Secondary lymphoid organ development

Lymph nodes, and other secondary lymphoid organs such as the spleen, are important for the specific immune response against pathogens. In these organs, different types of white blood cells are located of which lymphocytes are the most important cells for the specific long-term defense against dangerous pathogens. This response of the immune system results in immunological memory, which leads to a more specific immune response after a next encounter with the same pathogen.

Amongst white blood cells that are involved in this reaction are B- and T-cells. In lymph nodes, B-cells are located primarily in the cortex, while T-cells can be found in the paracortex. A separation in B- and T-cell areas is also found in the white pulp of the spleen, and in Peyer’s patches, lymphoid organs located in the intestines. But also in inflamed tissue of a part of the patients suffering from chronic inflammation diseases such as rheumatoid arthritis (RA) or Chron’s disease B- and T-cell areas can be found. Thereby, the inflamed tissue has characteristics of secondary lymphoid organs formed naturally. While the existence of secondary lymphoid organs is beneficial for the body because the immune reaction partially takes place at these locations, the presence of lymphoid tissue at sites of inflammation is regarded as a part of the chronic inflammation disease. Despite the research in lymphoid organ development of the last fifteen years, little is known about the first steps in the formation of secondary lymphoid organs. The aim of this research is therefore to further unravel the molecular and cellular components of secondary lymphoid development.

More than a century ago, Florence Sabin studied the development of lymph nodes. In her model, lymph nodes arose from so called lymph sacs; sinuses covered with lymphatic endothelial cells in which in a later stage mesenchymal tissue would grow in to, to eventually form the lymph nodes. It would take half a century however before it became clear that lymph nodes, and also other lymphoid organs such as the spleen, were the most important locations in the body where the antigen-specific reaction of the immune system takes place.

Where the immune system is located, and how the antigen-specific immune reaction works on a cellular level, only became clear from the late fifties. At that moment it was already known that the body makes antibodies upon vaccination. Research in chickens by Glick showed that these antibodies were formed by lymphocytes. This was followed by investigations of Cooper, by which it became clear that different subsets of lymphocytes exist, namely B- and T-lymphocytes, which have their specific function in the immune reaction. In addition it was shown that these subsets were formed at different places in the body. B-cells in the Bursa of Fabricius (a specific organ in birds; later it would appear that in mammals B-lymphocytes are formed in the bone marrow) and T-lymphocytes in the thymus. Gowans and Knight then displayed that both T- and B-cells migrate to lymph nodes via blood vessels after their generation.
Summary

The underlying mechanism of the immune system and the importance of lymphoid organs for the immune reaction thus became more and more exposed. But research in the lymphoid organ development was relatively rare. Only in the early nineties, hundred years after the investigations of Sabin, the field of lymphoid organ development was boosted by the emergence of molecular biology. After the characterization of the lymphotoxin α (LTα) deficient mice in 1994 it appeared that in the absence of LTα, lymph nodes and Peyer's patches (lymphoid organs in the intestine) did not develop. Together with the discovery of 'lymphoid tissue inducer cells' (LTi cells), specific blood cells that are crucial for the development of lymph nodes and Peyer's patches, this research formed the basis of an extension of the hundred year old model for lymph node development of Sabin. An historical overview of research in the development of the immune system and in lymphoid organ formation is given in chapter 2.

To get more insight into the first phases of lymph node development, we studied the murine lymph node primordium during embryonic development. Therefore we elucidated the role of distinct cell types and molecules. In chapter 3 we describe our study in the subpopulations of cells that form the lymph node primordium at embryonic day 16.5 (E16.5) in the mouse, which is approximately 3 days before birth. At this time point, different cell populations appear present in the developing lymph node. Most prominent are LTi cells and stromal cells that later play an important role in the organization of the lymph node, in specific in the organization of B- and T-cell areas. We can now subdivide these stromal cells into two groups based on their cell surface molecules. These two groups have a specific gene expression pattern. The distribution of the two stromal cell subsets appears to differ in mesenteric lymph nodes when compared to peripheral lymph nodes. Apart from these two stromal cell types, distinct types of endothelial cells appear to be present in the developing lymph node.

