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Disease Progression in a Murine Model of *bcr/abl* Leukemogenesis

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We have developed a system for expressing *bcr/abl* genes in the mouse hematopoietic system utilizing retroviral gene transfer and bone marrow transplantation. Expression of the P210*bcr/abl* gene in mice gives rise to a spectrum of hematological malignancies, most prominently a myeloproliferative syndrome which closely resembles human chronic myelogenous leukemia (CML). Studies of this system and related systems in other laboratories have begun to yield insights into the pathophysiology of the human *bcr/abl* leukemias. The CML-like syndrome appears to be a consequence of infection of a multipotential hematopoietic progenitor target cell. The leukemic clone is difficult to transplant to secondary recipients, but undergoes evolution to acute leukemia. The P190 form of *bcr/abl* appears to be more potent in leukemogenesis than P210, but may also be associated with a CML-like picture upon infection of a multipotential target cell. There may be a spectrum of different chronic phase duration associated with different Bcr/Abl proteins, with *bcr* sequences influencing the rate of disease progression. In mice, duplication or alterations of the *bcr/abl* gene itself may constitute a major mechanism of disease progression.

KEY WORDS: Murine model Bcr-Abl leukemia disease progression

INTRODUCTION

The *bcr/abl*-associated leukemias are among the most intensively studied human malignancies, and provide perhaps the best available system for understanding leukemogenesis. The *bcr/abl* translocation is found in leukemic cells from nearly every patient with the myeloproliferative disease chronic myelogenous leukemia (CML), and is also present in approximately 30% of cases of adult acute lymphoblastic leukemia (ALL), 10% of pediatric ALL, and about 1% of adult acute myelogenous leukemia (AML). A great deal has been learned about the biochemistry and genetics of transformation by *bcr/abl* genes by expressing these genes in various tissue culture systems, but full understanding of their leukemogenic properties can only come from studying their expression within the hematopoietic system of a living animal. We have recently described a system for expressing activated *abl* genes in mouse bone marrow utilizing retroviral gene transfer and bone marrow transplantation. We have used this system to express one of the activated *abl* genes, the $P210^{bcr/abl}$ gene of the Philadelphia chromosome of human CML, in the hematopoietic system of mice and observed a lethal myeloproliferative syndrome with a striking resemblance to the chronic phase of human CML. Studies in this system and by other laboratories in related systems have begun to yield insights into the molecular pathophysiology of the human *bcr/abl* leukemias. Here I will summarize some recent results from expressing *bcr/abl* in mice which pertain to disease progression.

It now appears clear that the essential pathophysiological feature of CML, that of elevation of myeloid cells with preservation of differentiation, can be recapitulated in mice by retroviral transfer of the $P210^{bcr/abl}$ gene.[1–3] The bone marrow target cell for the induction of a myeloproliferative syndrome appears to be an early multipotential hematopoietic progenitor cell, perhaps the pluripotent stem cell itself. As with human CML, there is a paradox in that the presence of *bcr/abl* in stem or multipotential cells is required, but the major phenotypic abnormality is restricted to the neutrophil compartment. This remains unexplained, but suggests a requirement for *bcr/abl* action very early in myeloid development. P210 appears to have the ability to provide a growth stimulus

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to the malignant clone without causing the profound block in differentiation associated with acute leukemia. This is borne out by studies in several *in vitro* and *in vivo* systems, demonstrating the P210 can transform (and possibly immortalize) lymphoid[4] and myeloid,[5,6] progenitors without blocking the ability of these lineages to differentiate. In mice, the CML-like syndrome can be transplanted to secondary recipients, but with a low efficiency, suggesting that the *bcr/abl* stem cell clone is a minor part of the stem cell compartment or that it is at a competitive disadvantage in repopulation after transplantation.[7] These data suggest that approaches involving high-dose conventional chemotherapy, possibly combined with autografting, deserves further investigation in human CML patients who are not candidates for allografting.

While we have not yet observed a transition to acute leukemia in primary animals with the CML-like syndrome, all such animals appear to die of the overwhelming granulocytosis, and may not survive long enough for disease progression to take place. It is possible that treatment of these animals with myelosuppressive agents such as hydroxyurea may prolong the so-called "chronic phase" and allow the appearance of acute leukemias. However, we have observed the development of acute leukemias in secondary mice which are recipients of bone marrow from primary animals with the CML-like syndrome.[7] These leukemias may be of lymphoid or myeloid origin, and share the same proviral integration marker as the original CML clone, proving that they are derived from the same clone. Thus, these events represent the progression of the leukemic clone itself, propagated by serial transplantation, to blast crisis.

