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Studying the pathogenesis of *BCR–ABL*$^+$ leukemia in mice

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Animal models of *BCR–ABL*$^+$ leukemias have provided important new knowledge about the molecular pathophysiology of these diseases, and answered questions that are difficult or impossible to address using *BCR–ABL*-expressing cell lines or primary Ph$^+$ leukemia samples from patients. The power of mouse models lies in their ability to recapitulate precisely the phenotypes of *BCR–ABL*$^+$ leukemias *in vivo*, but this comes at the price of significant complexity. Here I review recent studies of leukemias induced in mice by *BCR–ABL* with an emphasis on the intricate nature of these diseases and the need for careful pathological and molecular analysis.
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Introduction

The molecular biology of *BCR–ABL* and its protein product, the Bcr–Abl tyrosine kinase, have been intensively analysed in cell culture systems since the discovery of the fusion oncogene nearly 18 years ago. Through these studies, we know that Bcr–Abl has increased tyrosine kinase activity relative to c-Abl (Ilaria and Van Etten, 1995; Lugo *et al*., 1990) and has gained the ability to transform fibroblasts (Lugo and Witte, 1989), cytokine-dependent hematopoietic cell lines (Daley and Baltimore, 1988; Hariharan *et al*., 1988), and primary bone marrow B-lymphoid cells (McLaughlin *et al*., 1987). The primary structures of the Bcr and Abl polypeptides have been analyzed and dissected in great detail, with the elucidation of an oligomerization domain (McWhirter *et al*., 1993), Grb2 binding site (Pendergast *et al*., 1993b; Puil *et al*., 1994), serine kinase activity (Maru and Witte, 1991) and regulatory phosphorylation sites (Liu *et al*., 1996; Wu *et al*., 1998), SH2 binding domain (Pendergast *et al*., 1991) and Dbl homology in Bcr and description of N-terminal SH3 (Franz *et al*., 1989; Jackson and Baltimore, 1989; Van Etten *et al*., 1995), SH2 (Ilaria and Van Etten, 1995; Mayer *et al*., 1992) and catalytic domains (Pendergast *et al*., 1993a) and C-terminal adapter protein binding sites (Ren *et al*., 1994), nuclear localization signals (Wen *et al*., 1996) and DNA-binding (Kipreos and Wang, 1992) and actin-binding (McWhirter and Wang, 1993; Van Etten *et al*., 1994) domains in Abl. Studies in cultured cells have identified many signal transduction pathways activated by Bcr–Abl, including activation of Ras (Sawyers *et al*., 1995), MAPK (Cortez *et al*., 1997), JNK/SAPK (Raitano *et al*., 1995), phosphatidylinositol-3 kinase (Skorski *et al*., 1995a; Varticovski *et al*., 1991), NF-κB (Reuther *et al*., 1998), and STAT pathways (Carlesso *et al*., 1996; Ilaria and Van Etten, 1996; Shuai *et al*., 1996). Studies with inhibitors and dominant-negative mutants have suggested that several of these pathways contribute to transformation of fibroblasts or hematopoietic cells by Bcr–Abl *in vitro* (Dickens *et al*., 1997; Nieborowska-Skorska *et al*., 1999; Reuther *et al*., 1998; Sawyers *et al*., 1992, 1995; Raitano, 1995; Skorski *et al*., 1995a,b).

Although much has been learned about the biology of *BCR–ABL* through these studies, a complete understanding of the pathophysiology of *BCR–ABL*-associated leukemias requires the expression of the oncogene in the hematopoietic system of a living organism. This is because the complex nature of leukemia cannot be adequately modeled in any currently existing cell culture system. Although cell lines do exist that recapitulate some aspects of hematopoietic differentiation *in vitro*, these may not be appropriate systems for the analysis of *BCR–ABL* activity. For example, although the hallmark of human chronic myeloid leukemia (CML) is an overproduction of myeloid cells with preservation of myeloid differentiation, expression of *BCR–ABL* in 32D cells, which can undergo terminal granulocytic differentiation in response to G-CSF, blocks the ability of these cells to differentiate (Laneuville *et al*., 1991). Furthermore, while murine embryonic stem (ES) cells can undergo differentiation *in vitro* to all myeloid lineages, studies of *BCR–ABL* activity in ES cells have not provided major insights into leukemogenesis. Expression of *BCR–ABL* in ES cells alters the balance of *in vitro* erythroid differentiation towards myeloid and multipotential progenitors (Era and Witte, 2000) and permits multilineage engraftment of irradiated recipient mice by differentiated ES cells (Perlingeiro *et al*., 2001), but the hematologic disease that develops in recipients is not an accurate representation of human CML (Peters *et al*., 2001).

In order to express *BCR–ABL* directly in the hematopoietic system of mice, both transgenic and

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retroviral transduction approaches have been employed. Despite a large effort, there is no realistic transgenic model of *BCR–ABL*-induced CML (Van Etten, 2001). However, the retroviral expression system does provide such a model, and will be the focus of this review.

