REVIEW

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Key factors in experimental mouse hematopoietic stem cell transplantation

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Abstract

The first mouse model of hematopoietic stem cell transplantation (HSCT) was developed more than 50 years ago. HSCT is currently being widely used in a broad range of research areas, which include studies of the engraftment process, the pathogenesis of graft-versus-host disease and possible ways of its treatment and prophylaxis, attempts to use the graft-versus--leukemia/tumor effect in treating hematological and oncological malignancies, cancer vaccine development, induction of transplanted organ tolerance, and gene therapy. However, although this model is widely distributed, many laboratories use different protocols for the procedure. There are a number of papers discussing different HSCT protocols in clinical work, but no articles summarizing mouse laboratory models are available. This review attempts to bring together different details about HSCT in the mouse model, such as the types of transplantation, possible pretreatment regimens and their combinations, methods and sources of graft harvesting and preparation for the transplantation procedure, the influence of graft cell dose and content on the engraftment process, the transplantation method itself, possible complications, symptoms and techniques of their prophylaxis or treatment, as well as follow-up and engraftment assessment. We have also tried to reflect current knowledge of the biology of the engraftment.

Key words: hematopoietic stem cell transplantation, mouse models, engraftment, chimerism.

Abbreviations: BMT – bone marrow transplantation, BMC(s) – bone marrow cell(s), FACS – fluorescence-activated cell sorting, FNPB – near-term fetal and newborn peripheral blood, G-CSF – granulocyte colony-stimulating factor, GVHD – graft--versus-host disease, GVL – graft-versus-leukemia (effect), GVT – graft-versus-tumor (effect), Gy – Gray (=100 rad), HSC(s) – hematopoietic stem cell(s), HSCT – hematopoietic stem cell transplantation, KTLS – c-kit^{+}Thy1.1^{low}Lin$^{-/low}$Sca-1^{+} cells, LTRA – long-term repopulation ability (cells), MHC – major histocompatibility complex, NK – natural killer, NST – non-myeloablative stem cell transplantation, MPBC(s) – mobilized peripheral blood cell(s), MPBCT – mobilized peripheral blood cell transplantation, STRA – short-term repopulation ability (cells), TBI – total body irradiation, TLI – total lymphoid irradiation

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INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) in the mouse model has been used for several decades. It was shown in the 1950's that mice given lethal doses of irradiation could be saved by bone marrow infusion. The first experience of this procedure was connected with experimental bone marrow transplantation (BMT) and studies of the possibility of post-irradiation and post-chemotherapy rescue in a laboratory model [94]. HSCT is currently a widespread method used in a broad range of scientific research areas, such as engraftment process studies, graft-versus-host disease (GVHD) pathogenesis investigations, attempts at using the graft--versus-leukemia/tumor (GVL/GVT) effect in treating hematological malignancies and solid tumors, cancer vaccine development, and transplanted organ tolerance induction. During the past decades many researchers have chosen this method due to the availability and wide utilization of mice in laboratory investigations. However, although this model is widely distributed, many laboratories use different protocols for the procedure as well as different sources of stem cells. The impact of all the factors of transplantation on the biolo-

gy of engraftment and outcome is not always clearly understood.

There are number of papers discussing different HSCT protocols in clinical work, but no articles summarizing experience with mice laboratory models are available. This review does not intend to establish a unified protocol for HSCT: this would be impossible due to the diverse subjects and goals of using this model by researchers. The main purpose of this article is to summarize briefly the current knowledge of the biology of the hematopoietic engraftment process in laboratory mice and to describe the influence of various key factors on the outcome of the procedure.

In general, the procedure of HSCT consists of several steps in the mouse model, just as is the case in humans. There are various pretreatment regimens which have a major influence on the extent and the type of the engraftment, post-transplantation complications, and survival. The sources of the hematopoietic stem cells (HSCs) as well as recipient and donor status are also important and therefore discussed in the paper. Graft harvesting, processing, and optional enrichment are different in the models used nowadays and have their impacts on the procedure outcome as a whole. Finally, in this review we also tried to highlight post--transplantation mice survey, support therapy, complications, and chimerism assessment.

THE BIOLOGY OF HSCs IN MICE

HSCs possess two fundamental features distinguishing them from the more mature hematopoietic cells. First, they are able to reconstitute all lineages in the hematolymphoid system. Second, they possess the unique ability of self-renewal and can maintain hematopoiesis for the entire life of the organism. HSCs constitute a very small proportion of the population of cells and extensive studies were conducted during the past two decades to determine the phenotype of these cells. A number of surrogate *in vitro* and *in vivo* assays assessing HSC purity and functions have been developed. The most frequently used are *in vivo* colony-forming units in spleen assay [137], *in vitro* colony-forming cell [57], long--term culture-initiating cell [132], and cobblestone area--forming cell [92] assays. However, these deal mainly with the more mature progenitor cells. Therefore the *in vivo* long-term reconstitution assay is the most stringent test in evaluating the properties and self-renewal potential of HSCs [144, 154].

The isolation and description of mouse HSCs has greatly improved our understanding of the biology of the engraftment process in mice. The primitive mouse HSCs could be divided into three major subpopulations with distinct phenotypes and biological properties: the earliest long-term repopulation ability stem cells (LTRA, c-kit$^+$Thy1.1^{low}Lin$^{-/low}$Sca-1$^+$Flk-2$^-$), which give rise to short-term repopulation ability stem cells (STRA, c-kit$^+$Thy1.1^{low}Lin$^{-/low}$Sca-1$^+$Flk-2$^+$), and the most mature committed progenitor cells, which maintain multipotency but not self-renewal potential (c-kit$^+$Thy1.1$^-$Lin$^{-/low}$Sca-1$^+$Flk-2$^+$) [10, 17, 166]. The LTRA and STRA subpopulations constitute approximately 0.05–0.08% of total bone marrow cellularity and are often referred to as KTLS cells (c-kit$^+$Thy1.1^{low} Lin$^{-/low}$Sca-1$^+$) [83, 127, 140]. Despite numerous attempts, the unique cellular marker characterizing HSCs has not yet been found. Therefore, as noted above, mouse primitive HSCs are separated nowadays by KTLS phenotype [10, 12, 166] using the fluorescence--activated cell sorting (FACS) with antibody-based approach. New markers, which could be potentially used in mouse HSCs for further purification steps, were also reported recently: CD38 [99], CD201 [5], CD105 [15], Flk-2 [17], and CD49b [148].

There are also additional approaches for HSCs purification. The fluorescent DNA-binding dye Hoechst 33342 was introduced for this purpose by Goodell et al. [47]. Due to the highest efflux of this dye from the most primitive HSCs, these cells form a small but distinct side population when analyzed with flow cytometry simultaneously at two emission wavelengths (red and blue). This population revealed high reconstitution potential *in vivo* and contributed to both lymphoid and myeloid lineages when engrafted [71]. The vast majority of cells selected with Hoechst 33342 were shown to be quiescent primitive HSCs by independent antigen staining, and this method could therefore be useful even alone for purification [47, 89].

Rhodamine-123 (Rh123) is another fluorescent dye which is often used for further purification of HSCs selected by the antibody-based approach [47]. Rh123 distinguishes cell populations based on metabolic activity by staining the mitochondria in cells [59]. It was shown that the low-metabolizing Rh123^{low} KTLS cell population is highly enriched in LTRA cells, whereas Rh123^{high} KTLS cells could be characterized as STRA cells [65]. However, Rh123 alone, without antibodies, is not enough for HSCs purification, because the stained stem cell population is not distinct from the rest of the bone marrow as is the case with the Hoechst SP [47]. The methods of purification described above are not mutually exclusive and could be used together to obtain a population of highly enriched HSCs with potent long--term reconstitution abilities [154].

Studies with purified populations by Cheshier et al. [16] revealed that the majority, about 75%, of the most primitive HSCs are quiescent in terms of the cell cycle, i.e. rest in the G_0 stage. Only about 5% are in S/G_2/M and another 20% in G_1 phase. In the study, with bromodeoxyuridine incorporation, the same authors estimated that approximately 8% of primitive HSCs asynchronously entered the cell cycle per day and 99% of them divided regularly, on average every 57 days [16]. It is important to consider the cell cycle status of HSCs used for transplantation, especially if *ex vivo* cytokine expansion of donor HSCs was used [48]. A large amount of literature data provides strong evidence that quies-

cent purified HSCs engraft successfully, whereas the same cells in $S/G_2/M$ phase revealed defective long-term engraftment [48, 98, 129, 153]. This phenomena was first illustrated by Fleming et al. [39]. In their elegant experiment, just 100 highly enriched G_0/G_1 HSCs rescued 90% of lethally irradiated mice, whereas the same amount of $S/G_2/M$ HSCs protected only 25% of recipients. However, it was also shown that this lack of long-term repopulative ability of $S/G_2/M$ HSCs is reversible as soon as they return in the G_0/G_1 phase [48]. The reduced long-term engraftment of these cells was explained by impaired homing, since it was shown that HSCs have decreased expression of adhesion receptors in $S/G_2/M$ phases of cell cycle [95].

The primitive quiescent HSCs in bone marrow are believed to reside in niches which maintain a special microenvironment. This concept of a definitive cellular space for HSCs was initially proposed by Schofield [115] in 1978. The matrix and supporting cells surrounding HSCs constitute the niche, localized in close proximity to the endosteal surface [46]. It was shown recently that osteoblasts are the key cells in the niche structure. They provide both mechanical support and intrinsic factors for maintaining HSC fate [9, 134, 164]. As an HSC becomes committed, it loses its quiescence, enters the cell cycle, leaves its niche, moves toward the bone marrow central region, and begins to differentiate [130]. Another possibility for an HSC to leave its niche is the physiological migration from the bone marrow to the peripheral blood and back [156]. These processes are a part of normal homeostasis of the hematopoietic system, and Bhattacharya et al. [7] revealed that approximately 0.1–1.0% of niches are vacant at any given time. It was also shown by Wright et al. [156] that although only about 100 HSCs are present in the mouse peripheral blood at any given time, the total turnover is quite high. The daily flux through the blood was estimated as approximately 30,000 HSCs [7]. However, this process could be greatly accelerated following mobilization procedures, which will be described later.

It was thought for a long time that engraftment of donor HSCs would not take place in the case of a non-myeloablated recipient, where there are not enough available niches in the recipient bone marrow [18, 70, 94, 116]. However, a number of studies have provided evidence that myeloablation is not strictly obligatory, at least for syngeneic HSCT, and could be compensated by using mega doses and repetitive injections of HSCs [101, 128, 157]. A good illustration of this concept was the series of experiments performed by Stewart et al. [128]. In their model, non-myeloablated female hosts received 40×10^6 male cells per day for 5 consecutive days. Chromosome banding techniques showed that 5–46% of marrow cells were of donor origin 3–9 months post transplantation. These data and a confirmative study by Wu and Keating [157] further support the theory that some of the niches are available at any time for immediate engraftment of donor stem cells. In addition, Askenasy and Farkas [3] have shown that the primary adhesion of HSCs in bone marrow is not restricted by the availability of free space in stroma or antigen disparity between donor and host.

Zhong et al. [165] raised the question of whether the incomplete engraftment in non-myeloablated mice is the result of a space shortage for graft homing or a lack of proliferation of donor cells. They implanted labeled donor HSCs directly into the femur cavity of myeloablated and non-myeloablated mice and then studied their distribution and proliferation profiles. It was found that the homing of donor HSCs was roughly equivalent in myeloablated and non-myeloablated mice. Thus the incomplete engraftment in non-myeloablated mice with the same homing efficiency was considered by Zhong et al. [165] as the result of a proliferation halt of the donor stem cells. In contrast, in studies by Hendrikx et al. [51] it was shown that homing to non-irradiated marrow was even 2.5 times higher than to irradiated bone marrow. The set of mechanisms controlling this proliferation is quite complex and still largely unknown. It seems that the rapid expansion of donor cells in myeloablated mice is primarily due to the production of stimulatory factors induced by myelodepletion and low competition from the host HSCs [94, 165]. Donor stem cells transplanted following the myeloablation could play a major role in hematopoiesis of the recipient, resulting in a higher level of donor engraftment [101, 165]. In view of these results, a model of competitive stem cell engraftment was proposed which suggests that in the absence of immunological barriers, the final percentage of donor chimerism is determined by the ratio of host to donor stem cells rather than by the extent of the space for engraftment opened by preparative regimens [19, 95, 101, 129]. In other words, syngeneic donor stem cells compete with the host counterparts for niches and could engraft even in a non-myeloablated recipient, but their impact on overall hematopoiesis will be minimal due to high competition from the host stem cells. However, as mentioned above, administration of large stem cell doses could increase the competitive potential of the donor HSCs [128, 157]. The competitive repopulation assay, proposed by the Harrison [49] in 1980 for the first time, is based on this principle and is often used in studies comparing different subsets of HSCs. In this assay, several populations of HSCs easily distinguishable by some marker are mixed in known proportions and their repopulation potential is then assessed by the relative contribution in the hematopoietic reconstitution of the myeloablated recipient. The practical details concerning this assay and its modifications can be found in the recent review by van Os et al. [144].

