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Hematopoietic Stem Cell Properties and Transplantation Preconditioning Studied by Competitive Repopulation of the Hematopoietic Tissue

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TABLE OF CONTENTS:

Summary in Czech.. 1

Summary in English..2

Introduction..3

Aims of the study..4

Materials and methods...4

Results..5

Discussion...11

Conclusions...13

References...13

List of publications..15

SOUHRN V ČEŠTINĚ

Hemopoetické kmenové buňky (HSCs) jsou primitivní buňky schopné nahradit terminálně diferencované buňky v průběhu života. HSCs jsou charakterizovány schopností pluripotence a sebeobnovy. Proces, během kterého kmenové buňky dávají vznik terminálně diferencovaným buňkám nastává přes stádia progenitorových buněk v mikroprostředí konstní dřeně (BM). Místo, kde HSCs spočívají *in situ* se označuje jako niche. Pro úspěšnou transplantaci krvetvorné tkáně (BMT) je nutné vychytání v kostní dřeni, uhnízdění a přihojení v niche. Existuje řada důkazů o tom, že se mezi sebou liší fetální kmenové buňky a kmenové buňky z dospělých jedinců..

Experimentální práce probíhala v několika fázích. Nejprve byl testován a hodnocen jeden z nejčastěji užívaných myších transplantačních systémů, Ly5.1/Ly5.2. Dále byl tento myší kongenní transplantační model použit ke studiu chování kmenových buněk, které byly transplantovány příjemcům s různými kombinacemi přípravného režimu cyklofosfamid (CY) – celotělové ozáření (TBI) nebo busulphan (Bu). Byla studována citlivost hemopoetického mikroprostředí k transplantaci po systémové aplikaci látek ovlivňujících kostní metabolismus (bifosfonáty). Dále byla studována rozdílná hematopoetická mikroprostředí (fetální, neonatální, dospělé) ve smyslu získání citlivosti kmenových buněk k estrogenu.

Ke studiu vlastností kmenových buněk byla použita metoda kompetitivní repopulace. Procento dárcovských buněk přítomných v krvi příjemce (po-transplantační chimerismus) byl hodnocen za použití průtokové cytometrie (FACs Calibur).

Kongenní myší transplantační model Ly5.1/Ly5.2 je spolehlivý. Výsledky získané jak z kmene Ly5.1, tak Ly5.2 jsou dobře reprodukovatelné a nejsou závislé na kmeni nebo pohlaví dárců, resp. příjemců. U myší s vysokým celkovým chimerismem jsou hlavní subpopulací bílých krvinek ve štěpu B-lymfocyty, stejně jako u normálních myší. U myší s nízkým celkovým chimerismem jsou hlavní subpopulací štěpu Gr/Mac^{+} buňky.

Při použití přípravného režimu cyklofosfamid-celotělové ozáření závisí přihojení dárcovských buněk na časovém intervalu mezi první podanou dávkou cyklofosfamidu a ozářením. Prodloužení tohoto intervalu ze 2 na 5 nebo 7 dní vede k signifikantnímu vzestupu dárcovského chimerismu. Příprava režimem cyklofosfamid-busulfan vede k myelosupresi srovnatelné s režimem CY-TBI. Zvyšování dávky záření ve skupině CY-2 nevede k vzestupu dárcovského chimerismu.

B-lymfopoéza původem z fetálních jater zůstává signifikantně méně citlivá k supresi estrogenem ve srovnání s B-lymfopoézou neonatálního nebo dospělého původu. Zůstává to její trvalou vlastností. Mikropstředí v pozdním stádiu fetálního vývoje se zdá být nutné pro budoucí získání citlivosti B-lymfocytů k estrogenu.

Bisfosfonáty u myší hemopoézu neovlivňují. U myší se zdají být bezpečné z hlediska efektu na hematopoetickou tkáň.

SUMMARY IN ENGLISH

Hemopoietic stem cells (HSCs) are primitive cells capable of replacing terminally differentiated cells throughout life. HSCs are defined as pluripotent cells able to give rise to a number of different functional cell types and they posses a huge self-renewal capability. The process during which stem cells give rise to terminally differentiated cells occurs through a number of committed progenitor cells in the bone marrow (BM) microenvironment. The place where HSCs reside in situ is called a niche. Successful bone marrow transplantation (BMT) involves homing, seeding and engraftment of HSCs in the niche. More factors, for instance chemotherapeutics and irradiation, can influence the effect of BMT. There are indices that HSCs differ between fetal and adult life.

