Diversity of Human Leukemia Xenograft Mouse Models: Implications for Disease Biology

Lüder Hinrich Meyer and Klaus-Michael Debatin

Abstract

Over the past decade, xenografting human leukemia cells into mice with different levels of immunodeficiency, with or without preconditioning, has provided an important tool to study various aspects of leukemia biology and to identify distinct clinical risk groups for evaluation of novel therapeutic strategies, as well as the possibility of amplifying human leukemia cells in vivo. Interestingly, these models using human acute lymphoblastic leukemia and acute myeloid leukemia cells as xenografts recapitulate many clinical features of the disease. Similar to the human environment (for example, in the bone marrow), transplanted leukemia cells in the murine setting are exposed to both favorable and unfavorable conditions for engraftment that may exert a distinct pressure for selection of subclones. Thus, results obtained in these models may vary depending on the experimental setup. The impact of in vivo growth of human leukemia cells on the background of a more or less hostile murine environment for leukemia biology and the course of the disease in patients are discussed in the context of the diversity of xenograft models. Cancer Res; 71(23); 7141–4. ©2011 AACR.
the distinct models used: different recipients, such as SCID (showing low overall engraftment of 15% in this study; ref. 12) versus NOD/SCID (9); a relatively short observation time (12 weeks) of the SCID mice in contrast to 20 weeks’ follow-up in our study; and the use of cell bank versus fresh samples. However, when cryopreserved cell bank samples were transplanted in our model, the distinct engraftment phenotypes of primary leukemias associated with early relapse were still maintained. We monitored some leukemias for longer observation times and detected leukemia manifestation up to 10 months after transplantation, suggesting that, ultimately, almost all samples would engraft and show in vivo disease manifestation. In line with our findings, superior patient survival has been reported if acute myeloid leukemia (AML) cells could not repopulate NOD/SCID mice, in contrast to in vivo proliferation associated with inferior outcome (13).

Interestingly, a distinct engraftment phenotype upon leukemia xenotransplantation was also recently observed in different, albeit much more permissive, models.

Cryopreserved ALL samples transplanted intraosseously showed rapid or prolonged engraftment, although no significant association with patient outcome was found in NK cell–depleted, irradiated NOD/SCID mice (14). However, using NSG mice, an association between engraftment and risk stratification of patients was observed (15). Further evidence that engraftment and proliferation are determined by the aggressiveness of the leukemia itself has been provided by in vivo treatment studies. Time to reappearance of ALL after chemotherapy of leukemia-bearing mice was associated with remission duration and patient outcome (5). However, because we observed significantly different engraftment phenotypes of leukemia cells isolated at diagnosis from so-far untreated patients, this difference does not seem to be sufficiently explained by distinct sensitivity or resistance to treatment. Moreover, when transplanting different doses of TTLshort and TTLlong leukemia cells, engraftment properties are retained independently of the number of cells transplanted and, thus, are an intrinsic feature of the leukemia cells. Similarly, the engraftment capability of AML cells into NOD/SCID mice was shown to be intrinsic to the leukemia cells independent of the cell numbers transplanted (13).

The biology and clinical course of leukemia in the xenograft model is not only characterized by the recipient mouse strain used but also determined by a number of other factors contributing to successful transplantation and overall engraftment capacity of the respective model: (i) source and processing of leukemia cells, (ii) transplantation procedure and conditioning of the recipient, (iii) the recipient itself, and (iv) definition of leukemia manifestation (Fig. 1). Due to the known high rate of spontaneous apoptosis in ALL cells upon removal from bone marrow, the shipping, cryopreservation, storage, and preprocelling of cells are of crucial relevance. Leukemia cells that have surface-bound antibodies after sorting are prevented from engraftment if transplanted onto NOD/SCID mice (16). Upon transplantation of frozen cell bank samples in comparison to freshly isolated cells, we and others observed a longer latency to leukemia (9, 17). Furthermore, the route of injection determines engraftment.

