Interplay between Flt3L and IL-7 in early haematopoietic development

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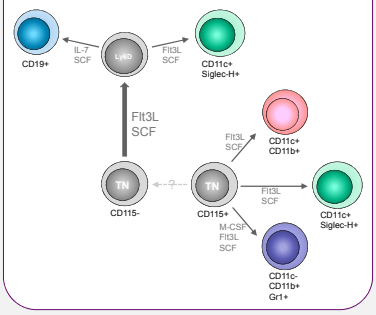
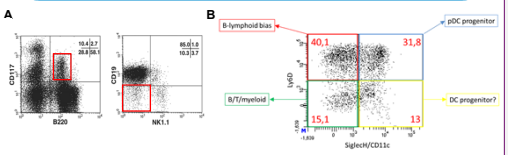
Abstract

The long standing model of haematopoiesis describes single routes of differentiation towards individual fates and decisions were seen to be irrevocable. However, latest research points towards a more flexible decision making process. We are especially interested in early events in haematopoietic progenitor cell specification with focus on the EPLM cell population – early progenitors with lymphoid and myeloid potential – which are defined as cKit^{low}B220^{+}CD19^{-}NK1.1^{-}. Using the cell surface markers Ly6D, Siglec-H, CD11c, and CD115 we were able to distinguish five subpopulations with certain differentiation biases. Now we are studying their individual developmental potentials and possible precursor-product relationships using different *in vitro* as well as *in vivo* approaches. While the Ly6D single-positive population seems to be biased towards the B-lymphoid lineage and retains pDC potential, the CD115^{+} portion of Ly6D^{-}SiglecH^{-}CD11c^{-} cells shows efficient myeloid, cDC, and pDC differentiation. The CD115^{-} counterpart gives rise to B cells and pDCs. Additionally, we assess the potential instructive role of the cytokines Flt3L and IL-7 in the decision-making process. For this purpose we generated a complete set of knock-out and transgenic mice (and the respective combinations). We found that the B-cell deficiency of IL-7 knock-out mice is rescued by increased levels of Flt3L and Bcl-2, indicating that IL-7 acts mainly as proliferation factor.

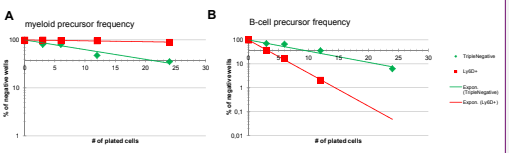
Hypothesis for EPLM differentiation



EPLM – Early Progenitor with Lymphoid and Myeloid Potential



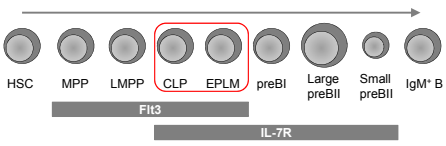
(A) The EPLM are characterised as CD117^{low}B220^{+}CD19^{-}NK1.1^{-}. They constitute about 0,1% of nucleated BM cells. (B) The cell surfers markers Ly6D, CD11c, as well as Siglec-H further subdivide them into four different subpopulations. More recently we found within the triple negative cells equal fractions of CD115^{+} and CD115^{-} cells.



In vitro as well as *in vivo* assays revealed diverging differentiation biases of the two CD11c/Siglec-H negative subpopulations. In limiting dilution on ST2 (A) or OP9 (B) stroma cells + IL-7 , respectively, the triple negative compartment had some lymphoid and strong myeloid potential, whereas the Ly6D single-positive EPLM lost the latter and almost exclusively gave rise to lymphoid cells.

➡ *possible precusor-product relationship with the triple negative EPLM giving rise to the Ly6D^{+} ones*

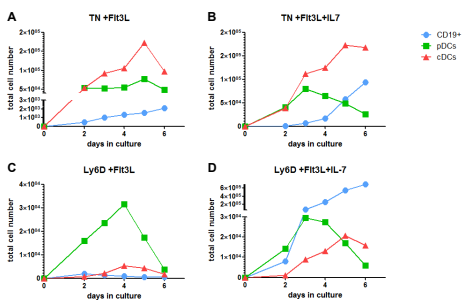
Cytokine effects on EPLM



To analyse the functional role of the cytokines Flt3L and IL-7 we generated a complete set of knock-out and transgenic mice, and the respective combinations.