First, we have evaluated and tested a one of the frequently used murine transplantation systems. Further, we have used this murine congenic transplantation model to study the behavior of HSCs transplanted into recipients conditioned with cyclophosphamide (CY) – total body irradiation (TBI) regimen ort busulphan (Bu). Finally, we have studied the succeptibility to transplantation of HSC niche after drugs affecting its cellular components (biphosphonates), and to investigate different types of hematopoietic microenvironment (fetal, neonatal and adult) in terms of aquisition of HSCs succeptibility to estrogen. Competitive repopulation assay was used to study the behavior of HSCs. The percentage of donor cells in recipient mice after transplantation (chimerism) was determined by flow cytometry.

The congenic Ly5.1/Ly5.2 murine transplantation model is reliable. Results obtained from both systems are well reproducible and do not rely on strain and sex combinations. In mice with high total chimerism, the main WBC subpopulation of the graft are B-lymphocytes, as in normal mice. In mice with low total chimerism, the main contribution of graft to WBC is made up by Gr/Mac^+ cells.

In cyclophosphamide-total body irradiation preconditioning regimen, donor cells engraftment depends on the time interval between the first cyclophophamide administration and irradiation. Prolongation of the interval from 2 days to 5 or 7 days leads to significant increase in donor chimerism. Cyclophosphamide-busulphan preconditioning ensures myelosuppression comparable with CY-TBI regimen. Increasing irradiation in CY-2 group does not lead to an increase in donor cells engraftment.

The B-lymphopoiesis derived from fetal liver remained significantly less sensitive to suppression by estrogen compared to that of adult and neonatal origin. It remained its permanent feature. Exposure of HSCs to the late-stage FL microenvironment seems to be critical and mandatory for gaining later sensitivity to estrogen.

Bisphophonates do not affect hematopoiesis in the mouse. They seem to be safe in regard to the effect on hematopoietic tissue in mice.

1. INTRODUCTION

Hemopoietic stem cells (HSCs) are primitive cells capable of replacing terminally differentiated cells throughout life (Mazurier F et al., 2003). HSCs are defined as pluripotent cells able to give rise to at least ten different functional cell types and they posses a huge self-renewal capability (Dexter TM et al., 1984). The process during which stem cells give rise to terminally differentiated cells occurs through a number of committed progenitor cells in the bone marrow (BM) (Golde DW, 1991). During this process stem cells undergo proliferation and sequential differentiation, which is accompanied by gradual loss in self-renewal capacity and capability to produce mature cells. It has been estimated that stem cells are very low in number and that they divide relatively rarely (Abkowitz JL et al., 2002).

Recently it has been demonstrated that it is possible to reprogramme adult murine and human cells to pluripotent state. These cells are called induced pluripotent stem cells (iPS cells). They are genetically modified by the integration of up to four viral vectors bearing DNA-transcription factors (Oct3/4, Sox2, c-Myc, and Klf4) into adult cell genome. IPS cells can be induced from a variety of adult cells. However, since they are modified with viruses, there is a potential activation of oncogenes at the moment (Takahashi K et al., 2006).

Several markers are used for the identification of stem cells. Most commonly, the HSCs are identified in the absence of all lineage markers (Lin^-). In the experimental setting, the murine HSCs were first described to be in $c\text{-}Kit^+$ $Thy1^{lo}$ Lin^- $Sca\text{-}1^{hi}$ population (Spangrude GJ et al., 1988). Now, SLAM family markers are used for identification of HSCs. HSCs were shown to be CD150+, CD48- (Yilmaz OH et al. 2006). The most interesting fraction of bone marrow cells capable of long-term repopulation is present within this $Thy1^{lo}$ fraction (Christensen JL and Weisman IL, 2001). Additionally, the most concentrated population of HSCs resides in the so-called SP fraction. This population is characterized according to the extrusion of Hoechst 3342 from the cells and is largely enriched for HSCs. A number of other markers could be used to identify HSCs, such as CD10, CD7, IL-3R-α-low (Ziegler BL et al., 1999).

