Lineage Ambiguity, Infidelity, and Promiscuity in Immunophenotypically Complex Acute Leukemias

Genetic and Morphologic Correlates

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The laboratory characterization of acute leukemia has evolved as new technologies have emerged and been applied in the routine evaluation of these hematologic malignancies. With the use of morphology, cytochemistry, flow cytometry, cytogenetics, and molecular genetics, it is possible to characterize acute leukemias in an extremely sophisticated and (ideally) therapeutically rational manner. Such routine approaches have unraveled, in part, the extreme and sometimes bewildering heterogeneity of the acute leukemias, with the identification of increasingly numerous subtypes. Much of this complexity is defined by the underlying genetic lesions, the determination of which is assuming an even greater role in the definition of specific entities. For example, more than 200 different recurrent cytogenetic abnormalities have been described in one broad category of acute leukemia, the acute myeloid leukemias (AMLs).1 Of note, these karyotypically detectable abnormalities occur in just more than half of all AMLs, with the remaining approximately 45% of these cases having normal cytogenetics. Molecular genetic insights into the latter category have accelerated in the past 5 years, with up to 80% of cases having normal karyotypes revealing fascinating submicroscopic molecular aberrations.2 This will yield additional tiers of complexity, accompanied, hopefully, by clarity of the underlying pathogenic mechanisms and, ultimately, improved therapy.

Nevertheless, the major initial decision to be made when assessing an acute leukemia is whether it is lymphoid or myeloid because this usually straightforward assignment of lineage is central to guiding remission induction chemotherapy and subsequent therapy. Whereas morphologic studies and cytochemistry were originally the primary tools used in such decisions, they have been largely if not completely superceded by immunophenotypic analysis, most often performed by flow cytometry. This allows the vast majority (>95%) of acute leukemias to be definitively classified as AML or acute lymphoblastic leukemia (ALL), with the latter quite easily further assigned to B- or T-cell lineage.3

However, the immense diagnostic power of flow cytometry may not always be absolute, and the data it provides are not always, at first pass, absolutely clear. Thus, the following become evident: (1) morphology retains its value, not only in the initial current “gold-standard” detection and enumeration of blasts, but also with an astute microscopist being able to recognize morphologic features that herald an immunophenotype or genotype; (2) immunophenotypic analysis by multiparametric flow cytometry has sometimes revealed greater complexity than what may have been desired or required for initial diagnostic purposes. Indeed, at least 20% each of AMLs and ALLs coexpress lymphoid or myeloid antigens, respectively,3 in what may be termed aberrant or cross-lineage expression. Such scenarios should not be (but unfortunately sometimes are) confused with the fewer than 5% of acute leukemia cases that have more profoundly complex immunophenotypes and that have been referred to by a variety of not always synonymous terms, including acute leukemia of ambiguous lineage, acute mixed-lineage leukemia, hybrid acute leukemia, biphenotypic acute leukemia, and acute bilineal leukemia.

Ultimately, of course, it is the genetic abnormality that largely defines the morphologic, immunophenotypic, clinical, and prognostic phenomena and, hence, the need to appreciate that acute leukemias, as with cancers in general, are genetic diseases. In fact, some morphologic findings and immunophenotypic profiles, with or without so-called cross-lineage antigen
expression, are quite intimately associated with specific underlying genetic abnormalities Table 1.\textsuperscript{4,8}