Figure 1. Diversity of human leukemia xenograft mouse models. Xenograft mouse models in which primary patient cells are transplanted into immunodeficient recipients are increasingly used to study leukemia biology. Distinct proliferation of individual leukemia samples reflects disease progression in the patient and shows prognostic impact. A variety of distinct factors and experimental conditions define engraftment and in vivo leukemia growth of xenotransplanted leukemia cells and, thereby, determine the outcome of the experiment. However, preferable conditions for analyzing leukemia biology by means of in vivo xenograft growth mimicking the situation of the patient are (i) unprocessed patient cells (ii) systemically injected into (iii) animals with remaining immunity and then (iv) assessment of full-blown leukemia. ab, antibody; hu, human; i.f., intrafemoral; i.p., intraperitoneal; i.t., intratibial.

Direct transfer of cells into the bone marrow niche, circumventing the homing process, leads to higher engraftment frequencies, in contrast to systemic i.v. injection (18). Preconditioning regimens using sublethal irradiation modulate the murine environment and improve engraftment. However, successful ALL engraftment has been shown without conditioning prior to transplantation (6), and strikingly, irradiation has been shown to have a negative impact on homing of ALL cells (19). Residual murine immunity may affect engraftment efficacy not only on the level of immune responses against human leukemia cells but also on the level of cytokine production upon encounter of human ALL cells with the murine environment. Additionally, cross-species reactivity of homing molecules and growth factors (murine on human and vice versa) influences xenograft growth (20), and humanized models transgenic for human cytokines are developed to increase engraftment and differentiation of hematopoietic cells (21). Accordingly, recipients treated with or transgenic for human growth factors show increased AML engraftment (22–24); however, whether leukemia subtypes or subclones are selectively stimulated is unclear. Comparing recipients with different grades of immunodeficiency by parallel transplantation, aggressive ALL with rapid leukemia onset in permissive (NK cell–depleted NOD/SCID or NSG) mice also engrafted in less permissive NOD/SCID mice, whereas samples with prolonged NSG engraftment did not engraft in NOD/SCID recipients within 24 weeks (14). Similarly, transplantation of high-risk or relapsed ALL samples revealed...
accelerated leukemia onset in NSG versus NOD/SCID mice (25). Preconditioning treatment as used in these studies will attenuate the residual immunity of the recipients, leading to a diminished selection pressure on leukemia cells independent of the mouse strain used. However, despite preconditioning, AML samples that failed to engraft in NOD/SCID mice also did not engraft in more immunocompromised recipients (13). On the other hand, each patient ALL sample that engrafted in NOD/SCID mice will engraft in more permissive NSG mice, although it remains to be investigated whether ALL samples that failed to engraft in NOD/SCID mice will succeed in a less hostile microenvironment and engraft in NSG recipients. It can be expected that samples with failed or prolonged engraftment in low permissive mice (e.g., 10 months observed in our NOD/SCID model) will be engrafted at higher rates or shorter times in NSG mice. Furthermore, the age and gender of the recipient affect xenografting. Young age is associated with superior engraftment (26), and newborn mice have been used to avoid the effect of innate immunity (27). Most interestingly, the gender of the recipient has significant impact with more than 10 times superior engraftment in female versus male mice (28). Finally, the endpoint of the observation time (engraftment in a given time versus leukemia manifestation) and estimation of leukemia onset will be pivotal for the outcome of the experiment.