However, there is at least one further point of concern in allogeneic as distinct from syngeneic HSCT. To break through the antigen disparity barrier, an effective immunosuppressive component has to be introduced to overcome graft rejection by the host immune system [18, 94]. Confirmative data obtained by Colson et al. [18] and Quesenberry et al. [96] in irradiation-based models suggest that there are two distinct populations which

play a role in syngeneic and allogeneic engraftment. Since there is no problem of immunological rejection of donor cells in syngeneic HSCT, it is only necessary to raise their competitive potential over host stem cells. Elimination of the first recipient's population, mostly primitive HSCs with radiosensitivity of about 1–2 Gy, serves this goal and therefore results in marked syngeneic donor engraftment [94]. The second population, host immune cells with radiosensitivity of about 5.5–7 Gy, is responsible for the rejection of allogeneic donor HSCs and has to be removed for effective prevention of graft rejection in the allogeneic model. The problem of graft rejection could also be solved by utilization of a costimulation blockade, which has recently been used more extensively [94, 97].

The engraftment rate in the mouse model is quite rapid if rejection can be effectively prevented, and donor cells could be detected in bone marrow already several hours after intravenous injection [94, 165]. It is important that the impact of the HSC populations described above on the engraftment process is different and was determined in studies with purified HSCs. Hematopoietic reconstitution after HSCT occurs in two phases. There is a rapid repopulation by STRA and late donor progenitor cells with reduced self-renewal capacity in the initial stage. Transient chimerism at this period can be confirmed within two or three weeks [10, 64, 138]. However, sustained long-term engraftment in the later stage is conducted by LTRA, the most primitive HSCs [10, 85].

It is still an issue of debate whether HSCs home to bone marrow or could also engraft in other organs, particularly the spleen. Two groups, Plett et al. [91] and Cao et al. [10], have recently shown that the majority of donor cells home to bone marrow already within 20 h after transplantation, albeit there were substantial populations of donor cells also in the spleen. In the entire bone marrow compartment the primary homing loci of donor HSCs were in the spine (approximately 50%) [10]. This fact is in agreement with results obtained recently by Colvin et al. [19] which show that the spine constitutes approximately half of the entire bone marrow compartment in mice. Plett et al. [91] have also tested functional differences between donor cells which home to bone marrow and to the spleen. They found that bone-marrow-homed cells were significantly enriched in cells capable of secondary multilineage hematopoiesis in mice undergoing serial transplantation compared with spleen-homed cells. Consequently, it was considered that most primitive HSCs home mainly to bone marrow [19, 91], due to the specificity of their adhesion receptors [94]. However, Cao et al. [10] explained the relative possibility of homing HSCs to a specific locus just by the impact of the engrafted organ on the entire hematopoietic system. Since the bone marrow compartment in adult mice is the main site of hematopoiesis, the majority of cells engrafted there. These authors also noted that the final overall contribution of individual hematopoietic stem cells to chimerism does not appear to depend on whether the initial engraftment occurs in the spleen or in the bone marrow [10].

PRETREATMENT REGIMENS USED IN THE MOUSE HSCT MODELS

The type and the intensity of the pretreatment are the key factors in the entire transplantation procedure. The majority of the protocols were designed with the thought in mind that a pretreatment regimen should empty space for competitive donor cells and suppress the host immune system to prevent graft rejection [18, 97]. Pretreatment regimens can be divided into three principal groups: radiation-based, chemotherapy-based, and mixed. The statement "the more intensive, the better" was dominant in the science for a long time, but the complications caused by the severity of the strict myeloablation and immunosuppression protocols, as well as better understanding of the biology of the engraftment process, shifted both clinical and experimental HSCT toward developing milder, so-called non-myeloablative stem cell transplantations (NSTs), which are also mentioned below.

Traditional irradiation regimens are total body irradiation (TBI) and total lymphoid irradiation (TLI) in different doses and/or combinations [64]. Due to the high radiosensitivity of immune and HSCs, TBI is both immunosuppressive and myeloablative and was historically the first method of the preparative regimen [18, 94]. The myeloablating effect of irradiation was shown to result from the induction of apoptosis in both primitive HSCs and mature progenitors [74] due to DNA damage. The ability of irradiation to ablate the recipient's stem cells and eliminate host immune cells to prevent transplant rejection is known to be dose dependent [72, 104]. A single dose of 7–8 Gy is considered to be lethal and ablative enough in the majority of mouse models [14, 20, 43, 45, 63, 111, 142], but there are also reports of 9 Gy [73, 161], 10 Gy [44, 68], 11 Gy [4, 88], 12 Gy [139], and 13 Gy [60] doses. Without rescue HSCT, mice die within 11–18 days after irradiation, depending on the dose received [13, 44, 73, 166]. It was also documented that radiosensitivity is dependent on the age and strain of the experimental mice [22, 145, 162].

The impact of total irradiation dose and the pattern of its administration on the transplantation process was extensively studied in syngeneic and allogeneic, both major histocompatibility complex (MHC)-matched and MHC-mismatched, BMT models. It was clearly demonstrated by Down et al. [34] and further by van Os et al. [145] that the success of donor hematopoietic engraftment is critically dependent on the irradiation dose. Starting already from 2 Gy doses, mixed chimerism was completely transformed into full chimerism in syngeneic models when a single-dose irradiation protocol was applied. In the allogeneneic MHC-matched transplanta-

tion, poor or no engraftment at all was seen at doses below 5.5 Gy, but data at doses of 6 Gy and above were comparable with the syngeneic engraftment curve. As for allogeneneic MHC-mismatched transplantation, even higher doses were needed. This fact was explained by the speculation that only a dose of about 6 Gy and higher is able to effectively suppress immune-mediated rejection in allogeneic HSCT. Similar dose-dependent effects of irradiation on allogeneic engraftment have been confirmed by others [18, 97].

When comparing the same total doses of irradiation, the single-dose schedule has the larger cytoreductive effect on the recipient's stem and immune cells when compared with multiple-dose schedules. Dividing the irradiation into smaller multiple fractions and extending the intervals between them results in diminished engraftment and higher rejection rates in the allogeneic model. Therefore, higher total irradiation doses are needed to induce a level of engraftment similar to that of a single-dose protocol [22, 34, 110, 147]. Applying the single-dose protocol or increasing the dose rate and fractional dose induce engraftment enhancement due to more effective host immunosuppression and myeloablation, but frequently result in a reduction in the recipient survival rate due to the higher overall toxicity of the procedure [34, 110, 147]. However, the decreased myeloablative efficacy of low-irradiation protocols could be compensated by an increased number of stem cells transplanted [110]. Investigators usually balance the intensity of the irradiation and graft stem cell dose to select the optimal protocol depending on the subject of the study and its endpoint. High-irradiation schedules are used in case strong myeloablation is needed and low-irradiation protocols are more applicable if there is a desire to lower the risk of irradiation-induced toxicity and complications.

TLI is an alternative, immunosuppressive, but non-myeloablative radiation protocol in which, in order to suppress host immunity and avoid systemic toxicity of the irradiation, only lymphoid tissue is irradiated. It was shown that this preparative regimen has fewer toxic effects than TBI, reduces the incidence of GVHD and secondary tumor appearance, and can potentially induce stable, long-term engraftment of even fully allogeneic HSCs [123, 125]. The irradiation protocol for TLI often consists of multiple daily fractions with a total dose of up to 34 Gy [125]. Lower doses of TLI could be used if combined with the myeloablative effect of low-dose TBI [121]. It has also been documented that both the thymus and the peripheral lymphoid tissues have to be included in the irradiation field to gain the full immunosuppressive effect of TLI and to prevent graft rejection, especially in allogeneic HSCT [116].

The use of TLI allowed safer administration of much more effective doses of irradiation to the lymphoid tissues while sparing non-lymphoid organs. Thus it appears that non-myeloablative conditioning could result in durable engraftment of even fully mismatched donor stem cells with the induction of permanent and specific transplantation tolerance to all donor tissues while avoiding GVHD [120]. Indeed, as was shown by Slavin et al. [125], BMT following TLI conditioning resulted in stable mixed chimerism, the basis for bilateral unresponsiveness of host-versus-graft and graft-versus-host. The mechanism of unresponsiveness was due primarily to central clonal deletion and natural suppressor cells [79, 124]. It was shown that the host natural killer (NK) T cells act as regulatory natural suppressor cells and hamper the development of GVHD in recipients following TLI [61, 62]. The concept of using TLI as part of a pretreatment regimen to reduce the incidence of GVHD has been transferred into clinical practice [67]. The role of donor alloantigen persistence in maintaining antigen-specific unresponsiveness was also documented, which could explain, in part, the crucial role of mixed chimerism in the induction and maintenance of tolerance [78].

Chemotherapy is another pretreatment regimen which has been introduced in the attempt to avoid irradiation toxicity [29]. To substitute irradiation in allogeneic HSCT successfully, chemotherapy-based regimens must have both myeloablative and immunosuppressive properties [18]. Busulfan (1,4-butanediol dimethanesulfonate, busulphan, myleran, BU) and its homologue dimethylbusulfan (dimethylmyleran) are the myeloablative but non-immunosuppressive agents which are used most widely [29, 64, 161]. The activity of these agents is based on their unique ability to eliminate the most primitive quiescent HSCs in the bone marrow [1, 29, 33, 64, 70, 72]. Although BU is known to be an alkylating agent which is not cell cycle phase specific, the exact mechanism of its selective action against primitive HSCs is not well understood. BU and its analogs administered in several fractions have a more profound effect on stem cells than when given in one large dose [152]. There is a dose-dependent effect of these agents on the degree of myeloablation and therefore the extent of engraftment [1, 161]. Other drugs, namely 5-fluorouracil (5-FU), cyclophosphamide (CY), melphalan, and bischloroethylnitrosourea, are also used. However, Down et al. [31, 33] showed that these non-BU-based agents have only a transient cytoreductive effect on mature dividing cells with little, if any, effect on the population of primitive HSCs. The activity of 5-FU is based on the elimination of mature and cycling progenitors [100]. Therefore, the use of these chemotherapeutic agents alone in HSCT, without introducing an additional myeloablative component into the regimen, is rather limited.

The success of HSCT is highly dependent on suppression of the recipient immune system to prevent graft rejection by host immunocompetent cells. The risk of such rejection is all the higher the more immunological incompatibility there exists between donor and recipient [18]. It has been well known for a long time that BU cannot be used as a single agent for the preparation regimen due to its profoundly myeloablative effect and lack of immunosuppressive properties [161]. The combina-

tion of BU with CY was a treatment of choice not only for allogeneic, but even for autologous transplantations [107]. However, some literature data make it clear that BU may be successfully applied alone in cases where total suppression of the host immune system is not necessary. Such cases include autologous, syngeneic [107, 111], congeneic [1, 161], and sex-mismatched [70] transplantations. As regards allogeneic and, especially, xenogeneic transplantations, BU alone is not enough for a pretreatment regimen, and some additional immunosuppressive therapy must be perfomed [6, 107, 152, 161]. The most frequently applied protocols include myeloablative agents in combination with CY or other immunosuppressive drugs, depending on the type of transplantation [29, 158].