It is within this context that the study by De and colleagues\textsuperscript{9} in this issue of the Journal is of interest. The expression of B lymphoid–associated antigens, in particular CD19 and CD79a, with \textit{RUNX1-RUNX1T1} (the current approved terminology for \textit{AML1-ETO}/t(8;21)+ AMLs) is well recognized, as is the likelihood of a \textit{KIT} mutation as a “second-hit”/cooperating mutation. However, not all \textit{RUNX1-RUNX1T1}+ AMLs are CD19+, and the absence of expression of this antigen is now shown to correlate with the presence of a \textit{KIT} mutation. This suggests that other second-hit mutations (such as \textit{FLT3} internal tandem duplications in \textit{PML-RARA}/t(15;17) AML and \textit{RAS} mutations in \textit{CBFB-MYH11}/inv(16) AML) might perhaps be associated with the characteristic, but not invariably present, cross-lineage features of these AMLs (Table 1).\textsuperscript{4-8} In addition to the interesting, and sometimes still elusive, pathobiology, the immunophenotypic (and morphologic) hint that these additional genetic lesions may be present is not clinically trivial, because such additional genetic abnormalities may affect prognosis. Thus, data yielded from microscopy and flow cytometry may have a role in prompting the performance of additional assays to determine whether these often cryptic but therapeutically relevant genetic abnormalities are present.

Another noteworthy facet of this study is that it highlights the ever-expanding contexts in which CD56 is expressed in hematopathologic scenarios. Thus, it is no longer appropriate to simply term CD56 as an “NK-cell antigen” because its expression is well recognized in numerous and quite diverse contexts. These include neoplasms of plasmacytoid dendritic cells (so-called hematodermic neoplasms with their characteristic CD4+/CD56+/CD123+ immunophenotype\textsuperscript{10}), plasma cells (in which the expression of CD56 is typically expected in plasma cell myeloma, but its absence portends aggressive disease and an inferior prognosis\textsuperscript{11}), and monocytes (in which aberrant CD56 expression can help facilitate a diagnosis of a monocytic leukemia\textsuperscript{12}).

Because a significant minority of acute leukemias demonstrate the expression of antigens that are unanticipated for their designated lineage, diagnostic dilemmas are certain to occur. At what point then does one distinguish a true biphenotypic acute leukemia (BAL) from one that is unilineage but with “aberrant” coexpression of antigens traditionally characteristic of another? (The term aberrant is used here to indicate the expression of antigens that are not known to be physiologically expressed by those cells, with the assumption that their unscheduled expression is a manifestation of the neoplastic phenotype. However, this is likely to be a naive and oversimplified assumption. For example, chronic lymphocytic leukemia

<table>
<thead>
<tr>
<th>Genetic Abnormality</th>
<th>Lineage- and Maturation-Associated Antigens</th>
<th>Cross-Lineage Antigens\textsuperscript{7}</th>
<th>Associated “Classical” Morphologic Features</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute myeloid leukemia \textit{t}(8;21)/q22</td>
<td>CD13+, CD33+, CD34+, HLA-DR+</td>
<td>CD19, CD56, CD79a</td>
<td>Myeloblasts with single fine Auer rods and numerous salmon-pink azurophilic granules</td>
<td>4</td>
</tr>
<tr>
<td>\textit{t}(15;17)/q22</td>
<td>CD13+, CD33+, CD34–, HLA-DR–</td>
<td>CD2, cCD3</td>
<td>Leukemic promyelocytes, in particular, microgranular forms with cottage-leaf nuclei</td>
<td>4</td>
</tr>
<tr>
<td>inv(16)/p13</td>
<td>CD13+, CD33+, CD34–, CD56–</td>
<td>CD65</td>
<td>Regular nuclear contour, increased number of Pelger-like cells</td>
<td>5</td>
</tr>
<tr>
<td>Normal karyotype/FLT3 ITD/NPM1 mutations NOTCH1 mutations/CEBPA silencing/CEBPA mutations</td>
<td>CD13+, CD33+, CD34+, HLA-DR–</td>
<td>CD2</td>
<td>Abnormal eosinophils with basophilic granules, together with an admixture of myeloblasts and monoblasts</td>
<td>4</td>
</tr>
<tr>
<td>B-lineage acute lymphoblastic leukemia \textit{t}(4;11)/q21; q23</td>
<td>CD10–, CD19+, CD33+, Tdt+</td>
<td>CD15, CD65</td>
<td>NWD</td>
<td>4</td>
</tr>
<tr>
<td>\textit{t}(12;21)/q13; q22</td>
<td>CD10–, CD19+, CD33+, Tdt+</td>
<td>CD13, CD33</td>
<td>NWD</td>
<td>4</td>
</tr>
<tr>
<td>\textit{t}(9;22)/q34; q11</td>
<td>CD10–, CD19+, CD33+, Tdt+</td>
<td>CD13, CD33</td>
<td>NWD</td>
<td>4</td>
</tr>
<tr>
<td>\textit{t}(11;19)/q13; p13</td>
<td>CD10–, CD19+, CD33+, cμ, Tdt–</td>
<td>None</td>
<td>NWD</td>
<td>4</td>
</tr>
</tbody>
</table>

