Environments of haematopoiesis and B-lymphopoiesis in foetal liver

K. Kajikhina[1], M. Tsuneto[1,2], F. Melchers[1]

[1]*Max Planck Fellow Research Group on "Lymphocyte Development", Max Planck Institute for Infection Biology, Berlin, Germany;*
[2]*Department of Stem Cell and Developmental Biology, Mie University Graduate School of Medicine, Tsu, Japan.*

Katja Kajikhina
Motokazu Tsuneto, PhD
Fritz Melchers, PhD

Please address correspondence to:
Fritz Melchers,
Max Planck Fellow Research Group on "Lymphocyte Development",
Max Planck Institute for Infection Biology, Chariteplatz 1,
D-10117 Berlin, Germany.
E-mail: melchers@mpiib-berlin.mpg.de

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ABSTRACT

In human and murine embryonic development, haematopoiesis and B-lymphopoiesis show stepwise differentiation from pluripotent haematopoietic stem cells and multipotent progenitors, over lineage-restricted lymphoid and myeloid progenitors to B-lineage committed precursors and finally differentiated pro/preB cells. This wave of differentiation is spatially and temporally organised by the surrounding, mostly non-haematopoietic cell niches. We review here recent developments and our current contributions on the research on blood cell development.

Introduction

Human and murine haematopoietic stem cells generate their progeny, erythroid, megakaryocytic, myeloid and lymphoid lineage cells continuously throughout life in bone marrow. These progenitors and differentiated lineage cells are guided in their development by microenvironments (niches) consisting of mostly non-haematopoietic stromal cells. Because of this continuous cell development during life bone marrow contains a mixture of all haematopoietic cell stages in their environments at any given time (1-3). Furthermore, the three-dimensional complexity of the cavities, in which marrow is localised in bone, complicates histological analyses of spatial ordering of haematopoietic niches. Foetal liver is easily accessible for histological sectioning and, therefore, for the localisation of haematopoietic niches and their relationship in time of development (4).

Embryonic development of the mouse starts to generate haematopoietic progenitors with limited erythroid/myeloid capacities in yolk sac at E8.5 (5, 6), while pluripotent haematopoietic stem cells (pHSC) are generated in the aorta-gonad-mesonephros area of the embryo at around E10.5 (7-9), multipotent myeloid/lymphoid progenitors (MPP), and their immediate progeny, common myeloid progenitors (CMP) and common lymphoid progenitors (CLP) soon thereafter (10). The first T- or B-lymphoid lineage-directed progenitors appear at E12.5-13.5, for T-lymphocytes in the developing thymus (11), for B-lymphocytes in foetal liver (12). Time in development, therefore, separates and orders these different developmental haematopoietic stages. Three-dimensional imaging of progenitors and precursors indicates that stem cells are mainly found inside the embryonic blood vessel, and are attracted to vascular endothelium at the early stage of tissue colonisation at E10.5-E11.5 (13).

Haematopoietic stem cells and progenitors on their way to B cells

We have chosen foetal liver as an embryonic site, where myeloid and B-lymphoid cells develop in a timely ordered fashion (4). Our analyses of the composition and kinetics of $CD45^+$ haematopoietic cells show that the foetal liver is colonised with less than 1000 pHSC at E13.5, which are characterised as $lin^-c\text{-}kit^+Sca1^+CD150^+CD48^+$ or $CD48^-$ cells, while lin^- $c\text{-}kit^+Sca1^+Flt3^+CD48^+$ MPP and $lin^-c\text{-}kit^+IL7R\alpha^+$ CLP are 10-fold, and $lin^-c\text{-}kit^+IL7R\alpha^-c\text{-}fms^+$ myeloid progenitors (MP) 100-fold as frequent. It is notable that the absolute numbers of MPP, MP and CLP increase only slightly with increasing time of gestation between E13.5 and E16.5. At the same time the numbers of differentiated SLC^+CD19^+preB cells, the immediate precursors of SLC^-IgM^+ B cells, undergo an over 400-fold expansion within 4 days (Fig. 1).