An accurate and quantitative model of CML in mice

Human CML can be faithfully modeled in mice by retroviral transduction of the *BCR–ABL* gene into mouse bone marrow cells, followed by transplantation into irradiated syngeneic mice (Daley *et al*., 1990; Kelliher *et al*., 1990). When high-titer virus stock is employed, this procedure induces CML-like myeloproliferative leukemia in all recipients within 4 weeks after transplantation (Li *et al*., 1999; Pear *et al*., 1998; Zhang and Ren, 1998). Mice with *BCR–ABL*-induced CML-like disease exhibit massive polyclonal expansion of maturing myeloid cells, principally neutrophils, which express Bcr–Abl protein and infiltrate spleen, liver, and lungs. Although neutrophils are the predominant hematopoietic lineage overproduced in murine CML-like disease, macrophages, erythroid progenitors, B-lymphocytes and sometimes T-lymphocytes from diseased mice carry the same spectrum of *BCR–ABL* proviral clones as the granulocytes, demonstrating that the cells initiating the CML-like leukemia are early multipotential progenitors (Li *et al*., 1999). The CML-like disease is efficiently transferred by transplantation of bone marrow or spleen cells from a primary animal to irradiated secondary recipients (Li *et al*., 1999; Pear *et al*., 1998; Zhang and Ren, 1998). Interestingly, of the many different *BCR–ABL*-transduced clones that contribute to the leukemia in the primary mouse, only a small subset are capable of generating day 12 spleen colonies and of engrafting and inducing CML-like disease in secondary recipients (Li *et al*., 1999; Zhang and Ren, 1998), suggesting that the cells initiating CML-like disease are heterogeneous for self-renewal. Serial transplantation leads to evolution of the leukemic process into clonal acute myeloid or more often lymphoid leukemia, representative of blast crisis (Daley *et al*., 1991; Pear *et al*., 1998). Murine CML-like leukemia is therefore an accurate and faithful model of human CML that has proven useful for analysis of the molecular pathophysiology of this disease (Li *et al*., 2001; Li *et al*., 1999; Million and Van Etten, 2000; Roumiantsev *et al*., 2001).

Multiple distinct leukemias originate from *BCR–ABL*-transduced marrow

All recipients of *BCR–ABL*-transduced marrow develop CML-like disease when bone marrow donors are pretreated with 5-fluorouracil (5-FU) before harvest. This is consistent with the origin of these leukemias from early progenitor/stem cells, whose transduction is favored by 5-FU treatment. However, other *BCR–ABL*-transduced progenitors are present in the bone marrow population and can induce other forms of leukemia if recipients do not first succumb to CML-like disease. This can be seen with decreases in virus titer (Daley *et al*., 1990), alterations in transduction conditions (Elefanty and Cory, 1992), or by employing marrow from donors not treated with 5-FU (Li *et al*., 1999). These other malignancies include acute B-lymphoid and T-lymphoid leukemia/lymphoma, erythroleukemia, and histiocytic tumors (sarcomas) arising from the myelomonocytic lineage. The latter disease most closely resembles one of the human histiocytoses such as malignant histiocytic reticuloendotheliosis (Groopman and Golde, 1981), which does not typically have a Ph chromosome.

These multiple leukemias can compete within the bone marrow of recipient mice and lead to confusing clinicopathological syndromes under certain conditions. In recipients of *BCR–ABL*-transduced marrow from non-5-FU-treated donors, a mixture of CML-like myeloproliferative disease, B-cell acute lymphoblastic leukemia (B-ALL), and histiocytic sarcoma develops, with some recipients having two or even all three diseases simultaneously (Li *et al*., 1999). Such multi-leukemic mice have the cardinal clinicopathological features of each disease independently and can be recognized by careful histological analysis and by the demonstration of distinct proviral clones in the different leukemic cells. The effect of competing leukemias can also be observed with mutations in *BCR–ABL* that attenuate the induction of CML-like disease, such as point mutations in the Src homology 2 (SH2) domain. The R1172K mutation in p210 *BCR–ABL* eliminates phosphotyrosine binding by SH2 (Ilaria and Van Etten, 1995) and recipients of p210 R1172K-transduced marrow all succumb to B-ALL (Roumiantsev *et al*., 2001), suggesting that the SH2 domain is absolutely required for induction of CML-like disease by *BCR–ABL*.

However, the cells initiating the B-ALL have restricted differentiation potential (Li *et al*., 1999) and phenotypic characteristics of early B-lymphoid progenitors (D Krause and RA Van Etten, unpublished observations). Depletion of these progenitors from the p210 R11722K-transduced marrow allows CML-like disease to reemerge in recipients after a delay, demonstrating that loss of Bcr–Abl SH2 function merely attenutates the development of CML-like leukemia but does not eliminate it (Roumiantsev *et al*., 2001). Defining the nature of the bone marrow progenitors that initiate distinct *BCR–ABL*-induced leukemias and developing methods to model each disease separately are major goals for future work. Importantly, B-ALL can be efficiently induced in the absence of CML-like disease by direct transduction and transplantation of marrow from non-5-FU-treated donor mice (Roumiantsev *et al*., 2001), allowing this *BCR–ABL*-induced disease, which is an accurate representation of human Ph$^+$ acute B-lymphoblastic leukemia, to be quantitatively modeled in mice.