*ITD, internal tandem duplication; NWD, not well described; Tdt, terminal deoxynucleotidyl transferase; +, positive; –, negative; ++, brightly positive; +/–, often positive, may be negative; +/-, often negative, may be positive.*

Only a simple and fairly characteristic immunophenotypic expression is noted; however, this is not necessarily absolutely pathognomonic and is neither specific nor sensitive for the genotype.\textsuperscript{1}

\textsuperscript{1}Typically “lymphoid” antigens in acute myeloid leukemia or “myeloid” antigens in acute lymphoblastic leukemia.

\textsuperscript{2}Cases with \textit{NPM1} mutations also more specifically associated with aberrant localization of the NPM protein to the cytoplasm.

\textsuperscript{3}A wide spectrum of morphologic findings is seen in cases with \textit{FLT3} and/or \textit{NPM1} mutations; however, the presence of cup-like intranuclear inclusions is strongly associated with both of these genetic lesions.
was, until not so long ago, referred to as a B-cell leukemia with aberrant expression of the T-cell antigen CD5; it is now known that a normal subset of physiologic B cells are normally CD5+, with chronic lymphocytic leukemia presumably a neoplasm of such cells.)

Attempts to codify what determines that an acute leukemia is biphenotypic were proposed in 1995 by the use of a scoring system in which points (2, 1, or 0.5) are assigned depending on the power of each antigen’s lineage association.13 By using this European Group for the Immunological Characterization of Leukemias (EGIL) system, a score of more than 2 for 2 lineages is indicative of BAL. The 2001 World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues alludes to these criteria, without specifically endorsing them. Unfortunately, in the second printing of this definitive and authoritative WHO work, a typographical error crept in, with more than 2 (≥2) points being replaced with 2 or more (≥2) points.14

This error had led to the mistaken appearance of the criteria for designating BAL being relaxed. For example, in a recent report on BAL,15 the number of cases more than tripled (from 10 to 31) by applying the (incorrect) WHO criteria rather than the EGIL criteria! Even when the correct criteria are applied, there is the potential for the diagnostic waters distinguishing BAL from a well-defined AML coexpressing lymphoid antigens (or an ALL expressing myeloid antigens) to become confusingly muddied. Indeed, the strict application of the EGIL criteria to some of the specific entities in Table 1 might theoretically lead to the surely inappropriate designation of t(8;21)+ AML or t(15;17)+ acute promyelocytic leukemia (APL) (can you get an AML more myeloid than these?) as BAL. Furthermore, a provocative recent proposal using a minimalistic approach, employing only 6 antibodies, that was able to discriminate AML, B-cell ALL, and T-cell ALL from one another in 97% of cases, considered 2 major EGIL markers each assigned the maximum individual score of 2 (myeloperoxidase and CD79a) to be unnecessary.16

It is desirable (and anticipated) that the updated WHO tome, due to be published in 2008, will move away from the strict adherence to a scoring system for BAL and, perhaps, even introduce yet another term to accommodate rare trilineage cases. This diagnostic complexity and potential definitional confusion aside, one useful advantage is that the identity of aberrant acute leukemia immunophenotypes greatly facilitates the distinction of such cells from normal, physiologic hematopoietic precursors (such as blasts), and, thus, they have major value in the specific and sensitive determination of minimal residual disease.

