In vivo modelling of normal and pathological human T-cell development

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Chapter 8

English summary
Nederlandse samenvatting
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List of publications

English summary

A single hematopoietic stem cell (HSC) can make up all the different cells of the immune system. Most of the different cell lineages of the immune system develop in the bone marrow. T cells, however, develop in the thymus where a specialized environment is present. Within the thymus, the T-cell progenitors are selected; T cells that do not recognize antigens and autoreactive T cells are deleted. T cells are needed to fight virus infections, provide help to B cells during bacterial infections and can also be reactive towards tumors.

A multipotent progenitor migrates from the bone marrow, via the bloodstream, to the thymus. After engraftment in the tissue, this progenitor will start to proliferate and develop towards more mature cell types. Once committed to the T-cell lineage, it will start to rearrange different parts in the DNA, T-cell receptor loci that can form a T-cell receptor. This rearrangement provides the great diversity that is present within the T-cell receptors expressed on mature T cells to ensure recognition of many different antigens of, for instance, pathogens.

T-cell development follows a defined path during which the thymus seeding cell will proliferate and differentiate. Most of the knowledge of this development stems from studies performed in mice. The availability of many transgenic mouse strains makes it possible to study the influence of signaling pathways on the development of T cells. Knowledge on the development of human T cells is mainly derived from descriptive studies in which *ex vivo* human thymus material is studied by cellular phenotyping and gene expression analysis or *in vitro* assays as described in **chapter 1**.

There are different diseases in which patients suffer from abnormal T-cell development from which insights can be gained into the pathways regulating human T-cell development. Such diseases include on the one hand Severe Combined Immunodeficiency (SCID), in which there is a block in T-cell development leading to absence of functional T cells, and on the other hand T-cell acute lymphoblastic leukemia (T-ALL) where patients suffer from an accelerated malignant growth of T cells. As it is difficult to study the mechanisms behind both these forms of pathological T-cell development, an optimized *in vivo* model for the study of human T-cell development has been developed that is described in **chapter 2**. As the NOD/Scid-Il2rg$^{-/-}$ (NSG) mice are severely immunodeficient, caused by the absence of NK cells, B cells and T cells, they allow the engraftment of human cells. A short culture of isolated human hematopoietic stem and progenitor cells (HSPCs) allows for possibility of genetically modifying these HSPCs while maintaining robust engraftment of human cells and the development of a human immune system in this mouse. The B cells and T cells that develop within in these mice are able to mount an immune response to an endogenous antigen demonstrating their functionality. The HSCs present in the transplant do engraft in the bone marrow of the mouse and are able to self-renew as illustrated by the fact that bone marrow from primary recipients can fully engraft secondary recipients. In addition, the optimized protocol also leads to good engraftment of HSPCs obtained from human bone marrow and development of the different lymphoid cell types. Together with the finding that thymi from mice engrafted with human hematopoietic cells show comparable human T-cell development to *ex vivo* analyzed human thymus biopsies opened the possibility to study patient material from biobanks.

This idea has been exploited in **chapter 3** to study the stages of arrest in human T-cell development for different types of SCID. As from most of the SCID patients no thymic biopsies are taken, the T-cell developmental blocks have remained elusive. Previously, extrapolations have been made from knockout mice. However, it was found that the blocks in human T-cell development for different types of SCID reside at earlier stages then anticipated from these models illustrating the differences between human and murine T-cell development. From the obtained data, a model was proposed that shows and early need for cytokine-driven proliferation directly after seeding of the thymus by progenitors. In addition, the point of β-selection could be attributed to an earlier stage then previously described as demonstrated by the block in development observed for Artemis-SCID. As this study illustrated the power of the model in detecting developmental arrests in development of human lymphoid cells, HSPCs from a patient with atypical presentation of SCID were transplanted in these immunodeficient mice. In **chapter 4** the results of this study are described that confirmed that the patient indeed suffered from $T^-B^+NK^+$-SCID. The arrest in development was found to reside in the $CD4^+CD8^+$ double positive stage. As none of the known SCID-causing genes contained a genetic aberration, whole exome sequencing of the patient and both parents was performed leading to the detection of a heterozygous *de novo* mutation in *VPS4B* in this patient. This gene has not been previously associated with SCID.

Chapter 5 described the severe restriction of hematopoietic clones during human T-cell development demonstrating two restriction points. Despite the severe hematopoietic clonal restriction this limited number of clones can still make up a very diverse polyclonal T-cell receptor repertoire, which is needed to fight different pathogens that are encountered on a daily basis.

In contrast to **chapter 3 and 4**, in which gene deficiencies were studied, the effect of overexpression of *LMO2*, a known oncogene in T-ALL, on human T-cell development was studied. *LMO2* was overexpressed in human HSPCs isolated from umbilical cord blood and these cells were transplanted in the severe immunodeficient NSG mice as described in **chapter 6**. In gene therapy trials for immunodeficiencies, T-ALL did develop in several patients due to insertional mutagenesis. Often the insertion of the therapeutic vector was in or near *LMO2* and resulted in overexpression of this gene causing the development of T-ALL in some patients. Transgenic mouse models for *Lmo2* demonstrated blocks in immature T-cell progenitors and the development of leukemia with a long latency. Using the humanized NSG mouse model, it was demonstrated that *LMO2* overexpression causes aberrant human T-cell development by three different mechanisms; the delayed development as also observed in transgenic mice and *in vitro* assays, an accumulation of $CD4^+CD8^+$ double positive cells and accelerated development leading to a higher frequency of mature T cells.

Altogether, this thesis describes novel insights in human T-cell development by transplanting human HSPCs in severe immunodeficient NSG mice. Thereby a model is provided that can further aid both in fundamental studies and can be used for optimization of gene therapy approaches and stem cell expansion protocols. Furthermore, it illustrates (see **chapter 7**) the need for more in depth understanding of human T-cell development, which could help further improve T-cell reconstitution after transplantation in patients and might aid in the diagnosis and future treatment of patients with T-ALL.