While P210 is found in leukemic cells from all human patients with chronic phase CML, some patients with Ph[1]-positive ALL have recently been shown to have a distinct Abl-related protein of molecular weight 185 to 190 kilodaltons.[8] Molecular cloning and sequencing of cDNAs from these patients have revealed a novel form of the *bcr/abl* translocation, with the breakpoint on chromosome 22 in the first intron of the *bcr* gene instead of the usual 5.8 kb breakpoint cluster region.[9] This leads to expression of a 190 kDa fusion protein consisting of sequences from the first exon of the *bcr* gene joined to the second *abl* exon, differing from P210, which contains sequences from the first 11 or 12 exons of *bcr* in the hybrid protein. Like P210, $P190^{bcr/abl}$ is highly active as a tyrosine kinase. Because the P190 form of *bcr/abl* has been found in cases of Ph[1]-positive AML, it appears that the P190 protein is not specifically linked to lymphoid leukemia. However, it may be that the P190 form of *bcr/abl* is associated only with cases of *de novo* acute leukemia, since P190 is rarely if ever seen in the chronic phase of CML. While all documented cases of CML have traditional breakpoints in the 5.8 kb bcr region giving rise to the P210 form of *bcr/abl*, some patients who present with Ph[1]-positive ALL and AML also have traditional bcr breakpoints and express P210. As a rule, these patients have persistence of the Ph[1] chromosome in remission, and are probably cases of CML presenting in blast crisis after an unrecognized chronic phase. In contrast, most ALL patients with the more 5′ breakpoint become Ph[1]-negative during remissions, and do not exhibit the additional cytogenetic abnormalities typical of CML blast crisis, suggesting that they represent transformation of a cell type which is more restricted in its differentiation potential than a pluripotent stem cell. However, patients who appear to violate these rules have been described.[10,11] A central unanswered question is whether the difference in disease spectrum associated with the two forms of *bcr/abl* is due to some intrinsic difference in the properties of the proteins themselves.

Expression of the P190 gene in a bone marrow infection system in the presence of helper virus gives rise to the same spectrum of hematological malignancies seen with the P210 gene, but with a shorter latency period, again suggesting that P190 is more potent than P210 in leukemogenesis.[12] When P190 is expressed in murine bone marrow by a helper-free retroviral vector, it very rapidly and efficiently induces pre-B lymphoma and tumors of monocyte/macrophage origin. The target cells for the induction of these disease are lineage-restricted and probably represent committed progenitors. A small number of animals appear to have had stem cell infection by P190, and exhibit a transient myeloproliferative syndrome followed by rapid evolution of the infected clone to acute leukemia, mimicking blast crisis.[13] These results suggest that P190 may be able to induce CML when expressed in a stem cell, but rapid progression to acute leukemia may preclude identification of human patients with chronic phase CML who express P190. Thus, the two forms of *bcr/abl* have similar growth stimulatory and transforming properties when expressed in the hematopoietic system, but the P190 protein, by virtue of differences in bcr sequences, gives rise to a very short "chronic phase." This suggests that the propensity of the *bcr/abl* clone to progress to acute leukemia is a function of *bcr* sequences present, and that some sequences present in P210 but missing in P190 confer a longer chronic phase dura-

tion. This concept is supported in part by studies of chronic phase duration in CML patients with different bcr breakpoints. Some studies have suggested that patients with 3′ breakpoints, which produce a slightly larger form of P210 protein containing sequences from bcr exon 3, may have a shorter chronic phase duration than patients with 5′ breakpoints who produce a slightly shorter form of P210,[14–16] although other studies have not found significant differences between these two groups.[17] It is not clear mechanistically how the different forms of *bcr/abl* would affect the duration of chronic phase. In a classical view of multistep carcinogenesis, the progression of the malignant clone from chronic phase to acute leukemia represents the acquisition of additional mutations, involving both dominantly and recessively acting oncogenes, by the malignant clone.[18] Formally, different bcr/abl proteins may accelerate this process either by influencing the size of the target population for secondary mutations, or by accelerating the rate of acquisition of secondary mutations. Further studies will be necessary to distinguish between these possibilities.

The nature of secondary mutations involved in the progression of human CML to blast crisis has been the extensively investigated in humans. Studies of blast crisis patients have implicated mutations in N-*ras*, c-*myc*, and p53 genes in disease progression. Mouse model systems may prove useful in identifying additional novel mutations involved in CML disease progression which would be difficult or impossible to obtain from human studies. Disease progression in humans may be associated with the appearance of additional nonrandom chromosomal abnormalities by the malignant clone, including duplication of the Philadelphia chromosome. In the mouse model system, we have observed both duplication of the *bcr/abl* provirus and alterations in the *bcr/abl* gene itself in progression to acute leukemia, suggesting that alterations in the *bcr/abl* gene copy number or structure may have a major influence upon disease progression. It is possible that this is somewhat artefactual and due to the small size and instability of the provirus, as there is no definite evidence that duplication, amplification, or overexpression of the existing *bcr/abl* gene is a uniform feature of disease progression in humans.[19–21] Indeed, cases of blast crisis have been reported where loss of the Ph[1] chromosome or deletion of *bcr/abl* sequences have been documented, suggesting that the expression of P210 may be dispensable once the leukemic clone has progressed to blast crisis.[22,23] However, based on the mouse data, the structure of the *bcr/abl* locus in human blast crisis patients may deserve further investigation with modern molecular methods such as PCR and fluorescence *in situ* hybridization.

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