Comment on Jones et al, page 5920

Stem cells in Hodgkin lymphoma?

Randy D. Gascoyne BRITISH COLUMBIA CANCER AGENCY

In this issue of *Blood*, Jones and colleagues take advantage of an important yet largely overlooked observation from 20 years ago that described the presence of small B cells in the Hodgkin lymphoma cell line, L428.

When these cells were plated out as single cells, they gave rise to pleomorphic large cells resembling what we recognize as Hodgkin Reed-Sternberg (HRS) cells. The work presented in this issue of *Blood* provides compelling evidence for the presence of clonotypic B cells in the peripheral blood of patients with classic Hodgkin lymphoma (cHL), building on this seminal observation by Newcom et al in 1988.[1]

Hodgkin lymphoma is unique among human cancers because the malignant cells typically comprise only 0.1% to 1.0% of the total cells in a biopsy.[2] Thus, most of what the clinician feels when palpating the abnormal lymph nodes of patients with Hodgkin lymphoma are non-neoplastic cells. This paucity of large neoplastic HRS cells has hampered our understanding of the nature of these cells, made it difficult to establish permanent cell lines, and precluded the development of useful animal models. The advent of laser-capture microdissection has proven instrumental in overcoming this obstacle to more detailed study of the molecular biology of this disease. Now, relatively purified HRS cells can be obtained from frozen sections of clinical samples and studied using molecular genetic and even genome-wide approaches following nucleic acid amplification.

Briefly, HRS cells in cHL are unique B cells that bear little phenotypic resemblance to any normal counterpart in the immune system. They do harbor clonal immunoglobulin (IG) heavy chain gene (*IGH*) rearrangements but fail to express surface IG and have essentially extinguished the B-cell transcription program. More recent evidence suggests that this is in part the result of epigenetic silencing.[3] Sequence analysis of the *IGH* rearrangements from HRS cells shows somatic mutations, thus linking the cell of origin to the germinal center. Typically, these cells express CD15 and CD30, but only a fraction show dim and variable CD20 expression.

Jones et al study 2 Hodgkin lymphoma cell lines, L428 and KM-H2, using flow cytometry and reconfirm the presence of a small population of $CD20^+$ B cells in these cultures.[4] Furthermore, they show that these B cells are clonal (lambda) and demonstrate phenotypic features consistent with memory B cells ($CD27^+$). Moreover, these cells also express $ALDH^{high}$, a reliable marker of stem cells/repopulating cells. Importantly, a limiting dilution strategy with the replating of these B cells supported the growth of both $CD20^+$ B cells and HRS cells, establishing that these cells can give rise to the characteristic HRS cells of Hodgkin lymphoma. Armed with this information, the authors then studied the biopsies and the matched peripheral blood specimens of Hodgkin lymphoma patients to determine whether similar cells are present. Using cell enrichment techniques, they demonstrate that a small percentage of $CD19^+$ clonal B cells can be found in the blood of patients with cHL. In addition, following sequencing of the *IGH* from both the blood and the HRS cells from biopsies, they showed that these cells are clonally identical. These data thus establish that clonotypic small B cells can be found in the blood of Hodgkin lymphoma patients and, in the small number of patients studied, this finding appeared to be independent of clinical stage. However, although the authors clearly establish that these cells are clonotypic, they were not able to demonstrate that they are clonogenic and thus have not met the burden of proof required to suggest that they constitute "cancer stem cells."

What are the next steps? First, these experiments should be validated by other investigators. Second, in vitro strategies need to be found that allow for the isolation of sufficient numbers of these clonotypic B cells, ideally from diagnostic biopsies, to enable proof-of-principle studies in immunodeficient mice demonstrating these cells are indeed clonogenic. Third, the clinical relevance of these cells will need to be studied from a number of perspectives, including their relationship to clinical outcome, their role in minimal residual disease detection, and the possibility that they might represent important targets for novel therapies. Jones et al raise the possibility of a link between their findings and the reported efficacy of anti-CD20 (rituximab) in cHL.[5] Although this may be a relevant mechanism in Hodgkin lymphoma, recent gene expression profiling studies in cHL suggest that targeting $CD20^+$ B cells in Hodgkin lymphoma may be counterproductive.[6] Further experiments will be necessary to resolve these potentially conflicting observations. Regardless of the outcome of such studies, the Jones et al article in this issue represents an important new step toward an improved understanding of the biology of the enigmatic cancer we call Hodgkin lymphoma.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Newcom SR, Kadin ME, Phillips C. L-428 Reed-Sternberg cells and mononuclear Hodgkin's cells arise from a single cloned mononuclear cell. Int J Cell Cloning. 1988; 6:417-431.

2. Kuppers R. The biology of Hodgkin's lymphoma. Nat Rev Cancer. 2009;9:15-27.

3. Ushmorov A, Leithauser F, Sakk O, et al. Epigenetic processes play a major role in B-cell-specific gene silencing in classical Hodgkin lymphoma. Blood. 2006;107:2493-2500.

4. Jones RJ, Gocke CD, Kasamon YL, et al. Circulating clonotypic B cells in classic Hodgkin lymphoma. Blood. 2009;113:5920-5926.

5. Younes A, Romaguera J, Hagemeister F, et al. A pilot study of rituximab in patients with recurrent, classic Hodgkin disease. Cancer. 2003;98:310-314.

6. Chetaille B, Bertucci F, Finetti P, et al. Molecular profiling of classical Hodgkin lymphoma tissues uncovers variations in the tumor microenvironment and correlations with EBV infection and outcome. Blood. 2009;113:2765-2775.