Autocrine and paracrine effects in *BCR–ABL* leukemogenesis

Another intricacy of *BCR–ABL*-induced leukemia is that *BCR–ABL* can induce the secretion of multiple cytokines. This was appreciated initially from studies in the myeloid cytokine-dependent FDCP-1 cell line, where expression of *BCR–ABL* induces secretion of IL-3 in an SH2-dependent fashion (Anderson and Mladenovic, 1996; Hariharan *et al*., 1988). However, the ability of *BCR–ABL* to transform IL-3-dependent hematopoietic cell lines to become independent of exogenous IL-3 for survival and growth does not involve an autocrine mechanism (Daley and Baltimore, 1988; Hariharan *et al*., 1988; Ilaria and Van Etten, 1995).

In bone marrow transplant experiments, cytokine secretion by *BCR–ABL*-expressing cells can lead to expansion of hematopoietic cell populations in recipient mice that do not express the oncogene. A good example is the histiocytic sarcoma induced by *BCR–ABL*. Small areas of perivenular histiocytic infiltration are frequently observed in livers of mice with CML-like leukemia (Figure 1a), and analysis of proviral integration patterns demonstrates these cells are derived from the same multilineage progenitors that generate the neutrophils in this disease (Li *et al*., 1999). However, *BCR–ABL*-induced histiocytic malignancies can exist independently of classic myeloproliferative disease (Figure 1b), where they are characterized by slow accumulation of malignant macrophages that involve liver, mesentery, peritoneum and often associated with ascites (Daley *et al*., 1990; Elefanty *et al*., 1990). Mice with primary histiocytic sarcoma can exhibit increased levels (from $10-50\times10^3$ per mm^3) of neutrophils in the peripheral blood, suggestive of myeloproliferative disease; however, molecular analysis demonstrates that these neutrophils do not contain the retroviral provirus and hence are not a direct part of the malignant process (Daley *et al*., 1990; Elefanty *et al*., 1990; Scott *et al*., 1991). Mice with histiocytic sarcoma contain increased levels of circulating G-CSF and GM–CSF that are likely produced directly by these tumors and may responsible for the secondary increase in neutrophils (Elefanty *et al*., 1990). This illustrates that one must be extremely careful about diagnosing myeloproliferative disease in mice that harbor histiocytic sarcoma.

This phenomenon was responsible for some initial confusion about the leukemogenic properties of v-*abl*, the transforming gene of Abelson murine leukemia virus, when expressed in murine bone marrow. It was first reported that v-*abl* also induced CML-like disease in recipients of transduced marrow (Kelliher *et al*., 1990), and others subsequently described chronic myeloproliferative disease induced by v-*abl* in similar experiments (Chung *et al*., 1991; Han *et al*., 1991). However, these mice develop a complex mixture of B-lymphoid, mast cell, and histiocytic tumors and although some recipients have increased circulating neutrophils, genomic DNA from these cells lack the retroviral provirus and hence likely arise from paracrine stimulation by secreted cytokines (Scott *et al*., 1991). The inability of v-*abl* to induce CML-like myeloproliferative disease in the current high-efficiency retroviral bone marrow transduction/transplantation model system was confirmed by two recent studies (Gross and Ren, 2000; Million and Van Etten, 2000).

