Transplant Immunology 34 (2016) 54–59

Contents lists available at ScienceDirect

Transplant Immunology

journal homepage: www.elsevier.com/locate/trim

# Quantitative evaluation of the transplanted lin⁻ hematopoietic cell migration kinetics

Vytautas Kašėta [a,b,*], Aida Vaitkuvienė [a], Aušra Liubavičiūtė [a], Rūta Maciulevičienė [b], Arūnas Stirkė [b], Genė Biziulevičienė [a]

[a] State Research Institute Center for Innovative Medicine, Žygimantų str. 9, LT-01102 Vilnius, Lithuania
[b] Center for Physical Sciences and Technology, A. Goštauto Str. 11, LT-01108 Vilnius, Lithuania

ARTICLE INFO

*Article history:*
Received 21 September 2015
Received in revised form 10 November 2015
Accepted 17 November 2015
Available online 18 November 2015

*Keywords:*
Mouse bone marrow
Lin⁻ hematopoietic cell
Inflammation
Migration

ABSTRACT

Stem cells take part in organogenesis, cell maturation and injury repair. The migration is necessary for each of these functions to occur. The aim of this study was to investigate the kinetics of transplanted hematopoietic lin⁻ cell population (which consists mainly of the stem and progenitor cells) in BALB/c mouse contact hypersensitivity model and quantify the migration to the site of inflammation in the affected foot and other healthy organs. Quantitative analysis was carried out with the real-time polymerase chain reaction method. Spleen, kidney, bone marrow, lung, liver, damaged and healthy foot tissue samples at different time points were collected for analysis. The quantitative data normalization was performed according to the comparative quantification method. The analysis of foot samples shows the significant migration of transplanted cells to the recipient mice affected foot. The quantity was more than 1000 times higher, as compared with that of the untreated foot. Due to the inflammation, the number of donor origin cells migrating to the lungs, liver, spleen and bone marrow was found to be decreased. Our data shows that transplanted cells selectively migrated into the inflammation areas of the foot edema. Also, the inflammation caused a secondary migration in ectopic spleen of hematopoietic stem cell niches and re-homing from the spleen to the bone marrow took place.

© 2015 Elsevier B.V. All rights reserved.

## 1. Introduction

Stem cells take part in organogenesis, cell maturation and injury repair. Stem cell migration, defined as directed and oriented cell movement to a specific site in the organism, is necessary for each of these functions to occur [1].

Based on literature data, the donor origin hematopoietic stem cells (HSCs) following the allogeneic transplantation, can be found almost in all organs and tissues of the recipient: CNS, skin, endothelium, nails, liver, intestine, womb, lungs and muscles [2–6]. The donor origin cells were not detected only in the recipient's hair follicle [7]. The data of different authors revealed that the amounts of the donor origin cells varied from 0.04% to 72.9% in different organs of the recipient [8,9]. Pathological processes, subjected to the foreseen cell transplantation, are usually accompanied by inflammation reactions; therefore, it is very important to elucidate stem cell migration during the inflammation. The SDF-1/CXCR4 axis is presupposed to be the main regulator for the stem cells [10]. The study on the HSC migration in the renal injury model showed that the inhibition of the SDF-1/CXCR4 association does not influence the transplanted HSC migration to the site of injury [11]. Under the influence of cytokines, the HSC mobilization reaches its peak following 5–6 days. However, the chemokine induced HSC mobilization lasts from 30 min to several hours [10]. Stem cells can migrate to the site of injured tissue but their migration mechanisms are still not clear.

It is supposed that physiological, as well as transplanted cell migration processes, are regulated by general mechanisms. However, the migration kinetics still needs to be elucidated as the results of different investigators are rather contradictory. The following parameters should be better identified: the number of transplanted HSC at the injury site; their amount in other organs following the transplantation and the amount change during inflammation. The data on the properties of different HSC populations in vivo at different pathologies, followed by inflammation, is scarce. The effect of inflammation on the migration of stem cells is undefined. On the other side, the influence of the stem cells on the inflammation process is not clear either. The aim of this study was to investigate the kinetics of transplanted hematopoietic lin⁻ (predominantly of stem and progenitor) cells in BALB/c mouse contact hypersensitivity model and quantify their migration to the site of inflammation in the affected foot and other healthy organs. The studies on migration of these cells in case of inflammation are important for a more effective and safe cell therapy.

