REVIEW ARTICLE

The Cellular Biology of the Reed-Sternberg Cell

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FOR STUDENTS OF the history of medicine, the evolution of our understanding of Hodgkin's disease (HD) has long proved a compelling subject. Traced in the chronology of this disorder, from its first descriptions, through the refinements in its classification, to its medical and radiotherapeutic cure, is a more general outline of the development of our knowledge of malignant lymphomas. Yet, recently advances in cell and molecular biology have afforded a better comprehension of the pathogenesis of non-Hodgkin's lymphomas (NHLs) than of HD itself. In fact, historically long-standing enigmas regarding the nature of HD continue to defy understanding.

The most prominent of these enigmas concerns the origin of the Reed-Sternberg (RS) cell itself. It has been variously identified as a cell deriving from lineages related to macrophages or histiocytes, interdigitating reticulum cells, dendritic reticulum cells, or granulocytes. It has been postulated to result from fusions between lymphocytes, lymphocytes and reticulum cells, or virus-infected cells. However, the weight of recent evidence supports the thesis that the Reed-Sternberg cell originates with a B or T lymphocyte.

Advances in our understanding of the derivation of the RS cell have been driven by developments in five areas of cell biology. (1) Immunophenotyping of fixed tissues and cell lines has facilitated the characterization of the RS cell's surface, and allowed precise subclassification and identification of phenotypically distinct, but perhaps related, disorders (eg, nodular lymphocyte predominance HD, Ki-1 anaplastic large-cell lymphoma, and lymphomatoid papulosis). (2) Genotyping, both by way of cytogenetic and molecular techniques, has provided some genetic correlates to immunophenotyping data. (3) The cultivation of cell lines derived from HD specimens has enabled the development of reliable and specific monoclonal antibodies (MoAbs) and has allowed detailed study in vitro of the putative malignant cells. (4) The finding of an association between HD and Epstein-Barr virus (EBV) infection has suggested a potential pathogenetic relationship between the two. (5) Descriptions of numerous cytokines produced in HD lesions have provided insight into the histogenesis and pathophysiology of the disorder.

These lines of research have at last begun to coalesce into a uniform body of knowledge relating to the cellular biology of the RS cell. In the discussion that follows, we will review the recent developments in each of these areas in turn.

IMMUNOPHENOTYPING

The antigenic characteristics of RS and Hodgkin cells and variants (RS-H cells) have been investigated in numerous studies. The most useful reagents have been MoAbs with well-defined cell and tissue specificities. An additional useful class of MoAbs produced by immunization with an HD cell line are Ki-1 and related antibodies. Various groups using these reagents have compiled a detailed description of the RS-H cell surface. However, studies differ as to its precise features; no completely uniform phenotype has emerged. Moreover, no precursor cell or benign counterpart to the phenotype of the antigenically defined RS-H cell has yet been identified.

Many studies have defined a set of epitopes common to most RS-H samples in pathologic specimens. A significant fraction of RS-H cells express surface markers consistent with a B- or T-lymphocyte lineage. Markers expressed on cells of myeloid or monocytic derivation are usually not identifiable on RS-H cells, with the prominent exception of Leu-M1, which is found on a high proportion of HD samples. The cells usually express a number of activation antigens, including the interleukin-2 (IL-2) receptor (CD25), Ki-1 (CD30), the transferrin receptor (OKT 9, CD71), and HLA DR epitopes.

Lymphocytic markers. T-cell marker expression by RS-H cells is variable. Some studies report negative or equivocal results, but others demonstrate expression of various T-cell markers. Kadin et al showed expression of CD2, CD3, or CD4 in up to 40% of examined cases; expression was limited to primarily nodular sclerosis (NS) or mixed cellularity (MC) subtypes. Several studies found frequent expression of CD1, CD2, CD3, and CD4 in lymphocyte-depleted (LD), NS, and MC histologies. Other workers, using plastic embedding, have confirmed a large proportion of T-cell marker positivity. Additional studies have found variable T-cell marker positivity. The consensus is that a significant fraction of RS-H cells do in fact carry pan-T-cell antigens on their surface.

The expression of B-cell markers has been similarly explored. As for the T-cell markers, some studies report negative findings, but most groups report that some pathologic samples exhibit staining with B-lymphocyte markers, albeit less commonly than with T-cell reagents. Ig heavy or light chains are usually not expressed by RS-H cells, although occasionally individual cases that stain positive are noted. In addition, although in one study in situ hybridization failed to detect Ig light chain mRNA synthesis in RS cells, a recent report using in situ techniques detected κ or λ light chain mRNAs in H-RS cells in 50% of nodular lymphocyte predominant (LP) HD cases. B-cell–restricted antigens CD19, CD20, and CD22 are expressed in a small fraction of cases of all histologies, although in some series
they are expressed in the majority of cases.30 The B-cell antigen BLA.36 has been shown to be expressed on some RS cells.31 Expression of the B-cell–specific J chain has also been examined, and shown to occur exclusively in the nodular LP subtype.32 This variant of HD also exhibits nearly uniform positivity for the CD20 antigen, although a small proportion of malignant cells in up to 50% of non-LP HD cases also stain for CD20.33 Nodular lymphocyte predominance HD is now largely accepted as an immunophenotypically distinct B-cell subtype of this malignancy.34 The immunophenotypic features of nodular LP HD are contrasted with other subtypes in Table 1.

