MIXED LINEAGE LEUKEMIA: THE IMPLICATIONS FOR HEMATOPOIETIC DIFFERENTIATION

To the Editor:

Greaves and colleagues' recently reviewed the evidence for the violation of lineage fidelity in the human leukemias. They propose a model suggesting that "apparent cases of lineage infidelity do not reflect genetic misprogramming but rather a transient phase of limited promiscuity of gene expression occurring in normal bipotentia1 or multipotent progonitors and able to be preserved as a relic in leukemic blast cell populations that are in maturation arrest." While acknowledging that "aberrant expression may well occur in leukemia," the authors conclude that such cases occur "with insufficient regularity to be of general significance."

The simultaneous expression of genes encoding for myeloid and lymphoid differentiation demonstrated by the reactivity of monoclonal antibodies (MoAbs) with surface antigens might be criticized as antibody recognition of a cross-reacting epitope, or as a MoAb identifying an antigen that is widely expressed and not restricted to a single lineage. However, we have eliminated the possibility of a cross-reacting epitope for the E-rosette-associated antigen (T11 molecule) by immunoprecipitating this molecule from myeloblasts. Nor can the possible difficulty of widely expressed antigens explain our detection of as many as four myeloid-associated antigens on blasts from patients with acute lymphoblastic leukemia (ALL). Moreover, some cell surface antigens such as T3 normally appear late in differentiation and their presence on myeloblasts is difficult to explain on the basis of mosaic expression of genes during early differentiation.

In an attempt to determine the frequency and clinical significance of acute leukemia displaying both lymphoid and myeloid characteristics we undertook a prospective study using a large panel of lineage-associated markers which included DNA analysis. We identified myeloid-associated features in 19% of 95 consecutive cases of otherwise typical ALL and lymphoid-associated features in 25% of 28 consecutive cases that met standard criteria for acute myeloid leukemia (AML). Dual staining techniques including microsphere-conjugated monoclonal antibodies and flow cytometric analysis demonstrated that individual blast cells were simultaneously expressing lymphoid- and myeloid-associated antigens, with some cases simultaneously expressing T cell, B cell, and myeloid characteristics. We have termed such cases "acute mixed-lineage leukemia." We have now studied a total of 260 consecutive children with acute leukemia, and our early results documenting the frequency and spectrum of mixed lineage expression are being confirmed. Furthermore, we have extended our findings to the DNA level and have documented mixed gene expression in typical cases of acute leukemia and in cases of mixed lineage leukemia.

Earlier studies by McCulloch and colleagues indicated that acute leukemia with mixed-lineage characteristics confers a poor prognosis. We believe the clinical importance of acute mixed lineage leukemia is now beginning to emerge. For patients with otherwise typical morphologic features of ALL, the expression of specific myeloid-associated surface antigens correlates with a poor response to induction therapy. Furthermore, we have treated four patients with AML whose blasts expressed the T11 antigen (Tp50 molecule) that failed myeloid-directed therapy and then responded dramatically to treatment generally used for ALL.

We concur with Greaves that pluripotential progenitor cells may be able to coexpress lymphoid and myeloid differentiation-linked genes before they commit to a particular lineage, and have suggested that leukemic transformation of such cells offers an explanation for cases of mixed-lineage leukemia. In fact, mosaic expression of differentiation-associated surface antigens which function as receptors (eg, T11) may provide the means for external factors to affect lineage commitment. However, evidence suggests that other mechanisms may contribute to the high frequency of anomalous phenotypes and genotypes that we and others have identified in acute leukemias. One of these mechanisms may involve rearrangements of DNA (ie, reciprocal translocations, inversions, or partial deletions) that potentially could disrupt the sequences that normally control transcription of genes encoding specific differentiation antigens. Yusuf, for example, found chromosomal abnormalities in virtually all cases of AML he examined, while Williams et al recently reported such changes in 94% of 116 cases of ALL. In our patients expressing mixed lineage characteristics we commonly identified complex cytogenetic abnormalities. Thus an almost infinite variety of cell lineage-related phenotypes and genotypes might result from this mechanism, whether or not a pluripotent stem cell was the target of leukemic transformation.

This model of aberrant differentiation predicts that a high proportion of cases with specific chromosomal translocations that disrupt genetic control would be expected to show features of mixed-lineage leukemia. In a relatively large series of cases with the t(4;11) translocation we found mixed-lineage characteristics whenever the blast cells could be extensively analyzed. Individual blasts coexpressed myeloid- and lymphoid-associated surface antigens as well as ultrastructural characteristics of both lineages. Others have found that cases with t(1;22) abnormalities express mixed phenotypic characteristics. This information combined with our data from the t(4;11)(q21;q23) cases suggests that the q23 breakpoint on chromosome 11 may be important in the simultaneous expression of myeloid and lymphoid characteristics. Further identification of cases with...
specific chromosomal abnormalities other than 11q23 that are associated with mixed lineage leukemia would provide further support for a model based on the loss of genetic control.

Collectively, our findings indicate that unusual combinations of myeloid and lymphoid cell lineages are more common in acute leukemia than has been generally recognized or suspected, and that more than one mechanism may be responsible for their genesis. We propose the general term "mixed-lineage leukemia" in place of "biphenotypic leukemia" (implying two populations of cells), "hybrid phenotype" (suggesting a fusion of elements), or "lineage infidelity" (which suggests aberrant gene expression). Extension of mixed-lineage characteristics to the genomic level in a number of articles limits the accuracy of earlier terms such as "mixed lymphoid-myeloid phenotype" or "polyphenotypic leukemia." Since lineage promiscuity makes no provision for genetic misprogramming, we feel it adds an unnecessary term to an already complex literature and would object to its application to cases such as ours. Because we believe both mechanisms, genetic misprogramming and mosaic gene expression, may be responsible for the genesis of mixed-lineage leukemia, molecular studies of such cases might provide important information about hematopoietic differentiation.

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REFERENCES

8. Kitchingman GR, Bunin N, Mirro J, Murphy SB, Goorha RM: Rearrangement of the γ- and β-chain gene of the T cell receptor in acute leukemias of non-T cell lineages. (submitted)

To the Editor:

Our review article1 was written to provoke debate and we welcome the response from Mirro and colleagues,2 particularly because they have contributed much of the primary data relevant to the subject under discussion. There are three distinct issues which are somewhat intertwined in the letter from Mirro et al; we feel it is important to consider them separately. First, Mirro et al prefer the term "mixed lineage" leukemia to describe acute leukemias in which markers of more than one lineage are expressed on individual blast cells, and they disapprove of our term "promiscuity"2 in this context. Of course, we never suggested in all seriousness that such leukemias should be labeled as "promiscuous" acute leukemias; this was, as most will realize, a tongue-in-cheek term coined in response to the infidelity argument3,4 and was used to help encapsulate a potential biological explanation for mixed phenotypes. We currently refer to such leukemias operationally as "unclassifiable" and use the term "mixed lineage" for those cases in which two or more leukemic cell populations belonging to different lineages coexist.

Second, Mirro and colleagues emphasize that "mixed lineage" leukemias may have an inferior prognosis as suggested earlier by Smith et al.4 From the data presented this seems likely to be correct for those cases diagnosed as AML but not (overall) for ALL with "myeloid" markers.4 We acknowledged in our review1 that this association might well exist but emphasized that it has no bearing on the issue of whether "mixed lineage" gene expression reflects aberrant or normal differentiation.

Third, Mirro et al conclude, as we did, that multiple mechanisms
Mixed lineage leukemia: the implications for hematopoietic differentiation [letter]

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