Depending on the aim of the experiment, different xenograft models may be suitable for different purposes. To achieve high engraftment rates and cell amplification (and for cells with limited viability, e.g., shipped and/or frozen samples), highly permissive models supporting as unrestricted xenograft growth as possible will be preferred. In contrast, if features of leukemia biology are characterized by means of in vivo growth of xenografted cells mimicking the situation of the patient, careful adaptation of an appropriate model is required. Unmodified cells with the highest functional integrity and viability possible (i.e., directly from the patient) should be favored. Although the topical origin of leukemia is largely unknown, precursor or initiating cells are hematogenously spread during leukemogenesis and home into the supportive (bone marrow) environment, subsequently leading to full-blown disease. This homing process with xenograft–host interaction, adhesion, and migration will be maintained by systemic i.v. injection, but it is bypassed if cells are transplanted intraosseously directly into their niches. In the patient, leukemia cells originate and develop in a microenvironment that is, with its cytokines and immune cells, both supportive and hostile and most certainly exerts selection pressure. Thus, an in vivo model in which leukemia cells have to deal with and ultimately overcome residual immunity in a xenograft setting will be advantageous in resembling human leukemogenesis and disease progression compared with a model of widely unrestricted proliferation under highly permissive conditions. In patients, leukemia is generally diagnosed at the stage of fully developed disease. In a xenotransplantation setting, assessment of leukemia engraftment identifying low percentages of xenografted cells in the recipient might not mirror the composition and biology of the different populations present at the patient’s diagnosis, which, in contrast, will be reflected at onset of full-blown leukemia in the recipient mouse. However, for this strategy, careful daily investigation of the recipient and immediate termination of the experiment upon signs of leukemia-related morbidity should be ensured. Thus, to analyze leukemia biology reflecting the situation of patients, the following conditions seem appropriate: (i) direct transplantation of unmanipulated cells by (ii) systemic injection into (iii) recipients bearing residual immunity, with (iv) onset of overt leukemia as the end point.

The interplay between leukemia aggressiveness on one side and hostility of the model on the other side determines engraftment and in vivo proliferation and has a profound impact on our understanding of leukemia biology. Thus, the repopulating activity of xenografted tumor cells is a characteristic of initiating or stem cells, and different models may affect the frequency of these cells. A hierarchical concept of only a few immature cells possessing stem cell properties at the apex of a differentiation tree has been challenged by the proposal of a stochastic model in which a high proportion of cancer cells, including cells with more mature phenotypes, harbors stem cell activity. It is striking that the paradigmatic change of the ALL stem cell definition from rarely present, immature cells to more frequent, mature cells may depend on the permissiveness of the xenograft model employed. Although ALL stem cells have been characterized as infrequently present and phenotypically immature cells using classical NOD/SCID mice (29, 30), the use of recipients with increased immunodeficiency (like NSG mice) has identified more mature cells at higher frequencies to have leukemogenic potential (27, 31, 32). Even more evident, in malignant melanoma with only rare cancer stem cells as assayed in NOD/SCID mice, as many as 1 stem cell in 4 melanoma cells was identified using NSG mice (33). In this context, leukemia progression and evolution of ALL subclones analyzed in xenograft models will also depend on the model used. Recent studies reported clonal diversity of BCP-ALL cells when serially transplanted intraosseally into NSG mice (14, 15, 34), suggesting a lack of selection pressure supporting proliferation of different subclones. Thus, in addition to the intrinsic oncogenic potential of transplanted leukemia cells, the selection pressure put on human leukemia cells by transplantation conditions is expected to have a profound impact on the "true frequency" of leukemia-initiating cells, on clonal evolution, and on other functional features.

Taken together, xenograft models are diverse, not only characterized by recipient permissiveness but also by several additional factors that, in summary, will determine engraftment outcome. The model used is of critical relevance if aspects of leukemia biology are analyzed based on xenograft in vivo growth and clinically relevant conclusions are drawn from these experiments. The question of which xenograft model will most precisely reflect the patient situation ultimately may depend on the focus of the experiment. Thus, highly permissive xenograft models providing as much unrestricted engraftment and in vivo proliferation as possible might be superior or inferior compared with less permissive models, which probably more closely reflect the patient setting.
Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

34. Received May 23, 2011; revised August 1, 2011; accepted August 2, 2011; published OnlineFirst November 16, 2011.
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