**Myelomonocytic markers.** HD specimens are usually negative for myeloid and monocytic antigenic markers. In an excellent review by Drexler et al,35 a tabulation of various studies showed that, of 196 samples tested for CD11b (Mo1) and CD14 (Mo2), none was positive. Other MoAbs, such as antimonocyte antibodies 20.2 and 20.3, antimacrophage marker EBM11,35 and anti-lysozyme,36 fail to stain HD specimens.

However, taken against the body of negative immunophenotyping data is the evidence from histochemical studies. Because of technical differences, these studies are often difficult to interpret. Yet, some groups find that HD samples stain weakly positive for two markers generally taken to characterize histiocytes, nonspecific esterase and acid phosphatase.5,6 To these investigators, the histochemical evidence suggests a shared lineage with interdigitating reticulum cells. The finding of exogenous polyclonal Ig in RS-H cells is felt to be consistent with a macrophage lineage.5

Stein et al initially reported staining of RS-H cells by MoAbs including T9 and 3C4, which react with granulocytes. Hsu and Jaffe later reported RS-H cells to be positive for Leu M1. These antibodies recognize CD15. Although initially it was hoped that Leu M1 might prove to be a specific and sensitive HD reagent, this has not proven to be the case. A number of studies have shown CD15 positivity in NHLs, primarily of T-cell origin,38,41 although some reports are negative.42 CD15 has also been shown to be expressed in nonhematopoietic malignancies.39 Conversely, the nodular LP subtype is uniformly negative for CD15.32,33,38

The significance of the expression of CD15 and of the histochemical findings noted is not clear. Few investigators feel that the RS-H cell is derived from myelomonocytic precursors. However, that fact that CD15 is expressed on both RS-H cells and a small proportion of T-lymphocyte malignancies, when taken in the context of the pattern of lymphocyte marker expression on RS-H cells discussed earlier, may suggest common origins for these tumors.

Recently, polymerase chain reaction analyses of reverse transcribed mRNA (RT-PCR) from single RS-H cells lends has provided some support to this hypothesis. mRNA coding for both the hck family of tyrosine kinases and other genes common to myelomonocytic cells, as well as mRNA coding for the 1ck family of tyrosine kinases and other genes common to T cells, have been shown in individual RS-H cells.43,44

**The CD30 antigen.** The Ki-1 antibody was produced in an attempt to isolate specific markers for HD. It was raised against the HD cell line L428,15 and initial studies on biopsy material suggested that it detected an antigen (later designated CD30) restricted to RS-H cells. Subsequently, the antigen was found to be expressed on both T cells46,47 and B cells48 when activated in vitro by a variety of stimuli. In addition, CD30 was detected in a significant proportion of peripheral T-cell lymphomas,49 in angioimmunoblastic lymphadenopathy,50 in lymphomatoid papulosis,51,52 and in anaplastic large-cell lymphoma (ALCL).53-55 The latter tumor, by virtue of its Ki-1 positivity, has been regarded as a distinct entity with some pathologic features of carcinoma or histiocytosis, and usually derives from activated T cells.56 Table 1 contrasts the immunophenotype of ALCL with that of HD.

Ki-1 is one of several antibodies51,54 that recognize the CD30 antigen. This marker is expressed primarily on lymphocytes, although its expression on differentiated macrophages in culture has been reported.50 The Ki-1 reagent has been used to assay for soluble CD30 in sera of patients with HD, and levels have been demonstrated to reflect disease activity.50 Other anti-CD30 antibodies have been linked to ricin for use as immunotoxins to image HD and treat refractory HD.54,57,58 Antibodies to CD30 linked to ricin can eradicate primary HD xenografts propagated in severe combined immunodeficient (scid) mice.60 The CD30 transcript has been cloned.61 It codes for a protein that is homologous to members of the nerve growth factor receptor superfamily of proteins. In addition, CD30 was recently found to function as a signal transducing molecule in a specific subset of CD45RO+ T cells.62 These findings suggest a potential role for CD30 in the genesis of HD via disturbances in its expression or function. Although a causal relationship between such CD30 alterations and RS-H transformation has yet to be proven, the exciting prospect of a molecular understanding of this aspect of the pathogenesis of HD now exists.

**Activation markers.** Several other antigens, although not specific for HD, are commonly expressed on RS-H cells in biopsy specimens. These include the IL-2 receptor (CD25), HLA-DR (Ia), and OKT9 (CD71, the transferrin receptor).16-18,23,25,26,28,30,63,64 Ki-67, a nuclear proliferation marker, is usually present.21,22,65 Findings with antibodies to leucocyte common antigen (CD45, T200), a hematopoietic and lymphoid antigen, are variable. Some studies report no expression of this marker,17,30,64,65 whereas others demonstrate

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* Although most workers regard HD as LCA negative, data are conflicting; see text.
† The majority of ALCL are T-cell malignancies, but B-cell cases comprise 20% in some series.
virtually uniform positivity.\textsuperscript{16,18,21} The positive findings likely reflect more sensitive immunocytochemical techniques\textsuperscript{16} and support the lymphoid origin of the RS-H cell. Of note, all HD cell lines express CD 45 (see below).

