To the Editor:

Recently, Naumovsky et al. characterized a cell line, SUP-HDI, derived from Hodgkin’s disease (HD). They reported that SUP-HDI cells exhibit some features associated with B or T lymphocytes. We agree with the authors’ suggestion that the SUP-HDI cell line closely resembles three other HD-derived cell lines: L428, HDLM-2, and KM-H2. L428 cells exhibit both T-cell receptor (TcR)-β and immunoglobulin (Ig) gene rearrangements (GR) and express a B-cell antigen (CD19); HDLM-2 cells have TcR-β GR and express CD2; and KM-H2 display IgH GR and express CD4. However, whether these cultured H-RS cells are derived from lymphoid cells is the subject of considerable debate. I wish to call attention to the fact that tumor cells may express markers that would not be expected to be present, and that, on the other hand, tumor cells may not express some markers that are expected to be present. Such aberrant or inappropriate expression is not unusual in the leukemias, and it can also occur in lymphomas.

We have done extensive studies on the cultured H-RS cell lines KM-H2 and HDLM-1, which is similar, if not identical, to HDLM-2. Since the expression of a few markers and the presence of GR are not absolutely specific when used for determination of the lineage of tumor cells, we wanted to study the properties of H-RS cells after they had undergone differentiation. Differentiation of cells is a process that involves multiple genes, but does not result from the action of a single gene. Thus, the evidence for cell lineage that is obtained from differentiation studies should be considered to be much more specific than that obtained from phenotypic study alone. Finding a combination of reagents that can maximally induce the differentiation of H-RS cells should provide a path that leads to an answer regarding the origin of HD. We used a combination of phorbol ester, retinoic acid (RA), and extracellular matrix (ECM) to induce the maximal degree of differentiation in the H-RS cell lines HDLM-1 and KM-H2. When these H-RS cells were treated in culture, they clearly showed a number of characteristics of histio-

Fig 1. HDLM-1 (A through C) and KM-H2 (D through F) cells can differentiate into macrophage-like cells after induction with TPA, RA, and ECM. A and D, before induction; B and E, after induction. Note the smaller nuclei and abundant cytoplasm, as well as the ruffled surface flaps or ruffles, in the induced cells. The differentiated cells are similar cytologically to cultured macrophages. Expression of monocyte/macrophage markers (eg, CD68) is clearly detectable in these cells (C and F). Original magnification: C and F, 250×; others, 1,000×.

cytes or histiocyte-like cells:

1. After differentiation, both types of cells were indistinguishable cytologically from cultured macrophages (Fig 1). The cell surface displayed rugae and elongated microvilli and projections. Also, primary and secondary lysosomal granules were detected in KM-H2 cells.

2. These differentiated cells expressed numerous markers associated with monocytes/histiocytes, such as CD11b, CD11c, CD13, CD14, CD15, CD33, CD66 (Fig 1), CD74, LN-5, M387, IEG, lysozyme, and α1-antitrypsin. They remained negative for most of the more than 25 T- and B-cell markers tested.

3. Especially after differentiation, these cells secreted several cytokines, including interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), macrophage colony-stimulating factor (M-CSF), and possibly granulocyte (G)-CSF. These cells did not produce IL-2, IL-3, GM-CSF, or interferon gamma (IFN-γ). Expression of M-CSF receptor (c-fms) was observed in early passages of KM-H2 cells. Both HDLM-1 and KM-H2 cells expressed p55 IL-2 receptors, but not p75 IL-2 receptors. This cytokine profile has been confirmed for H-RS cells in tissues.

4. Expression of cyclooxygenase was detected in KM-H2 cells and in H-RS cells in tissues. KM-H2 cells produce large amounts of prostaglandin E2. These differentiated cells expressed numerous markers associated with antigen-presenting dendritic cells. IL-2, IL-3, GM-CSF, or interferon gamma (IFN-γ). Expression of M-CSF receptor (c-fms) was observed in early passages of KM-H2 cells. Both HDLM-1 and KM-H2 cells expressed p55 IL-2 receptors, but not p75 IL-2 receptors. This cytokine profile has been confirmed for H-RS cells in tissues.

5. The H-RS cells form spontaneous T-cell rosettes, a property that is characteristic of antigen-presenting dendritic cells.

6. H-RS cells in culture and in tissues expressed ICAM-1, HLA-Dr, and LFA-3, a property shared with antigen-presenting dendritic cells. These antigens serve as receptors for either cell-associated or cell-free, LFA-1, CD3/CD4/TcR-β, and CD2 molecules, respectively.

