The handle [http://hdl.handle.net/1887/43187](http://hdl.handle.net/1887/43187) holds various files of this Leiden University dissertation

**Author:** Quispel, W.T.
**Title:** Langerhans cell histiocytosis: genetic and immunologic fingerprinting
**Issue Date:** 2016-09-22
The main goal of this thesis was to gain insight to the pathogenesis of LCH by studying the genetic (Chapter 2) and immunologic ‘finger-prints’ (Chapter 3-6) of therapy-naive LCH lesions.

A significant proportion of LCH-cells display mutations in BRAF, MAP2K1 and MAP3K1 that drive the constitutive activation of the MAPK-pathway. This explains their putative resistance to apoptosis, enhanced survival and proliferation and chemotaxis capacity. The unexpected earlier reported detection of MAPK-activated kinases in LCH-cells lacking BRAF mutations suggests other mutations drive the constitutive MAPK-pathway activation. In collaboration with our colleagues from the Dana Farber Cancer Institute in Boston we investigated in Chapter 2 the presence of additional MAPK-pathway-activating mutations. We addressed genetic abnormalities expressed by lesional CD1a⁺ LCH-cells that were flow-sorted from unfixed, frozen LCH biopsies. LCH-cell derived DNA along with genomic DNA from granulocytes or buccal swab collected from the same patient was subjected to whole exome sequencing. We found that lesional LCH-cells from a BRAF⁰野生¹ patient displayed a somatic mutation in ARAF, another member of the MAPK-pathway. This occurred in the kinase activation domain of ARAF. We showed that this mutation, similar to the activity of BRAF¹⁶⁰E, MAP2K1 and MAP3K1, lead to the constitutive intracellular kinase activation of the MAPK-pathway. It was sensitive towards the BRAF-inhibitor vemurafenib, which is FDA approved for clinical applications. Although not eligible according to currently applied inclusion criteria, this offers new possibilities to increase treatment options for patients without BRAF¹⁶⁰E mutations.

An unsolved issue in LCH is how and why the (somatically mutated) LCH-cells also end up in other tissues than the typical LC homing sites. The chemokine-chemokine-receptor pair CXCL12-CXCR4 plays a key role in both the migration of normal immune cells and in cancer-cell metastases. In Chapter 3 we demonstrate that CXCR4-expression on LCH-cells guides their homing and retention to CXCL12-expressing LCH tissues. We found ‘LCH-like’ cells in peripheral blood and bone marrow samples that had a similar phenotype as tissue-resident CD1a⁺ LCH-cells. A key role for CXCR4 was also demonstrated in the migration of these circulating ‘LCH-like’ cells towards CXCL12. Of clinical importance, patients who initially presented with a single lesion containing CXCR4⁺ LCH-cells had a higher probability to develop additional LCH lesions. This observation qualifies CXCR4 as a candidate prognostic factor for less favourable disease outcome. Pathophysiological, this could be explained by the capacity from tissue resident CXCR4⁺ LCH-cells to migrate to non-conventional LC-homing sites that normally express CXCL12 and to form distant lesions.

Along with the LCH-cells, particular CD₃⁺ T-lymphocytes infiltrate LCH lesions. Lymphocyte recruitment into tissues is mediated via HEVs and through the release of various chemo attractant molecules. Given that lymphoid aggregates in non-lymphoid tissues display similar histological characteristics as classic LN they are classified as TLS.
SUMMARY

This prompted us to investigate whether similar elements are present in LCH lesions (Chapter 4). T-cell attractive elements were detected in around 20% of the lesions which was associated with the presence of high T-cell numbers. LCH lesions contained variable degrees of lymphoid aggregation, which were classified as TLS in at least 8 patients. The highest aggregation degree, lymphoid-follicular aggregation, were most frequently detected in patients presenting with unifocal LCH. Those patients had the lowest risk to develop new LCH lesions. The co presence of TLS and HEVs in LCH lesions displaying high T-cell frequencies indicates that these aggregates somehow control primary LCH manifestation and prevent the dissemination of LCH-cells.

The putative intralesional promotion of protective T-cell-mediated immune responses, prompted us to study the activation status of the present T-cells in LCH lesions in relation to their microenvironment. In Chapter 5, we demonstrate that T-cells in all lesions have an activated phenotype. Activated T-CONV were particular present in lesions that contained proteins and costimulatory molecules that facilitate intralesional T-cell activation. We however also reported the presence of cytokines and costimulatory molecules that facilitate the inhibition of T-CONV and the induction of T-REG. Each LCH lesion could be classified according a distinct immune suppressive cytokine profiles. T-REG were present in all LCH immune suppressive cytokine profiles while specialized T-REG were highly prevalent in lesions containing costimulatory-LCH-cells. As in this study neither an activated or immunosuppressive environment in LCH lesions was associated with a particular LCH manifestation form or outcome demonstrates that immune-fingerprinting lesions for these elements is clinically not relevant.

One cytokines which is key to proper effector T-cell function is the inflammation-promoting cytokine Interferon-gamma (IFN-γ), which is part of the type 1 cytokine pathway. In addition, poly-ostotic LCH patients are clinically difficulty to distinguish from patients who display particular mutations in the gene encoding the IFN-γR1 (ADIFNGR1 patients). This made us investigate whether a non-functional type I cytokine pathway underlies LCH pathogenesis and if it could distinguish LCH from ADIFNGR1 patients (Chapter 6). We demonstrate staining tissue specimens for CD1a and crucial components of the type 1 cytokine pathway is not meaningful for discrimination between those diseases. None of genotyped LCH patients displayed genomic variations in the most commonly affected exons of the gene encoding IFN-γR1. This may explain the ever functionally intact IFN-γ-signalling loop in LCH patients. In contrast to PBMC of ADIFNGR1 patients who are unresponsive to IFN-γ. Our study indicates that simple overnight functional assays quickly and correctly distinguish LCH patients from ADIFNGR1 patients at each moment during the diagnostic process and even after the start of treatment.

The research described in this thesis support the concept that LCH lesions should be seen as a ‘cocktail’ of genetically aberrant LCH-cells which accumulate at sites with a mixed immunological background.