A recent report identified a novel B-cell activation antigen recognized by MoAb FUN-1.\textsuperscript{90} FUN-1 recognizes a 75-kD protein present on activated B cells, EBV-transformed B cells, large B-cell lymphomas, ALCL, and RS-H cells. Resting lymphocytes, activated T cells, acute lymphocytic leukemia (ALL), and low-grade B-cell leukemias do not react with the antibody. This finding also lends support to a B-lymphoid origina of some ALCL and RS-H cells.

\textbf{Proliferation of the H-RS cell.} Although H-RS cells express proliferative markers such as Ki-67, their proliferative potential was, until recently, unclear. Initially, it was thought that mononuclear H cells fused into terminally differentiated multinucleated RS cells in a process similar to multinucleated giant cell formation by cells of monocyte/macrophage origin.\textsuperscript{89} However, cultures of HD explants showed an increase in 6-thymidine uptake in multinucleated RS cells, challenging this view.\textsuperscript{91} Recent results have confirmed this latter view. Using immunoperoxidase techniques, 50% to 95% of H-RS cells stain for proliferating cell nuclear antigen (PCNA), a marker of cell division.\textsuperscript{92-94} In addition, expression of PCNA in H-RS cells correlates with expression of another activation antigen, Ki-67.\textsuperscript{95} Finally, Drexler et al\textsuperscript{92} showed that the multinuclear RS cells form in culture from mononuclear HD cell lines through nuclear division without cytokinesis, or cell division. Thus, the RS cell is a truly neoplastic cell with defects in cytokinesis.

\textbf{Cytoskeletal markers.} H-RS cells immunohistochemically stain for vimentin, which may be a general indicator of neoplastic growth\textsuperscript{96} or may indicate EBV integration into the H-RS cell with latent membrane protein (LMP) expression.\textsuperscript{97} More recently, a novel cytoskeletal protein, expressed only in H-RS cells and ALCL, has been described.\textsuperscript{98} Restin is a 160-kD protein encoded by a cDNA isolated from in vitro cultured peripheral blood mononuclear cells (PBMs).\textsuperscript{99} Restin is associated with the intermediate filament cytoskeletal network, and has an \(\alpha\)-helical rod structure similar to intermediate filament proteins.\textsuperscript{75,76,100} The function of restin is unknown, but it is tempting to speculate that abnormalities of restin expression may underlie the abnormalities in cell division and cytokinesis seen in the RS cell. The gene for restin has been mapped to chromosome 12p24.3 by in situ hybridization.\textsuperscript{77}

\section*{GENOTYPING}

Investigations of both the cytogenetics and molecular genetics of HD have been productive. These studies have been particularly difficult to perform in fresh biopsy specimens, largely because of the paucity of malignant RS-H cells in involved tissues; they generally constitute approximately 2% of cellular material in biopsied lymph nodes. Nonetheless, genotypic studies have been central to establishing the clonality of the HD disease lesion. They have also shown that no uniform HD genotype is distinguishable. Instead, the general patterns of chromosomal and immunogenetic abnormalities suggest, as do the immunophenotypic findings, that the RS-H cell may derive from the lymphocyte lineage.

\textbf{Cytogenetics.} Chromosome abnormalities were initially shown to characterize human malignancies by Nowell and Hungerford\textsuperscript{78} in 1960; shortly thereafter, RS-H cells were examined for characteristic abnormalities.\textsuperscript{79} However, the cytogenetics of HD presented particular problems. Malignant lesions have few cells, low mitotic indices, exhibit a complex karyotype, and are difficult to band.\textsuperscript{80} Thus, in Rowley's series of 25 involved lymph nodes, 10 nodes showed abnormal and only four of these yielded clones, none of which could be completely karyotyped.\textsuperscript{81} Yet, recent technical improvements have afforded an improved description of RS-H cell cytogenetics.

HD lymph nodes exhibit clonal karyotypic changes. Teerenhovi et al\textsuperscript{82} first showed that clonal chromosomal abnormalities are carried by cells expressing Leu-M1 and CD30, ie, RS-H cells. More extensive series confirmed the presence of clonal abnormalities and showed that numerical abnormalities of virtually all of the chromosomes characterize most HD cases.\textsuperscript{82-84} Structural changes also occur, although no single translocation or other abnormality predominates. Cabanillas et al\textsuperscript{85} reported cytogenetic findings consistent with those of lymphoid malignancies; abnormalities of bands 9q22-24, 11q23, and 14q32 were seen. They also reported finding a t(14;18) typical of follicular B-cell lymphoma.\textsuperscript{85} Tilly et al\textsuperscript{86} also found abnormalities typical of lymphomas, but suggested that the findings in HD more closely resemble T-cell malignancy. More recently, Poppema et al\textsuperscript{87} showed abnormal metaphases in 23 of 28 HD cases. 14q32 abnormalities were found in 6 cases, but t(14;18) was infrequent. Another recent study also showed a low frequency of t(14;18) in HD metaphases.\textsuperscript{88}

\textbf{Immunogenetics.} The same features that have made the analysis of cytogenetic changes in HD difficult have hindered molecular genetic study as well. The variable and usually minor proportion of RS-H cells in biopsy specimens has impeded investigation of their molecular pathology. Furthermore, no uniform cytogenetic abnormality has provided a convenient clue to the location of a genetic lesion, as has been the case for a number of other lymphomas.\textsuperscript{8} As a result, our understanding of the molecular genetics of HD is limited.