The controversial question is: which regimen is better, chemotherapy or irradiation? The data of Samlowski et al. [111] obtained in a syngeneic transplantation mouse model indicated that both preparative treatments delay the reconstitution of the peripheral lymph node T cell populations; however, immune functions recovered more quickly in BU-treated mice. The comparative studies by the Mauch et al. [72] and Yeager et al. [161] showed that both TBI and BU are effective as pretreatment agents and their myeloablative potencies were found to be approximately equal. Down and Ploemacher [33] performed another experiment in which they investigated the influence of three conditioning regimens: TBI, BU-based, and 5-FU-based, on the extent of erythroid engraftment. They considered the myeloablative effect of a dose of 50 mg/kg BU to be roughly equivalent to that of a single dose of 6–8 Gy TBI. As for 5-FU, it induced only a transient chimerism lasting about 10 weeks.

It is hard to compare these results since there are some differences in chemotherapeutic and irradiation doses which can also influence the data. Due to this fact and the lack of long-term follow-up of the recipient mice treated with both regimens, the question of which regimen is preferable is still unclear, just as in humans. It was shown by Down et al. [30] that many effects of preparative regimens could become evident at a late post-transplantation time. Ratajczak [102] further confirmed the importance of long-term evaluation in his study on the biochemical and constitutional changes in mice receiving syngeneic HSCT after either irradiation--based or BU-based regimens. It was shown that one year after transplantation the body weight of irradiated mice was lower than that of animals treated with BU. However, significant differences in a number of serum biochemical parameters between the two experimental groups were not found up to 60 days post-transplantation. Delayed effects are worthy of long-term evaluation and comparison to discover a better preparative method, but it could be speculated that the BU regimen is the more suitable, at least in syngeneic/congeneic models, because of its mostly myeloablative, rather than immunosuppressive, effect. It also results in faster immunological reconstitution [111]. Furthermore, less damage to host tissue can result in a lower level of inflammatory cytokine release and a reduced incidence of acute GVHD development [33, 111, 159].

Over the last two decades, a trend has become apparent to develop new, less myeloablative regimens and to bring experimental achievements into the clinic. There are two main ideas behind such NST protocols. The first is to ablate the host HSCs to a certain degree to raise the competitive potential of donor inoculum. The second, which is important in allogeneic HSCT, is to suppress the host immune system to just as low as is necessary to prevent allogeneic donor HSC rejection. These regimens are usually much milder than the "classical" ones [1, 116, 129]. The most interesting directions of research are the new analogs of known immunosuppressive and myeloablation agents [152], costimulatory blockade [1, 8, 97, 151], and different combinations of supra-lethal doses of irradiation and/or chemotherapeutic drugs [52, 61, 62, 69, 158]. Induction of alloantigen-primed lymphocyte depletion was recently documented to be an effective method for tolerance induction across MHC using non-myeloablative conditioning as well [93].

One of the most promising of these low-toxic regimens is costimulatory blockade. Wekerle et al. [151] demonstrated that treatment of naive mice with a high dose of fully MHC-mismatched allogeneic bone marrow cells (BMCs) followed by one injection of anti-CD154 monoclonal antibody and cytotoxic T lymphocyte antigen 4 immunoglobulin resulted in multi-lineage hematopoietic macrochimerism that persisted for up to 34 weeks without any cytoreductive therapy. Moreover, long-term chimeras also established donor-specific tolerance. Costimulation blockade-based regimens were recently reviewed by Wekerle et al. [150].

SOURCES OF HSCs IN MOUSE MODELS AND THEIR CHARACTERIZATION

As in humans, there are several sources of hematopoietic stem cells in mice. One can harvest them from bone marrow or peripheral or umbilical cord blood, depending on the type of intended transplantation. Due to differences in the cell content of the sources, their relative repopulating potential, and the course of the engraftment process, the impact of the HSC source on the outcome of the transplantation should be considered in mouse HSCT [45].

The method of bone marrow harvesting is quite simple and well described in the literature [1, 8, 34, 82, 145, 147]. In experimental mouse models this method is probably used most frequently due to the high concentration of HSCs in bone marrow. The majority of researchers usually harvest bone marrow from the hind legs only [22, 33, 69, 73, 82, 97, 158], but some use both the front and hind leg bones, the humeri, femurs, and tibias, to collect more cells [1, 8, 13, 34]. The marrow is harvested by flushing the bones with an appropriate medium or PBS. All of the more or less solid clumps of

debris should be homogenized by passage through a needle or by washing twice with D-galactose [142]. Then the cell suspension is usually filtered and washed twice with medium, sometimes supplemented with antibiotics to prevent possible infectious complications [8, 18, 20, 52, 152]. The entire procedure is conducted under aseptic conditions. It is worth noting that different mouse strains have distinct, genetically determined HCS frequencies and reconstitutive potentials as well as overall cellularity of the bone marrow, and this should also be taken into account before selecting an appropriate donor strain for transplantation [10, 19, 25, 82].

In the two last decades, mobilized peripheral blood cells (MPBCs) have been increasingly used to replace BMCs as a source for HSCT. The successful protocols of mobilized peripheral blood cell transplantation (MPBCT) with high recipient survival rate, long-term hematopoietic chimerism, and low incidence of GVHD were also established in a mouse model [160]. Due to the fact that the peripheral blood of the normal mouse is deficient in HSCs, donor mice have to be stimulated to mobilize these cells from the marrow to the blood. Mobilization is usually performed by granulocyte colony-stimulating factor (G-CSF) or/and cytotoxic drugs, mainly CY [85, 133, 160]. The importance and advantages of different methods of MPBC mobilization were challenged by Neben et al. [85]. The authors investigated three methods: intraperitoneal administration of a single dose (200 mg/kg) of CY one week before cell harvesting, subcutaneous administration of G-CSF (250 μg/kg/d) twice daily for 4 days before cell harvesting, and a combined regimen which included both CY and G-CSF. They determined the optimal timing for harvesting stem cells from the blood during their pilot studies. The primitive stem cell content of stimulated blood was significantly increased following all three mobilization methods, approaching normal bone marrow levels, but was highest in the group treated with the CY + G-CSF combined regimen. The efficacy of a CY + G-CSF combined mobilization protocol identical to the one described above was confirmed in studies by Wierenga et al. [153].

Mice intended to serve as donors ought have their spleens removed under general anesthesia at least 2–4 weeks before donation to avoid pooling of MPBCs in the spleen [45, 153, 160]. After an appropriate mobilization regimen and donor mouse anesthetization, approximately 1 ml of blood has to be harvested from the orbital plexus [153, 160] or by cardiac puncture [85]. An alternative method suggested that if donor survival is not obligatory, blood could be collected under sterile conditions by dissection of both carotid arteries after mouse anticoagulation with heparin and sacrifice by cervical dislocation [45]. After lysis of erythrocytes and adjusting the cell number, the graft can be used for HSCT [153, 160]. There is also a report by Pan et al. [88] about using G-CSF-mobilized splenocytes from the donors as the source for transplantation in the experimental model of GVHD. Transplantation of splenocytes mobilized by G-CSF after TBI as pretreatment regimen resulted in inhibition of the development of GVHD in recipients, but did not abrogate it completely. Therefore, the spleen as a potential source of transplantation inoculum should be treated with caution due to high T cell inoculum. However, in another recent study by Shirasugi et al. [117] it was found that allogeneic barriers could be overcome by the combination of BU, costimulation blockade, and transplantation of allogeneic MHC-mismatched splenocytes. Recipients in this study showed high survival and a significant level of hematopoietic chimerism (5–40%) and developed stable immunological tolerance as confirmed by the skin graft survival.

It is well known that MPBCs and BMCs differ significantly in their HSC compartments. Despite the dramatic increase in the number of HSCs in peripheral blood during the donor pretreatment regimen [153, 160], normal bone marrow still contains a higher percentage of HSCs [45, 140]. Uchida et al. [140] showed that KTLS HSCs constitute approximately 0.08% of total steady-state bone marrow cellularity, whereas even in mobilized peripheral blood there are not more than 0.02–0.04%. Moreover, there are not only quantitative, but also qualitative differences between the two sources, because HSCs from mobilized blood were shown to contain a lower percentage of LTRA cells in comparison with bone marrow [166]. Indeed, Glass et al. [45] reported that BMC transplantation resulted in higher, faster, and more reliable engraftment when the potentials of MPBCs and BMCs were compared with identical numbers of nucleated cells in the mice model. However, a deficiency of HSCs in peripheral blood could be compensated by higher total cell doses of donor MPBC inoculum [45]. It is also worth noting that mobilized peripheral blood contains a higher percentage of T cells than does bone marrow, but GVHD incidence and mortality were shown to be approximately the same in some BMT and MPBCT models [45, 160]. This could be explained by the fact that pretreatment of MPBC donors with G-CSF polarizes T cells toward the type-2 response in a dose-dependent manner, and that this polarization is associated with increased secretion of protective IL-4 and IL-10 cytokines, inhibition of GVHD development, and reduced inflammatory cytokine production [80, 88].

Another difference between HSCs from bone marrow and from mobilized peripheral blood is that most of the HSCs in the mobilized peripheral blood are found to be quiescent [108]. These data were further confirmed by Uchida et al. [140], who showed that only 3% of KTLS HSCs from MPBCs were in $S/G_2/M$ stage, whereas the numbers of cycling KTLS HSCs from the bone marrow and spleens of mobilized animals were determined as 24–26% and 5–7%, respectively. The percentage of HSCs actively dividing in the peripheral blood of the mobilized animals was even lower than that in steady-state bone marrow from untreated animals (approximately 5% of KTLS cells in $S/G_2/M$ [16]). It was suggested and further confirmed that virtually all HSCs

enter the cell cycle in response to mobilization [84]. Afterwards, actively cycling cells migrate to the blood just after the M phase and prior to the next S phase, when they are in G_0/G_1 [155]. Additionally, the presence of HSCs in the blood was revealed to be brief; they selectively migrate mainly to the spleen, liver, and back to bone marrow, where they enter the next cell cycle [155]. Mobilization is also connected with the reciprocal expression of CD34 and CD38, as was shown by Tajima et al. [135]. The majority of mouse HSCs in steady-state adult mouse bone marrow was found to be $CD34^-CD38^+$, but they change phenotype to $CD34^+CD38^-$ after mobilization into the blood. However, after returning to the bone marrow they reverted their phenotype back to the $CD34^-CD38^+$ expression.

Umbilical cord blood transplantation is not a widespread model of mouse HSCT, probably due to its relative complexity. Different sources of HSCs were proposed as the surrogate of human umbilical cord blood: mouse near-term fetal and newborn peripheral blood (FNPB) [26, 27, 50, 54, 114], umbilical cord blood [76], and fetal liver cells [50, 133]. Successful transplantations of FNPB in syngeneic [114] and both MHC-matched [27] and MHC-mismatched [54] allogeneic adult mice with long-term hematopoietic chimerism and very low or even absent incidence of GVHD are described in the literature. Decreased incidence of GVHD was explained by the fact that the majority of T cells from FNPB are of immature phenotype [26]. Conversely, FNPB was also shown to have poorer radioprotective capacity, slower allogeneic engraftment, and impaired immune reconstitution compared with adult bone marrow and fetal liver cells [13, 50]. This could be explained by the fact that FNPB contains a lower number of HSCs than adult bone marrow and fetal liver [50]. However, the long-term repopulative abilities of HSCs themselves appeared to be similar, whether they were from adult bone marrow or from FNPB [50]. It was reported recently by Migishima et al. [76] that mouse umbilical cord blood cells could also fully reconstitute the hematopoietic system of lethally irradiated recipients.

Cells from fetal liver were shown to have an approximately 7-fold increased percentage of HSCs compared with adult bone marrow [81] and a significantly higher capacity for long-term engraftment compared with mobilized peripheral blood, FNPB, and adult bone marrow [50, 103, 133]. The mouse fetal liver is the primary site of hematopoiesis during the middle part of gestation, but after day 15 the number of HSCs in the liver declines very fast [81]. The phenotype of fetal liver HSCs was shown to be similar to their adult bone marrow counterparts (KTLS cells) [81, 105]. However, a number of distinct markers are also expressed on them, namely Mac-1 [81, 105], AA4.1 [58, 105], and CD34 [112]. The latter is considered to be the developmental marker, since the majority of mouse HSCs are $CD34^+$ until the 10th week of life, and after that time the expression of this marker on the HSCs from stable adult bone marrow becomes approximately 20% [56]. However, as noted above, it should not be forgotten that it is also expressed on HSCs from mobilized peripheral blood [135]. Therefore, the expression of CD34 in mouse HSCs is dependent on the source used. It was also shown that HSCs from fetal liver cycle more actively and contain twice as many cells in $S/G_2/M$ phase as the HSCs from adult bone marrow [39].