*Abbreviations:* CNS, central nervous system; HSC, hematopoietic stem cells; Lin⁻, lineage-negative cell fraction; DNFB, 2,4-Dinitrofluorobenzene; TSPY, Testis-specific protein Y-linked.

* Corresponding author at: State Research Institute Center for Innovative Medicine, Department of Stem Cell Biology, Žygimantų str. 9, LT-01102 Vilnius, Lithuania.
*E-mail address:* Vytautas.Kaseta@gmail.com (V. Kašėta).

http://dx.doi.org/10.1016/j.trim.2015.11.003
0966-3274/© 2015 Elsevier B.V. All rights reserved.

## 2. Materials and methods

### 2.1. Laboratory animals

BALB/c mice (weighing 22–24 g), obtained from the Laboratory Animal Center of State Research Institute Centre for Innovative Medicine (Lithuania), were used. Animals were maintained in an environment of controlled temperature (23 ± 1 °C). Food and water were provided ad libitum. All procedures were approved by the Lithuanian Ethics Committee on the Use of Laboratory Animals under State Veterinary Service.

### 2.2. $Lin^-$ cell populations

Bone marrow cells were isolated by flushing femur and tibiae of BALB/c mice 7 weeks of age. Isolation of a low-density cell fraction was performed as described by Juopperi [12] with some modifications. The low-density cell fraction was obtained by separation of mouse bone marrow cells in a Percoll (GE Healthcare BioSciences AB, Buckinghamshire, UK) density gradient (1.077–1.081–1.087 g/ml). The cell fraction was harvested from the 1.081/1.087 interface. Lineage-negative cell fractions were purified using magnetic cell sorting techniques and the expression of the HSC markers (CD117 and $Sca^{-1}$) (all from BD Biosciences, San Jose, CA, USA) was established by flow cytometry, according to the previously described procedure [13] with the similar results (over 74% of cells were $CD117^+$ $Sca\text{-}1^+$ and less than 8% did not express any of these markers) [13,14]. In the experiment a BALB/c mouse bone marrow lineage-negative cell fraction ($lin^-$) was used, the $lin^-CD117^+Sca\text{-}1^+$ phenotype was predominant in this population.

### 2.3. Contact hypersensitivity model

Contact hypersensitivity reaction was induced with 2,4-dinitrofluorobenzene (DNFB) (Sigma-Aldrich Co., St. Louis, MO, USA) by Hiltz and Lipton [15] with some modifications. All animals were sensitized by application of 25 μl of 0.5% DNFB onto the shaved abdomen. After 3 days, the mice paws were challenged with 10 μl of 0.1% DNFB. The DNFB solution was prepared just prior to the application of the acetone–olive oil mixture (4:1). Cell transplantation was performed 24 h after the DNFB challenge. A total of $10^6$ cells/mouse in 100 μl PBS were intravenously injected. The control group mice received 100 μl injections of phosphate buffered saline (PBS). The reference group was treated intraperitoneally with prednisolone (100 mg/kg of animal weight). Differences in the weight of the injected versus the uninjected paw were evaluated after the treatment.

### 2.4. Real-time qPCR study of migration

DNFB challenged mice were randomly allocated into groups ($n = 5$ in each). Experimental BALB/c mice were placed into 7 groups, according to the biopsy specimen collection time (1, 4, 8, 12, 24, 48, 72 h after cell transplantation). A total of $10^6$ cells/mouse of $lin^-$ hematopoietic cells collected from male mice in 100 μl PBS were injected into the tail vein of the female mice study group. Following an appropriate time after cell transplantation, mouse bone marrow, spleen, liver, lung, kidney, untreated paw and paw edema specimens of the recipient mice were collected and cryopreserved. Preparations were evaluated by the real-time PCR analysis. This experiment was performed in triplicate. Statistical significance between the data groups was calculated.