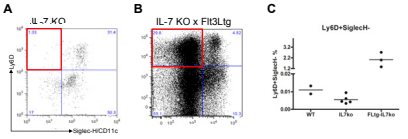
	WT	FLtg	FL$^{-/-}$	IL7tg	IL-7$^{-/-}$	IL7$^{-/-}$ FLtg	IL7$^{-/-}$ Bcl2tg	FL$^{-/-}$ Bcl2tg	FL$^{-/-}$ IL7tg	FLtg IL7tg
Flt3L	+	+++	-	+	+	+++	+	-	-	+++
IL-7	+	+	+	+++	-	-	-	+	+++	+++
EPLM	+	+++	-	+	+	+++	+		-	+++
Ly6D+	+	+++	-	+	-	+++	-/+		-	+++
preBI	+	+	-/+	+++	-	+	+		+	+++

Differentiation of the EPLM subpopulations upon cytokine stimulation

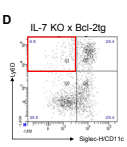


Upon sorting the triple negative and Ly6D single positive EPLM (of B6xFlt3Ltg mice) gave rise to committed B cells, cDCs, and pDCs with different efficiencies and kinetics. 5x10^{4} triple negative or Ly6D^{+} single positive EPLM were stimulated with 0,1µg/ml SCF, 0,05µg/ml Flt3L + 10% IL-7. The cultures were analysed daily for the generation of CD19^{+} B cells, CD11c^{+}CD11b^{+} cDCs, and CD11c^{+}Siglec-H^{+} pDCs. B cells arise late and in low numbers from triple negative EPLM (A), and IL-7 seems to be needed for their expansion (B). Ly6D^{+} EPLM were most potent in generating CD19^{+} cells upon addition of Flt3L and IL-7 (D). cDCs were obtained almost exclusively from TN EPLM (A and B) and Flt3L was sufficient. pDCs derived from TN and Ly6D+ EPLM even upon addition of Flt3L only (A to D).

Excess Flt3L and Bcl-2 rescue the IL-7 KO B-cell deficiency



The lymphopenic IL-7 knock-out mice are specifically lacking the Ly6D single positive EPLM compartment (A), which we think is the direct precursor of CD19^{+} committed B cells. Excess Flt3L rescues this deficiency in numbers as well as in terms of B-cell precursor frequency (B, C, and E).

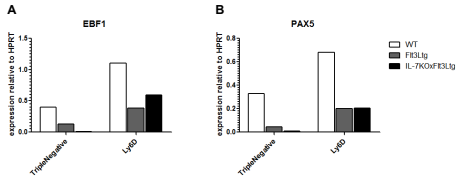


Overexpression of the pro-survival factor Bcl-2 didn't increase the number of Ly6D single positive EPLMs significantly (D). However, the number of B-cell precursors is restored (E).

E B-cell progenitor frequency within the Ly6D single-positive EPLM compartment

	WT	Flt3Ltg	Bcl2tg	IL-7KO	IL-7KO Flt3Ltg	IL-7KO Bcl2tg
B-cell progenitor frequency [1 in x]	6,19	8	8	380	4	4,75

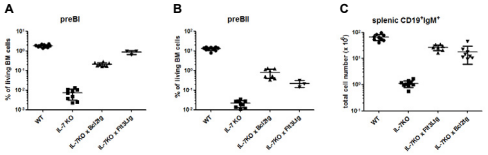
Excess Flt3L rescued B-cell specific transcription factor expression



EBF1 and PAX5 are indispensable transcription factors for B-cell commitment and development. In EPLM of IL-7 KO mice, their expression level ranges below the qPCR detection limit. However, upon overexpression of Flt3L, expression becomes detectable again.
We are now addressing the question why the Ly6D single positive EPLM of Flt3L transgenic mice have lower levels of PAX5 compared to WT.

➡ *excess Flt3L rescues the B-cell deficient phenotype of IL-7 KO mice*

Excess Flt3L or Bcl-2 rescued committed B-cell stages



Overexpression of Flt3L or Bcl-2 in IL-7 knock-out mice rescued early committed B-cell stages in the BM. (A) cKit^{+}CD19^{+} preBI and (B) cKit^{-}CD19^{+}IgM^{-} preBII cells were readily detectable again. (C) Peripheral B-cell receptor expressing cells were restored as well.

Conclusions

1. *EPLM consist of five subpopulations showing differentiation biases*
   a. *TripleNegative CD115^{-} EPLM show B-cell as well as pDC developmental potential with Flt3L and IL-7*
   b. *TripleNegative CD115^{+} EPLM give rise to myeloid cells, cDCs, as well as some pDCs*
   c. *Ly6D EPLM are lymphoid biased – B cells and pDCs*
2. *Excess Flt3L or Bcl-2 rescue the B-cell deficient phenotype of IL-7 knock-out mice*
   - ✓ *Ly6D single positive EPLM*
   - ✓ *B-cell specific transcription factor expression*
   - ✓ *Early committed B cells*
   - ✓ *Peripheral B-cell receptor positive B cells*
3. *IL-7 is not necessarily required for B-cell commitment, but it acts as proliferation factor making B cell development more efficient*

Acknowledgements

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