Most molecular analyses have involved examination of Ig and T-cell receptor (TCR) loci.\textsuperscript{89} Because these genes rearrange during lymphocyte ontogeny, they afford ready markers both for lineage and for clonality.\textsuperscript{90} A number of studies have shown that a small proportion of HD cases carry clonal rearrangements of one or more Ig or TCR loci.

The demonstration of TCR-\(\beta\) rearrangement in the HD cell line L428 first suggested that such an approach might be promising.\textsuperscript{91} Subsequently, Weiss et al\textsuperscript{92} analyzed fresh tissues for rearrangements. They initially found Ig heavy (H) and light (L) chain rearrangement in one of 16 cases of HD; however, when cases were selected for larger proportions of RS-H cells, 6 of 7 cases showed IgH or IgL rearrangements.

Other investigators have found Ig rearrangements as well, although the proportion of cases exhibiting clonal re-
arrangements varies widely, from about 10% of cases in some series to approximately 50% of cases in others. Rearrangements of IgH, IgL, or both have been found. Notably, several studies have found an IgL rearrangement carried with a germ-line IgH locus, an unusual pattern of Ig rearrangement also seen in ALCL. Some studies are negative for Ig rearrangements. It has been suggested that negative studies reflect a low proportion of RS-H cells in samples, and one group reports that enrichment of RS-H cell populations using density gradient centrifugation yields identifiable clonal Ig rearrangements. However, the intensity of rearranged bands on Southern blots often does not correlate with the size of the population of RS-H cells, and tissues with prominent RS-H cell populations are often without Ig rearrangements. It thus seems likely that the variable finding of Ig rearrangement in RS-H cells is biologic and not technical in nature.

The situation is much the same with regard to TCR rearrangements. Giessner et al first reported finding faint clonal TCR-β rearrangements in 4 of 8 cases of HD. No Ig rearrangements were seen. Later, the same group found that, in 22 cases of HD, 4 TCR-β and 15 TCRγ rearrangements were present. TCRγ rearrangement has been shown by other investigators as well, as has TCR-β.

The significance of these Ig and TCR rearrangements is unclear. Most investigators agree that these studies demonstrate clonal populations of cells, possibly corresponding to RS-H cells, in some biopsy specimens. Yet, these cells are not simply B or T lymphocytes. Although two studies have suggested that Ig rearrangements occur primarily in RS-H cells having B-cell immunophenotypes, additional studies have shown this not to be the case. At best, it can be stated that the finding of antigen receptor rearrangements in a proportion of RS-H cells supports the hypothesis that these cells derive from a lymphocytic cell.

In this light, the recent demonstration, using sensitive RT-PCR, that RS-H cells do not express the recombinase activating genes RAG-1 and RAG-2 of interest. This suggests that RS-H cells, if of lymphocytic origin, are derived from a more differentiated lymphocyte precursor that has already undergone Ig gene rearrangement.

Oncogene alterations. Analyses looking for abnormalities in a number of oncogenes have been performed. Stetler-Stevenson et al first showed rearranged bcl-1 genes caused by (14;18) translocations in 17 of 32 cases of HD. Subsequently, several other workers replicated this finding. Gupta et al found the translocation in 20% of cases; sequence analyses demonstrated the PCR products to represent bcl-2/IgH fusion in all cases. Similarly, Reid et al showed the translocation in 9% of cases. Recently, the translocation has been found in a series of cases of HD occurring in combination with follicular lymphoma. However, a number of groups have been unable to detect t(14;18) translocations by Southern or PCR analysis. This discrepancy is not understood, but is possibly the result of technical factors, including variables in the preparation of pathologic specimens and the relative underrepresentation of RS-H cells in tumors. One report failed to show consistent bcl-2 expression is RS-H cells, and suggested that bcl-2 positivity of HD cases may simply reflect the presence of small bystander B lymphocytes carrying the bcl-2 rearrangement that are found in other reactive lymphoid tissues such as tonsils. Thus, the precise frequency of bcl-2 translocations in HD is at this time not clear. Nonetheless, the association of the t(14;18) with HD is a potentially significant finding. It further supports the lymphoid derivation of RS-H cells. It also has implications for the role of EBV infection in HD, as discussed below. Its potentially important role in the pathogenesis of HD needs to be further elucidated.

The expression of other oncogenes has also been examined. The c-myc and ras proteins have been detected immunohistochemically in RS-H cells, and samples from two patients carried activated N-ras genes. No mutations were detected in 25 biopsy samples in another study. Although a number of oncogene products have been found in HD cell lines, including c-myc, p53, c-jun, c-ras, N-ras, and others, no characteristic pattern of expression has emerged.