The niches are specific microenvironments in which stem cell reside in situ. The concept of stem cell niche was first proposed for human hemopoietic system in the 1970ś (Schofield R, 1978). The niche tightly regulates the fate of the stem cells, it controls the balance between commitment and self-renewal. The osteoblasts are a critical component of adult BM niche (Calvi LM et al., 2003). They are bone-forming cells derived from mesenchymal stem cells (Yasuda H et al., 1998). The cells of the niche produce factors that maintain HSCs within the microenvironmental compartment. The most important chemokine for localization of HSCs in BM is the stromal derived factor-1 (SDF-1) and its receptor CXCR4 (Arai F et al., 2002).

Several processes are involved in successful transplantation of HSCs. They include homing, seeding and engraftment. Homing of HSCs by extravasation into the microenvironment of bone marrow and stem cell niche is thought to be a

pivotal process, because niche is the only site that normally hosts durable and multilineage differentiation of HCSc (Stein J et al., 2005). Seeding of HSCs is determined by adhesive interaction with the stroma (Askenasy N and Farkas DL, 2002). Engraftment means proliferation and differentiation of donor HSCs within the BM niche (Askenasy N et al., 2003).

Mechanisms leading to HSCs mobilization are less well understood. Similarly to homing and retention, important roles of the adhesion molecules in the mobilization has emerged (Lévesque JP et al., 2002). Few distinct mechanisms may lead to mobilization: administration of IL-8 (Laterveer L et al., 1996), administration of cytokines, such as granulocyte colony-stimulating factor (G-CSF or GM-CSF) (Sato N et al., 1994), administration of monoclonal antibodies against VLA-4 or VCAM-1 (Papayannopoulou T et al., 1993; Craddock CF et al., 1997).

It has successively become evident that the process of blood cell formation is very different between fetal and adult life (Kincade PW et al., 2002). This is particularly the case for lymphopoiesis, where the properties of progenitors and the differentiation pathways change dramatically in a developmental age-dependent manner. There are indices that fetal stem cells are intrinsically unique and do not efficiently acquire adult characteristics when transplanted to adult bone marrow (Igarashi H et al., 2001).

2. AIMS OF THE STUDY

1. To examine in detail murine congenic transplantation model Ly5.1/Ly5.2. To evaluate dependence of engraftment rate on cell dose transplanted to both lethally and sublethaly irradiated recipients. To study influence of mouse strain and sex on postransplantation chimerism.

2. To study microenvironment/hematopoietic niche succeptibility to transplantation after preconditioning regimens consisting of cyclophosphamide and total body irradiation or busulphan. In CY-TBI host regimen the aim was to study influence of different time intervals between CY and TBI, different irradiation doses, effect of two CY doses and on the level of post-transplantation chimerism. To study the effect of replacing total body irradiation with busulphan on both the levels of myeloablation and post-transplantation chimerism.

3. To study the succeptibility to transplantation of hematopoietic stem cell niche after drugs affecting its cellular components (biphosphonates), and to investigate different types of hematopoietic microenvironment (fetal, neonatal and adult) in terms of aquisition of HSCs succeptibility to estrogen.

3. MATERIALS AND METHODS

3.1 Mice

C57BL/6 mice (B6-Ly 5.2) and their congenic strain (B6-Ly5.1) were used in the experiments.

3.2 Irradiation

Irradiation was delivered from a ^{60}Co source 0.8Gy/min.

3.3 Chemotherapeutics:

Busulphan was dissolved in DMSO:oil solution (0.35:0.65) and injected s.c. in a dose 25mg or 50mg.

Cyclophosphamide was dissolved in PBS and injected i.p. in dose of 135 mg/kg of cyclophosphamide.

3.4. Drugs:

Bisphosphonates incorporated in animal diet in dose of 70 og/kg/day.

Estrogen male mice treated with 250 ug/2 weeks.

3.5 Competitive repopulation:

Behavior of HSCs was studied by competitive repopulation assay after transplantation into lethally or sublethally irradiated mice.

3.6 Analysis of recipients

Flow cytometry was done on FACS Calibur.

RT-PCR was performed on LightCycler.

3.7 Statistical analysis

Group of 8 mice given the same treatment were used to calculate the mean values and standard deviations. These values were used to calculate P values using the two-tailed Studentś *t*-test. ANOVA was used for comparison of multiple groups. Linear regression was used for analyses of experiments with expected linear increase in chimerism.