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On the other side, the microenvironments, in which pHSCs, MPPs, MPs

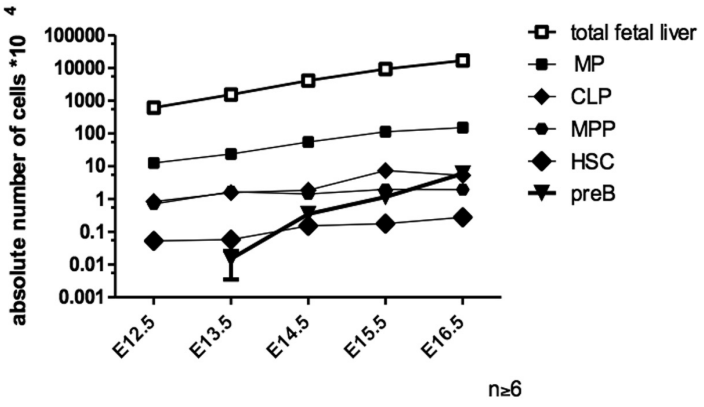

Fig. 1. Development of haematopoietic progenitor subsets and B cell precursors in wild-type murine foetal livers from E12.5 to E16.5

and CLPs, precursor B (preB) and immature and mature B-lymphocytes develop in foetal liver during embryonic development of the mouse between E13.5 and 16.5, are still largely unknown. We analysed enzymatically dissociated $CD45^+Ter119^+$ haematopoietic and $CD45^-TER119^-VCAM\text{-}1^+$ non-haematopoietic foetal liver cells by flow cytometry. Two subpopulations among the non-haematopoietic cells were identified that were either $CD105^{high}$ LYVE-1^{high} or $CD105^{low}$-$ALCAM^{high}$ (14). The LYVE-1^{high} cells were shown to co-express the endothelial markers CD31, Tie-2 and VEGFR3. The $ALCAM^{high}$ cells displayed hepatic and mesenchymal markers DLK1, BP-1 and CD133.

By RT-PCR and histological analysis of IL7-GFP reporter mice (15) we checked in these two types of non-haematopoietic cells for gene expression programs that could be involved in the chemokine mediated progenitor migration, and for their cytokine profiles for support of proliferation, survival and differentiation of haematopoietic and B-lymphoid progenitors.

At E13.5 and E14.5 LYVE-1^{high} endothelial cells produce CXCL10. The LYVE-1^{high} endothelial cells do not produce M-CSF, one major myeloid cytokine, nor IL7, the major lymphoid cytokine. They express SCF, the ligand for c-kit, which is expressed on pHSCs, MPPs, MPs and CLPs, and they also express Flt3L, active with Flt3 on the surface of MPPs, MPs and CLPs, and required for further myeloid and lymphoid differentiation (16).

At the same developmental stage the early progenitors are seen to migrate towards CXCL10 *in vitro* and to express the proper chemokine receptor CXCR3 and, furthermore, to migrate to CXCL12, since these cells co-express the receptor CXCR4.

At the same early times the $ALCAM^{high}$ non-haematopoietic liver mesenchymal cells produce CXCL10 and CXCL12, as well as the cytokines SCF, Flt3L, M-CSF and IL-7. Therefore these non-haematopoietic stromal cells produce the chemokines necessary to attract the progenitors to this second site, as well as the cytokines required to induce further differentiation along the myeloid and lymphoid pathways. It is well known that B cell development in the bone marrow as well as in foetal liver requires IL-7 and CXCL12 (17), whereas M-CSF drives myeloid lineage choice and differentiation (13,14). Therefore, $ALCAM^{high}$ cells appear to be capable to support both B-lymphoid and myeloid differentiation of early progenitors.