Quantitative and qualitative differences between available sources of HSCs are still a point of major concern. There are often conflicting results about their reconstitution potential, the frequency of most primitive stem cells, and the risk of GVHD development. Further studies are needed to clarify the relative usefulness of HSCs from these sources and their biological properties. Another promising direction is designing HSCs from embryonic stem cells and using them in transplantation. Embryonic stem cells are truly pluripotent and have the unique ability to become virtually any tissue. There have been remarkable advances in this area during the last decade and initial results are hopeful. This topic is beyond the scope of this paper, and interested readers are strongly encouraged to read the recent comprehensive review by Olsen et al. [86].

Another critical factor which could influence the outcome of HSCT is the age of both donor and recipient. Older mice (10 and more months old) were shown to have decreased bone marrow cellularity [19], impaired homing [66], lower frequency of the most primitive HSCs, and reduced self-renewal of these cells [60]. This fact results in defective engraftment of HSCs harvested from aged donors [60] and slower hematopoietic recovery of the recipients [66]. Furthermore, GVHD mortality, morbidity, and severity were all shown to be worse in aged recipient mice, probably due to upregulation of host antigen-presenting cells [87].

HSC DOSE, STATUS, MANIPULATION, AND TRANSPLANTATION

Irrespective of the type of pretreatment regimen used, most researchers prefer to inject HSC inoculum within 24 h after TBI [4, 11, 63, 111, 153], TLI [125], or the last administered fraction of irradiation [22] or chemotherapy [64, 111]. The collected graft cells have to be injected intravenously into either the tail vein [18, 45, 52] or paraorbital sinus [10, 82, 147].

The success of HSCT and the speed and extent of engraftment of donor HSCs are highly dependent on the number of cells transplanted [43, 45, 77, 129, 141]. The optimal dose of cells depends on the relatedness of the donor and recipient as well as the myeloablative and immunosuppressive therapy conducted before transplantation [1, 64, 110, 118, 158]. It was shown in autologous and syngeneic models that 10^6–10^7 unseparated nucleated BMCs or MPBCs are enough for full regeneration of the host hematopoietic system [45, 111]. For allogeneic MHC-matched and, especially, MHC-mismatched transplantations, 10^7–10^8 or even higher doses

of unseparated cells should be used [8, 20, 43, 45, 142, 151]. In other words, the general principle is: the more histoincompatibility and the less intense the myeloablation preparative regimen, the higher the cell dose needed, since it was shown that even a small difference in cell antigen expression between donor and recipient mice could impact on the extent of the engraftment [18, 34, 69, 138, 145, 146]. The dependence between the degree of histoincompatibility and graft cell dose [118] can easily be explained. Even in immunosuppressed hosts, the residual immune cells act against graft inoculum if there is significant genetic disparity between donor and recipient. Therefore, the risk of graft rejection increases together with the degree of histoincompatibility.

The importance of preparative regimen intensity and its connection with optimal graft cell dose was clearly illustrated by Stewart et al. [129] in sex-mismatched models of BMT. In their studies, recipient mice without TBI did not develop marked chimerism regardless of the HSC dose transplanted (2, 10, or 40×10^6). After irradiation of recipients with 100 cGy, the extent of engraftment was clearly dose dependent, with the highest at 40×10^6 dose. However, when a TBI dose of 700 cGy was used, recipients transplanted with all HSCs doses experienced the same high level of donor chimerism (near 100%). These studies further confirm that engraftment is most dependent on the HSC dose when the preparative regimen is suboptimal [69]. These data are in agreement with the concept of competitive engraftment, described previously in the chapter on the biology of HSCs. Therefore, to induce a high level of donor chimerism, one needs to increase the competitive potential of the donor HSCs in comparison with residual host HSCs. This could be done either by raising the intensity of the preparative regimens or by increasing the number of donor HSCs transplanted [104, 141].

Thanks to the identification of markers of mouse primitive stem cells, it is possible nowadays to transplant purified donor HSCs. These cells have the highest reconstitutive potential, and much smaller doses could be utilized to effectively reconstitute the host with donor cells [65, 141]. Moreover, the more purified (primitive) they are, the lower the cell dose needed, since successful multilineage engraftment from even a single HSC is described in the literature [10, 71]. However, even using purified HSCs there is a dependency between cell dose and the speed of donor reconstitution, at least to some extent [12, 141].

Purification of HSCs is usually performed by magnetic cell-separation methods, FACS methods using the antibody-based or/and fluorescent dye-based approaches, and density gradient centrifugation. These procedures are described in detail in a recent comprehensive review [154]. The above methods are not equal in purification efficacy and are often used in combination to ensure a higher purity of the final HSC population. Density gradient centrifugation allows discarding red blood cells and granulocytes and results in several-fold enrichment. Because of its low productivity, this method could only be considered as an initial stage of purification. Using the fluorescent dyes Hoechst 33342 and Rh123 to select the side population and $Rh123^{-/low}$ cells, respectively, is more productive, especially for the former dye [47]. These methods result in approximately 1000-fold enrichment and allow us to select populations which already contain significant percentages of primitive HSCs [47, 130]. However, antibody-based FACS and magnetic cell-separation are the most effective methods of purification. In mice they utilize positive ($c\text{-}kit^+$, $Thy1.1^{low}$, $Sca\text{-}1^+$) as well as negative (Lin^-, depleted of CD3, B220, Gr1, Mac1, Ter119 cells) selection markers, each resulting in approximately 10- to 20-fold enrichment [154].

When dealing with unpurified populations of HSCs, many investigators prefer to remove immunocompetent cells, such as different subsets of T cells, from transplant material. This removal has a strong inhibitory effect on the development of GVHD, one of the most dangerous complications of HSCT [126, 138, 139]. However, not all lymphoid subsets have such a negative effect on the outcome of transplantation. It was shown that donor alloreactive NK cells actually protect recipient mice from GVHD development by eliminating recipient antigen-presenting cells [109]. This protective effect was shown to be TGF-β-dependent, since it was completely abrogated by the treatment of the recipients with the anti-TGF-β monoclonal antibody [2].

It should not be forgotten that GVL/GVT effects are considered to be induced by T cells [36, 90, 122]. Although numerous studies suggest that GVHD could be separated from GVL/GVT [2, 36, 44, 45, 55, 113, 139], there is likely to be an overlap between them [138]. This connection was recently confirmed by the observation that the host antigen-presenting cells, which play the major role in T cell activation and the development of GVHD [35, 75, 119], are also crucial for the GVL response [106]. The GVL/GVT effects and their utilization in immunotherapy are beyond the scope of this review, and we refer interested readers to recent and comprehensive papers discussing the subject [90, 122]. It is worth noting that mouse bone marrow contains significantly fewer T cells (1–3%) compared with human bone marrow and this could account for the somewhat lower risk of GVHD development in the murine model [1, 126, 145]. Another explanation is that T cells in mouse bone marrow were shown to contain a high proportion of NK T cells, which are actually suppressive for GVHD [163]. Spleen or lymph node cells are routinely added to transplant inoculums if an increased T cell compartment is required for a special purpose [63, 126, 138]. Different antibodies, in combination with complement, were historically used to eliminate immunocompetent cells or their specific subsets from the graft in experiments demanding T cell depletion. The most frequently used monoclonal antibodies were anti-CD90, anti-CD4/CD8, and anti-CD3 [1, 12, 29, 69, 138, 142]. However, the immunomagnetic depletion of undesirable cells is utilized nowadays more often due to the convenience of the method [45, 113].

The influence of immunocompetent cell depletion on the engraftment process itself has been a controversial subject of inquiry during the past two decades. Lapidot et al. [63] obtained interesting results in an experiment performed on nude mice at the beginning of the 90's. It was shown that to ensure the success of the engraftment, a large inoculum of nude bone marrow must be supplemented with a trace quantity of donor T cells, whereas a small bone marrow dose requires a much larger number of T cells. The authors considered that the engraftment of allogeneic bone marrow is T cell dependent. Soderling et al. [126] concluded on the basis of their investigations that engraftment failure is partly determined by a dynamic equilibrium between T cells in the donor graft and the surviving host HSCs in the conditioned recipients, especially if a low number of donor HSCs was infused. More intensive conditioning is needed for T cell-depleted HSC engraftment. However, there are some data showing that T cell depletion does not significantly alter the rate of immunohematopoietic reconstitution in allogeneic MHC-matched and MHC--mismatched transplantations in mice [32, 43, 142]. These seemingly contrary results could be explained by the differences in the pretreatment regimens, because Truitt and Atasoylu [138] showed that the impact of donor T cells on the engraftment is most critical in models utilizing suboptimal conditioning. It is generally agreed nowadays that the graft rejection rate is higher in T cell-depleted HSCT due to the lack of a "protective" effect from the residual immune system of the recipient and an abrogation of hematopoietic growth factor release by donor T cells [4, 36, 37]. This effect was shown to be mediated by facilitating cells, two subpopulations of which are characterized by the $CD8^+TCR^+$ and $CD8^+TCR^-$ phenotypes [41]. The latter cells share phenotypic and functional similarities with the plasmacytoid precursor dendritic cells [40].

POST-TRANSPLANTATION MICE SURVEY AND COMPLICATIONS

It is generally agreed that it is better to maintain the recipient mice in particularly pathogen-free conditions after transplantation and provide them with sterile food and acidified water due to the high risk of infection-related mortality resulting from prolonged immunosuppression after the preparative regimen and the transplantation itself [11, 73, 138, 153, 161]. Antibiotics are often added to the mice's diet for 2–4 weeks post-transplantation as an additional method of infection prevention [4, 10, 20, 45, 82, 126].

Another serious and frequent complication is GVHD, which occurs when immunocompetent cells from the donor are introduced into an immunoincompetent recipient and start immunological reactions against host alloantigens [36, 143]. Acute GVHD occurs in the early period after transplantation and manifests by general wasting, weight and fur loss, skin ulcers, hunched posture, perianal dermatitis, diarrhea [8, 18, 42, 52, 69, 138, 139, 158], as well as by histological signs of acute inner organ injury, such as acantholysis and pyknosis of the nuclei in the epidermis, single-cell necrosis of the bowel epithelium with focal cellular infiltration (I–II stage), thinning of the epidermis, degeneration and loss of hair follicles, crypt abscesses of the bowel, cellular infiltration, and lowering of cellularity of spleen (stages III–IV) [11, 138, 159]. The main target organs for acute GVHD are skin, gastrointestinal tract, lungs, liver, and spleen [20, 143]. Chronic GVHD develops later, is not necessarily preceded by the acute stage, and is characterized by pathological changes in multiple organs resembling autoimmune syndrome.

The extent of histoincompatibility between donor and host and the residual number of T cells in the graft have a major impact on the development of GVHD [138, 139, 159]. The type and the intensity of the conditioning regimen are also important factors [24, 159]. Hill et al. [52] showed increased GVHD severity after intensification of the TBI dose. The authors identified irradiation intensity as an independent factor of GVHD development. More intensive radiation causes greater damage to the mucous membrane of the gastrointestinal tract and therefore greater amounts of microbial endotoxins entered the systemic circulation, inducing an increase in the level of TNF-α, a potent inductor of the GVHD. The importance of bowel decontamination and administration of probiotic microorganisms in the prevention of GVHD was also established [37, 42]. Furthermore, it was shown that administration of endotoxine antagonist to the recipient early after transplantation inhibits the secretion of TNF-α and reduces the GVHD manifestations [21]. The leading role of inflammatory cytokines in acute GVHD was recently confirmed in the study by Teshima et al. [136]. Altogether, the observations above are consistent with the "cytokine storm" paradigm, which suggests that a cascade of inflammatory cytokines, caused both by the conditioning regimen and microbial endotoxins leaking through damaged intestinal mucous membrane, induces the allogeneic T cell activation at the onset of acute GVHD and also attacks target cells directly. The current concept of the mechanism of T cell activation and the role of different cytokines are described in detail elsewhere [37, 38, 53].

