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B-lymphopoiesis gains sensitivity to subsequent inhibition by estrogens during final phase of fetal development

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ABSTRACT

Adult B-lymphopoiesis is suppressed by the inhibitory effects of elevated estrogens during pregnancy. At the same time, hematopoietic cells in the fetal liver are resistant to this suppression by estrogens and ensure active production of B-cells. We investigated whether this unresponsiveness to estrogens of fetal cells also applies to cells obtained from a newborn liver and projects into the adult hematopoiesis when fetal liver cells are transplanted to adult mice. Mixtures of fetal liver (E14.5), neonatal liver (P0.5) and adult bone marrow (BM) cells were co-transplanted into adult primary and secondary recipients treated with high doses of estrogen in the Ly5.1/Ly5.2 congenic mouse model. Total chimerism as a proportion of all nucleated blood cells, chimerism as a proportion of B220+ B-cells, and of other blood cell lineages as well, were determined by flow cytometry. B-lymphopoiesis derived from fetal liver (E14.5) stem cells remained resistant to estrogen after transplantation into both primary and secondary adult recipients, for up to 280 days. In contrast, B-lymphopoiesis derived from neonatal liver (P0.5) stem cells was resistant to estrogen only for approximately 50 days after the primary transplantation to the adult BM microenvironment. These results provide further evidence for a critical developmental period of B-lymphopoiesis during its fetal liver stage. In the mouse, critical developmental events that allow for the subsequent expressed sensitivity of B-lymphopoiesis for suppression by estrogens after sexual maturation appear to occur during the period of late-stage fetal liver hematopoiesis before its migration to the bone marrow.

1. Introduction

B-lymphopoiesis occurs in the bone marrow (BM), lymph nodes and in the spleen throughout life in both humans and mice (Kalis et al., 2007; Medina et al., 1993). It depends on specific events controlling gene transcription (Bryder and Sigwardsson, 2010; Ramírez et al., 2010) and passes through developmental stages distinguishable according to certain surface markers and their IL-7 dependence (Hardy and Hayakawa, 1991; Igarashi et al., 2002; Kalis et al., 2007; Kikuchi and Kondo, 2006; Pelayo et al., 2006). A specific feature of the adult B- lymphopoiesis lies in its sensitivity to suppression by estrogens (Medina et al., 2001). During pregnancy, the elevated estrogen production induces a down-regulation of B-lymphopoiesis in mice (Medina and Kincade, 1994; Medina et al., 1993) and also inhibits T-cell development in the thymus (Rijhsinghani et al., 1996). This might contribute to protecting the fetus from attack by the mother's adaptive immune system. Interestingly, during fetal development, an intensive B-lymphopoiesis occurs in the middle- and late-stage fetal liver (FL) despite high estrogen levels (Chang et al., 2005; Strasser et al., 1989). This has been explained by the lack of estrogen receptors in B-cell progenitors and precursors during FL hematopoiesis (Igarashi et al., 2001). Hematopoietic stem and progenitor cells (HSPC) derived from the middle-stage FL still lacked estrogen receptors even four weeks after transplantation to irradiated RAG-1-deficient mice (Igarashi et al., 2001).

An increasing body of evidence has provided information on the differences between FL and adult-BM hematopoietic cells (Bowie et al., 2007; Hardy and Hayakawa, 1991; Hardy et al., 2007; Irion et al., 2010; Medina and Kincade, 1994; Montecino-Rodriguez et al., 2006; Siggs et al., 2011). Kikuchi et al. (2005) have demonstrated that regarding the IL-7 receptor expression, the conversion from FL phenotype to adult phenotype occurs in mice during the first few weeks after birth. Previously, we had shown that the late-stage fetal liver microenvironment is required for the full conversion of the FL B-lymphopoiesis phenotype into the adult BM phenotype, characterized by sensitivity to suppression by estrogen (Pelichovská et al., 2008).

Abbreviations: HSPC, hematopoietic stem and progenitor cells; FL, fetal liver; BM, bone marrow; WBC, white blood cells; E14.5, embryonic day 14.5; P0.5, postnatal day 0.5.

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In this study, we investigated whether HSPC from the liver of newborn mice differ from HSPC collected from the middle-stage FL in terms of gaining sensitivity to estrogens after their transplantation to adult mice. Our results strongly suggest that the late stage FL microenvironment determines some features of adult B-lymphopoiesis, specifically its sensitivity to suppression by estrogens after sexual maturation, and thus represents a critical developmental period in B-lymphopoiesis ontogeny.

2. Materials and methods

2.1. Animals

C57BL/6. mice (Ly5.2 and Ly5.1) 8- to 12-weeks-old were maintained in a clean conventional animal facility with a light–dark cycle of 12 h and fed ad libitum. To set up mating, females were examined in the afternoon, and those in estrus were placed in cages with males (two females with one male). The morning after mating, the females were separated and checked for the presence of a copulation plug in the vagina. This day was designated embryonic day 0.5 (E0.5). Newborn mice were those delivered the previous night and they were designated postnatal day 0.5 (P0.5). Eight- to 12-weeks-old male Ly5.1 or Ly5.2 mice served as recipients in transplantation experiments.