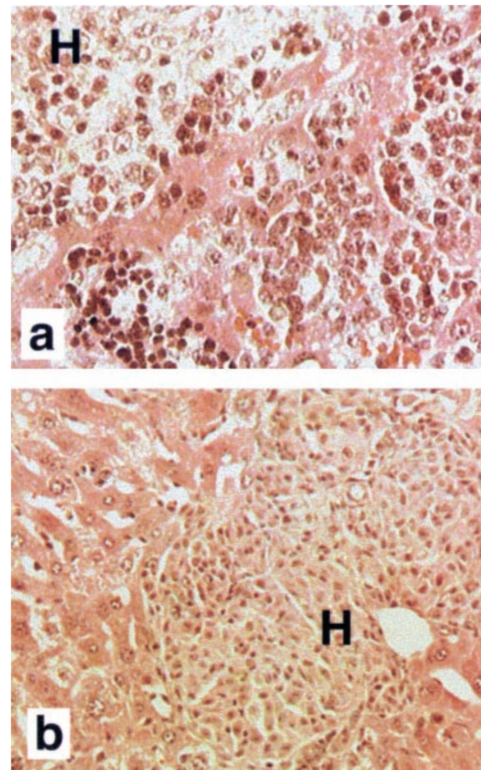

Figure 1 *BCR–ABL*-induced histiocytic sarcoma can exist independently of myeloproliferative disease. (**a**) Photomicrograph of hematoxylin and eosin (H&E)-stained mouse liver section from a recipient of *BCR–ABL*-transduced marrow from a 5-flurouracil-treated donor that developed CML-like myeloproliferative disease. Mice with CML-like disease exhibit small periportal aggregates of histiocytes with abundant vacuolated cytoplasm (H), accompanied by extensive sinusoidal infiltration with maturing myeloid and erythroid cells. Magnification 400×. (**b**) Photomicrograph of H&E-stained liver section from a recipient of *BCR–ABL*-transduced marrow from a normal donor that developed exclusively histiocytic sarcoma. Large hepatic, mesenteric, and peritoneal tumors (H) develop in these animals and can produce secondary elevation in circulating neutrophils through secretion of G- and GM–CSF. Magnification 200×

To complicate matters further, mice with *BCR–ABL*-induced CML-like disease exhibit a modest increase in circulating interleukin 3 (IL-3) (Li *et al*., 1999; Zhang and Ren, 1998), and perhaps in granulocyte-macrophage colony-stimulating factor (GM–CSF) as well (Zhang and Ren, 1998). The increase in IL-3 is particularly interesting because of the recent observation that primitive Ph$^+$ progenitors from human chronic phase CML patients express aberrant transcripts for IL-3 and exhibit autonomous *in vitro* growth that is partially inhibited by anti-IL-3 antibodies (Jiang *et al*., 1999). These observations

suggested that autocrine production of IL-3 might contribute to the pathogenesis of both human and murine CML. However, when mice with homozygous inactivation of the *Il3* or both the *Il3* and *Gmcsf* genes are used as donors in the retroviral bone marrow transduction/transplantation model, recipients of *BCR–ABL*-transduced marrow efficiently develop myeloproliferative disease (Li *et al*., 2001), demonstrating that neither cytokine is required for the pathogenesis of CML-like disease in this model system. Interestingly, increased circulating IL-3 is still observed in wild-type recipients of *BCR–ABL*-transduced marrow from $Il3^{-/-}$ donors but not when the host is of $Il3^{-/-}$ genotype (Figure 2), demonstrating that the source of increased IL-3 is the recipient, not the *BCR–ABL*-expressing donor cells (Li *et al*., 2001). Levels of circulating IL-3 are higher in recipients of marrow transduced with *BCR–ABL* retroviral vectors that co-express *A. victoria* green fluorescent protein (GFP) at high levels from an internal ribosome entry site than with vectors expressing a neomycin phosphotransferase gene at low levels from an internal promoter (Figure 2), while there is no increase in IL-3 in recipients of marrow transduced with an empty retrovirus. Collectively, these results suggest that the elevation in IL-3 represents an immunological reaction of the recipient to transplantation of bone marrow expressing a foreign protein.

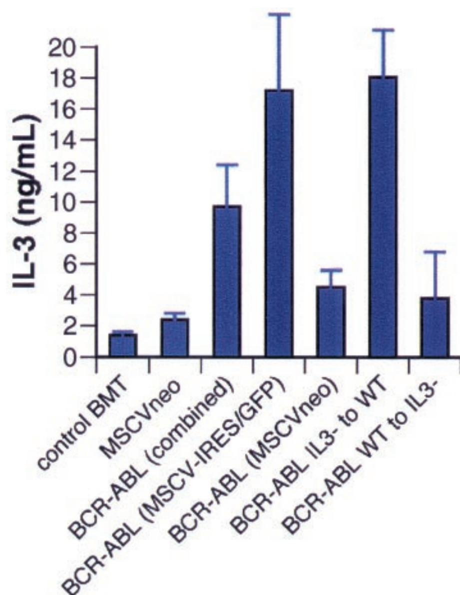

Figure 2 Recipients of *BCR–ABL*-transduced marrow produce IL-3 in reaction to GFP. Plasma IL-3 levels in transplanted mice were measured with an ELISA assay that detects nanogram quantities of this cytokine in biological fluids (Li *et al*., 2001). Leukemic recipients of *BCR–ABL*-transduced marrow (BCR–ABL (combined)) exhibit increased levels of circulating IL-3 relative to mice transplanted with untransduced marrow (control BMT) or with marrow transduced with empty vector (MSCVneo). Recipients of marrow transduced with retrovirus co-expressing high levels of *A. Victoria* green fluorescent protein (MSCV-IRES/GFP) exhibit significantly greater increases in circulating IL-3 than with BCR. Use of donors (BCR–ABL IL3- to WT) or recipients (BCR–ABL WT to IL3-) with homozygous inactivation of the *Il3* gene demonstrate that the source of IL-3 is the recipient, not the *BCR–ABL*-expressing donor cells