### 2.5. Biopsy preparation for DNA extraction

#### 2.5.1. Mouse bone marrow samples

Bone marrow cells were obtained by flushing with 500 μl sterile TE buffer through one of the femoral epiphyses, using a syringe needle (27-gauge). 200 μl cell suspension was used for further DNA extraction.

#### 2.5.2. Mouse tissue samples

Spleen, liver, lung, kidney, untreated paw and paw edema specimens were prepared using the tissue homogenizer — TissueLyser2 (Life Technologies Co., Calsbad, CA, USA). 40–50 mg of tissue was placed in a special test tube with a 4.5 mm diameter steel ball and a 400 μl TE buffer; shaken for 2 min at 30 Hz. 200 μl cell suspension was further used for DNA extraction.

### 2.6. DNA extraction

Total DNA was isolated from the cell suspensions using "Genomic DNA purification kit" (Fermentas, Vilnius, Lithuania), according to the manufacturer's instruction. DNA concentration was determined by measuring the absorbance (A) at 260 nm wavelength. DNA purity was defined by calculating the $A^{260}$ and $A^{280}$ absorbance ratio. DNA purity is considered sufficient if the $A^{260}:A^{280} > 1.5$. The measurements were performed using the Synergy 2 (BioTek Instruments, Inc., Vinooski, VT, USA) plate reader.

### 2.7. Real-time PCR

The reaction was performed using the Rotor-Gene 6000 (Corbett Research, Mortlake, Australia) rotary analyzer. The primers and probes used were designed according to Wang [16], to detect the mouse TSPY (mouse testis-specific Y-encoded protein) (forward: 5′-GAG AAC CAC CTT GGT GAT TCT CT-3′; reverse: 5′-TCC TTG GGC TCT TCA TTA TTC TTA AC-3′) and mouse β-actin (forward 5′-ACG GCC AGG TCA TCA CTA TTG-3′; reverse: 5′-CAA GAA GGA AGG CTG GAA AAG A-3′) genes. TSPY pseudogene quantities were determined in the samples; this gene is only detected in the male mouse cells (Y chromosome). The TSPY gene amplification was normalized by mouse β-actin (detected in all mouse cells) amplification. Specific FAM (carboxyfluorescein) probes were used to quantify the appropriate amplification gene products. TSPY (5′-/56-FAM/-TCC TGG ATC-/ZEN/-AGA GTG GCT TAC CCA GG-/3IABkFQ/-3′) and β-actin (5′-/56-FAM/-CAA CGA GCG-/ZEN/-GTT CCG ATG CCC T-/3IABkFQ/-3). The reaction with the FAM-labeled probes was performed using the "Absolute Blue qPCR mix" (Thermo Fisher Scientific, Waltham, MA, USA). 100 ng of DNA sample, 200 μM of primers and 100 μM of probe were used per 15 μl of the reaction volume. An initial 15 min denaturation step at 95 °C followed by 40 cycles of 0.25 min denaturation at 95 °C, for 1 min annealing at 60 °C and 0.5 min elongation at 72 °C were performed. In each reaction group, different concentrations of the positive control (BALB/c male DNA from 0.0001 to 200 ng), the negative control (PCR reagents without DNA, PCR reagents with BALB/c female DNA) and calibration samples (various concentration combinations of BALB/c male and female DNA, per 100 ng of the total DNA ratio from 1/10 to 1/100,000) were used. Test samples were duplicated and two independent tests were performed. The quantity of the product was determined in each cycle by measuring the specific probe fluorescence (excitation 470 ± 10 nm, detection 510 ± 5 nm). The product size was verified by agarose gel analysis. The quantitative data normalization was performed according to the comparative quantification method [17,18]. For normalization of DNA levels, the housekeeping gene β-actin was used. Based on the calibrator and standard curve data, the sample product was normalized and the relative concentration was calculated.

### 2.8. Statistics

Statistical significance among the mean values was determined using a two-sided Student's t-test. $P < 0.05$ was considered significant. All calculations were performed using the SigmaPlot (version 11).

Download English Version:

https://daneshyari.com/en/article/3391995

Download Persian Version:

https://daneshyari.com/article/3391995

Daneshyari.com