Several approaches have been designed to reduce the incidence of acute GVHD. The effect of donor T cells on the development of this disease has already been underscored, so the most frequent ways to prevent GVHD are *ex vivo* removal of defined subsets or the entire T cell population from the marrow graft [36, 37, 149] or post-transplant immunosuppression by glucocorticoids [37], CY [69], cyclosporin A [20, 37], rapamycin [14, 29, 158], or methotrexate [11, 37]. Different alternative approaches to preventing experimental GVHD were also described: the use of specific monoclonal antibodies which inhibit donor T cell expansion [149], the administration of some cytokines (IL-4, IL-11,

IL-12) or inflammatory cytokine inhibitors [37], the addition of immunoregulatory cells inhibiting the development of GVHD ($CD4^+CD25^+$ regulatory T cells, regulatory dendritic cells, γ (δ) T cells, NK cells, veto cells, mesenchymal stem cells) [2, 4, 28, 36, 55, 61, 62, 109, 113], and therapy with specific immunosuppressive agents, such as T helper 1 inhibitor [68]. The critical role of host antigen-presenting cells in the development of the disease was already mentioned and targeting of these cells could therefore be a prospective strategy for the suppression of GVHD [35, 75, 119]. Comprehensive reviews discussing modern strategies of GVHD prevention and treatment have also been recently published [23, 24].

TYPE OF HEMATOPOIETIC CHIMERISM AND ITS ASSESSMENT

Full donor chimerism is the state where the donor's hematopoietic system has entirely replaced that of the recipient (near to 100% of donor cells). In mixed chimerism (also referred to as macrochimerism), both the donor's and the recipient's cells coexist and are usually detectable by flow cytometry analysis (between 1% and 100% of donor cells). Finally, if the donor's stem cells persist in the recipient at a low level, beyond the range of flow cytometry operation but detectable by molecular techniques, such a state is referred to as microchimerism (less that 1% of donor cells) [77, 144, 149]. In spite of some differences between clinical and experimental HSCT, in most mouse models stable mixed hematopoietic chimerism is strongly associated with both donor- and recipient-specific tolerance and higher resistance to GVHD compared with full chimeras [1, 29, 69, 77, 149]. Immunological tolerance in chimeric mice may be maintained by clonal deletion, anergy, or through active suppression [69, 149]. Split chimerism, the engraftment of only selected donor-lineage cells, is also noted in mouse models of HSCT [138].

Earlier signs of engraftment in peripheral blood can be obtained if molecular analysis is done using the polymerase chain reaction. Assessment performed up to one month after HSCT can already provide evidence of transient donor-recipient chimerism, but observation periods of three months and longer have to be applied if proof of long-term and stable engraftment is needed (Shimon Slavin, Hadassah University Hospital, Israel, personal communication).

The extent of engraftment is assessed nowadays mainly by FACS analysis of the recipient's peripheral blood, lymph nodes, and spleen. Depending on the donor and recipient relatedness, MHC antigen-specific anti-H-2 monoclonal antibodies [1, 8, 18, 22, 43, 64, 73, 116, 158, 159] or non-MHC antigen-specific ones, such as anti-CD45, anti-CD5 or anti-CD90 [45, 52, 69, 97, 116, 146, 161], are applied to determine the percentage of cells of donor origin among the whole population. The expressions of these markers are lineage dependent: CD5 and CD90 are suitable only for lymphoid engraftment assessment, whereas CD45 is expressed on all bone-marrow-derived cells except mature erythroid cells [146]. Moreover, several appropriate biochemical markers, mostly for erythroid engraftment, distinguishing donor- from host-derived cells by electrophoresis have also been described: glucose-phosphate isomerase, hemoglobin, and carbonic anhydrase [18, 33, 34, 50, 145–147, 152]. In a number of recent studies dealing especially with HSC homing and trafficking, the use of HSC-expressed fluorescent markers obtained from transgenic animals is reported. Such donor cells expressing green fluorescent protein [76] or another variant [131] could be monitored *in vivo* and greatly improve our understanding of HSC fate after transplantation. Alternatively, donor HSCs from normal mice could be labeled with fluorescent membrane linker dye [3, 51, 133]. The polymerase chain reaction is another highly sensitive approach, capable of detecting even low levels of chimerism [114, 157]. Among other methods used, complement-dependent microcytotoxicity assay [125], Southern blot [129], and fluorescent *in situ* hybridization [19, 98, 114, 129] can also be noted.

CONCLUSIONS

The success of HSCT is critically dependent on a number of key factors, which we highlighted in this review. First of all, there is no single population of HSCs, but rather a diversity of overlapping subsets with different degrees of maturity, self-renewal abilities, and repopulative properties. Their engraftment kinetics and impact on long-term reconstitution are not equal and this fact should be taken into account. Similarly, HSCs from the different sources available nowadays are also distinct in their phenotypes and hematopoietic properties. The degree of histoincompatibility, inoculum cell dose, and the intensity of the preparative regimen are all interconnected and together have a strong impact on the extent of engraftment, risk of graft rejection, and GVHD incidence. Graft manipulations (T cell depletion, *ex vivo* expansion, purification of HSCs) are also important to consider. Among others, the age of both donor and recipient, the status of gut flora, genetic differences between HSC compartments in the strains available, and the type of GVHD prophylaxis should also be noted.

Summarizing, the entire process of HSCT could be presented as a biological model with various, strongly interrelated factors. A change in one variable most likely results in changes in others. For example, to illustrate this concept, an increase in the preparation regimen intensity results in a higher degree of allogeneic engraftment, but also provokes GVHD. T cell depletion decreases GVHD incidence, but also increases the risk of graft rejection. The possibility of rejection can be lowered by increasing the HSC dose transplanted or by a further increase in the preparation regimen intensity,

and so on. Finally, different endpoints are important for the HSCT model and could be used in its assessment: survival rate, incidence of GVHD, the speed and extent of engraftment, lymphoid and myeloid reconstitution, immunological tolerance development, and the frequency of graft rejection.

The first mouse model of HSCT was developed more than 50 years ago. During the past decades of intensive research we have gained much knowledge about the biological basis of the engraftment process in mice, transplantation benefits and complications, and the possibilities of using this model in experimental models preceding clinical trials in humans. However, there are still many issues of debate in this area and we hope that this paper can help to point towards further directions for research.

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REFERENCES

1. Adams A. B., Durham M. M., Kean L., Shirasugi N., Ha J., Williams M. A., Rees P. A., Cheung M. C., Mittelstaedt S., Bingaman A. W., Archer D. R., Pearson T. C., Waller E. K. and Larsen C. P. (2001): Costimulation blockade, busulfan, and bone marrow promote titratable macrochimerism, induce transplantation tolerance, and correct genetic hemoglobinopathies with minimal myelosuppression. J. Immunol., **167**, 1103–1111.
2. Asai O., Longo D. L., Tian Z. G., Hornung R. L., Taub D. D., Ruscetti F. W. and Murphy W. J. (1998): Suppression of graft-versus-host disease and amplification of graft-versus-tumor effects by activated natural killer cells after allogeneic bone marrow transplantation. J. Clin. Invest, **101**, 1835–1842.
3. Askenasy N. and Farkas D. L. (2002): Antigen barriers or available space do not restrict *in situ* adhesion of hemopoietic cells to bone marrow stroma. Stem Cells, **20**, 80–85.
4. Bachar-Lustig E., Reich-Zeliger S. and Reisner Y. (2003): Anti-third-party veto CTLs overcome rejection of hematopoietic allografts: synergism with rapamycin and BM cell dose. Blood, **102**, 1943–1950.
5. Balazs A. B., Fabian A. J., Esmon C. T. and Mulligan R. C. (2006): Endothelial protein C receptor (CD201) explicitly identifies hematopoietic stem cells in murine bone marrow. Blood, **107**, 2317–2321.
6. Basch R. S., Quito F. L., Beh J. and Hirst J. A. (1997): Growth of human hematopoietic cells in immunodeficient mice conditioned with cyclophosphamide and busulfan. Stem Cells, **15**, 314–323.
7. Bhattacharya D., Rossi D. J., Bryder D. and Weissman I. L. (2006): Purified hematopoietic stem cell engraftment of rare niches corrects severe lymphoid deficiencies without host conditioning. J. Exp. Med., **203**, 73–85.
8. Blaha P., Bigenzahn S., Koporc Z., Schmid M., Langer F., Selzer E., Bergmeister H., Wrba F., Kurtz J., Kiss C., Roth E., Muehlbacher F., Sykes M. and Wekerle T. (2003): The influence of immunosuppressive drugs on tolerance induction through bone marrow transplantation with costimulation blockade. Blood, **101**, 2886–2893.
9. Calvi L. M., Adams G. B., Weibrecht K. W., Weber J. M., Olson D. P., Knight M. C., Martin R. P., Schipani E., Divieti P., Bringhurst F. R., Milner L. A., Kronenberg H. M. and Scadden D. T. (2003): Osteoblastic cells regulate the haematopoietic stem cell niche. Nature, **425**, 841–846.
10. Cao Y. A., Wagers A. J., Beilhack A., Dusich J., Bachmann M. H., Negrin R. S., Weissman I. L. and Contag C. H. (2004): Shifting foci of hematopoiesis during reconstitution from single stem cells. Proc. Natl. Acad. Sci. USA, **101**, 221–226.
11. Cetkovic-Cvrlje M., Roers B. A., Waurzyniak B., Liu X. P. and Uckun F. M. (2001): Targeting Janus kinase 3 to attenuate the severity of acute graft-versus-host disease across the major histocompatibility barrier in mice. Blood, **98**, 1607–1613.
12. Chen B. J., Cui X., Sempowski G. D., Domen J. and Chao N. J. (2004): Hematopoietic stem cell dose correlates with the speed of immune reconstitution after stem cell transplantation. Blood, **103**, 4344–4352.
13. Chen B. J., Cui X., Sempowski G. D., Gooding M. E., Liu C., Haynes B. F. and Chao N. J. (2002): A comparison of murine T-cell-depleted adult bone marrow and full-term fetal blood cells in hematopoietic engraftment and immune reconstitution. Blood, **99**, 364–371.
14. Chen B. J., Morris R. E. and Chao N. J. (2000): Graft-versus-host disease prevention by rapamycin: cellular mechanisms. Biol. Blood Marrow Transplant., **6**, 529–536.
15. Chen C. Z., Li M., de Graaf D., Monti S., Gottgens B., Sanchez M. J., Lander E. S., Golub T. R., Green A. R. and Lodish H. F. (2002): Identification of endoglin as a functional marker that defines long-term repopulating hematopoietic stem cells. Proc. Natl. Acad. Sci. USA, **99**, 15468–15473.
16. Cheshier S. H., Morrison S. J., Liao X. and Weissman I. L. (1999): *In vivo* proliferation and cell cycle kinetics of long--term self-renewing hematopoietic stem cells. Proc. Natl. Acad. Sci. USA, **96**, 3120–3125.
17. Christensen J. L. and Weissman I. L. (2001): Flk-2 is a marker in hematopoietic stem cell differentiation: a simple method to isolate long-term stem cells. Proc. Natl. Acad. Sci. USA, **98**, 14541–14546.
18. Colson Y. L., Wren S. M., Schuchert M. J., Patrene K. D., Johnson P. C., Boggs S. S. and Ildstad S. T. (1995): A nonlethal conditioning approach to achieve durable multilineage mixed chimerism and tolerance across major, minor, and hematopoietic histocompatibility barriers. J. Immunol., **155**, 4179–4188.
19. Colvin G. A., Lambert J. F., Abedi M., Hsieh C. C., Carlson J. E., Stewart F. M. and Quesenberry P. J. (2004): Murine marrow cellularity and the concept of stem cell competition: geographic and quantitative determinants in stem cell biology. Leukemia, **18**, 575–583.
20. Contassot E., Robinet E., Angonin R., Laithier V., Bittencourt M., Pavy J. J., Cahn J. Y., Herve P. and Tiberghien P. (1998): Differential effects of cyclosporin A on the alloreactivity of fresh and *ex vivo*-expanded T lymphocytes. Bone Marrow Transplant., **22**, 1097–1102.
21. Cooke K. R., Gerbitz A., Crawford J. M., Teshima T., Hill

