T-cell development in the fetus and the neonate begins with immigration of fetal liver-derived stem cells into the thymus. After intrathymic T-cell maturation is complete, RTEs exit the thymus and enter the lymphoid periphery. RTEs are marked by green fluorescence in mice carrying a transgene-encoding green fluorescent protein driven by the RAG2 promoter. RTEs in the neonate enter a lymphopenic periphery and constitute the majority of peripheral T cells. In the adult, stem cells arise from the bone marrow, and after completing intrathymic maturation, the resulting RTEs exit the thymus and enter a lymphocyte-periph-ery, where they are surrounded by a majority of mature T cells.

...the functional defects allow the individual to purge self-reactive T-cells by permitting new emigrants to scan the periphery for tissue-specific antigens without the danger of eliciting autoimmunity? Neatons must uniquely cope with lymphopenia and the absence of mature peripheral T cells. Given that T-cells undergoing homeostatic proliferation adopt a memory cell phenotype and heightened function, the IL-7-driven proliferation of neonatal RTEs may both help fill up empty space and provide a population of memory-like T-cells. Clearly, much remains to be learned about how the youngest peripheral T-cells cope with their adolescence and successfully transition into adulthood.

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**DII1 and DII4: similar, but not the same**

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The VEGF-DII1-Notch1 signaling cascade has taken center stage in angiogenesis, but it now appears that DII1 ligands have precedence in arteries and even seem to control VEGF signaling.

**A**rteries and veins are structurally and functionally different types of vessels, and they display independent molecular signatures. A genetic program imposes arterial or venous fate onto embryonic precursors prior to formation of patent vessels and onset of blood circulation. This has been shown most elegantly in zebrafish and seems to hold true...
for mammals as well. However, there is enormous plasticity in this system, allowing complete cell fate changes in response to external stimuli during early embryogenesis. This property is progressively lost with developmental age.

Blood vessels in the embryo are first formed by vasculogenesis, the coalescence of precursors to form vascular tubes and meshworks followed by remodeling and sprouting. The current paradigm of sprouting angiogenesis holds that vascular endothelial growth factor (VEGF) signaling through VEGF receptor 2 (VEGFR2) is limited to Sprouty1 in migrating tip cells of newly forming sprouts, inducesDll4 ligands. Dll4 in turn activates Notch signaling in following stalk cells, making them refractory to further VEGF-induced sprouting, in part by reducing VEGFR2 and its coreceptor Nrp1. Dll4 also induces expression of arterial markers like Hey1/2 bHLH transcription factors and Efnb2 ligands, and it promotes maturation and lumen formation. This is paralleled by repression of vein endothelial markers such as Nrp2, EphB4, and Coup-TFI1, with the latter being involved in reciprocal antagonism with the Notch pathway components Hey1 and Hey2 to determine arterial versus venous cell fate.

Although this model of sprouting angiogenesis and arterialization has gained broad experimental support, there is increasing evidence that other components may be equally important. Limbourget al showed that Dll1, a related Notch ligand, is essential for postnatal angiogenesis with heterozygous Dll1+/− mice exhibiting strongly impaired reperfusion after experimental hindlimb ischemia. A complete Dll1 knockout is lethal with bleeding around day 11 (E11) of embryonic development, but this seems to be due to defects in surrounding tissues and not related to intrinsic vascular functions. The availability of hypomorphic and conditional knockout alleles of Dll1 now allow Sørensen and colleagues to pinpoint a novel embryonic vascular defect that predicts a revised hierarchy of signaling.

While Dll4 mutations (as well as mutations of Notch receptors or Hey1/2 bHLH effectors) show lethality after day 9.5 of embryonic development, Dll4 hypomorphs or mice with endothelial-specific Dll1 deletion survive until birth. Nevertheless, careful analysis of their blood vessels demonstrated that arterial identity is progressively lost from larger vessels starting at day 13.5, the time point when endothelial Dll1 expression should begin. Surprisingly, Dll4 is still expressed in these vessels, suggesting the existence of a switch making Dll4 incapable to sustain Notch activity and expression of arterial markers like Efnb2, Nrp1, or Hey1 and permitting expression of the vein marker Coup-TFI1.

Interestingly, the reduction of Nrp1 expression and thus the impaired capacity of endothelia to respond to VEGF in these mice is not due to up-regulation of the venous regulator Coup-TFI1, which gets expressed only later. Sørensen et al instead provide strong evidence that in arteries Nrp1 and perhaps VEGFR2 are directly induced by activated Notch receptors, which are absent from Dll1 negative or hypomorphic endothelia. Thus, VEGF cannot be placed upstream of the Dll/Notch cascade but rather it appears that Notch signaling enables VEGF responsiveness of these cells.

What is striking is the differential severity of endothelial defects in Dll1 versus Dll4 KO mice. While a lack of Dll4 leads to very early defects in angiogenesis and arterialization, this is much less so with Dll1. Although arterial markers are lacking and venous markers are up-regulated, this is still compatible with embryonic development. The perinatal lethality of the conditional endothelial Dll1 knockouts may be due to vascular problems, but this awaits further study. Nevertheless, it seems that arterialization defects may be tolerated in later development or adults for some time, at least as long as the animals are not subject to additional stress. This is reminiscent of the rather limited arterialization of grafted veins in human coronary arteries.

The present study clearly shows that there are parallel and noncompensating Dll-Notch signals in endothelial cells. Dll4 may be more important early on in the capillary bed, while Dll1 preferentially acts in arteries. Yet, the molecular basis for the differential function of these very similar ligands acting on the same Notch1 receptor remains unresolved. The principle of a VEGF-Dll-Notch signaling cascade in endothelia may have to be revised for arteries with Dll1-Notch1 acting not as a target, but as a facilitator of VEGF sensitivity.

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