7. Using 12-O-tetradecanoylphorbol-13-acetate (TPA)-treated H-RS cells as immunogen, we obtained a monoclonal antibody that restrictively reacted with interdigitating reticulum cells in lymphoid tissues.

The presence of one or more markers and GR, as well as the cytokines/growth factors listed above, is not completely specific when used for lineage determination. However, if we combine all of the above properties, we can conclude that HDLM and KM-H2 cells have characteristics close to those of cells of histiocyte and antigen-presenting dendritic-cell lineage. The capacity of both types of cultured H-RS cells (IgH-GR-positive and TcR-GR-positive) to differentiate along the histiocyte pathway must be viewed as indicating that H-RS cells are derived from cells of histiocyte lineage rather than of lymphoid lineage. Similarly, great caution must be applied before one can conclude that SUP-HD1 cells are related to lymphoid cells. It is inaccurate to conclude that SUP-HD1 cells are not related to histiocytes/dendritic cells based on phenotypic analysis with only three monoclonal antibodies.

The authors also speculated that the elaboration of IFN-γ by H-RS cells may be responsible for the unique clinical and pathologic features of HD. However, the production of IFN-γ by H-RS cells in tissues has not yet been confirmed. I want to comment that the HDLM-2-derived substance that is responsible for the differentiation of myelomonocytic cell lines is TNF, not IFN-γ. This differentiation effect can be neutralized with monoclonal anti-TNF antibody. Furthermore, no IFN-γ production is detected in KM-H2 and L428 cells in our laboratory.

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The literature on HD is filled with speculation about the derivation of the Reed-Sternberg (R-S) cell from lymphoid, myeloid, monocyte/macrophage, or interdigitating reticulum cells. We agree with S.-M. Hsu that HD is a controversial topic and most likely represents a spectrum of diseases.

We recently established and characterized a new HD-derived cell line SUP-HD1, which has many properties of lymphocytes based on analysis of the cell line with monoclonal antibodies, GR, and gene expression studies. Dr. Hsu writes "the presence of one or more markers, GR, as well as the cytokines/growth factors listed above, is not completely specific when used for lineage determination." He favors a histiocyte-like cell origin for the R-S cells based on differentiation studies, because "evidence for cell lineage that is obtained from differentiation studies should be considered to be much more specific than that obtained from phenotypic study alone." We suggest that differentiation studies themselves may be misleading in certain cases since tumor cells may differentiate along unexpected pathways. While differentiation studies have not been conducted on the SUP-HD1 cell line, we caution that the results of such studies may not be specific but rather dependent upon the inducing agents used. Recently, a mature T-lineage leukemia was induced to differentiate by different growth factors into either myeloid and monocyte cells or cytotoxic T lymphocytes.

Dr. Hsu presents persuasive evidence that the R-S cells in HD-derived cell lines KM-H2 and HDLM-2 may be derived from a histiocyte/dendritic cell. However, most recent studies on HD are consistent with a lymphoid origin for the R-S cells:

1. Epstein-Barr virus (EBV) genomes have been detected in R-S cells. B cells are known to be infected with EBV, but macrophages are not.
2. Improved immunophenotyping showed 19 cases of HD were negative for two different antigens found on dendritic reticulum cells.
3. Enzyme studies of HD-derived cell lines are consistent with a lymphoid origin and argue against a monocyte-histiocyte origin for these cell lines.
4. Interleukin-5 mRNA (normally produced by activated T cells) is present in R-S cells from HD with eosinophilia. We had speculated that lymphokines such as interferon gamma (found in the SUP-HD1 cell line and also normally produced by activated T cells) may be involved in the unique features of HD.
5. Gene rearrangement studies of immunoglobulin and T-cell receptor genes are most consistent with a lymphoid origin.

There is clinical and pathologic heterogeneity in HD. We speculate (as have others) that there is heterogeneity in the R-S cells and the HD-derived cell lines. The situation in HD may be analogous to that in acute lymphoblastic leukemia, where heterogeneity of the "cell of origin" of this disease (precursor B-cell, Pre B-cell, T-cell, mixed lineage cell) was elucidated after extensive studies. One of the main problems in studying HD is the minimal involvement of the biopsied tissue by the malignant cells. We hope to circumvent this problem by developing multiple cell lines representative of patients with HD to provide an unlimited number of cells for biologic studies.

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The never-ending controversies in Hodgkin's disease [letter; comment] [see comments]

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