CHAPTER 1

General Introduction

General Introduction

Histiocytic disorders, also called histiocytoses, are characterized by an abnormal activation and accumulation of cells of the mononuclear phagocyte system, which comprises dendritic cells (DCs), monocytes and macrophages (Mφ) (*1*). According to the WHO classification, the histiocytic disorders are divided into two groups; those of varying biological behavior and those that are truly malignant (*2*) (Table 1). The first group can be further divided in DC-related and Mφ-related diseases, and includes Langerhans cell histiocytosis (LCH). In the second group, a distinction is made between DC-related, Mφ-related, and monocyte-related disorders.

Histiocytic disorders are rare and the clinical presentation of the different entities is very heterogeneous. The etiology and/or pathogenesis of most of these diseases are, therefore, still unknown. Researchers and clinicians in this field are united in the Histiocyte Society and are putting much effort in elucidating the pathological mechanisms of these diseases and finding rational therapies for the histiocytoses.

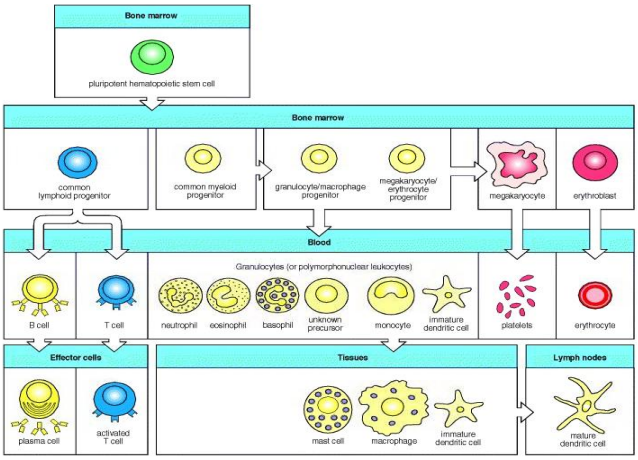

Figure 1. Lymphocytes of the immune system.

Hematopoietic stem cells in the bone marrow divide into a common lymphoid progenitor cell that gives rise to T and B lymphocytes, and a common myeloid progenitor cell that gives rise to different types of leukocytes, erythrocytes, and megakaryocytes. T and B lymphocytes are distinguished by their sites of encounter with antigen. B cells differentiate into antibody-secreting plasma cells, whereas T cells differentiate into effector T cells with a variety of functions. A third lineage of lymphoid-like cells, the natural killer cells, derive from the same progenitor but lack the antigen-specificity that is the hallmark of the adaptive immune response (not shown). The leukocytes that derive from the myeloid progenitor cell are the monocytes, the dendritic cells, and the granulocytes (basophils, eosinophils, and neutrophils). Monocytes enter tissues, where they differentiate into macrophages. Macrophages are the main tissue-resident phagocytic cells of the innate immune system. Immature dendritic cells travel via the blood to enter peripheral tissue where they ingest antigens. When they encounter a pathogen, they mature and migrate to lymphoid tissues, where they activate antigen-specific T cells. Granulocytes are recruited to sites of infection or (allergic) inflammation where they involved in defending against bacteria or parasites. Mast cells arise from precursors in bone marrow and complete their maturation in tissue and are important in allergic responses. Adapted from reference (*3*).

Table 1. Classification of Histiocytic Disorders*

Disorders of varied biological behavior
DENDRITIC CELL-RELATED
Langerhans cell histiocytosis
Secondary dendritic cell processes
Juvenile xanthogranuloma and related disorders
Solitary histiocytomas of various dendritic cell phenotypes
MACROPHAGE-RELATED
Primary hemophagocytic lymphohistiocytosis (familial and sporadic)
Secondary hemophagocytic syndromes
• Infection-associated
• Malignancy-associated
• Other
Rosai-Dorfman disease (Sinus histiocytosis with massive lymphadenopathy)
Solitary histiocytoma with macrophage phenotype
Malignant Disorders
DENDRITIC CELL-RELATED
Histiocytic sarcoma (localized or disseminated)
• Follicular dendritic cell
• Interdigitating dendritic cell
• Other
MACROPHAGE-RELATED
Histiocytic sarcoma (localized or disseminated)
MONOCYTE-RELATED
Leukemias
• Monocytic leukemia (M5A and B)
• Acute myelomonocytic leukemia (M4)
• Chronic myelomonocytic leukemia
Extramedullary monocytic tumor or sarcoma