Recent reports have investigated the tumor suppressor gene p53 in RS-H cells. High-level immunoreactivity for p53 protein using monoclonal antisera is found in RS-H cells of all HD subtypes, except nodular LP, in approximately 50% to 40% of cases. p53 overexpression did not correlate with bcl-2 expression in RS-H cells in one study. Point mutations in exons 5 and 8 of the p53 gene have been demonstrated by DNA sequencing in 1 of 6 HD cell lines, and mutation of p53 gene codon 246 has been demonstrated by single cell PCR in 5 of 7 RS-H cells examined from 4 patients with NS HD. Interestingly, in a study of Ki-1–positive ALCL, high-level p53 expression in most cases was not accompanied by mutations in p53 exons 5 through 9, suggesting alternate mechanism of increased p53 expression. The significance of these findings is unclear, but they suggest that alterations of p53 protein expression, by several mechanisms, are common in HD and ALCL.

CELL LINES DERIVED FROM HD

The establishment of cell lines from pathologic specimens of HD has, for several reasons, been technically difficult. Again, the relative dearth of malignant RS-H cells in involved tissues makes their in vitro cultivation problematic. A number of methods have been devised to enrich or purify populations of RS-H cells, including cell lines, and their salient features, are described in Table 2.

General features. The various cell lines share several characteristics. Most of them are derived from advanced cases of HD in patients who were previously treated with radiation, chemotherapy, or both. Nine of the cases were of nodular sclerosing histology. The lines have largely been
Gene rearrangement studies show that, in most cases, immunogenotype is consistent with the cell surface phenotype. The cell lines L428 and Sup HD1 exhibit rearrangement of both Ig and TCR loci (although reports differ with regard to Ig light chain rearrangement in L428). Other lines demonstrate B-lymphocyte surface phenotypes with Ig rearrangement or T-cell surface phenotypes with TCR rearrangements. Receptor gene expression is variable in B-lymphoid lines, but all of the T-lymphoid lines express TCR mRNA.

Conclusions from cell lines. For a number of reasons conclusions must be drawn from the above data with caution. The cell lines, primarily nodular sclerosing variants, many from effusions, have been isolated from samples not fully representative of the clinical spectrum of HD. They vary one from another and, taken together, they fail to define a single cell type. However, in general, the cell line data are consonant with data derived from study of RS-H cells in pathologic specimens. The cell lines express activation antigens as do RS-H cells in situ. One of these, Ki-1, was identified first in the L428 line, and subsequently shown to be widely expressed in HD tissue. With regard to lymphoid markers, pathologic specimens express B-cell antigens in a small fraction (up to 15%) of cases, and T-cell antigens more often (in up to 67% of cases), whereas each cell line expresses B- or T-lymphoid markers. Moreover, study of fresh HD specimens shows Ig or TCR rearrangements in a fraction of cases. All the cell lines exhibit one or the other.

The lymphoid characteristics of the putative HD cell lines are their most striking features. Although unequivocal proof that these lines do in fact represent cultured RS cells is unavailable, this is strongly suggested by the above comparisons. The HD cell lines thus further support the proposition that RS-H cells represent components of the lymphoid lineage.

**CYTOKINES IN HD**

Aside from the identity of the cell giving rise to the RS-H cell, several other aspects of HD remain enigmatic. Its histogenesis is not understood. Involved tissues often consist of only 2% RS-H cells, with a predominant reactive lymphocyte population, eosinophils, and varying degrees of fibrosis or sclerosis. The commonly observed paraneoplastic phenomena including B symptoms of fever, sweats, and cachexia; generalized pruritis; and immune abnormalities consisting in impaired T-cell immunity (reviewed in HJannman et al.); and a proclivity for disorders of autoimmunity such as immune thrombocytopenia all remain to be explained.

It is possible that many of these clinical features of HD are mediated through elaboration of cytokines by either the malignant H-RS cells or by the lymphocyte populations infiltrating the involved tissues. A number of cytokines have been shown to be present in H-RS cells in pathologic specimens or produced by HD cell lines.

**IL-1.** IL-1, first shown to be produced by monocytes and to facilitate lymphocyte proliferation, consists of IL-1α and IL-1β. These are produced by monocytes, lymphocytes, fibroblasts, epithelial, and endothelial cells. IL-1 is required for the induction of T-cell activation and plays a role in B-cell regulation. It may also regulate fibroblast proliferation and function as a pyrogen.

IL-1 was detected by immunoperoxidase in 20 cases of HD, representing all four histologies: no expression in B- or T-cell lymphomas was detected. Excised tumor nodules from spleens of HD patients produced IL-1 when cultured...
for periods of up to 2 months. Two of the HD cell lines, HDLM-1 and KM-H2, also produce IL-1. Using in situ hybridization, IL-1 mRNA was detected in H-RS cells in 12 of 19 cases examined in one study. Although IL-1 expression in HD has been taken as evidence of an interdigitating reticulum cell or macrophage origin for H-RS cells, this cytokine is also produced by activated B cells as well as other tissues. Thus its detection in HD tissues may not clarify the origin of the H-RS cell. IL-1 may, however, play a role in lymphocyte recruitment, in lymph node sclerosis, and in the production of fever in HD patients. However, in one study, immunohistochemical positivity of HD tissues for IL-1 did not correlate with symptoms.

**IL-2 receptor (IL-2R).** IL-2R has also been detected in HD. The receptor consists of two subunits: a low-affinity α subunit (CD25), a 55-kD glycoprotein expressed by activated T cells; and the intermediate affinity β subunit, consisting of two glycoproteins, 70 and 75 kD, that mediate membrane signal transduction and IL-2R internalization.

