Clonal Remissions in Acute Nonlymphocytic Leukemia: Evidence for a Multistep Pathogenesis of the Malignancy

By Philip J. Fialkow, Johannes W.G. Janssen, and Claus R. Bartram

Two types of clinical remission have been identified in acute nonlymphocytic leukemia (ANL). In one form, remission is characterized by repopulation of the marrow by normal stem cells; in the other type, the abnormal clone found at diagnosis persists throughout remission. Among the explanations for the latter occurrences ("clonal remissions"), the hypothesis we favor is that in some patients ANL has a multistep pathogenesis with a preleukemic phase. Induction chemotherapy ablates overtly leukemic cells, thereby inducing a clinical remission. However, the marrow is not entirely repopulated by normal progenitors but by varying numbers of clonal, "preleukemic" stem cells.

Two other explanations have been advanced for clonal remissions. The first is that they are not truly clonal, but result from non-neoplastic selective overgrowth of one population of cells. Two sets of observations make this hypothesis very unlikely to be correct: (1) extreme selective overgrowth of blood cells is uncommon in normal people. We found evidence for it in only 2 of 241 women without hematopoietic disease (and Fialkow PJ: unpublished observations). (2) Clonal remissions are not rare in ANL. In the three reported studies, clonal remissions were found in 4 of 13 patients, 3 of 13 patients, and 8 of 31 patients (Bartram CR, Janssen JWG: unpublished observations).

A second postulated explanation for clonal remissions is that they result from repopulation of the marrow by one of a relatively few normal stem cells that survive the chemotherapy used to induce the leukemia remission. Data from studies of retrovirally marked cells in a murine transplantation model demonstrate that the marrow can be reconstituted by one or a few stem cells in mice. However, studies in humans indicate that after marrow transplantation many stem cells contribute to hematopoiesis. The purpose of this communication is to review data that indicate that clonal remissions in patients with ANL do not result from repopulation of the marrow by one or a few normal stem cells that survive the induction therapy.

The systems used to study clonality exploit the naturally occurring cellular mosaicism present in all females by virtue of X-chromosome inactivation. Early in embryogenesis, with the exception of a small region, one of the two X chromosomes in each XX somatic cell becomes inactivated. The determination of which X will be inactive is made at random but, once it is made, it is fixed in all offspring of the cell. Therefore, adult women are composed of two cell populations: in one population the paternally derived X (Xp) is active, whereas in the other the maternally derived X (Xm) is active. By definition, a clonal leukemia develops from a single cell and, therefore, consists entirely of Xp or Xm cells, whereas normal hematopoietic tissue consists of a mixture of Xp and Xm cells.

In most of the studies conducted by Fialkow et al., the X-chromosome-linked enzyme glucose-6-phosphate dehydrogenase (G6PD) was used as the cell "marker." Because the G6PD locus undergoes inactivation, females who are heterozygous for the usual gene, Gda, and the common variant, Gdb, have two cell populations: in one of them, Gdb is active and type B G6PD is made; in the other, Gda is active and type A enzyme is produced. A neoplasia that develops from a single cell (i.e., a clonal tumor) in a Gdb/Gda heterozygote shows only one G6PD, whereas the tissue from which it arises manifests both G6PDs.

A similar rationale underlies the studies conducted by Fearon et al. and Bartram et al., but rather than using G6PD (a gene product) as the cell marker, polymorphisms in the DNA sequences of the genes themselves are used. The strategy, which is more complex but more widely applicable than the G6PD method, uses recombinant DNA techniques to detect polymorphisms in DNA sequences known as restriction fragment length polymorphisms (RFLP). The two X-linked genes that thus far have been used most frequently in X-chromosome inactivation mosaicism clonality studies code for the enzymes phosphoglycerate kinase (PGK) and hypoxanthine phosphoribosyl transferase (HPRT), respectively.