* Adapted from reference (*2*)

Historical overview of LCH

The description of patients with LCH in the medical literature started 145 years ago, and the history of LCH is a good example of how the history of medicine evolved in the past century (4). In 1865, Smith described a patient with skin lesions and holes in the cranium (5). His publication appeared before the invention of X-ray imaging, but his drawings of the skull defects suggest LCH (6). A couple of years later Hand published a case report entitled “Polyuria and tuberculosis” on a child with polyuria and exophthalmus (7). In 1905, Kay reported on a child with exophthalmos, diabetes insipidus and bone defects (8). Around 1920, Schuller and Christian described several patients with diabetes insipidus, exophthalmus, and skull defects (9, 10). Comparison of the above mentioned publications led to the eponym Hand-Schuller-Cristian disease for the clinical triad of diabetes insipidus, exophthalmus, and skull lesions. Unfortunately, Kay was not acknowledged as his publication was overlooked. A couple of years later, a report by Siwe was published in which he described several cases, including one previously reported by Letterer (11), of bone tumors, hyperplasia of non-lipid storing macrophages, organomegaly, lymphadenopathy, secondary anemia, and a hemorrhagic tendency (12). This combination of symptoms would later be named Letterer-Siwe disease. Around the same time as the appearance of Siwe’s publication, reports on eosinophilic granuloma of the bone were published by Mignon (1930) (13) and Fraser (1935) (14) and in 1940 by Otani and Ehrlich (15) as well as by Lichtenstein and Jaffe (16). In 1941, Farber concluded that Hand-Schuller disease, Letterer-Siwe disease, and eosinophilic granuloma of the bone represented manifestations of the same disease process (17). In 1953, Lichtenstein published his classic paper in which he introduced the term Histiocytosis X for this disease process (18). Birbeck found in 1961 the characteristic granules present in the cells of LCH lesions, which became a distinctive recognition marker and therefore a diagnostic marker for LCH (19). In 1973, Nezelof was the first to report that proliferating pathological Langerhans cells (LC) were the cause of Histiocytosis X (20). In relation to this finding, Risdall, and coworkers at the University of Minnesota, suggested 10 years later that Histiocytosis X should be changed into LCH (21). In 1985, the Histiocyte Society was established after the first workshop on histiocytosis was organized by D’Angio. Nezelof, a pediatric pathologist at the Necker-Enfants Malades Hospital in France, who contributed tremendously to the

development of the field of Pediatric Pathology, became the first president of this Society. Two years later, the morphological, immunohistochemical, and clinical criteria for LCH were outlined by the Writing Group of the Histiocyte Society (22). This year, the Histiocyte Society celebrated its 25th anniversary. In these 25 years, the number of participants has increased from 15 to over 200. The dedication of all these members to achieve a common goal, which is to improve the lives of patients with histiocytosis, has led to a uniform classification of the histiocytic disorders, standardized diagnostic criteria, and guidelines for patients' evaluation and follow up. Furthermore, the efforts of clinical study groups and basic researchers have led to improved evidence-based therapies for histiocytosis as well as increased knowledge on the pathophysiology of these rare entities. From my own experience, I would like to mention that the Annual Meetings of the Histiocyte Society are very informative and that the ambiance is very good. Attending the Meetings twice has encouraged me even more to contribute to the research in this field.

Clinical presentation of LCH

LCH is the most common form of the histiocytic disorders and affects patients of any age. In the peadiatric age group there are 2 good epidemiologic studies. A British and a Danish study reported incidences of 2.6 per million children in the age of 0-14 years (*23*) and 3-5 per million children in the age of 0-15 years (*24*), respectively. Based on data of the Dutch Childhood Oncology Group (DCOG), approximately 17 children per year are diagnosed with LCH in the Netherlands. In patients older than 18 years of age, this number is comparable. Since the localized form of LCH is often misdiagnosed or even undiagnosed (*25*), the real incidence in both groups is most likely higher.