4. RESULTS

4.1 The influence of chimerism on WBCs subpopulation distribution

The differential leukocyte count is mainly consisting of B-lymphocytes, followed by CD3+ cells and Gr/Mac+ cells in mice. B-lymphocytes (B220+ cells) encompass about two thirds of the WBC population. There is no significant difference in the distribution of the subpopulations between Ly5.1 and Ly5.2 mice (Fig. 1).

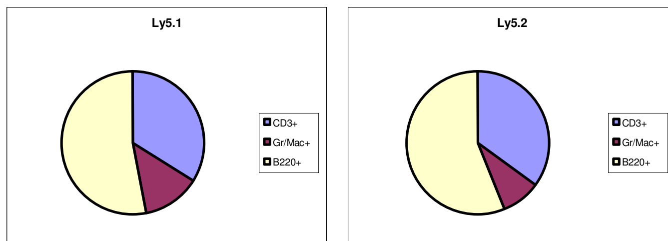
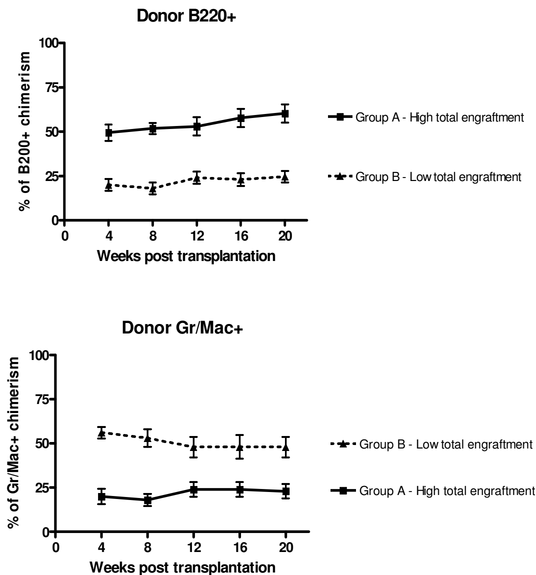


Fig. 1: Distribution of white blood cell subpopulations in Ly5.1 and Ly5.2 mice.

It has been studied whether different levels of HSCs engraftment are related to different distribution of WBC subpopulations using Ly5.1/Ly 5.2 model. Recipient mice were sublethally irradiated with 4 Gy and transplanted 2 hours later with 5 (Group A) or 20 million (Group B) congenic bone marrow cells. Ly 5.1/Ly 5.2 mice were reciprocally used. Transplantation of 5 million of donor cells lead to approximately 30% of donor hematopoiesis. Transplantation of 20 million cells lead to almost total donor chimerism. In mice with high level of donor engraftment (Group B), the main of donor derived WBCs are B220+ cells. Gr/Mac+ cells are relatively suppressed (Fig. 2). Low levels of donor cell engraftment (Group A) are associated with B220+ suppression and relatively higher contribution of Gr/Mac+ in donor derived part of WBC. Total distribution of WBC remained unchanged from normal mouse, only relative contribution of donor cells to B- and Gr/Mac-subpopulation depends on the level of engraftment.



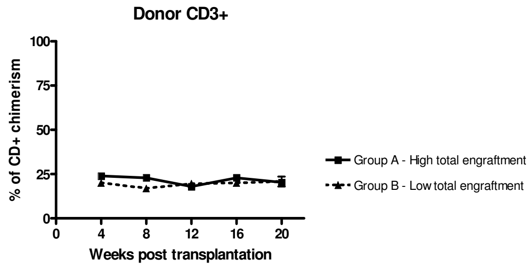


Fig. 2: WBCs subpopulation (B220+, Gr/Mac+, CD3+) distribution in mice with high and low total chimerism.

4.2 Importance of the time interval between CY dose and total body irradiation delivery of hosts

Mice were treated with CY (135mg/kg i.p.) in different time intervals before TBI
(4 Gy).Control mice were irradiated only. 2 hours after irradiation mice were transplanted with 4 x 10^6 bone marrow cells from congenic mice strain.