Immunoperoxidase studies show IL-2R in the cytoplasm of H-RS cells and on a high fraction of surrounding lymphocytes in HD tissue sections. Other investigators find similar IL-2R expression. Interestingly, in some HD cell cases, IL-2Rα (CD25), but not IL-2Rβ, positive cells formed clusters and aggregates in the primary tissue. Of note, the IL-2R is also expressed in B- and T-cell lymphomas and on scattered histiocytes in HD lesions. The HD cell line L540 similarly expresses IL-2R, as do HDLM-2 and KM-H2.

Increased serum levels of IL-2R have been found in a number of B- and T-cell malignancies, including NHL, acute lymphoblastic leukemia, chronic lymphocytic leukemia, hairy cell leukemia, adult T-cell leukemia, and Sézary syndrome. Soluble IL-2R is also elevated in HD. The serum level in children has been shown to correlate with stage, B symptoms, and, independently, with treatment failure. IL-2 itself has not been found to be expressed in HD tissues or cell lines.

**IL-3.** IL-3 is poorly studied, but in a single investigation was found to be rarely expressed by HD cell lines or RS cells.

**IL-4.** The cytokine IL-4 (BSF-1) is a T-cell–derived 68-kD glycoprotein that acts on B and T cells, natural killer (NK) cells, and monocytes. It is expressed on the HD cell line L428 and stimulates L428 cell proliferation. However, it is not expressed by two other HD cell lines. In HD tissues, one study demonstrated expression in 2 of 13 samples.

**IL-5.** Eosinophils are often a prominent component of the cellular infiltrate in tissues involved by HD. IL-5 is produced by activated T cells and may stimulate eosinophils in murine and human systems. In one study, in situ hybridization showed expression of IL-5 mRNA in the cytoplasm of H-RS cells in 16 of 16 cases of HD with eosinophils. HD cell lines also produce IL-5. These data suggest an explanation for the eosinophilia associated with HD, and also suggest a functional similarity between H-RS cells and activated IL-5-producing T lymphocytes.

**IL-6.** IL-6 is also T-cell–derived; identified originally as BSF-2, it is a 21-kD protein involved in maturation of B lymphocytes, in myeloma proliferation, and in T-cell activation, and is produced by B and T lymphocytes, monocytes, epithelial cells, and fibroblasts. IL-6 expression was found in 50% to 70% of HD tissues and in 4 of 6 HD cell lines tested. Receptors for IL-6 were expressed in 5 of 6 HD cell lines tested. Recently, IL-6 mRNA was detected by in situ hybridization in H-RS cells in 19 of 23 primary HD specimens.

**IL-9.** Recently, IL-9 has been shown to be produced in HD cells and KI-1 large cell anaplastic lymphoma. IL-9 is produced by activated CD4+ T cells and induces CD4+ T-lymphocyte proliferation. Mess et al analyzed 29 cases of B- and T-cell NHL without finding expression of IL-9; however, 6 of 13 cases of HD exhibited expression.

**Other cytokines.** Additional cytokines have been studied in relation to HD, although their expression is generally less characterized. These include IL-8, E-rosette inhibiting factor, macrophage colony-stimulating factor (M-CSF; CSF-1) and c-fms, granulocyte-macrophage CSF (GM-CSF), interferon-γ, transforming growth factor β (TGFβ), and tumor necrosis factor α (TNFα). Lymphotixin and TNFα immunostaining as well as mRNA expression can be found in H-RS cells in primary HD tissues as well as in HD cell lines; however, only LT protein is found in HD cell line supernatants. The significance of these findings is not yet well defined. The studies of cytokine expression in HD suggest several conclusions. The finding that lymphokines, including IL-2R, IL-4, IL-5, IL-6, and IL-9, are produced by HD tissues and cell lines supports the argument that RS-H cells derive from lymphocytes. Moreover, the expression of these molecules may explain several histologic peculiarities (IL-1 and fibrosis; IL-5 and eosinophilia; IL-9 and CD4+ T-cell infiltration) as well as systemic manifestations (IL-1, TNF, and B symptoms) of the disease. Ultimately, a clearer understanding of the cytokine interactions involved in HD may offer insight into the pathogenesis of this disease as well as potential strategies for therapeutic interventions.

**HD AND EBV**

The issue of the possible infectious etiology of HD is a long-standing one. Clinically, the disease may present with features of seemingly infectious origin, with fever, leukocytosis, eosinophilia, sweats, adenopathy, and hepatosplenomegaly. Thus, for many years after its description, clinicians and pathologists debated the view that HD is a response to an exogenous pathogen and not a truly neoplastic disorder. Most investigators now agree that HD indeed has neoplastic characteristics. Infectious and malignant etiologies are not mutually exclusive. A number of features of the epidemiology of HD suggest that the disease might result from infectious cofactors. Gutensohn and Cole have argued that the incidence patterns of HD reflect an uncommon consequence of a common infection. Features of the disease supporting this view include its bimodal age distribution, varia-
tions in its geographic distribution, its relation to social class,\textsuperscript{183} and its relation to childhood social environment.\textsuperscript{184}