Because the development of the lymph node anlage depends on the interaction between LTi cells and stromal cells, we investigated which molecules are involved. It was already known that triggering of the lymphotoxin-beta receptor (LTβR) plays an elementary in lymph node development and that LTi cells stimulate stromal cells via this receptor which leads to differentiation of stromal cells into 'lymph node organizer cells'. In chapter 4 we show that stimulation of this receptor does not only lead to an elevation of the expression of cell surface molecules and chemokines, but also in up-regulation of cytokines and the growth factor VEGF-C. This growth factor is important for the development of lymphatic endothelium. Signaling via the LTβR can therefore control the differentiation of distinct cell types.

On the one hand, lymphatic endothelium appears to be present apart from vascular endothelium at E16.5 in murine lymph node primordia. This is in line with the hundred year old model of Sabin, in which the development of lymph sacs by lymph vessels marked the first stage of lymph nodes. On the other hand, signaling via the LTβR leads to an increased expression of VEGF-C, by which LTi cells via activation of stromal cells thus can regulate the formation of lymphatic endothelium. To investigate whether the very first clustering of LTi cells depends
on previously formed lymph sacs, we studied mice deficient for the transcription factor Prox1. In these animals, lymph sacs and lymphatic endothelium are not formed. Our results (described in chapter 5) show that in these animals a normal clustering of LTi cells can still occur, and clusters can be found at locations were lymph nodes form normally. The location of the lymph nodes is thus not completely controlled by lymph sacs. For a normal development of lymph nodes, the formation of lymphatic endothelium is however indispensable, because the capsule and sinuses that are crucial for a proper function of the lymph node are formed by lymphatic endothelium.

We thus studied the effect of signaling via the LTβR, which is necessary for the differentiation of stromal cells in the lymph node primordium, and we also studied the expression of Prox1, which is required for the differentiation of lymphatic endothelium that next to lymph vessels also forms parts of the lymph node. To further unravel the mechanism of lymph node organ development, we also examined the role of the enzyme C5-epimerase in this process. This enzyme is important for a correct modification of heparan sulfate proteoglycans (HSPGs); sugar structures coupled to specific proteins displayed on cell surfaces and in the extracellular matrix. These sugar structures can bind a large variety of factors, including chemokines, cytokines, and growth factors. In chapter 6 we describe that in absence of C5-epimerase all previously described cell types can develop, but that in C5-epimerase deficient mice, lymphoid organ primordia are more often aberrant. With respect to spleen primordia, aberrations were even found in all C5-epimerase deficient mice, resulting in a severe decrease in spleen size.

The white pulp of the spleen is similar to lymph nodes with respect to the presence of B- and T-cell areas, but important differences can also be found. For instance, in the spleen a capsule of lymphatic endothelium is lacking. In chapter 7 we show that also in development of the splenic white pulp there are important differences compared to lymph node development, in specific in the activation of local stromal cells. In lymph nodes, LTi cells express the ligand of the LTβR by which they can induce the differentiation of stromal cells. In stead of LTi cells, in the spleen B-cells primarily express lymphotoxin on their cell surface. Therefore in the development of splenic white pulp, these cells appear to take over the role of LTi cells in the induction of stromal cell differentiation.

The results of these and other recent studies in the development of lymphoid organs and implications for the clinic we described in chapter 8. Here we also show for lymph node development. In this model, lymph node development is depicted in five phases, which is also described for other organs. The model can be a basis for future research into the first steps of lymph node development in which the role of other cell types such as vascular endothelium in the development of lymph nodes is further unraveled.

Summarizing, the research of this thesis concerns the early developmental stages of secondary lymphoid organs. To study these early stages, the cellular and molecular composition of the developing lymph node en splenic white pulp was characterized, and the importance of distinct molecules during the formation
Summary

was shown. Our results enabled the integration of the functions of LTi cells, stromal cells and lymphatic endothelium into a five-step model of lymph node development. In splenic white pulp, the makeup of cell types that play a role during development is clearly different when compared to this model, underlining the role of B-cells as inducers of stromal cell differentiation.