The biologic mechanisms underlying the apparently inappropriate expression of antigens are enigmatic. Two alternative theses have been proposed. In one, termed lineage infidelity, this is considered to be a manifestation of the abnormal genetics associated with leukemogenesis. In another, the similarly interestingly termed model of lineage promiscuity, it is suggested that the leukemia is an expansion of a (? physiologic) bipotential or multipotential progenitor. Although these putative, and not necessarily mutually exclusive, mechanisms remain to be fully resolved, a number of recent studies have begun to shed some light.

APL, which, as noted, is traditionally construed of as a prototypic myeloid leukemia, evinces numerous features consistent with some commitment to T-cell differentiation.17 This is most evident in the hypogranular variant, M3v, with the presence of TRG@ gene rearrangements, expression of pre-Tα (PTCRA) and sterile TRA@ and TRG@ transcripts, and occasional expression of cCD3 protein. Of note, some of these T-cell–associated transcripts are expressed at even higher levels in these APLs than in T-cell ALLs. Furthermore, cCD3 earns a score of 2 in the EGIL system, being, along with myeloperoxidase and CD79a for myeloid and B-cell lineage, respectively, one of the highest-ranked T-cell markers. Another mechanism for AMLs to manifest T-cell features is via aberrantly activated Notch signaling, through activating NOTCH1 mutations.8 PAX5, a quintessential B-cell transcription factor, is characteristically, but not specifically or invariably, expressed in t(8;21) AML, suggesting that the aberrant expression of the B-cell antigens CD19 and CD79a in these cases is a PAX5-dependent phenomenon.18 CD79a may also be apparently inappropriately expressed in other AMLs, including t(15;17) cases and acute megakaryoblastic leukemia.19

Cross-lineage antigen expression is certainly not restricted to AML. A number of genetically well-defined B-cell ALLs are also associated with quite characteristic, although not absolutely specific, expression of myeloid antigens (Table 1). Furthermore, up to 15% of T-cell ALLs can coexpress myeloid antigens such as CD13, CD33, and/or CD117. In contrast with the scenarios with many of the AMLs noted in Table 1, however, these ALLs with characteristic and/or cross-lineage antigenic profiles are not associated with distinctive morphologic features. The fact that cross-lineage antigen expression and, indeed, bona fide BALs are much more likely to reflect myeloid/B-cell or myeloid/T-cell profiles rather than B-cell/T-cell profiles is intriguing and potentially raises questions about canonical models of hematopoiesis or at least how they may be dysregulated in malignant states.

It has thus become increasingly evident that antigens initially understood to be lineage-restricted are subsequently shown to have a much broader distribution. Whether this reflects genetic reprogramming (infidelity) and/or genetically based neoplastic transformation of a versatile progenitor cell (promiscuity) is unclear. What is becoming clear, however, is that we are gaining increasing insights into the fascinating genetic basis of acute leukemia. But, at a time when molecular
diagnostics may be construed to be adding (too much) complexity and/or potentially providing a possible threat to conventional microscopy and when immunophenotypic analysis may seem to be confounding established dogmas and fostering ambiguity regarding lineage, the role of morphologic studies ought not to be underestimated.20 Indeed, it is a testament to the sagacity of the insightful French-American-British group that more than 30 years ago identified morphologic characteristics of now genetically defined acute leukemias. It remains to be determined what roles nascent approaches such as expression profiling and proteomics will have in the evaluation of acute leukemias; depending on one’s perspective, they may add complexity or provide remarkable clarity. For now and the foreseeable future, the approach toward the diagnosis and classification of acute leukemia has to be multimodal, with the knowledge that it is the disease-defining genetic dysregulation that underlies much of the often characteristic immunophenotypic hallmarks (a term perhaps preferable to that of lineage ambiguity) and sometimes classical morphologic features.

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