CY given to recipients two days prior to TBI (CY-2) significantly decreased both short- and long-term engraftment of donor HSCs compared to control mice that were only irradiated. On the other hand, CY administered five (CY-5) or seven (CY-7) days before TBI significantly increased donor-derived chimerism. Administration of CY three (CY-3), ten (CY-10) or fourteen (CY-14) days before TBI lead to intermediate engraftment, achieving higher percentage than in control mice, but not reaching the level of CY-7/5 group (Fig. 3).

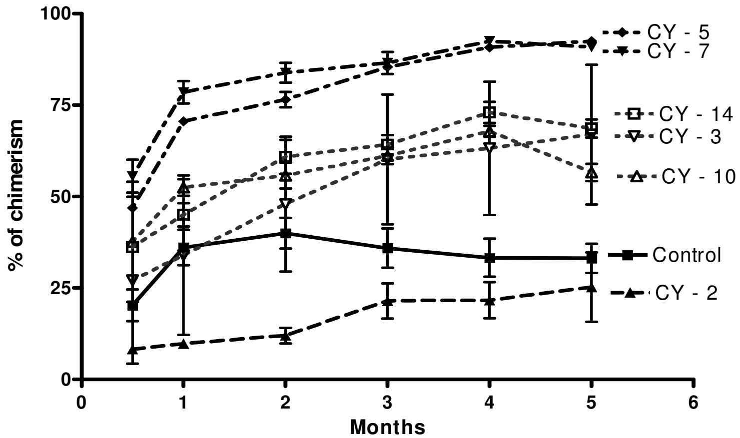


Fig. 3: Donor-derived engraftment (% of chimerism) achieved in recipient mice in particular preconditioned groups.

Month	0.5	1	2	3	4	5
CTRL	20 ± 4.3	36 ± 4.8	40 ± 4.2	36 ± 5.4	33 ± 5.2	33 ± 4.0
CY-2	8.2 ± 1.0 **	9.8 ± 1.2 **	12 ± 2.2 **	21 ± 4.8 **	22 ± 4.9 *	25 ± 9.5 **
CY-3	27 ± 12.8 ns	34 ± 12.2 ns	48 ± 5.8 *	60 ± 10.8 **	63 ± 11.8 *	67 ± 12.0 *
CY-5	47 ± 1.0 **	71 ± 0.8 ***	77 ± 2.1 ***	85 ± 1.3 ***	91 ± 1.1 ***	92 ± 0.4 ***
CY-7	56 ± 4.5 ***	78 ± 3.0 ***	84 ± 2.7 ***	86 ± 3.0 ***	92 ± 1.2 ***	91 ± 1.2 ***
CY-10	38 ± 1.6 **	52 ± 2.3 *	56 ± 3.6 *	61 ± 2.3 **	68 ± 1.5 **	57 ± 2.3 **
CY-14	36 ± 1.0 **	45 ± 3.2 *	61 ± 4.6 **	64 ± 2.6 **	73 ± 2.9 **	69 ± 2.5 **

Table 6: Statistical analysis. Values are expressed as mean ± SEM. Significant difference from controls: (*) $p<0.05$, (**) $p<0.01$, (***) $p<0.001$.

4.3 Sensitivity of B-lymphopoiesis to estrogen of fetal liver origin and newborn liver origin cells after co-transplantation into lethally irradiated mice and after secondary transplantation

Lethally irradiated male Ly5.2 mice were transplanted with a mixture of five million of Ly5.1 FL E14.5 cells and Ly5.2 P0.5 cells or Ly5.2 FL E14.5 cells and Ly5.1 P0.5 cells. Half of the recipients were treated with estrogen. There was a stable chimerism in peripheral blood for the whole observed period after transplantation. Chimerism in the BM corresponded to the chimerism in peripheral blood at the time of sacrifice. 20 weeks after the primary transplantation there were approximately 75% FL E14.5 cells in estrogen treated mice and 55% in control mice (Fig. 4), when the mice were sacrificed and the bone marrow was transplanted into secondary lethally irradiated male recipients. A half of the secondary recipients were again treated with estrogen. Estrogen administration increased the proportion of FL 14.5 derived B220+ cells until 140 days after the secondary transplantation. P0.5 derived cells remained sensitive to suppression by estrogen (Fig. 5 and 6). Estrogen suppressed P0.5 derived cells more than E14.5 derived cells, which then dominated.