The use of such bicistronic retroviral vectors, which co-express *BCR–ABL* and GFP from a single mRNA *via* an internal ribosome entry site (IRES), facilitates titering of retroviral stocks and allows the identification by flow cytometric analysis of transduced hematopoietic cells in diseased mice (Pear *et al*., 1998). The presence of a large fraction of GFP^- myeloid cells in mice with *BCR–ABL*-induced CML-like disease has been taken as evidence of a significant paracrine effect in this model system (Zhang and Ren, 1998), but the persistence of this GFP^- $CD11b^+$ myeloid population in $Il3^{-/-}$ $Gmcsf^{-/-}$ recipients of *BCR–ABL*-transduced marrow from donors of the same genotype (Tomasson *et al*., 2001) demonstrates that IL-3 or GM–CSF are not responsible. The majority of these GFP^- cells are probably accounted for by mechanisms other than paracrine stimulation of normal marrow progenitors. While it is fairly certain that GFP^+ cells also express *BCR–ABL*, the converse is not true, and a large fraction of the GFP^- cells must contain the retroviral provirus and may also express *BCR–ABL*. This follows from the observation that myeloid cells from these mice contain the *BCR–ABL* provirus at levels that are greater than or equal to one proviral copy per diploid genome (Li *et al*., 1999), and indeed Southern blotting of genomic DNA from purified GFP^+ and GFP^- myeloid cell populations from mice with myeloproliferative disease induced by an oncogenic receptor tyrosine kinase, activated FLT3, demonstrates equivalent levels of the provirus in the two populations (Kelly *et al*., 2002). The explanation for the lack of detection of GFP in $provirus^+$ cells may involve loss of GFP due to cell damage during *in vitro* manipulation (Tomasson *et al*., 2001) and possibly to genetic or epigenetic mechanisms that impair IRES function after proviral integration.

These examples demonstrate that autocrine and paracrine effects of *BCR–ABL* expression are an inevitable complication of *in vivo* leukemogenesis model systems that must be considered during the analysis of hematologic malignancies induced by *BCR–ABL* in mice.

Not all myeloproliferative disease is created equal: the case of Tel–Abl

Fatal myeloproliferative leukemia develops in recipients of bone marrow transduced with retroviruses expressing a wide variety of dysregulated tyrosine kinases in addition to Bcr–Abl, including Tel–Jak2 (Schwaller *et al*., 1998), Tel-PDGFβR (Tomasson *et al*., 2000), and activated FLT3 (Kelly *et al*., 2002). However, the human hematologic diseases associated with these different kinases have some distinct features from classical Ph^+ CML, and careful histopathological and molecular analysis of the disease process in mice can yield valuable insights into differences in patho-

physiology. The leukemogenic activity of the Tel–Abl fusion tyrosine kinase provides a good illustration of this.

Fusion of the *ABL* gene to *TEL* (also called *ETV6*) on chromosome 12p13 has been reported in six patients with leukemia, three of whom had acute leukemia of B-lymphoid (Papadopoulos *et al*., 1995), T-lymphoid (van Limbergen *et al*., 2001) and undifferentiated myeloid (Golub *et al*., 1996) origin, the other three with atypical (Brunel *et al*., 1996) or typical (Andreasson *et al*., 1997; van Limbergen *et al*., 2001) CML. *TEL* encodes a ubiquitously expressed 452 amino acid protein with homology to the Ets family of transcription factors (Golub *et al*., 1994). Two different *TEL–ABL* fusions have been observed; in the patients with B-ALL and atypical CML, the first four exons of *TEL* were fused to *ABL* exon 2, while the other four patients had *TEL* exons 1–5 fused to *ABL* exon 2. The resulting chimeric Tel–Abl proteins contain Tel amino acids 1–154 or 1–336, respectively, fused to the same 1104 COOH-terminal amino acids of c-Abl that is found in the Bcr–Abl fusion proteins. Both Tel–Abl fusion proteins share an NH_2-terminal region of Tel (the PNT homology domain) that mediates homo-oligomerization (Golub *et al*., 1996; Jousset *et al*., 1997). The fact that Bcr contains a coiled-coil oligomerization domain that is also required for activation of Bcr–Abl kinase activity and transformation (McWhirter *et al*., 1993) has led to the suggestion that oligomerization of Abl is the critical event in the pathogenesis of these leukemias, and that other functions of the NH_2-terminal Abl fusion partner are unimportant. Consistent with this, Tel–Abl has been shown to transform Rat-1 fibroblasts (Golub *et al*., 1996), primary bone marrow B-lymphoid cells (Golub *et al*., 1996), and cytokine-dependent Ba/F3 hematopoietic cells (Golub *et al*., 1996; Hannemann *et al*., 1998) *in vitro* in a manner indistinguishable from Bcr–Abl. Furthermore, Tel–Abl and Bcr–Abl activate similar intracellular signaling pathways in cultured hematopoietic cells (Okuda *et al*., 1996; Voss *et al*., 2000).

