Chronic myelogenous leukemia: molecular and cellular aspects

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Abstract Chronic myelogenous leukemia (CML) originates in a pluripotent hematopoietic stem cell of the bone marrow and is characterized by greatly increased numbers of granulocytes in the blood. Myeloid and other hematopoietic cell lineages are involved in the process of clonal proliferation and differentiation. After a period of 4–6 years the disease progresses to acute-stage leukemia. On the cellular level, CML is associated with a specific chromosome abnormality, the t(9; 22) reciprocal translocation that forms the Philadelphia (Ph) chromosome. The Ph chromosome is the result of a molecular rearrangement between the c-ABL proto-oncogene on chromosome 9 and the BCR (breakpoint cluster region) gene on chromosome 22. Most of ABL is linked with a truncated BCR. The BCR/ABL fusion gene codes for an 8-kb mRNA and a novel 210-kDa protein which has higher and aberrant tyrosine kinase activity than the normal c-ABL-coded counterpart. Phosphorylation of a number of substrates such as GAP, GRB-2, SHC, FES, CRKL, and paxillin is considered a decisive step in transformation. An etiological connection between BCR/ABL and leukemia is indicated by the observation that transgenic mice bearing a BCR/ABL DNA construct develop leukemia of B, T, and myeloid cell origin. CML cells proliferate and expand in an almost unlimited manner. Adhesion defects in bone marrow stromal cells have been proposed to explain the increased number of leukemic cells in the peripheral blood. However, findings of our laboratory have shown that the BCR/ABL chimeric protein that is expressed in transfected cells may, under certain conditions, also increase the adhesion to fibronectin via enhanced expression of integrin. Our previous immunocytological studies on the expression of β1 and β2 integrins have found no qualitative differences between normal and CML hematopoietic cells in vitro. Even long-term-cultured CML bone marrow or blood cells continuously express those adhesion molecules that are characteristic of the cytological type. Recent experiments indicate that certain early CML progenitors may adhere to the stromal layer in vitro similarly to their normal counterparts. They cannot be completely removed by long-term culture on allogeneic stromal cells. At present, the only curative therapy is transplantation of allogeneic hematopoietic stem cells. Based on the molecular and cellular state of knowledge of CML, new therapies are being developed. BCR/ABL antisense oligonucleotides, inhibitors of tyrosine kinase, peptidespecific adoptive immunotherapy or peptide vaccination, and restoration of hematopoiesis by autologous stem cell transplantation following CML cell purging are examples of important approaches to improving CML treatment.

Key words Chronic myelogenous leukemia · Philadelphia chromosome · BCR/ABL fusion gene · BCR/ABL tyrosine kinase · Adhesion molecules · CML long-term culture

Abbreviations CML chronic myelogenous leukemia · ALL acute lymphoblastic leukemia · IL interleukin · FISH fluorescent in situ hybridization

Introduction

Chronic myelogenous leukemia (CML) is a proliferative disease of the hematopoietic system. It is characterized by clonal expansion of a primitive pluripotent stem cell that has the capacity to differentiate into the myeloid, monocyte, megakaryocyte, and erythrocyte lineages. Even B and T cells are likely to be involved.

CML is a multistage disease. In the initial chronic phase, granulocytes are increased in number 10- to 100-
fold in the blood. Immature cells appear in the accelerated phase, which may precede the terminal stage, an acute leukemic blast crisis. Generally, the median survival time after diagnosis is 4–6 years. The only curative therapy is transplantation of allogeneic bone marrow or peripheral stem cells from HLA-identical siblings or HLA-compatible unrelated donors. During disease progression, multiple genetic changes take place at the cellular level. At diagnosis in about 90% of patients, the majority of bone marrow metaphases show a karyotype marker, the Ph chromosome, which is the result of a translocation involving chromosomes 9 and 22. A fusion gene, BCR/ABL, is formed, which is probably causally related to CML. About 10% of patients with the hematological diagnosis CML do not show the Ph chromosome. In one-third of these patients, the BCR/ABL rearrangement or the gene products are detectable by molecular methods [Southern blotting, reverse transcription/polymerase chain reaction (CPCR), Western blotting, fluorescent in situ hybridization (FISH)]. The initial characteristics, clinical course and response to therapy do not differ between Ph-negative/BCR/ABL-positive and Ph-positive patients. Seven per cent of CML patients do not carry the Ph translocation. At diagnosis, BCR/ABL-negative patients have lower leukocyte, hemoglobin, and platelet counts, lack basophilia, and have more blasts in their peripheral blood and bone marrow than do BCR/ABL-positive patients. The median survival of BCR/ABL-negative patients is only about 1.5 years.

