American</td>
<td>70%-100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Africa</td>
<td>80%</td>
<td>80%</td>
<td>100%</td>
</tr>
<tr>
<td>China</td>
<td>60%</td>
<td>50%</td>
<td>90%-100%</td>
</tr>
<tr>
<td>Children</td>
<td>80%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>80%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 9. Hodgkin's lymphoma: association between EBV, epidemiology, and histology.

<table>
<thead>
<tr>
<th>Sex</th>
<th>EBV</th>
<th>MC</th>
<th>NS</th>
<th>LP*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Male</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
</tr>
</tbody>
</table>

and LMP-1 [109]. LMP-1 has transforming activity for B cells, and its expression should give a survival advantage to infected cells. Since EBV is capable of immortalizing B cells, and since all the cells in infected cases carry the same clone of EBV and express the most potent transforming protein (LMP-1), the obvious conclusion is that EBV must play some role in the pathogenesis of HL.

In addition, epidemiologic and serologic data also seem to favor a role for EBV in HL: there is a correlation between a history of infectious mononucleosis (IM) and HL, and HL occurs in the same socioeconomic groups that are at risk for infectious mononucleosis.

These observations give rise to the hypothesis that HL is, like IM, a consequence of late infection with EBV [80]. In addition, some patients with HL have unusually high titers of antibodies against EBV [105]. However, the fact that EBV is not present in all the cases leaves open the question of the pathogenesis of EBV-negative cases, and, more importantly, the question of whether EBV is important even in positive cases.

At least three hypotheses can be advanced to explain the EBV-negative cases. First, another virus could be involved in the these cases; however, studies to date have failed to identify another virus [110]. Second, EBV may be involved in all the cases, but is undetectable in some; this has been called the 'hit and run' theory. In this scenario, EBV infects the cell, alters the DNA in some way, and is then eliminated, leaving the cell either transformed or susceptible to transformation. Elimination of EBV with its immunogenic proteins might be expected to occur most commonly in patients with active immune systems, such as the young females with NSHL. However, a third possibility, and the one perhaps best supported by epidemiologic data, is that EBV is not important in the pathogenesis of any of the cases of HL, and that its presence in some cases simply reflects the presence of a larger reservoir of latently EBV-infected cells in these individuals.

Paradoxically, the group in which EBV had been predicted most likely to be involved – young females from high socioeconomic groups with NSHL – proved to have the lowest incidence of EBV in tumor tissue [94]. Furthermore, there is no correlation between a history of either infectious mononucleosis or unusually high titers of antibodies to EBV and detectable EBV in HL tissues [111]. In contrast to what one would expect if HL were caused by EBV, populations in which HL is common have EBV-negative HL (young females in affluent
societies) and populations in which HL is rare have EBV-positive HL (young children in underdeveloped countries and older individuals) [94]. These apparent paradoxes force us to at least consider the possibility that EBV may be merely an epiphenomenon in HL, reflecting a high incidence of EBV-infected B cells in the patient, rather than an etiologic factor.

The host immune system may be the most important factor in both the likelihood of developing HL and the likelihood that it will be EBV+. Patients with intact immune systems are thought to have low levels of EBV-infected B cells, while patients with impaired immunity have a larger reservoir of EBV-infected B cells, by virtue of having a less active immune system to eliminate them. If there are increased numbers of EBV+ B cells in these individuals with low immunity, they do not appear predisposed to become Hodgkin’s cells with increased frequency, given the low incidence of HL in these groups. However, when a B cell in such a patient does transform into a Hodgkin’s cell, it has an increased likelihood of being EBV+, simply because more of the patients’ B cells are EBV+. Some evidence for this hypothesis comes from the finding that EBV-specific cytotoxic T cells are deficient in lymph nodes with EBV+ HL compared with those from EBV-negative cases [112].

Conclusions

What have we learned from the ‘many faces of Hodgkin’s Lymphoma?’

The study of the immunophenotype of both the neoplastic cells and the background infiltrate has helped us to better define the disease and its subtypes, and it may help to predict prognosis in classical HL. It has also helped us to recognize and distinguish from HL two important new aggressive non-Hodgkin’s lymphomas – T-cell rich large-B-cell lymphoma and anaplastic large-cell lymphoma. From both immunophenotyping and molecular genetic studies come the answer to the question of what is the malignant cell – we know that it is a B cell of germinal center type in most cases of both LP and classical types. Why is there variation in different populations? It is possible that this is related to the status of the host immune system, which varies with age, sex, and socioeconomic status. Finally, is HL caused by a virus? Much evidence suggests that EBV is probably implicated in pathogenesis of many cases, although formal proof of this is lacking and the paradox of its distribution remains to be explained.

However, the study of the pathology of Hodgkin’s lymphoma leaves us with many more questions. What is the relationship of NLPHL to PTGC? Is lymphocyte-rich classical HL a distinct subtype? Are there pathologic features that will predict prognosis in classical HL? Are LPH and T-cell rich large B-cell lymphoma related or simply morphologically similar? What is the border-line between classical HL and ‘B-cell ALCL’? What is the genetic abnormality that turns a germinal center B cell into a RS cell? How does a B cell with crippling V-region mutations survive? Is there a T-cell HL? Does the host immune system affect the likelihood of developing HL, and if so, how? What is the real role of EBV, and is there another virus in the EBV+ cases? These and other questions will be the subject of the next decades of study.

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56


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