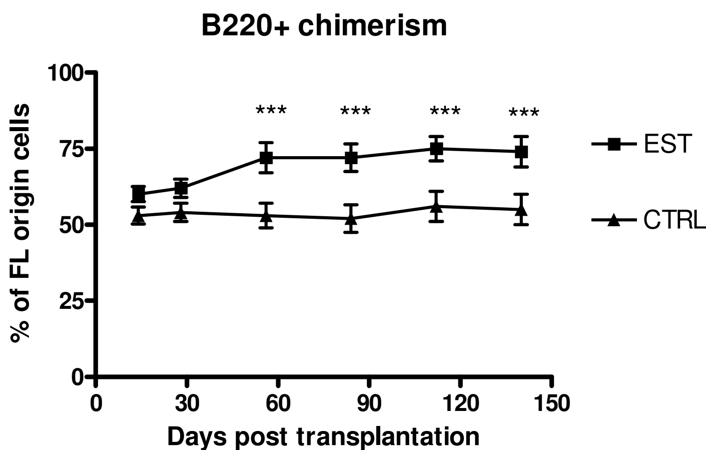


Fig. 4: Chimerism in PB of FL B-cells co-transplanted with P0.5 liver cells into primary recipients. B220+ chimerism; FL Ly5.1 (***) $p < 0.001$.

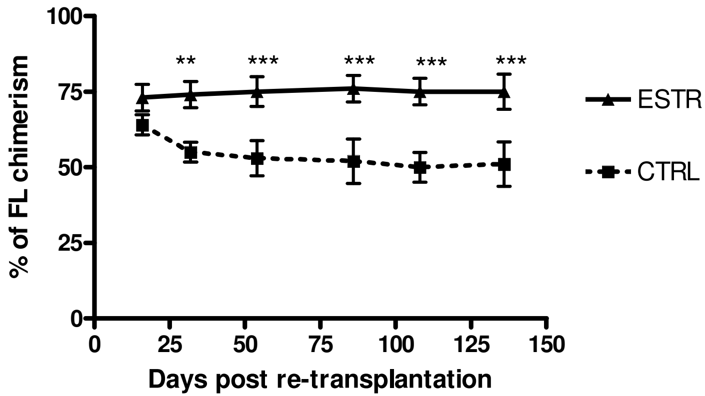


Fig. 5: Chimerism in PB of FL-origin B-cells co-transplanted with P0.5 liver cells (from Fig. 37) after transplantation into secondary recipients. Estrogen treated group. (***) $p < 0.001$, (**) $p < 0.01$

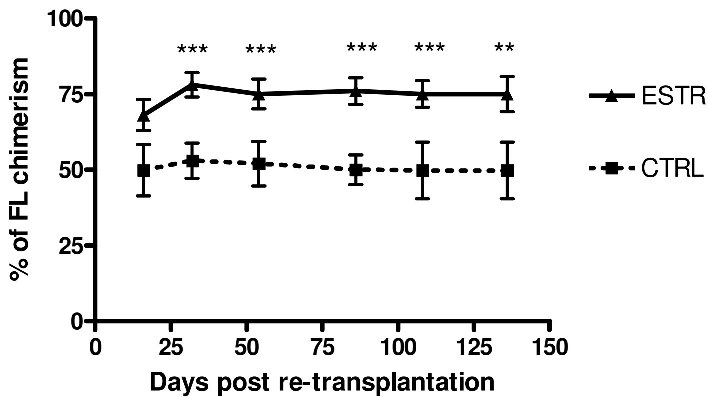


Fig. 6: Chimerism in PB of FL-origin B-cells co-transplanted with P0.5 liver cells (from Fig. 37) after transplantation into secondary recipients. Control group.

(***) $p < 0.001$, (**) $p < 0.01$

4.4 Influence of biphosphonates on the ability of HSCs to repopulate the host microenvironment

Adult male Ly5.1 mice were used as donors. Half of the mice were treated with biphosphonates in diet for two weeks. The administered dose was 70ug/kg/day. Bone marrow was then collected and $4x10^6$ bone marrow cells were transplanted into sublethally irradiated (6 Gy) male Ly5.2 mice. There was no significant difference in bone marrow cellularity in biphosphonate-treated and in control mice (Table 2). Biphosphonates did not effect transplantability of hematopoietic stem cells. There was also no difference in hematopoietic stem cell engraftment between the two groups (Fig. 7).