G. R., Tesolin A., Rossignol D. P. and Ferrara J. L. (2001): LPS antagonism reduces graft-versus-host disease and preserves graft-versus-leukemia activity after experimental bone marrow transplantation. J. Clin. Invest, **107**, 1581–1589.
22. Cui Y. Z., Hisha H., Yang G. X., Fan T. X., Jin T., Li Q., Lian Z. and Ikehara S. (2002): Optimal protocol for total body irradiation for allogeneic bone marrow transplantation in mice. Bone Marrow Transplant., **30**, 843–849.
23. Davies J. K. and Lowdell M. W. (2003): New advances in acute graft-versus-host disease prophylaxis. Transfus. Med., **13**, 387–397.
24. Deeg H. J. (2003): New strategies for prevention and treatment of graft-versus-host disease and for induction of graft-versus-leukemia effects. Int. J. Hematol., **77**, 15–21.
25. de Haan G., Szilvassy S. J., Meyerrose T. E., Dontje B., Grimes B. and Van Zant G. (2000): Distinct functional properties of highly purified hematopoietic stem cells from mouse strains differing in stem cell numbers. Blood, **96**, 1374–1379.
26. de La Selle V., Gluckman E. and Bruley-Rosset M. (1996): Newborn blood can engraft adult mice without inducing graft-versus-host disease across non H-2 antigens. Blood, **87**, 3977–3983.
27. de La Selle V., Gluckman E. and Bruley-Rosset M. (1998): Graft-versus-host disease and graft-versus-leukemia effect in mice grafted with peripheral newborn blood. Blood, **92**, 3968–3975.
28. Deng W., Han Q., Liao L., Li C., Ge W., Zhao Z., You S., Deng H. and Zhao R. C. (2004): Allogeneic bone marrow-derived flk-1+Sca-1- mesenchymal stem cells leads to stable mixed chimerism and donor-specific tolerance. Exp. Hematol., **32**, 861–867.
29. de Vries-van der Zwan, van der Pol M. A., de Waal L. P. and Boog C. J. (1998): An alternative conditioning regimen for induction of specific skin graft tolerance across full major histocompatibility complex barriers. Transpl. Immunol., **6**, 147–151.
30. Down J. D., Berman A. J., Warhol M., Van Dijken P. J., Ferrara J. L., Yeap B., Hellman S. and Mauch P. M. (1989): Late tissue-specific toxicity of total body irradiation and busulfan in a murine bone marrow transplant model. Int. J. Radiat. Oncol. Biol. Phys., **17**, 109–116.
31. Down J. D., Boudewijn A., Dillingh J. H., Fox B. W. and Ploemacher R. E. (1994): Relationships between ablation of distinct haematopoietic cell subsets and the development of donor bone marrow engraftment following recipient pretreatment with different alkylating drugs. Br. J. Cancer, **70**, 611–616.
32. Down J. D., Mauch P., Warhol M., Neben S. and Ferrara J. L. (1992): The effect of donor T lymphocytes and total-body irradiation on hemopoietic engraftment and pulmonary toxicity following experimental allogeneic bone marrow transplantation. Transplantation, **54**, 802–808.
33. Down J. D. and Ploemacher R. E. (1993): Transient and permanent engraftment potential of murine hematopoietic stem cell subsets: differential effects of host conditioning with gamma radiation and cytotoxic drugs. Exp. Hematol., **21**, 913–921.
34. Down J. D., Tarbell N. J., Thames H. D. and Mauch P. M. (1991): Syngeneic and allogeneic bone marrow engraftment after total body irradiation: dependence on dose, dose rate, and fractionation. Blood, **77**, 661–669.
35. Duffner U. A., Maeda Y., Cooke K. R., Reddy P., Ordemann R., Liu C., Ferrara J. L. and Teshima T. (2004): Host dendritic cells alone are sufficient to initiate acute graft-versus-host disease. J. Immunol., **172**, 7393–7398.
36. Edinger M., Hoffmann P., Ermann J., Drago K., Fathman C. G., Strober S. and Negrin R. S. 2003): CD4+CD25+ regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation. Nat. Med., **9**, 1144–1150.
37. Ferrara J. L., Cooke K. R., Pan L. and Krenger W. (1996): The immunopathophysiology of acute graft-versus-host-disease. Stem Cells, **14**, 473–489.
38. Ferrara J. L., Cooke K. R. and Teshima T. (2003): The pathophysiology of acute graft-versus-host disease. Int. J. Hematol., **78**, 181–187.
39. Fleming W. H., Alpern E. J., Uchida N., Ikuta K., Spangrude G. J. and Weissman I. L. (1993): Functional heterogeneity is associated with the cell cycle status of murine hematopoietic stem cells. J. Cell Biol., **122**, 897–902.
40. Fugier-Vivier I. J., Rezzoug F., Huang Y., Graul-Layman A. J., Schanie C. L., Xu H., Chilton P. M. and Ildstad S. T. (2005): Plasmacytoid precursor dendritic cells facilitate allogeneic hematopoietic stem cell engraftment. J. Exp. Med., **201**, 373–383.
41. Gandy K. L., Domen J., Aguila H. and Weissman I. L. (1999): CD8+TCR+ and CD8+TCR– cells in whole bone marrow facilitate the engraftment of hematopoietic stem cells across allogeneic barriers. Immunity, **11**, 579–590.
42. Gerbitz A., Schultz M., Wilke A., Linde H. J., Scholmerich J., Andreesen R. and Holler E. (2004): Probiotic effects on experimental graft-versus-host disease: let them eat yogurt. Blood, **103**, 4365–4367.
43. Glass B., Uharek L., Gaska T., Gassmann W., Loffler H. and Muller-Ruchholtz W. (1993): The influence of graft-versus-host reactivity, lymphocyte depletion, and cell dose on allogeneic bone marrow engraftment. Bone Marrow Transplant., **12** (suppl. 3), S41–S47.
44. Glass B., Uharek L., Gassmann W., Focks B., Bolouri H., Loeffler H. and Mueller-Ruchholtz W. (1992): Graft-versus-leukemia activity after bone marrow transplantation does not require graft-versus-host disease. Ann. Hematol., **64**, 255–259.
45. Glass B., Uharek L., Zeis M., Dreger P., Loffler H., Steinmann J. and Schmitz N. (1997): Allogeneic peripheral blood progenitor cell transplantation in a murine model: evidence for an improved graft-versus-leukemia effect. Blood, **90**, 1694–1700.
46. Gong J. K. (1978): Endosteal marrow: a rich source of hematopoietic stem cells. Science, **199**, 1443–1445.
47. Goodell M. A., Brose K., Paradis G., Conner A. S. and Mulligan R. C. (1996): Isolation and functional properties of murine hematopoietic stem cells that are replicating *in vivo*. J. Exp. Med., **183**, 1797–1806.
48. Habibian H. K., Peters S. O., Hsieh C. C., Wuu J., Vergilis K., Grimaldi C. I., Reilly J., Carlson J. E., Frimberger A. E., Stewart F. M. and Quesenberry P. J. (1998): The fluctuating phenotype of the lymphohematopoietic stem cell with cell cycle transit. J. Exp. Med., **188**, 393–398.
49. Harrison D. E. (1980): Competitive repopulation: a new assay for long-term stem cell functional capacity. Blood, **55**, 77–81.
50. Harrison D. E. and Astle C. M. (1997): Short- and long-term multilineage repopulating hematopoietic stem cells in late fetal and newborn mice: models for human umbilical cord blood. Blood, **90**, 174–181.

51. Hendrikx P. J., Martens C. M., Hagenbeek A., Keij J. F. and Visser J. W. (1996): Homing of fluorescently labeled murine hematopoietic stem cells. Exp. Hematol., **24**, 129–140.
52. Hill G. R., Crawford J. M., Cooke K. R., Brinson Y. S., Pan L. and Ferrara J. L. (1997): Total body irradiation and acute graft-versus-host disease: the role of gastrointestinal damage and inflammatory cytokines. Blood, **90**, 3204–3213.
53. Hill G. R. and Ferrara J. L. (2000): The primacy of the gastrointestinal tract as a target organ of acute graft-versus--host disease: rationale for the use of cytokine shields in allogeneic bone marrow transplantation. Blood, **95**, 2754–2759.
54. Huang S. L., Huang W. G., Xu H. G., Fang J. P., Wei J., Chen F. Y., Guo F. F. and Li S. N. (2002): Establishment of a murine model for allogeneic umbilical cord blood transplantation. Zhongguo Shi Yan. Xue. Ye. Xue. Za Zhi., **10**, 564–567.
55. Imamura M. and Tanaka J. (2003): Immunoregulatory cells for transplantation tolerance and graft-versus--leukemia effect. Int. J. Hematol., **78**, 188–194.
56. Ito T., Tajima F. and Ogawa M. (2000): Developmental changes of CD34 expression by murine hematopoietic stem cells. Exp. Hematol., **28**, 1269–1273.
57. Johnson G. R., Dresch C. and Metcalf D. (1977): Heterogeneity in human neutrophil, macrophage and eosinophil progenitor cells demonstrated by velocity sedimentation separation. Blood, **50**, 823–831.
58. Jordan C. T., Astle C. M., Zawadzki J., Mackarehtschian K., Lemischka I. R. and Harrison D. E. (1995): Long-term repopulating abilities of enriched fetal liver stem cells measured by competitive repopulation. Exp. Hematol., **23**, 1011–1015.
59. Kim M., Cooper D. D., Hayes S. F. and Spangrude G. J. (1998): Rhodamine-123 staining in hematopoietic stem cells of young mice indicates mitochondrial activation rather than dye efflux. Blood, **91**, 4106–4117.
60. Kim M., Moon H. B. and Spangrude G. J. (2003): Major age-related changes of mouse hematopoietic stem/progenitor cells. Ann. NY Acad. Sci., **996**, 195–208.
61. Lan F., Zeng D., Higuchi M., Higgins J. P. and Strober S. (2003): Host conditioning with total lymphoid irradiation and antithymocyte globulin prevents graft-versus-host disease: the role of CD1-reactive natural killer T cells. Biol. Blood Marrow Transplant., **9**, 355–363.
62. Lan F., Zeng D., Higuchi M., Huie P., Higgins J. P. and Strober S. (2001): Predominance of NK1.1+TCR alpha beta+ or DX5+TCR alpha beta+ T cells in mice conditioned with fractionated lymphoid irradiation protects against graft-versus-host disease: "natural suppressor" cells. J. Immunol., **167**, 2087–2096.
63. Lapidot T., Lubin I., Terenzi A., Faktorowich Y., Erlich P. and Reisner Y. (1990): Enhancement of bone marrow allografts from nude mice into mismatched recipients by T cells void of graft-versus-host activity. Proc. Natl. Acad. Sci. USA, **87**, 4595–4599.
64. Lapidot T., Terenzi A., Singer T. S., Salomon O. and Reisner Y. (1989): Enhancement by dimethyl myleran of donor type chimerism in murine recipients of bone marrow allografts. Blood, **73**, 2025–2032.
65. Li C. L. and Johnson G. R. (1995): Murine hematopoietic stem and progenitor cells: I. Enrichment and biologic characterization. Blood, **85**, 1472–1479.
66. Liang Y., Van Zant G. and Szilvassy S. J. (2005): Effects of aging on the homing and engraftment of murine hematopoietic stem and progenitor cells. Blood, **106**, 1479–1487.
67. Lowsky R., Takahashi T., Liu Y. P., Dejbakhsh-Jones S., Grumet F. C., Shizuru J.A., Laport G. G., Stockerl--Goldstein K. E., Johnston L. J., Hoppe R. T., Bloch D. A., Blume K. G., Negrin R. S. and Strober S. (2005): Protective conditioning for acute graft-versus-host disease. N. Engl. J. Med., **353**, 1321–1331.
68. Lu Y., Sakamaki S., Kuroda H., Kusakabe T., Konuma Y., Akiyama T., Fujimi A., Takemoto N., Nishiie K., Matsunaga T., Hirayama Y., Kato J., Kon S., Kogawa K. and Niitsu Y. (2001): Prevention of lethal acute graft-versus-host disease in mice by oral administration of T helper 1 inhibitor, TAK-603. Blood, **97**, 1123–1130.
69. Luznik L., Jalla S., Engstrom L. W., Iannone R. and Fuchs E. J. (2001): Durable engraftment of major histocompatibility complex-incompatible cells after nonmyeloablative conditioning with fludarabine, low-dose total body irradiation, and posttransplantation cyclophosphamide. Blood, **98**, 3456–3464.
70. Massa G., Wyllie J. P., Pratt A. M., Molineux G. and Schofield R. (1987): Marrow repopulation in mice treated with busulphan or isopropyl methane sulphonate and bone marrow. Br. J. Haematol., **66**, 11–14.
71. Matsuzaki Y., Kinjo K., Mulligan R. C. and Okano H. (2004): Unexpectedly efficient homing capacity of purified murine hematopoietic stem cells. Immunity, **20**, 87–93.
72. Mauch P., Down J. D., Warhol M. and Hellman S. (1988): Recipient preparation for bone marrow transplantation. I. Efficacy of total-body irradiation and busulfan. Transplantation, **46**, 205–210.
73. Mencacci A., Perruccio K., Bacci A., Cenci E., Benedetti R., Martelli M. F., Bistoni F., Coffman R., Velardi A. and Romani L. (2001): Defective antifungal T-helper 1 (TH1) immunity in a murine model of allogeneic T-cell-depleted bone marrow transplantation and its restoration by treatment with TH2 cytokine antagonists. Blood, **97**, 1483–1490.
74. Meng A., Wang Y., Brown S.A., Van Zant G. and Zhou D. (2003): Ionizing radiation and busulfan inhibit murine bone marrow cell hematopoietic function via apoptosis--dependent and -independent mechanisms. Exp. Hematol., **31**, 1348–1356.
75. Merad M., Hoffmann P., Ranheim E., Slaymaker S., Manz M. G., Lira S. A., Charo I., Cook D. N., Weissman I. L., Strober S. and Engleman E. G. (2004): Depletion of host Langerhans cells before transplantation of donor alloreactive T cells prevents skin graft-versus-host disease. Nat. Med., **10**, 510–517.
76. Migishima F., Oikawa A., Kondo S., Ema H., Morita Y., Nakauchi H., Yokoyama M., Song S. Y., Nishijima M., Okabe M. and Shinohara N. (2003): Full reconstitution of hematopoietic system by murine umbilical cord blood. Transplantation, **75**, 1820–1826.
77. Monaco A. P. (2003): Chimerism in organ transplantation: conflicting experiments and clinical observations. Transplantation, **75** (suppl. 9), 13S–16S.
78. Morecki S., Leshem B., Eid A. and Slavin S. (1987): Alloantigen persistence in induction and maintenance of transplantation tolerance. J. Exp. Med., **165**, 1468–1480.
79. Morecki S., Leshem B., Weigensberg M., Bar S. and Slavin S. (1985): Functional clonal deletion versus active suppression in transplantation tolerance induced by total-lymphoid irradiation. Transplantation, **40**, 201–210.