Migration from embryonic blood to foetal liver

The location of IL-7Rα^+ haematopoietic progenitors in the endothelial, LYVE-1^+ and mesenchymal $ALCAM^+$ microenvironments of foetal liver was investigated by immuno-fluorescence in histological sections between E13.5 and E16.5.

At E13.5, but not later, these analyses found three of four IL-7Rα^+-stainable ($CD19^-c\text{-}kit^+SLC^-$) progenitors, possibly a mixture of MPPs and CLPs, in contact with vascular endothelial-type LYVE-1^{high} cells. One day later, these $c\text{-}kit^+$IL-7Rα^+ cells developed to $CD19^-$ and $^+$, SLC-expressing, DHJH-rearranged pro and preBI cells. Less than 10% were still in contact with LYVE-1^{high} cells. By contrast, half of them were now in contact with mesenchymal $ALCAM^{high}$ liver cells (11).

This change in the histologically detectable neighborhood of the MPPs and CLPs, their chemokine receptor expression and migration responsiveness, and the chemokine and cytokine expression profiles of the non-haematopoietic cells suggested that transendothelial migration of these progenitors at E13.5 into foetal liver is mediated by CXCR3-CXCL10 interactions. MPPs and CLPs then migrate through the LYVE-1^{high} vascular endothelium to $ALCAM^{high}$ mesenchyme, attracted by CXCL10 and CXCL12 produced by this hepatic environment. Once in contact with mesenchymal cells MPPs, CLPs (possibly also MPs) and their progenitors become resident and begin their further differentiation toward B-lymphoid and myeloid cell lineages, induced by the cytokines M-CSF and IL7 produced in this $ALCAM^{high}$ niche. It is interesting that *in vitro* migration assays at E15.5 showed that differentiated B-progenitors had lost their migration capacity toward CXCL10, but remained sensitive to CXCL12, although they retained the expression of the corresponding receptor, CXCR3, on their surface. It needs to be clarified whether signalling via CXCR3 has been abandoned by preB cells, or whether the quality of signal transduction has changed from migration to residence, survival and proliferation at the second, mesenchymal site of B-lymphoid development.

For the development of B-lineage cells these observations suggest that MPPs and CLPs with Ig gene loci in germline configuration enter the developing foetal liver at E13.5 through the vascular endothelium and then migrate within a day to an $ALCAM^{high}$ liver mesenchymal cell microenvironment. It is known that proB and preB cells are chemoattracted *in vitro* towards CXCL12 and that non-haematopoietic stroma cells control the early stages of differentiation to DHJH-rearranging, surrogate light chain-expressing pro B and preBI cells (4, 20). We see this when, between E15.5 and E16.5, preBI cells expand 10-fold in continued contact with AL-

CAM^{high} cells, begin VH- to DHJH-rearrangements, but fail to express IgμH-chains and preB cell receptors before E17.5 (12).

Conclusion

Foetal liver harbours all distinct progenitor subset throughout full time course of gestation, promoting a rapid B cell differentiation wave at the same time. Early haematopoietic and B-lymphoid progenitors first migrate from embryonic blood through endothelium, guided by a specific set of chemokines made by the endothelial cells. Thereafter, they differentiate to CLPs, migrate to mesenchymal liver cells, again attracted by special chemokines produced there, become resident in the supporting niche where they are stimulated among others by the cytokine IL7 which drives their proliferation and differentiation. Finally, they begin DJ and V to DJ-rearrangements at the IgH chain loci to become first CD19^{+} preB and then IgM^{+} B cells.

Abbreviations

HSC: haematopoietic stem cell
MPP: multipotent progenitor
CLP: common lymphoid progenitor
CMP: common myeloid progenitor
SLC: surrogate light chain
ALCAM: activated leukocyte cell adhesion molecule
ICAM: intercellular adhesion molecule
LYVE: lymphatic vessel endothelial receptor
MP: myeloid progenitors
VCAM: vascular cell adhesion molecule

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