	Weight [g]	**Number of cells [million/femur]**
Biphosphonate-treated	23.85 (±1.24)	34.82 (±3.58)
Control	24.98 (±1.25)	34.80 (±1.79)

Table 2: Weight and bone marrow cellularity of biphosphonate-treated and control mice.

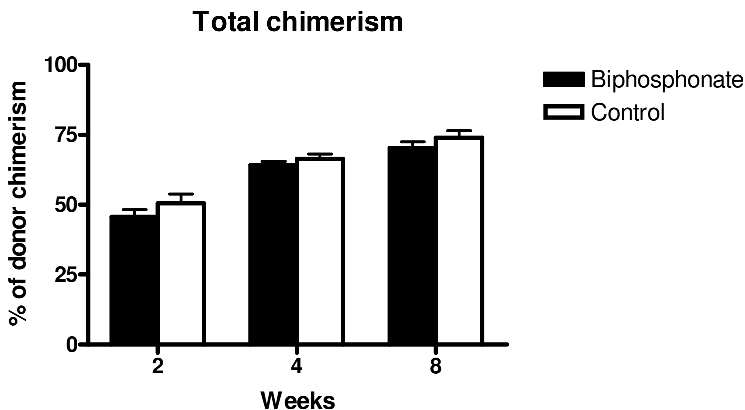


Fig. 7: Donor chimerism in recipient mice up to 8 weeks after transplantation. Donors bisphophonates treated.

5. DISCUSSION

5.1 Verification of murine congenic transplantation models

Congenic Ly5.1/Ly5.2 murine model is widely used in experimental hematology in functional studies of HSCs behavior (Soderling CC et al., 1985, van Os. R et al., 2001). Despite frequent use of this system, no methodological data were published so far describing reciprocity of Ly5.1/Ly5.2 strains, sex and dose dependency of chimerism. Van Os et al. (2001) found that engraftment following experimental BMT relies on the donor and host combination chosen; especially when minimal conditioning is applied. They ascribed it to T-cell-mediated immunity. As most our experimental data were based on chimerism measurement, we have done methodology experiments first to study in detail this transplantation model and to prove its validity for gaining reliable data.

We have studied the influence of sex and dose of BM cells transplanted into lethaly and sublethally irradiated recipients on chimerism. We have analyzed distribution of WBCs subpopulations in relation to the level of achieved chimerism.

We have proved that results obtained in this transplantation model are reliable and reproducible. There are no restrictions for recipients regarding sex of the donors. The post-transplantation chimerism reflected well the number of transplanted cells in both lethally irradiated (10 Gy) and sublethally irradiated (4 Gy) hosts. There is a strong correlation between total engraftment and WBCs subpopulation distribution. In case of a high total engraftment, the subpopulation distribution in the graft remains unchanged from normal mice.

5.2 Evaluation and comparison of different conditioning regimens on the level of engraftment of transplanted HSCs

The most frequent use of cyclophosphamide in clinical practice is as part of preconditioning regimen before BMT (Zhang J et al., 2006). We have studied the CY-TBI regimen from several perspectives and their influence on the level of engraftment of transplanted HSCs. Recipient preconditioning has been modified in respect of different time intervals between CY administration and TBI delivery, different radiation doses and application of two separate CY doses. Effect of CY administration on donor HSCs has been studied.

Using the same doses of CY and TBI in preconditioning and the same number of BM cells transplanted, just changing the time interval between CY application and TBI delivery, leads to pronounced differences in chimerism. When CY is administered 2 days prior to TBI, the chimerism after transplantation is significantly lower compared to only irradiated hosts. When CY is delivered 5 or 7 days before TBI, the chimerism is significantly increased. Regeneration of BM after CY administration follows a unique pattern (Šefc L. et al., 2003). It has been demonstrated by us that hematopoietic progenitors intensively proliferate one to three days after CY administration while they are quiescent five to seven days after CY administration (Šefc L. et al., 2003). Expression of SCF and SDF-1 is high in CY-2 group and low in CY-5/7. CY-2 induces regeneration of BM whereas CY-5/7 induces mobilization (Lévesque JP et al., 2002, Pšenák O et al., 2003).