Included in this analysis are all 12 patients with clonal remissions known to us who were heterozygous for G6PD or an RFLP and were tested both at diagnosis and in remission (and Bartram CR, Janssen JWG: unpublished observations). Clinical features and G6PD and DNA results for the 12 patients are given in Table 1. At the time of inactivation, a given cell has an equal chance to have an active Xm or an active X. Thus, in a G6PD heterozygous woman, on average, the probability in a given cell that Gdb is active (and, therefore, that G6PD A is synthesized) is 50%. Similar reasoning applies to the DNA markers. The hypothesis that the basis for clonal remissions is reconstitution of the marrow by one surviving normal stem cell that by chance alone has the same active G6PD gene (or DNA RFLP) as was present in the leukemic clone at diagnosis predicts that, on average, half of the time the G6PD (or DNA) types in remission at presentation will differ. In fact, the phenotypes of the clones were the same at presentation and during clonal remission in each of the 12
patients. The probability that this occurred by chance alone is <0.001 (1/2^25). Thus, the stem cell depletion hypothesis is very unlikely to be correct.

We think the explanation for clonal remissions in ANL is that, as in chronic myelogenous leukemia, ANL has a multistep pathogenesis with a preleukemic stage in some patients. The clearest evidence for this is provided by studies of patient 3. At presentation, the leukemic blast cells had a specific complex chromosomal aberration. As anticipated, this cytogenetic abnormality was not detected during remission; nonetheless, G6PD studies demonstrated that the remission was clonal. It seems likely that chemotherapy ablated the overtly leukemic, cytogenetically abnormal cells, but spared some preleukemic clonal stem cells that lacked the chromosome abnormality. The latter cells repopulated the marrow and differentiated to mature granulocytes, red blood cells, and platelets. Thus, at least two steps were apparently involved in the pathogenesis of this patient’s leukemia: an early step causing clonal proliferation of hematopoietic stem cells and a later step resulting in a chromosomal abnormality in a descendant of these progenitors. The cytogenetic aberration presumably conferred on that cell a proliferative advantage that led through subclonal evolution to overt leukemia in which the chromosomally abnormal cells dominate the marrow. It is likely that the proliferative advantage enjoyed by the chromosomally abnormal stem cell was related to oncogene activity.

Similar conclusions are suggested for another patient with a clonal remission on the basis of studies of the N-ras oncogene (Table 1, patient 5). At presentation, a mutation on N-ras was found in the leukemic cells, but during remission the mutant allele was not detected.* These findings suggest that occurrence of the ras mutation was a late event. Presumably, treatment used to induce remission ablated “ras-positive” overtly leukemic cells; surviving clonal, preleukemic “ras-negative” stem cells then repopulated the marrow and underlay the patient’s clonal remission.

The clinical implications of clonal remissions are not fully known and can best be elucidated by prospective studies of large numbers of patients. However, the data currently available suggest that clonal remissions are not always associated with rapid relapses (eg, patients 5 and 6) and that even restoration of a normal karyotype or a normal ras genotype in marrow cells may not be a sufficient criterion to define a true biologic remission (eg, patients 3 and 5, respectively).

**REFERENCES**


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Table 1. Findings in 12 Patients With Clonal Remissions of ANL

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (y)</th>
<th>FAB Class</th>
<th>Cell Sample</th>
<th>G6PD* or PGK† (active allele)</th>
<th>Cell Sample</th>
<th>Duration* (mo)</th>
<th>G6PD* or PGK† (active allele)</th>
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Abbreviations: FAB, French-American-British classification†; PB, peripheral blood; BM, bone marrow; CCR, complete continuing remission; unpubl., unpublished observations.

*The two types of G6PD are designated A and B.
†The two PGK alleles are designated 0.9 kb (kilobases) and 1.05 kb according to the length of the polymorphic DNA restriction fragment.
‡Months after initial diagnosis.
§This patient was also studied at months 1 through 5 with similar results.
∥This patient was also studied at months 1, 2, 3, 26.5, and 27.5 with similar results.


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PJ Fialkow, JW Janssen and CR Bartram