2.2. Cells for transplantation

Fetal livers from E14.5 embryos and livers from P0.5 newborn mice were collected. Bone marrow cells were flushed out from the femurs of adult mice. A single cell suspension was prepared in a phosphate-buffered saline (PBS) solution containing 0.5% albumin by repeatedly flushing the tissues through 18- and 27-gauge needles. Finally, cells were passed through a nylon mesh with a pore size of 70 μm (Falcon 2350, Becton, Dickinson Labware, Franklin Lakes, USA), counted and appropriately diluted for transplantation.

2.3. Irradiation

Mice were lethally irradiated with a dose of 10 Gy (5 Gy + 5 Gy with a 30 min interval, ^{60}Co, 0.8 Gy/min).

2.4. Transplantation to primary recipients

Recipient male mice were lethally irradiated. Two hours after irradiation, the mice were transplanted with a mixture of either.

- adult BM cells (Ly5.2) and E14.5-FL cells (Ly5.1) or
- adult BM cells (Ly5.2) and P0.5-newborn-liver cells (Ly5.1) or
- E14.5-FL (Ly5.1) and P0.5-newborn-liver cells (Ly5.2).

A total of 10^7 cells were injected intravenously into the retrobulbar plexus in a volume of 0.5 mL PBS with 0.5% bovine albumin. The cell mixtures were transplanted to both Ly5.1 and Ly5.2 recipients in order to exclude an engraftment preference for syngenic over congenic cells. Half of these mice were treated with estrogen. The presence of E14.5-FL-derived cells, P0.5-newborn-liver-derived cells and those derived from adult bone marrow was followed in the peripheral blood for up to 16 weeks.

2.5. Transplantation to secondary recipients

Lethally irradiated male mice were intravenously injected with 10^7 bone marrow cells obtained from the primary recipients – from the mice that had been previously lethally irradiated and transplanted with 10^7 E14.5-FL cells and P0.5-newborn-liver cells (1:1) 16 weeks earlier. Half of the secondary recipients were treated with estrogen. The presence of E14.5-FL- and P0.5-newborn-liver-derived cells in the peripheral blood was followed for up to 20 weeks in the secondary recipients.

2.6. Estrogen treatment

Estrogen (estradioli dipropionas; Estrofem, Novo Nordisk A/S, Bagsvaerd, Denmark) was delivered in 250 μg i.m. injections in two-week intervals starting one day before transplantation.

2.7. Analysis of recipients

2, 4, 8, 12, 16 and 20 weeks after transplantation, a sample of blood was obtained from the retrobulbar plexus. Aliquots (50 μL) from each blood sample were added to three separate tubes filled with 3 mL lysis buffer (0.15 M NH_4Cl, 0.035 M NaCl, and 0.1 mM EDTA), and red blood cells were lysed for 10 min. Cells were then washed twice in PBS, resuspended in PBS, and stained with phycoerythrin (PE)-conjugated anti-Ly5.1 and fluorescence isothiocyanate (FITC)-conjugated anti-Ly5.2 antibodies. They were simultaneously stained with biotinylated anti-B220 antibody, anti-CD3, or a mixture of anti-Gr-1/anti-Mac-1 antibodies. The secondary staining was done with streptavidin-PE-Cy-5. All antibodies and reagents were purchased from Pharmingen (San Diego, USA). Samples were analyzed on a FACS Calibur (Becton, Dickinson, San Jose, USA). Gating for Ly5.1+ and Ly5.2+ cells was performed, and Ly5.1+ Ly5.2+ artificial doublets were omitted from the analysis.

2.8. Statistical analysis

Eight recipient mice given the same treatment were used per group. Analysis of variance (two-way ANOVA with Bonferroni post-test) was used to calculate the significance of differences from the control group.

3. Results

3.1. Sensitivity of B-lymphopoiesis derived from E14.5 fetal liver or from P0.5 newborn liver to estrogen compared to those derived from adult bone marrow

Lethally irradiated male Ly5.2 and/or Ly5.1 mice were transplanted with a mixture of five million E14.5-FL cells or P0.5-newborn-liver cells, both of the Ly5.2 phenotype, combined with five million Ly5.1 adult BM cells. Half of the recipients received estrogen treatment. Estrogen administration significantly increased the fraction of FL-derived B220+ cells over those derived from the adult BM up to 90 days after transplantation (Fig. 1A). In contrast to this, the fraction of B220+ cells derived from P0.5 newborn liver was only increased by estrogen treatment up to six weeks after transplantation. Later on, starting approximately seven weeks after transplantation, the ratios of B220+ P0.5-newborn-liver-derived cells and those derived from adult BM were no longer affected by estrogen administration (Fig. 1B). Estrogen treatment also affected the ratio of FL- or postnatal-liver-derived versus adult-BM-derived total white blood cells (Fig. 2). The ratios of T-cells (CD3 positive WBC) and granulocytes–monocytes cells (Gr-1 and Mac positive WBC) were not affected by estrogen treatment (results not shown).