However, when the ability of the larger Tel–Abl fusion protein to induce myeloid leukemia in mice was tested in the retroviral bone marrow transduction/transplantation model, several distinct differences with Bcr–Abl-induced myeloproliferative disease were noted (Million *et al*., 2002). Some recipients of *TEL–ABL*-transduced bone marrow succumbed to CML-like leukemia that was very similar to that induced by *BCR–ABL* but with a significant increase in disease latency. However, most *TEL–ABL* recipients died abruptly around 4–5 weeks post-transplantation with moderate leukocytosis and splenomegaly (Figure 3a), but without evidence of the pulmonary myeloid infiltrates and hemorrhage that are the cause of morbidity and death in mice with classic CML-like disease. Histopathological evaluation of these mice revealed acute fatty liver and extensive neutrophilic infiltration and necrosis of the small bowel villi (Figure 3b,c). The hepatic picture is suggestive of endotoxin-induced injury, and indeed analysis of serum cytokine and chemistry profiles from premorbid mice revealed significant elevations in circulating endotoxin and TNFα with evidence of fulminant hepatic and renal failure (Figure 4). This distinctive fatal illness has been named small bowel syndrome (SBS). The precise pathophysiological mechanism of SBS and its distinct association with Tel–Abl are under investigation, but it is possible that abnormal homing of Tel–Abl expressing neutrophils to the gut and/or direct induction of TNFα expression by Tel–Abl are responsible (Figure 5). The relevance of Tel–Abl-induced SBS to human disease is suggested by the presence of ulcerative bowel disease in a patient with Tel–Abl-associated CML (van Limbergen *et al*., 2001).

A second distinct difference between *BCR–ABL* and *TEL–ABL*-induced leukemias is observed upon adoptive transfer of leukemic cells from primary diseased mice into secondary recipients. Similar to *BCR–ABL*, *TEL–ABL*-induced CML-like disease arises from multilineage progenitors capable of generating day 12 spleen colonies in secondary transplants, but neither *TEL–ABL*-induced CML-like disease nor SBS could be transferred to lethally irradiated recipient mice despite transplantation of large numbers of viable bone marrow and/or spleen cells (Million *et al*., 2002). In contrast, *BCR–ABL*-induced CML-like disease is successfully transferred to secondary recipients over 80% of the time under identical conditions. Most *TEL–ABL* secondary recipients succumbed to early or delayed graft failure, with others developing T-lymphoid or histiocytic tumors after long latent periods (Figure 6). Many of the former secondary mice had evidence of provirally marked cells in the spleen 2–3 weeks post-transplantation but these clones failed to radioprotect the recipients. These results suggest that *TEL–ABL* may act to expand a hematopoietic progenitor that lacks self-renewal as measured by secondary transplantation, or that *TEL–ABL* directly inhibits self-renewal of stem cells. Distinguishing between these possibilities will require further experiments and may have important implications for the treatment of these diseases by autologous stem cell transplantation.

Conclusions

The *BCR–ABL*+ leukemias are perhaps the most thoroughly understood of human malignancies, in part because of the development of accurate animal models for these diseases. In this review, I have tried to illustrate that the pathophysiology of these diseases must be understood in the most minute detail if the experimental results are to provide information useful for understanding human leukemia. A common mistake in analysis of murine leukemia experiments is to use survival as the primary endpoint of the study but fail to define precisely the cause of morbidity or death. As demonstrated by *TEL–ABL*, similar survival

