

## Summary of thesis

Vitamin A plays an important role in maintaining mucosal immune homeostasis. Dysregulation of this balance, for instance by impaired vitamin A metabolism or vitamin A deficiency results in the development uncontrolled inflammatory conditions. The studies described in this thesis focus on the different cell types involved in maintenance of the mucosal microenvironment and immune balance. Furthermore, the effects of vitamin A deficiency and differences in vitamin A metabolism on the mucosal immune system in health and disease were addressed.

**Chapter 2** describes the role of the LN microenvironment in providing signals for T cell tropism. Gut or skin draining LNs were transplanted into the popliteal fossa, so that DCs entering these LNs are bringing in antigen from the same peripheral site. These transplantations show that the microenvironment of mucosal mesenteric LNs (MLNs) and not peripheral LNs (PLNs), supports the induction of  $\alpha_4\beta_7$ , but not CCR9, on T cells upon their activation. Furthermore, MLN stromal cells showed expression of vitamin A converting enzymes allowing production of RA by these cells. While *in vitro* cultured MLN stromal cells were able to induce gut-homing tropism on activated T cells directly, addition of RALDH-low unpulsed bone marrow-derived DCs (BM-DCs) strongly enhanced the expression of gut-homing molecules  $\alpha_4\beta_7$  and CCR9. These results demonstrate a crucial role for MLN stromal cells in creating an instructive mucosal microenvironment in lymph nodes and that stromal cells, DCs and lymphocytes cooperate for efficient differential imprinting of tissue tropism.

In **chapter 3**, we have studied the capacity of the peripheral tissue microenvironment to imprint DCs in such a way that they can induce skin and small intestine homing receptors on activated T cells. Upon activation by Ag-pulsed BM-DCs, CD8<sup>+</sup> T cells up-regulated the skin homing receptor E-selectin ligand when co-cultured with dermal fibroblasts and gut homing receptors CCR9 and  $\alpha_4\beta_7$  when co-cultured with small intestinal epithelial cells. Soluble factors, such as RA, as well as cell-cell contact were essential for the induced tissue tropism imprinting capacity of DCs. This shows that peripheral tissue stromal and epithelial cells produce factors that license DC to induce tissue-specific homing receptors and thereby transmit information about their tissue of origin and the site of Ag capture to T cells.

In **chapter 4** we investigated how the expression of RALDH in MLN-DCs and MLN stromal cells is regulated postnatally. Studies with Trif mutant and MyD88<sup>-/-</sup> animals demonstrated that expression and activity of RALDH enzymes in MLN-DCs is independent of TLR signalling. On the contrary, dietary vitamin A appeared to be crucial for RALDH expression in MLN-DCs and MLN stromal cells. Furthermore, RA directly regulated the level of RALDH expression in BM-DCs as well as lymph node stromal cells, thereby regulating RALDH expression within the mucosal immune system and consequently maintaining mucosal immune homeostasis. These data establish that dietary vitamin A plays a crucial role in proper functioning of the mucosal immune system.

C57BL/6 and BALB/c mice are known as prototypical Th1- and Th2-type mice respectively, and RA has been described to skew T cells upon their activation towards the Th2-type profile. In **chapter 5** of this thesis, we therefore investigated whether C57BL/6 and BALB/c mice differ in their capacity to produce RA. We demonstrated that BALB/c mice expressed higher levels of RALDH enzymes and had more RA-mediated signaling in the intestines. Consequently, MLN-DCs displayed higher RALDH activity, which led to increased induction of gut-homing molecule expression on CD4<sup>+</sup> T cells and FoxP3 regulatory T cells. The enhanced capacity to induce gut-homing molecules correlated with an increased accumulation of T cells and B cells in BALB/c small intestines when compared to C57BL/6 small intestines. Also, secretion of IgA into the lumen of the small intestines was higher in BALB/c mice when

compared to C57BL/6 mice. Thus, these studies showed that the enhanced ability to convert vitamin A results in a better developed mucosal immune system.

In **chapter 6**, we investigated whether the differences in RA production and RAR signaling observed in BALB/c and C57BL/6 mice could indeed improve the response in an inflammatory setting such as colitis. BALB/c mice suffered from less severe DSS colitis compared to C57BL/6 mice and recovered more quickly. During colitis, BALB/c mice showed an increased ability to form tertiary lymphoid tissue, which could contribute to IgA production and increased numbers of regulatory T cells. RA has numerous beneficial effects on the mucosal immune system implicating that an increase in RA signaling could potentially improve the outcome of inflammatory disease and recovery from inflammation in BALB/c mice compared to C57BL/6 mice.

**Chapter 7** describes the investigation of LT<sub>i</sub> differentiation during LN development. We hypothesized that RA affects hematopoietic LT<sub>i</sub> differentiation in the embryo and demonstrated that oral supplementation of pregnant mothers with RA skewed the differentiation of hematopoietic precursors towards the final LT<sub>i</sub> phenotype. Consistently, administration of a vitamin A deficient diet to pregnant mothers led to a significant decrease of the LT<sub>i</sub> differentiation state in the developing LN of the embryo. Also, embryos from BALB/c mice, which displayed enhanced vitamin A metabolism when compared to C57BL/6 mice, had more mature LT<sub>i</sub> cells in their MLNs and adult BALB/c mice displayed larger mucosal lymphoid organs. Thus, RA is involved in local differentiation of LT<sub>i</sub> cells during LN development and we propose that vitamin A levels in the mother have a significant effect on the amount of LT<sub>i</sub> cells and the formation of LNs in the embryo.

Finally, in **chapter 8**, the findings described in this thesis are summarized and discussed in the context of recent developments in the research on vitamin A and the mucosal immune system. Potential future research directions are indicated, which may resolve unanswered questions. This will give further insight in the mechanism of how the mucosal immune system operates and how it can be influenced.