80. Morris E. S., MacDonald K. P., Rowe V., Johnson D. H., Banovic T., Clouston A. D. and Hill G. R. (2004): Donor treatment with pegylated G-CSF augments the generation of IL-10-producing regulatory T cells and promotes transplantation tolerance. Blood, **103**, 3573–3581.
81. Morrison S. J., Hemmati H. D., Wandycz A. M. and Weissman I. L. (1995): The purification and characterization of fetal liver hematopoietic stem cells. Proc. Natl. Acad. Sci. USA, **92**, 10302–10306.
82. Morrison S. J., Qian D., Jerabek L., Thiel B. A., Park I. K., Ford P. S., Kiel M. J., Schork N. J., Weissman I. L. and Clarke M. F. (2002): A genetic determinant that specifically regulates the frequency of hematopoietic stem cells. J. Immunol., **168**, 635–642.
83. Morrison S. J. and Weissman I. L. (1994): The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. Immunity, **1**, 661–673.
84. Morrison S. J., Wright D. E. and Weissman I. L. (1997): Cyclophosphamide/granulocyte colony-stimulating factor induces hematopoietic stem cells to proliferate prior to mobilization. Proc. Natl. Acad. Sci. USA, **94**, 1908–1913.
85. Neben S., Marcus K. and Mauch P. (1993): Mobilization of hematopoietic stem and progenitor cell subpopulations from the marrow to the blood of mice following cyclophosphamide and/or granulocyte colony-stimulating factor. Blood, **81**, 1960–1967.
86. Olsen A. L., Stachura D. L. and Weiss M. J. (2006): Designer blood: creating hematopoietic lineages from embryonic stem cells. Blood, **107**, 1265–1275.
87. Ordemann R., Hutchinson R., Friedman J., Burakoff S. J., Reddy P., Duffner U., Braun T. M., Liu C., Teshima T. and Ferrara J. L. (2002): Enhanced allostimulatory activity of host antigen-presenting cells in old mice intensifies acute graft-versus-host disease. J. Clin. Invest, **109**, 1249–1256.
88. Pan L., Delmonte J. Jr., Jalonen C. K. and Ferrara J. L. (1995): Pretreatment of donor mice with granulocyte colony--stimulating factor polarizes donor T lymphocytes toward type-2 cytokine production and reduces severity of experimental graft-versus-host disease. Blood, **86**, 4422–4429.
89. Pearce D. J., Ridler C. M., Simpson C. and Bonnet D. (2004): Multiparameter analysis of murine bone marrow side population cells. Blood, **103**, 2541–2546.
90. Plautz G. E., Cohen P. A. and Shu S. (2003): Considerations on clinical use of T cell immunotherapy for cancer. Arch. Immunol. Ther. Exp., **51**, 245–257.
91. Plett P. A., Frankovitz S. M. and Orschell C. M. (2003): Distribution of marrow repopulating cells between bone marrow and spleen early after transplantation. Blood, **102**, 2285–2291.
92. Ploemacher R. E., van der Sluijs J. P., Voerman J. S. and Brons N. H. (1989): An *in vitro* limiting-dilution assay of long-term repopulating hematopoietic stem cells in the mouse. Blood, **74**, 2755–2763.
93. Prigozhina T. B., Gurevitch O., Zhu J. and Slavin S. (1997): Permanent and specific transplantation tolerance induced by a nonmyeloablative treatment to a wide variety of allogeneic tissues: I. Induction of tolerance by a short course of total lymphoid irradiation and selective elimination of the donor-specific host lymphocytes. Transplantation, **63**, 1394–1399.
94. Quesenberry P. J., Colvin G. and Abedi M. (2005): Perspective: fundamental and clinical concepts on stem cell homing and engraftment: a journey to niches and beyond. Exp. Hematol., **33**, 9–19.
95. Quesenberry P. J., Colvin G. A., Abedi M., Lambert J. F., Moore B., Demers D., Greer D., McAuliffe C., Dooner M., Lum L. G., Badiavas E. and Falanga V. (2003): The marrow stem cell: the continuum. Bone Marrow Transplant., **32** (suppl. 1), S19–S22.
96. Quesenberry P. J., Stewart F. M., Becker P., D'Hondt L., Frimberger A., Lambert J. F., Colvin G. A., Miller C., Heyes C., Abedi M., Dooner M., Carlson J., Reilly J., McAuliffe C., Stencel K., Ballen K., Emmons R., Doyle P., Zhong S., Wang H. and Habibian H. (2001): Stem cell engraftment strategies. Ann. NY Acad. Sci., **938**, 54–61.
97. Quesenberry P. J., Zhong S., Wang H. and Stewart M. (2001): Allogeneic chimerism with low-dose irradiation, antigen presensitization, and costimulator blockade in H-2 mismatched mice. Blood, **97**, 557–564.
98. Ramshaw H. S., Rao S. S., Crittenden R. B., Peters S. O., Weier H. U. and Quesenberry P. J. (1995): Engraftment of bone marrow cells into normal unprepared hosts: effects of 5-fluorouracil and cell cycle status. Blood, **86**, 924–929.
99. Randall T. D., Lund F. E., Howard M. C. and Weissman I. L. (1996): Expression of murine CD38 defines a population of long-term reconstituting hematopoietic stem cells. Blood, **87**, 4057–4067.
100. Randall T. D. and Weissman I. L. (1997): Phenotypic and functional changes induced at the clonal level in hematopoietic stem cells after 5-fluorouracil treatment. Blood, **89**, 3596–3606.
101. Rao S. S., Peters S. O., Crittenden R. B., Stewart F. M., Ramshaw H. S. and Quesenberry P. J. (1997): Stem cell transplantation in the normal nonmyeloablated host: relationship between cell dose, schedule, and engraftment. Exp. Hematol., **25**, 114–121.
102. Ratajczak M. Z. (1990): Values of certain biochemical parameters in mouse serum after syngeneic bone marrow transplantation. Effect of various methods of myeloablation-immunosuppression preparation and recipient's age. Arch. Immunol. Ther. Exp., **38**, 299–308.
103. Rebel V. I., Miller C. L., Eaves C. J. and Lansdorp P. M. (1996): The repopulation potential of fetal liver hematopoietic stem cells in mice exceeds that of their liver adult bone marrow counterparts. Blood, **87**, 3500–3507.
104. Rebel V. I., Miller C. L., Spinelli J. J., Thomas T. E., Eaves C. J. and Lansdorp P. M. (1995): Nonlinear effects of radiation dose on donor-cell reconstitution by limited numbers of purified stem cells. Biol. Blood Marrow Transplant., **1**, 32–39.
105. Rebel V. I., Miller C. L., Thornbury G. R., Dragowska W. H., Eaves C. J. and Lansdorp P. M. (1996): A comparison of long-term repopulating hematopoietic stem cells in fetal liver and adult bone marrow from the mouse. Exp. Hematol., **24**, 638–648.
106. Reddy P., Maeda Y., Liu C., Krijanovski O. I., Korngold R. and Ferrara J. L. (2005): A crucial role for antigen-presenting cells and alloantigen expression in graft-versus--leukemia responses. Nat. Med., **11**, 1244–1249.
107. Robbin W. G., Ploemacher R. E. and Down J. D. (1998): Maintaining high levels of syngeneic bone marrow engraftment while omitting cyclophosphamide in busulfan-containing conditioning regimens. Blood, **92**, 1462–1463.
108. Roberts A. W. and Metcalf D. (1995): Noncycling state of peripheral blood progenitor cells mobilized by granulocyte colony-stimulating factor and other cytokines. Blood, **86**, 1600–1605.
109. Ruggeri L., Capanni M., Urbani E., Perruccio K., Shlom-