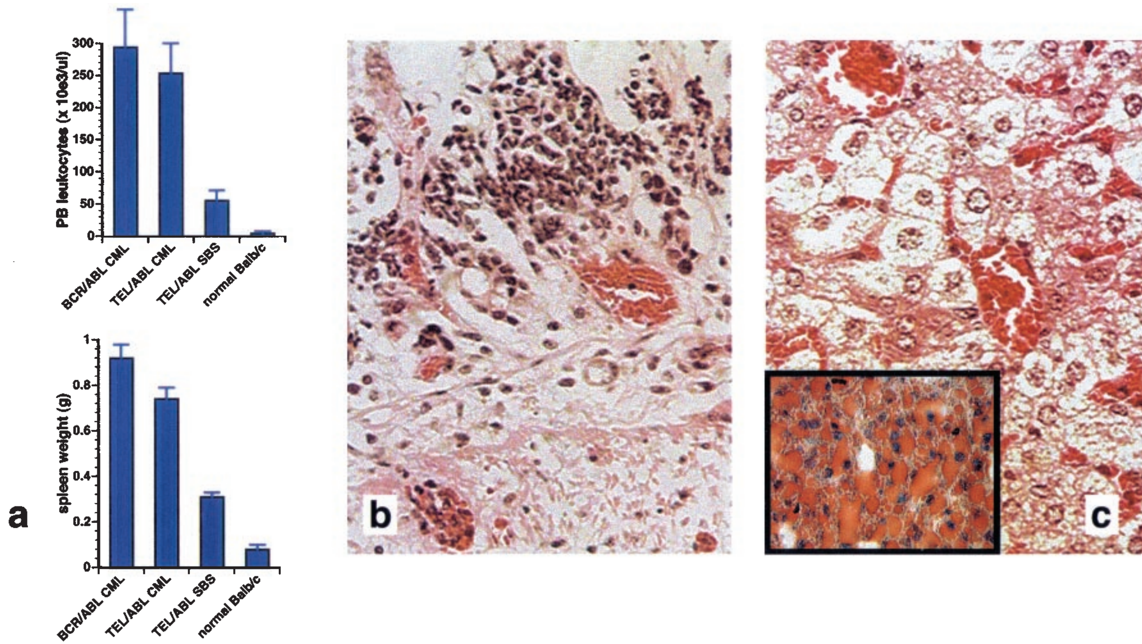

Figure 3 A novel fatal syndrome of small bowel myeloid infiltration and hepatic steatosis in recipients of *TEL–ABL*-transduced bone marrow. (**a**) Comparison of peripheral blood leukocyte counts (upper panel) and spleen weights (bottom panel) at time of morbidity or death of mice with *BCR–ABL*- or *TEL–ABL*-induced CML-like disease *versus TEL–ABL*-induced small bowel syndrome (SBS). Mice with SBS exhibit increased circulating myeloid cells and modest splenomegaly relative to normal controls but significantly lower than mice with classic CML-like myeloproliferative disease. (**b**) Photomicrograph of H&E-stained small intestine of a mouse with SBS sacrificed at the time of morbidity. Serosal surface is oriented to the bottom with villous surface upwards. Note the extensive infiltration of villi with neutrophils with concomitant necrosis. Magnification 200×. (**c**) Photomicrograph of H&E-stained liver from mouse with SBS, demonstrating lack of cellular infiltrate but with extensive microvesicular change and hepatocellular apoptosis. Similar findings are present in all mice with SBS but to a varying degree. Insert shows oil red staining of this liver, demonstrating that the vaculolar change is due to the accumulation of neutral lipids such as triglycerides. Magnification 400×

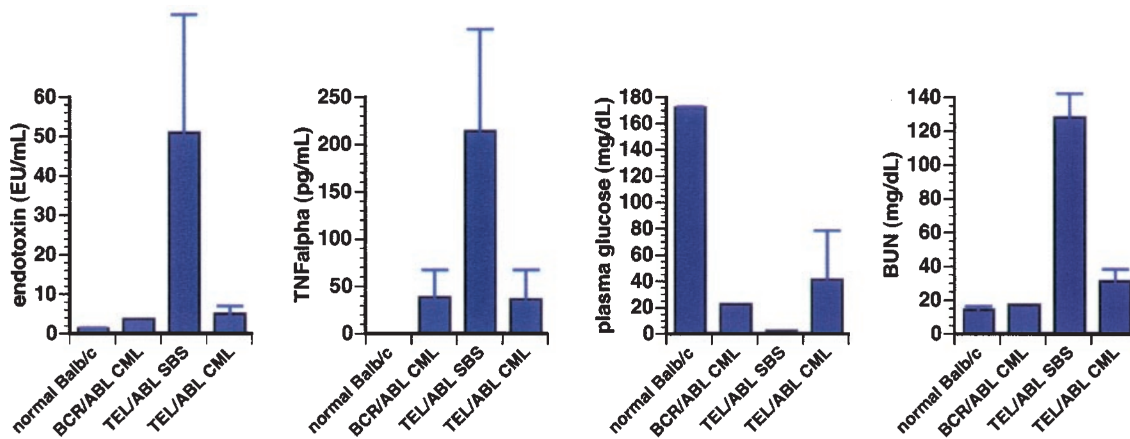

Figure 4 Increased circulating endotoxin and TNFα and hepatic and renal failure in mice with *TEL–ABL*-induced small bowel syndrome (SBS). Plasma endotoxin (far left) and TNFα (second from left) levels in mice with *BCR–ABL*- and *TEL–ABL*-induced CML-like disease were determined by commercial ELISA assays and compared with *TEL–ABL*-induced SBS and control recipients of untransduced marrow (normal Balb/c). Similarly, plasma glucose (third from left) and blood urea nitrogen (BUN, far right) levels were measured in these mice. Mice with SBS exhibit variable but significant increases in circulating endotoxin and TNFα, with marked hypoglycemia and increased BUN characteristic of severe liver and kidney dysfunction

curves can result from very different pathological processes. Conversely, the case of *BCR–ABL* SH2 mutants shows that minor changes in the physiology of what is essentially the same leukemic process can result in disparate survival outcomes. Careful and creative application of these model systems should continue to provide important new knowledge about the pathophysiology of *BCR–ABL*+ leukemia that cannot be obtained from analysis of cell lines or primary leukemic cells.