Several viruses have been proposed as the putative infectious cofactor for HD, including cytomegalovirus\textsuperscript{185} and herpesvirus-6 (HHV-6).\textsuperscript{186,187} The most prominent candidate is EBV. EBV is clearly implicated in the pathogenesis of Burkitt's lymphoma and nasopharyngeal carcinoma. Several lines of evidence indirectly suggest a role in HD as well. First, several large cohort studies demonstrate that the risk of HD in patients with a history of infectious mononucleosis is increased up to fivefold in excess over control populations.\textsuperscript{188-193} Second, a larger than expected proportion of patients with HD exhibit elevated antibody titers against EBV capsid antigens (VCA)\textsuperscript{185,194,195}; titers to early antigen, indicative of viral activity, can also be detected.\textsuperscript{185,196} A retrospective study has shown that raising anti-EBV titers antedate the development of HD.\textsuperscript{197} These indirect data provided the rationale for attempting to detect evidence of EBV in tissues involved by HD. Initial evidence for the presence of the virus in HD lesions consisted in demonstration of the EBV nuclear antigen (EBNA) in a single case.\textsuperscript{198} Subsequently, HD lesions were shown to express EBV antigens,\textsuperscript{199-202} to carry EBV DNA demonstrable by conventional Southern or in situ hybridization techniques\textsuperscript{203-207} as well as by PCR\textsuperscript{186,200,208-214} and to contain EBV-encoded RNA.\textsuperscript{202,215,216}

A number of EBV-associated antigens have been demonstrated in HD lesions. Poppema et al\textsuperscript{208} first demonstrated EBNA in the nuclei of RS-H cells. Subsequently, several groups showed that the EBV LMP is expressed in up to 49% of HD cases. LMP is a virally encoded 60-kD transmembrane protein.\textsuperscript{217} When transfected into rodent cells, it is transforming.\textsuperscript{218} When transplanted into human B cells, it protects them from apoptosis via augmented expression of bcl-2.\textsuperscript{219,220} The induction of bcl-2 by EBV may parallel its induction by t(14;18) in NHLs, and may explain the presence of bcl-2 immunohistochemical expression in the absence of bcl-2 chromosomal rearrangement described in one report.\textsuperscript{88,221} However, several studies fail to demonstrate a firm correlation between LMP expression, bcl-2 expression, and EBV expression in H-RS cells and HD tissues.\textsuperscript{222-224} LMP expression varies among subtypes, and is found in up to 96% of MC cases, 34% of NS cases, and 10% of LP cases.\textsuperscript{199-201} EBNA-2 expression was not found,\textsuperscript{199-201} nor was expression of the VCA, early antigen (EA), or membrane antigen (MA).\textsuperscript{201}

Using various methods to detect nucleic acids, up to 79% of HD specimens have been shown to carry EBV.\textsuperscript{216} Conventional DNA hybridization techniques have demonstrated the monoclonality of EBV-infected H-RS cells.\textsuperscript{203-207} The EBV encodes approximately 500-bp tandem repeats at each of its termini, and viruses are heterogeneous in the number of repeats they carry. Thus, probing for terminal fragments may show monoclonal or polyclonal viral populations.\textsuperscript{225} HD tissues carry monoclonal EBV populations.\textsuperscript{203-207} Furthermore, in situ hybridization shows EBV in the nuclei of RS cells.\textsuperscript{204,205,207} proving the clonality of these cells as well. Taken together with the Ig and TCR rearrangement studies cited earlier, these data provide strong evidence for the monoclonality of RS-H cells.

Other hybridization methods enhance the sensitivity of EBV detection. PCR generally detects EBV in 60% to 80% of cases.\textsuperscript{186,200,208-214} EBV also encodes small RNAs, transcribed at up to 10 copies per genome, called EBERs.\textsuperscript{216} These can be detected in 50% to 75% of HD cases.\textsuperscript{186,202-216}

What is the significance of the detection of EBV in HD? It has already been pointed out that epidemiologic studies suggest a possible infectious etiology for HD; EBV infection could be responsible. Indeed, underscore this point, in developing countries such as Peru, the incidence of HD associated with EBV is higher (95% in one study)\textsuperscript{226} than in the United States. The possible transforming role of EBV LMP has been underscored. But, EBV infection may also bear on the identity of the RS-H cell of origin. EBV has generally been taken to be a B-cell pathogen, infecting mature B lymphocytes via the C3d receptor.\textsuperscript{227} But recently, EBV has been shown to be present in T cells in Kawasaki disease,\textsuperscript{228} in T-cell lymphoma,\textsuperscript{229} and in T-cell lethal midline granuloma,\textsuperscript{230} as well as in epithelial cells in nasopharyngeal carcinoma (NPC).\textsuperscript{231} Thus the presence of EBV in itself does not necessarily imply that the infected cell is of B-cell, or even lymphoid, lineage. Of note, EBV in HD expresses LMP but not EBNA-2.\textsuperscript{199,200} In NPC, expression is similar.\textsuperscript{231} However, in vitro transformed B-cell lymphoblastoid cell lines (EBNA-1 through -6 positive, LMP positive), Burkitt's lymphoma (EBNA-2 negative, LMP negative), and T-cell midline granuloma (EBNA-2 positive, LMP positive)\textsuperscript{230} all differ from NPC and HD.\textsuperscript{232} Does this imply some aspect of differentiation shared by epithelial cells and H-RS cells, or does it reflect some component of the viral transforming or latency strategy?\textsuperscript{232} These are questions for active investigation.

