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Benign hematopoietic progenitors in chronic myeloid leukemia: current status and future prospects

Received 2 March 1994 / Accepted 7 June 1994

Summary Many patients with chronic myeloid leukemia (CML) retain a certain degree of normal hematopoiesis at disease presentation. This fact, suspected on the basis of cytogenetic findings, has been confirmed by long-term bone marrow cultures (LTBMC) and the combined use of phenotypic and molecular studies. Based on the lack of HLA-DR expression, it has been possible to recognize a benign subpopulation within the stem-cell compartment in CML. Different in vitro techniques have been developed for the selection of these benign progenitors, including LTBMC, marrow incubation with cytolytic drugs or interferon, positive selection based on their phenotypic characteristics, and exposure to synthetic antisense oligodeoxynucleotides. In vivo selection with interferon or intensive chemotherapy is also possible. The primary goal of the selection of benign hematopoietic progenitors is their use for autologous transplantation. To date, a few hundred CML patients have been submitted to the latter procedure using bone marrow or peripheral blood. The fact that the majority of them show evidence of persistent disease emphasizes the necessity for better selection methods of the benign progenitors, for intensifying the conditioning regimen to reduce the tumor burden as much as possible, and for the use of adjuvant therapy post-transplantation. Future trends include the refinement of positive selection methods, negative selection by taking advantage of the different stromal adhesiveness of the benign and malignant progenitors, or the use of autologous natural killer cells, antisense oligodeoxynucleotides, or specific antibodies to the bcr/abl junction region, and retroviral marking to determine the origin of relapse in autologous transplantation.

Key words Chronic myeloid leukemia · Benign hematopoietic progenitors · Autologous transplantation

Introduction

The fact that many patients with chronic myeloid leukemia (CML) have normal hematopoietic cells in their bone marrow and peripheral blood at diagnosis has only recently been well established. However, this has been known for many years on the basis of cytogenetic findings. The first evidence came from the observation that some bone marrow cells were Ph negative at CML presentation in some patients [7, 39, 49], and also from reports of isolated patients who became Ph negative, often for a prolonged time, following busulfan-induced bone marrow aplasia [32]. Later on, the analysis of large series of conventionally treated CML patients showed that approximately one quarter of them displayed a proportion of normal metaphases at routine bone marrow cytogenetic study [14, 63]. In most cases, Ph mosaicism was observed either at disease presentation or within 2 years from diagnosis, although in some instances the Ph-negative cells were detected in the more advanced chronic phase and even at blast crisis. The use of intensive chemotherapy in the treatment of CML led to the observation of cytogenetically normal marrow cells emerging in a proportion of patients [11, 38, 43, 45, 59, 62]. More interestingly, the study of one such patient, a woman who was also heterozygous for the glucose-6 phosphate dehydrogenase (G-6PD) enzyme, strongly suggested the nonclonal origin of the emerging Ph-negative cells [61]. Further evidence of the presence of normal hematopoietic stem cells in CML patients has resulted from treatment with interferon over the past 10 years, since this therapy often leads to a partial or, less frequently, complete restoration of Ph-negative hematopoiesis [67]. Finally, return of Ph-negative hematopoiesis, usually transient but sometimes prolonged, following autografting with un-
manipulated Ph-positive bone marrow or peripheral blood [8, 37, 44, 58] represents additional evidence that many CML patients retain a certain degree of normal hematopoiesis.

**Benign and malignant hematopoietic progenitors in CML**

CML is considered a clonal disorder arising in a pluripotent stem cell common to all hematopoietic lineages. Indeed, the Ph abnormality, characteristic of CML, can be found in all myeloid progenitors in the bone marrow, whereas additional G-6PD enzyme studies demonstrate the clonality of Ph-positive hematopoiesis [31]. Furthermore, B lymphocytes are part of the CML neoplastic proliferation [50], and it has been shown that some T lymphocytes may also originate from the malignant Ph-positive stem cell [29, 42]. Using long-term bone marrow cultures (LTBMC), the Vancouver group has demonstrated the existence of a previously undetectable population of cytotogenetically normal hematopoietic cells in CML [17]. Thus, when the bone marrow of newly diagnosed CML patients is submitted to LTBMC, a progressive decrease in Ph-positive progenitors is seen, leading to the final disappearance of the malignant cell population and the appearance of Ph-negative progenitors, which can be the only detectable population. Subsequent studies from the same investigators showed similar findings in some already treated CML patients [24]. Moreover, the study of benign progenitors emerging from LTBMC established with CML bone marrow demonstrated their nonclonal origin [24, 25, 40].