Variant cytogenetic forms and complex Ph translocations have been observed. Additional chromosomal abnormalities appear in 80% of blast crisis patients. That the BCR/ABL gene contributes to CML development is deduced from the observation that leukemia-like syndromes develop in mice transfected with BCR/ABL by retroviral constructs. There are two major forms of BCR/ABL proteins, p190 and p210, showing tyrosine kinase activity. They are associated with different forms of leukemogenesis. Expression of p190 is restricted to Ph+ acute lymphocytic leukemia (ALL) almost entirely whereas p210 may be present both in Ph+ ALL and in CML.

At the beginning of CML and in the early chronic phase, normal hematopoiesis coexists with the leukemic clone. The mechanism by which suppression of normal hematopoiesis occurs over time is not yet known. In vitro studies indicate that normal hematopoietic cells have a growth advantage over leukemic cells. Obviously CML stem cells adhere less well to stromal layers in tissue culture. Furthermore, CML cells are less dependent on growth factors. Altered adhesive interactions with stromal cells as well as other phenotypic differences between CML and normal progenitors may give rise to techniques that might allow separation in vitro of the two cell compartments. Purged autologous stem cells for transplantation would then allow a new therapeutic approach to treating CML.

Although CML belongs to the best-characterized neoplastic diseases, molecular and cellular studies have not yet achieved a decisive step towards the ex vivo removal of leukemic stem cells or to the large-scale production in vitro of normal hematopoietic cells.

In the following, a survey of selected data is presented on the characteristic features of Ph+ CML cells with specific relevance to normal hematopoiesis in CML. It should be emphasized that the sections that subdivide the article cover important but not all aspects of the broad field of CML biology.

**Clinical background**

Busulfan has been the principal drug in CML therapy for the past 40 years. The median survival times of treated patients ranged from 35 to 47 months. In a randomized study a statistically significant survival advantage for hydroxyurea-treated patients was observed in all groups at risk from CML, showing that hydroxyurea is superior to busulfan in the therapy of CML in the chronic phase. The median survival in the busulfan-treated group was 45 months, and 58 months in the group treated with hydroxyurea (Hehlmann et al. 1993). This observation was confirmed in a subsequent analysis of randomized studies (CML Trialists Collaborative Group 1997).

Cytoreduction of the leukemic clone to a degree comparable to that brought about by hydroxyurea can also be achieved by interferon α (IFN), which was introduced for CML therapy by the Talpaz group in 1983 (Talpaz et al. 1986). In a minority of patients durable remissions have been observed. More than 80% of patients achieving major cytogenetic responses1 are alive after 7 years (Kantarjian et al. 1995). Several studies and a meta-analysis2 of randomized IFN studies confirm that IFN prolongs survival time in CML patients by about 20 months (Tura et al. 1994; Hehlmann et al. 1994; Kantarjian et al. 1996; CML Trialists Collaborative Group 1997). The survival advantage conferred by IFN seems to be most apparent in low-risk patients (Hehlmann et al. 1997). Apparently the in vivo response to IFNz is a dominant treatment-associated prognostic factor. Although some progress can be seen, CML treatment by drugs and biological response modifiers remains non-curative. CML is heterogeneous with respect to clinical and biological characteristics.

**Expansion of the CML clone**

The clonal origin of CML was demonstrated by Fialkow et al. (1977), who used the X-linked glucose-6-phosphate

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1 Major cytogenetic responses include complete (0–1% Ph-positive metaphases) and partial (<35% Ph-positive metaphases in bone marrow specimens) cytogenetic responses

2 Meta-analysis in this context means a worldwide collaborative overview of several randomized clinical trials