We suppose that transplantation of hematopoietic cells into microenvironment supporting their proliferation stimulates donor stem cells into cycle

and decreases their ability for long-term repopulation. Transplantation into non-inductive microenvironment allows donor stem cells to seed and ensures a stable long-term engraftment.

5.3 Difference of fetal and adult HSCs with special emphasis on the development of sensitivity to estrogen

It has been demonstrated previously that HSCs/progenitors from different stages of FL development were ready to home and function in the environment of adult hematopoiesis (Chang KT et al., 2005). However, the process of blood formation is very different between fetal and adult life (Kincade PW et al., 2002). This is particularly the case for lymphopoiesis. An excellent example is pregnancy, during which adult B-lymphocytes are suppressed by high levels of circulating estrogen, but the B-lymphopoiesis from fetus is unaffected (Medina KL et al., 1993). Igarashi et al have demonstrated that FL-derived HSCs still lacked the receptors for sex steroid hormones four weeks after transplantation of FL into irradiated RAG-1-deficient mice and that the B-lymphopoiesis derived from these cells was less sensitive to inhibition by estrogens compared to that derived from adult BM (Igarashi H et al., 2001). We have examined the timing of conversion of the estrogen-resistant phenotype of FL B-lymphopoiesis into the sensitive phenotype of the adult BM B-lymphopoiesis. Our results provide the first *in vivo* functional confirmation of a differential responsiveness of FL- and adult BM-derived B-lymphopoiesis to suppression by estrogen (Pelichovská T et al., 2008). The B-lymphopoiesis derived from FL remained significantly less sensitive to inhibition by estrogen compared to that of adult BM origin. It remained its permanent feature as demonstrated in re-transplantation experiments. In the case of B-lymphopoiesis derived from neonatal liver its non-sensitivity to suppression by estrogen lasted only for about 50 days after transplantation to adult recipients. Later on, there was no difference from adult B-lymphopoiesis. An exposure of HSCs to the late-stage FL microenvironment seems to be critical and mandatory for the proper timing and a full conversion of the FL phenotype of the B-lymphopoiesis into that of the adult BM phenotype.

5.4 Influence of systemically administered drugs affecting bone marrow microenvironment on hematopoietic tissue

Bisphosphonates are commonly used in clinical settings for the treatment of osteoporosis (Mahakala A et al., 2003). It has been shown that new generation of bisphononates inhibits proliferation of endothelial cells *in vitro* (Yuasa T el at., 2007). Since endothelial cells are an important part of the BM microenvironment, knowledge of the effect of prolonged administration of bisphosphonates on HSCs and BM hematopoiesis is of outmost significance. Using murine transplantation model, we have tested the *in vivo* effect of bisphosphonates administration on HSCs, on their ability to repopulate the host microenvironment and their effect on host metabolism and ability to engraft donor cells. No difference between bisphosphonates-treated and control mice was observed in bone marrow cellularity or in the content of functional HSCs (Šefc L et al., 2007). There was no difference in hematopoiesis and in short-term as well as long-term engraftment between treated and control mice.

6. CONCLUSIONS

Part I – Congenic Ly5.1/Ly5.2 murine transplantation model is reliable. Results obtained from both systems are well reproducible and do not rely on strain and sex combinations. In mice with high total chimerism, the main WBC subpopulation of the graft are B-lymphocytes, as in normal mice. In mice with low total chimerism, the main contribution of graft to WBC is made up by Gr/Mac^+ cells.

Part II – In cyclophosphamide-total body irradiation preconditioning regimen, donor cells engraftment depends on the time interval between the first cyclophophamide administration and irradiation. Prolongation of the interval from 2 days to 5 or 7 days leads to significant increase in donor chimerism. Cyclophosphamide-busulphan preconditioning ensures myelosuppression comparable with CY-TBI regimen. Increasing irradiation in CY-2 group does not lead to an increase in donor cells engraftment.

Part III – The B-lymphopoiesis derived from fetal liver remained significantly less sensitive to suppression by estrogen compared to that of adult and neonatal origin. It remained its permanent feature. Exposure of HSCs to the late-stage FL microenvironment seems to be critical and mandatory for gaining later sensitivity to estrogen.

Part IV – Bisphophonates do not affect hematopoiesis in the mouse. They seem to be safe in regard to the effect on hematopoietic tissue in mice.

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