In recent years, the development of techniques for bone marrow cell separation has allowed the recognition within the CML stem-cell compartment of two populations, which can be separated on the basis of their different HLA-DR expression. Indeed, Verfaillie et al. [74] demonstrated that the bone marrow of CML patients contains a predominant CD34-positive/DR-negative cell population which has the morphological appearance of large blast cells, exhibits both the Ph chromosome and its bcr/abl molecular equivalent, and gives rise to Ph-positive hematopoietic colonies when plated in a LTBMC system. They also observed that a smaller population of CD34+ bone marrow cells exists which not only lack antigens associated with myeloid and lymphoid lineages, but also fail to express the HLA-DR antigen. These CD34+/HLA DR- cells are small lymphocyte-like blasts, thought to be benign since, unlike the malignant Ph-positive primitive progenitors, when submitted to LTBMC they result in the growth of secondary colonies harboring neither the Ph chromosome nor its molecular equivalent. Such benign progenitors, which have the same phenotype as the most primitive progenitors isolated from normal bone marrow [2, 64], share with the latter the capacity to generate benign secondary clonogenic progenitors (the so-called LTC-initiating cells or LTC-IC) when cultured in LTBMC, although in a significantly lower number. This latter finding has been explained through the possibility that benign CML progenitors could be in a more quiescent state than their normal counterparts, or that their number is actually reduced. A more recent study, however, has failed to find such a clear-cut separation between the malignant and benign CML hematopoietic cells on the basis of DR expression. Thus, Leemhuis et al. [48], using PCR techniques, observed bcr/abl-positive cells in nine CD34+/DR- post-sorted populations, as well as in two of five LTBMC initiated with these populations. It seems, therefore, that the benign hematopoietic progenitors in CML are preferentially located within the CD34+/DR- bone marrow population, and that selection for the latter may be an appropriate primary enrichment step for such progenitors.

Besides differences in phenotype and behavior in LTBMC, other differences have been demonstrated between malignant Ph-positive progenitors and both CML benign progenitors and normal hematopoietic progenitors [27, 36, 73]. Some of these differences probably account for the increased turnover of Ph-positive progenitors and possibly contribute to their expansion in vivo. Thus, it is now known that adhesion to bone marrow stroma may have a negative regulatory role in normal hematopoiesis [13]. Because of this, the defective adhesiveness found in the CML malignant progenitors may contribute to the lack of regulation of their growth by the marrow microenvironment. A decreased capacity to adhere to normal stroma layers [36, 73], fibronectin and its proteolytic fragments [73], and an increased adhesion to the basement membrane components laminin and collagen type IV have been shown in the Ph-positive progenitors [73]. Since they have a normal number of alpha 4, alpha 5, and beta 1 integrin receptors, important for the adhesion of normal progenitors to bone marrow stroma, the reduced stromal adhesion has been ascribed to an impaired receptor function [67]. According to recent studies, interferon might overcome this defective stromal adherence by changing the neuraminic acid composition of the abnormal layer [23], by increasing the expression of the surface cytoadhesion molecule termed lymphocyte function-associated antigen (LFA3), which is reduced in CML progenitors [21], or through the correction of the abnormal beta integrin receptor function [6]. On the other hand, and in contrast to what happens in normal individuals, in CML patients the Ph-positive clonogenic cells are localized predominantly in the peripheral blood rather than in the bone marrow [27]. Therefore, it seems reasonable to assume that the above-mentioned alterations in the adhesive properties of the Ph-positive progenitors may be responsible for their premature release into the peripheral circulation. As the behavior of CML benign progenitors parallels that of the normal hematopoietic progenitors with regard to adhesion to normal stroma, it would then be theoretically possible to take