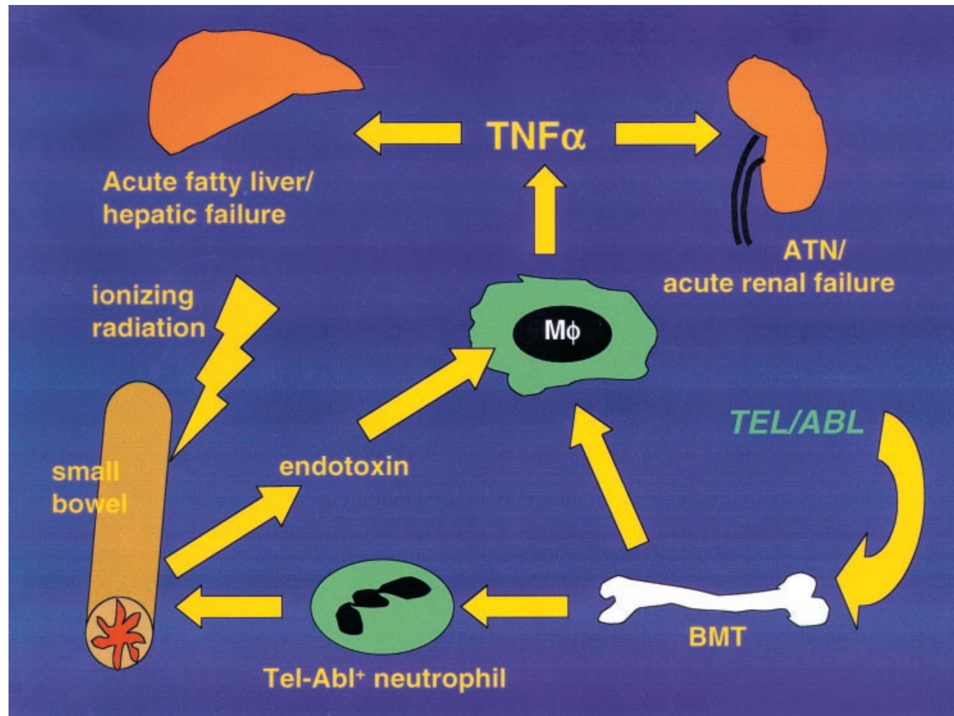

Figure 5 Possible pathophysiological mechanism of *TEL–ABL*-induced small bowel syndrome. Transplantation of *TEL–ABL*-transduced bone marrow into irradiated recipient mice (BMT) is followed by homing of Tel–Abl-expressing neutrophils to the small bowel with infiltration and necrosis. Because mice with *TEL–ABL*-induced CML-like disease do not exhibit significant infiltration of the small bowel despite large numbers of circulating Tel–Abl$^+$ neutrophils, it is possible that transient alterations in expression of leukocyte homing receptors in the gut from the radiation employed in the conditioning regimen contribute to the disease. Destruction of the bowel mucosal barrier leads to endotoxemia and stimulates TNFα production by monocytes, which is directly responsible for hepatic fatty change and apoptosis, leading to shock and renal acute tubular necrosis (ATN). It is also possible that direct induction of TNFα expression by Tel–Abl-expressing hematopoietic cells contributes to the pathogenesis of SBS

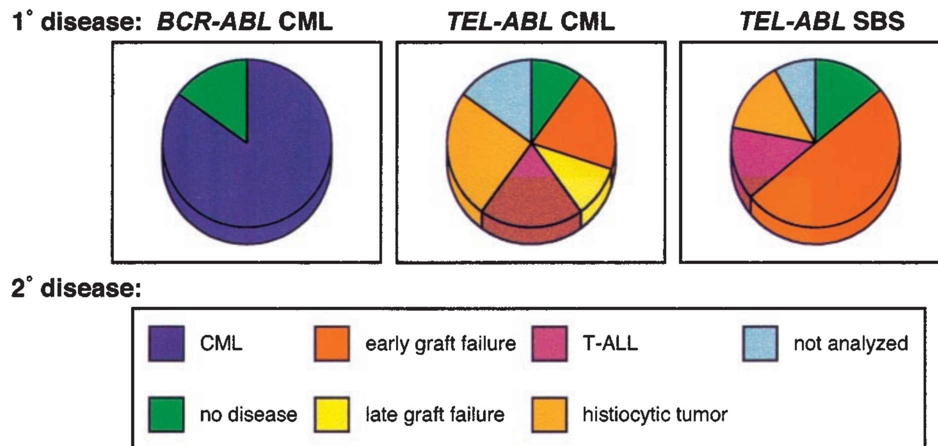

Figure 6 Neither *TEL–ABL*-induced CML-like disease nor SBS can be transferred to secondary recipients. Pie diagrams representing outcomes of transplantation of bone marrow and spleen cells from primary mice with *BCR–ABL*-induced CML-like disease (left), *TEL–ABL*-induced CML-like disease (middle panel), or *TEL–ABL*-induced SBS (right panel) into lethally irradiated secondary recipient mice. The phenotype of disease that developed in secondary recipients is indicated by the color code at the bottom

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