chik W. D., Tosti A., Posati S., Rogaia D., Frassoni F., Aversa F., Martelli M. F. and Velardi A. (2002): Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science, **295**, 2097–2100.
110. Salomon O., Lapidot T., Terenzi A., Lubin I., Rabi I. and Reisner Y. (1990): Induction of donor-type chimerism in murine recipients of bone marrow allografts by different radiation regimens currently used in treatment of leukemia patients. Blood, **76**, 1872–1878.
111. Samlowski W. E., Araneo B. A., Butler M. O., Fung M. C. and Johnson H. M. (1989): Peripheral lymph node helper T-cell recovery after syngeneic bone marrow transplantation in mice prepared with either gamma-irradiation or busulfan. Blood, **74**, 1436–1445.
112. Sanchez M. J., Holmes A., Miles C. and Dzierzak E. (1996): Characterization of the first definitive hematopoietic stem cells in the AGM and liver of the mouse embryo. Immunity, **5**, 513–525.
113. Sato K., Yamashita N., Yamashita N., Baba M. and Matsuyama T. (2003): Regulatory dendritic cells protect mice from murine acute graft-versus-host disease and leukemia relapse. Immunity, **18**, 367–379.
114. Scaradavou A., Isola L., Rubinstein P., Galperin Y., Najfeld V., Berlin D., Gordon J. and Weinberg R. S. (1997): A murine model for human cord blood transplantation: near-term fetal and neonatal peripheral blood cells can achieve long-term bone marrow engraftment in sublethally irradiated adult recipients. Blood, **89**, 1089–1099.
115. Schofield R. (1978): The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells, **4**, 7–25.
116. Sharabi Y. and Sachs D. H. (1989): Mixed chimerism and permanent specific transplantation tolerance induced by a nonlethal preparative regimen. J. Exp. Med., **169**, 493–502.
117. Shirasugi N., Emmanouilidis N., Pearson T. C. and Larsen C. P. (2004): Ability of donor splenocytes with costimulation blockade to induce mixed hematopoietic chimerism and transplantation tolerance. Transplant. Proc., **36**, 2423–2424.
118. Shizuru J. A., Jerabek L., Edwards C. T. and Weissman I. L. (1996): Transplantation of purified hematopoietic stem cells: requirements for overcoming the barriers of allogeneic engraftment. Biol. Blood Marrow Transplant., **2**, 3–14.
119. Shlomchik W. D., Couzens M. S., Tang C. B., McNiff J., Robert M. E., Liu J., Shlomchik M. J. and Emerson S. G. (1999): Prevention of graft versus host disease by inactivation of host antigen-presenting cells. Science, **285**, 412–415.
120. Slavin S., Fuks Z., Kaplan H. S. and Strober S. (1978): Transplantation of allogeneic bone marrow without graft--versus-host disease using total lymphoid irradiation. J. Exp. Med., **147**, 963–972.
121. Slavin S., Morecki S., Weigensberg M., Bar S. and Weiss L. (1986): Functional clonal deletion versus suppressor cell-induced transplantation tolerance in chimeras prepared with a short course of total-lymphoid irradiation. Transplantation, **41**, 680–687.
122. Slavin S., Morecki S., Weiss L. and Or R. (2003): Immunotherapy of hematologic malignancies and metastatic solid tumors in experimental animals and man. Crit Rev. Oncol. Hematol., **46**, 139–163.
123. Slavin S. and Seidel H. J. (1982): Hemopoietic activity in bone marrow chimeras prepared with total lymphoid irradiation (TLI). Exp. Hematol., **10**, 206–216.
124. Slavin S. and Strober S. (1979): Induction of allograft tolerance after total lymphoid irradiation (TLI): development of suppressor cells of the mixed leukocyte reaction (MLR). J. Immunol., **123**, 942–946.
125. Slavin S., Strober S., Fuks Z. and Kaplan H. S. (1977): Induction of specific tissue transplantation tolerance using fractionated total lymphoid irradiation in adult mice: long-term survival of allogeneic bone marrow and skin grafts. J. Exp. Med., **146**, 34–48.
126. Soderling C. C., Song C. W., Blazar B. R. and Vallera D. A. (1985): A correlation between conditioning and engraftment in recipients of MHC-mismatched T cell--depleted murine bone marrow transplants. J. Immunol., **135**, 941–946.
127. Spangrude G. J., Heimfeld S. and Weissman I. L. (1988): Purification and characterization of mouse hematopoietic stem cells. Science, **241**, 58–62.
128. Stewart F. M., Crittenden R. B., Lowry P. A., Pearson--White S. and Quesenberry P. J. (1993): Long-term engraftment of normal and post-5-fluorouracil murine marrow into normal nonmyeloablated mice. Blood, **81**, 2566–2571.
129. Stewart F. M., Zhong S., Wuu J., Hsieh C., Nilsson S. K. and Quesenberry P. J. (1998): Lymphohematopoietic engraftment in minimally myeloablated hosts. Blood, **91**, 3681–3687.
130. Suda T., Arai F. and Shimmura S. (2005): Regulation of stem cells in the niche. Cornea, **24** (suppl.), S12–S17.
131. Surdez D., Kunz B., Wagers A. J., Weissman I. L. and Terskikh A. V. (2005): Simple and efficient isolation of hematopoietic stem cells from H2K-zFP transgenic mice. Stem Cells, **23**, 1617–1625.
132. Sutherland H. J., Lansdorp P. M., Henkelman D. H., Eaves A. C. and Eaves C. J. (1990): Functional characterization of individual human hematopoietic stem cells cultured at limiting dilution on supportive marrow stromal layers. Proc. Natl. Acad. Sci. USA, **87**, 3584–3588.
133. Szilvassy S. J., Meyerrose T. E., Ragland P. L. and Grimes B. (2001): Differential homing and engraftment properties of hematopoietic progenitor cells from murine bone marrow, mobilized peripheral blood, and fetal liver. Blood, **98**, 2108–2115.
134. Taichman R. S. and Emerson S. G. (1994): Human osteoblasts support hematopoiesis through the production of granulocyte colony-stimulating factor. J. Exp. Med., **179**, 1677–1682.
135. Tajima F., Deguchi T., Laver J. H., Zeng H. and Ogawa M. (2001): Reciprocal expression of CD38 and CD34 by adult murine hematopoietic stem cells. Blood, **97**, 2618–2624.
136. Teshima T., Ordemann R., Reddy P., Gagin S., Liu C., Cooke K. R. and Ferrara J. L. (2002): Acute graft-versus--host disease does not require alloantigen expression on host epithelium. Nat. Med., **8**, 575–581.
137. Till J. E. and McCulloch E. A. (1961): A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat. Res., **14**, 213–222.
138. Truitt R. L. and Atasoylu A. A. (1991): Impact of pretransplant conditioning and donor T cells on chimerism, graft-versus-host disease, graft-versus-leukemia reactivity, and tolerance after bone marrow transplantation. Blood, **77**, 2515–2523.
139. Tsukada N., Kobata T., Aizawa Y., Yagita H. and Okumura K. (1999): Graft-versus-leukemia effect and graft--versus-host disease can be differentiated by cytotoxic

mechanisms in a murine model of allogeneic bone marrow transplantation. Blood, **93**, 2738–2747.
140. Uchida N., He D., Friera A. M., Reitsma M., Sasaki D., Chen B. and Tsukamoto A. (1997): The unexpected G0/G1 cell cycle status of mobilized hematopoietic stem cells from peripheral blood. Blood, **89**, 465–472.
141. Uchida N., Tsukamoto A., He D., Friera A. M., Scollay R. and Weissman I. L. (1998): High doses of purified stem cells cause early hematopoietic recovery in syngeneic and allogeneic hosts. J. Clin. Invest., **101**, 961–966.
142. Uharek L., Glass B., Gaska T., Gassmann W., Loeffler H. and Mueller-Ruchholtz W. (1994): Influence of donor lymphocytes on the incidence of primary graft failure after allogeneic bone marrow transplantation in a murine model. Br. J. Haematol., **88**, 79–87.
143. van Leeuwen L., Guiffre A., Atkinson K., Rainer S. P. and Sewell W. A. (2002): A two-phase pathogenesis of graft--versus-host disease in mice. Bone Marrow Transplant., **29**, 151–158.
144. van Os R., Kamminga L. M. and de Haan G. (2004): Stem cell assays: something old, something new, something borrowed. Stem Cells, **22**, 1181–1190.
145. van Os R., Konings A. W. and Down J. D. (1992): Radiation dose as a factor in host preparation for bone marrow transplantation across different genetic barriers. Int. J. Radiat. Biol., **61**, 501–510.
146. van Os R., Sheridan T. M., Robinson S., Drukteinis D., Ferrara J. L. and Mauch P. M. (2001): Immunogenicity of Ly5 (CD45)-antigens hampers long-term engraftment following minimal conditioning in a murine bone marrow transplantation model. Stem Cells, **19**, 80–87.
147. van Os R., Thames H. D., Konings A. W. and Down J. D. (1993): Radiation dose-fractionation and dose-rate relationships for long-term repopulating hemopoietic stem cells in a murine bone marrow transplant model. Radiat. Res., **136**, 118–125.
148. Wagers A. J. and Weissman I. L. (2006): Differential expression of {alpha}2 integrin separates long-term and short-term reconstituting Lin-/loThy1.1loc-kit+Sca-1+ hematopoietic stem cells. Stem Cells, **24**, 1087–1094.
149. Wekerle T., Blaha P., Koporc Z., Bigenzahn S., Pusch M. and Muehlbacher F. (2003): Mechanisms of tolerance induction through the transplantation of donor hematopoietic stem cells: central versus peripheral tolerance. Transplantation, **75** (suppl.), 21S–25S.
150. Wekerle T., Blaha P., Langer F., Schmid M. and Muehlbacher F. (2002): Tolerance through bone marrow transplantation with costimulation blockade. Transpl. Immunol., **9**, 125–133.
151. Wekerle T., Kurtz J., Ito H., Ronquillo J.V., Dong V., Zhao G., Shaffer J., Sayegh M. H. and Sykes M. (2000): Allogeneic bone marrow transplantation with co-stimulatory blockade induces macrochimerism and tolerance without cytoreductive host treatment. Nat. Med., **6**, 464–469.
152. Westerhof G. R., Ploemacher R. E., Boudewijn A., Blokland I., Dillingh J. H., McGown A. T., Hadfield J. A., Dawson M. J. and Down J. D. (2000): Comparison of different busulfan analogues for depletion of hematopoietic stem cells and promotion of donor-type chimerism in murine bone marrow transplant recipients. Cancer Res., **60**, 5470–5478.
153. Wierenga P. K., Setroikromo R., Kamps G., Kampinga H. H. and Vellenga E. (2002): Peripheral blood stem cells differ from bone marrow stem cells in cell cycle status, repopulating potential, and sensitivity toward hyperthermic purging in mice mobilized with cyclophosphamide and granulocyte colony-stimulating factor. J. Hematother. Stem Cell Res., **11**, 523–532.
154. Wognum A. W., Eaves A. C. and Thomas T. E. (2003): Identification and isolation of hematopoietic stem cells. Arch. Med. Res., **34**, 461–475.
155. Wright D. E., Cheshier S. H., Wagers A. J., Randall T. D., Christensen J. L. and Weissman I. L. (2001): Cyclophosphamide/granulocyte colony-stimulating factor causes selective mobilization of bone marrow hematopoietic stem cells into the blood after M phase of the cell cycle. Blood, **97**, 2278–2285.
156. Wright D. E., Wagers A. J., Gulati A. P., Johnson F. L. and Weissman I. L. (2001): Physiological migration of hematopoietic stem and progenitor cells. Science, **294**, 1933–1936.
157. Wu D. D. and Keating A. (1993): Hematopoietic stem cells engraft in untreated transplant recipients. Exp. Hematol., **21**, 251–256.
158. Wu T., Sozen H., Luo B., Heuss N., Kalscheuer H., Lan P., Sutherland D. E., Hering B. J. and Guo Z. (2002): Rapamycin and T cell costimulatory blockade as post--transplant treatment promote fully MHC-mismatched allogeneic bone marrow engraftment under irradiation--free conditioning therapy. Bone Marrow Transplant., **29**, 949–956.
159. Xun C. Q., Thompson J. S., Jennings C. D., Brown S. A. and Widmer M. B. (1994): Effect of total body irradiation, busulfan-cyclophosphamide, or cyclophosphamide conditioning on inflammatory cytokine release and development of acute and chronic graft-versus-host disease in H--2-incompatible transplanted SCID mice. Blood, **83**, 2360–2367.
160. Yamamoto Y., Yasumizu R., Amou Y., Watanabe N., Nishio N., Toki J., Fukuhara S. and Ikehara S. (1996): Characterization of peripheral blood stem cells in mice. Blood, **88**, 445–454.
161. Yeager A. M., Shinn C. and Pardoll D. M. (1991): Lymphoid reconstitution after transplantation of congenic hematopoietic cells in busulfan-treated mice. Blood, **78**, 3312–3316.
162. Yuhas J. M. and Storer J. B. (1969): On mouse strain differences in radiation resistance: hematopoietic death and the endogenous colony-forming unit. Radiat. Res., **39**, 608–622.
163. Zeng D., Lewis D., Dejbakhsh-Jones S., Lan F., Garcia--Ojeda M., Sibley R. and Strober S. (1999): Bone marrow NK1.1(–) and NK1.1(+) T cells reciprocally regulate acute graft versus host disease. J. Exp. Med., **189**, 1073–1081.
164. Zhang J., Niu C., Ye L., Huang H., He X., Tong W. G., Ross J., Haug J., Johnson T., Feng J. Q., Harris S., Wiedemann L. M., Mishina Y. and Li L. (2003): Identification of the haematopoietic stem cell niche and control of the niche size. Nature, **425**, 836–841.
165. Zhong J. F., Zhan Y., Anderson W. F. and Zhao Y. (2002): Murine hematopoietic stem cell distribution and proliferation in ablated and nonablated bone marrow transplantation. Blood, **100**, 3521–3526.
166. Zijlmans J. M., Visser J. W., Laterveer L., Kleiverda K., Heemskerk D. P., Kluin P. M., Willemze R. and Fibbe W. E. (1998): The early phase of engraftment after murine blood cell transplantation is mediated by hematopoietic stem cells. Proc. Natl. Acad. Sci. USA, **95**, 725–729.