CONCLUSION

Each of the lines of evidence we discussed above has afforded a detailed description of the characteristics of the RS cell. In some cases it expresses antigens found on T cells; less often those found on B cells; and usually activation antigens. It can carry rearranged TCR genes, rearranged Ig loci, both, or neither. It exhibits chromosomal abnormalities, but no unique and defining aberration, and may carry a rearranged bcl-2 gene. It can express a number of cytokines, but, again, in no unique pattern. EBV appears to infect the majority of cases. Cell lines, variously reflecting most of these features, have been established. Yet, despite the extent of these descriptive data, we lack a definitive understanding of the nature and origin of the RS-H cell. At best, these characteristics describe a cell having lymphoid features.

A final avenue of investigation that may offer further insight into the nature of the RS-H cell is the emerging relationship between HD and several closely related conditions. The B-cell variant of HD is clearly represented by the nodular lymphocyte predominant histology.\textsuperscript{34} This malignancy, as described earlier, expresses the J-chain\textsuperscript{32} and the B-lymphocyte marker CD20.\textsuperscript{33} The RS-H cells of nodular LP HD are clearly malignant B cells.

A corresponding T-cell variant of HD also likely exists. Observations on the relationship between HD, lym-
phomatoïd papulosis, mycosis fungoides, and Ki-1 ALCL\(^{48,51,52,233,234}\) have suggested that these entities might share a common pathogenesis. Ki-1 ALCL has been discussed earlier. It is usually a T-cell malignancy composed of large pleomorphic cells that express CD30 and activation antigens that frequently infiltrate the skin.\(^{51,52}\) RS cells may be present in involved tissues.\(^{51}\) Lymphomatoïd papulosis is also a cutaneous infiltration, with atypical T-lymphoid cells having a resemblance to RS-H or Sézary cells.\(^{48}\) Moreover, HD, lymphomatoïd papulosis, and mycosis fungoides occur in association more frequently than expected.\(^{233}\) For instance, a patient was described who developed lymphomatoïd papulosis, HD, cutaneous T-cell lymphoma, and ALCL in succession.\(^{48}\) Examination of tissues from the patient’s consecutive diagnoses showed that clonal TCR rearrangements and a t(8;9) translocation were consistently present in the successive lesions. An abnormal T-cell clone was therefore successively manifest as each of these disorders in this patient. Thus, Kadin and others\(^ {234}\) have suggested that these disorders are closely related and represent a spectrum of T-lymphocyte malignancy.

If HD disease is truly a unique lymphoid malignancy, it is tempting to draw analogies between its pathogenesis and that of the NHLs. In particular, immunosuppression, EBV, and bcl-2, each of which may play a role in the development of HD, together act as cofactors in the development of aggressive B-cell malignancies, including Burkitt’s lymphoma (BL).\(^ {239,240}\) BL occurs endemically in equatorial Africa in the setting of hyperendemic malaria and near uniform EBV infection.\(^ {240}\) EBV promotes the expansion of infected B-lymphocyte populations, possibly through upregulation of bcl-2 expression by the viral latent membrane protein. Suppression of these infected B-cell populations is probably abrogated through the T-cell immunosuppressive effect of malaria.\(^ {241}\) The result is that the expanded B-lymphocyte pool is subject to genetic errors that may eventually result in the characteristic translocations seen in BL, and ensuing malignancy.\(^ {242}\) It is noteworthy that, in other types of NHL, parallel factors are probably operative. Other forms of immunosuppression,\(^ {243}\) including transplantation-related iatrogenic immunosuppression and HIV infection,\(^ {244}\) are associated with an increased incidence of aggressive NHL. Furthermore, bcl-2 activation obviously need not result from viral infection; aggressive NHLs carry t(14;18) translocations,\(^ {245}\) and bcl-2 activation via translocation may precede the development of a second translocation that activates c-myc.\(^ {246}\)

It is intriguing that the same factors appear to operate in HD. The roles of EBV infection and of LMP and bcl-2 expression have been detailed already. In addition, it is well known that patients with HD exhibit abnormal T-cell immunity. It is possible that these factors recapitulate those described above for BL. That is, immunosuppression may precede the development of HD, and may set the stage for an abnormal response to EBV infection, bcl-2 activation, and subsequent malignancy. In this view, the abnormal T-cell function well-documented in HD is not a consequence, but a part of the cause, of the disease. The recent observation that HIV infection confers a fivefold elevated risk of HD on HIV carriers,\(^ {245,246}\) that there is a high frequency of EBV-positive H-RS cells in HIV-associated HD,\(^ {247}\) and that HD occurs with increased frequency in other immunosuppressive states,\(^ {248}\) supports this hypothesis. Thus, in the setting of impaired immune surveillance, the expansion of a lymphoid precursor to the H-RS cell might occur. This might be facilitated by bcl-2 activation, whether by translocation or EBV infection, which in turn would enlarge the pool or H-RS precursors subject to further genetic alterations and tumor progression. This hypothesis is represented in Fig 1.

![Fig 1. Schematic representation of one hypothesis for the pathogenesis of HD. The interrelationship between immunosuppression, EBV infection, bcl-2 activation or expression, and malignancy is diagrammed.](www.bloodjournal.org)
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