The clinical presentation of LCH varies widely, and although the features are well described in children, in adults they remain incompletely defined and sometimes misdiagnosed at first (*26*). LCH can manifest as a local lesion at a single site that can heal without any therapeutic intervention, but it can also present as a widespread disease, affecting multiple internal organs which can be life-threathening (*25*). The latter manifestation is termed multisystem LCH. Of children, those younger than 2 years are more often affected by multisystem disease (*27*). These patients present

with symptoms related to the affected organs, which can include lung, liver, spleen, gastro-intestinal tract, central nervous system including diabetes insipidus, and the hematopoietic system. A large retrospective study from the International registry of the Histiocyte Society reported that in adults multisystem disease represented 68.6% of the total cases (*28*). Involvement of bone, skin and lung was most frequently found. Multisystem LCH can be divided in a low-risk type and a high risk type, when the disease burden becomes so large that it leads to severe organ dysfunction (*29*).

The single system form of LCH is restricted to one organ and can present as a single lesion or as multiple lesions (multifocal). Single system LCH occurs mainly in skin, bone, and lymph nodes with seborrhea-like papules, painful swelling, and enlarged lymph nodes respectively as examples of symptoms. The sites of bone involvement in children differ from that in adults. In children the skull is more often involved while in adults the mandible is more often affected (*30*) (*31*) (*32*) (*33*). Another difference between children and adults is the occurrence of female genital LCH, which is exceptional in childhood LCH (*34*). Genital LCH can occur as a single system form as well as part of multisystem disease.

Of interest to note is that Edelbroek et al. reported that of a group of 18 adults that initially presented with skin LCH, 5 patients developed a secondary malignancy without being exposed to chemotherapeutic agents upfront (*35*).

Diagnosing LCH in elderly patients can be a challenge as few cases have been described and the clinical presentation is very variable. Lajolo et al. reported 2 cases of oral LCH in women over 70 years old (*36*).

Pulmonary LCH as a single site disease is mostly associated with smoking and is found in the age-group of 20-40 years (*37*). The single form of isolated pulmonary LCH is exceptional in childhood LCH, as pulmonary disease in this age-group is more often part of multi-system disease (*26*). Single system LCH in children most frequently occurs between the age of 5 and 15 years (*38*). Multifocal restricted single system LCH is mostly described in children between 2 and 5 years of age (*27*).

Diagnosis of LCH

The diagnosis of LCH is based on the presence of classical symptoms, which are related to the site(s) of the lesion(s), combined with the histological and immunohistochemical criteria (*25*). LCH cells in lesions were previously detected by the demonstration of Birbeck granules using electron microscopy. The discovery of Langerin, a type II lectin, and its role in the formation of Birbeck granules (*39*) has made the use of the electron microscopy technique redundant. The current method to detect LCH cells is by immunohistochemical staining for CD1a and/or Langerin expression (*29*), as $CD1a^{+}Langerin^{+}$, $CD1a^{+}Langerin^{-}$ (*40*) as well as $CD1a^{-}Langerin^{+}$ LCH cells (*41*) have been described. The differential diagnosis of LCH includes osteomyelitis, (non-) Hodgkin lymphoma, and immune-mediated dermatitis in case of skin involvement.

Treatment of LCH

The therapeutic options for LCH depend on the extent of disease. For single system skin disease topical application of corticosteroids is usually sufficient. For single system bone disease, biopsy, used as a diagnostic tool, can sometimes initiate the lesion to resolve. Otherwise, corticosteroids can be administered orally (*42, 43*). For multisystem disease, multicenter and international clinical trials are ongoing to compare treatment strategies and to find new treatment options with the goal of reduction of mortality and the prevention of permanent consequences, recurrences, and chronic disease (*44*).

The first therapeutic trials in LCH, AIEOP-CNR-HX 83 and DAL-HX 83/90, were conducted in the 1980's. They consisted of intensive chemotherapy regimens, based on the idea the LCH is a malignant process. In the AIEOP-CNR-HX 83 study, patients were divided in a good prognosis and a poor prognosis group, the latter including those with visceral organ involvement. In the good prognosis group, patients with a single lesion received local treatment. The rest of the patients received either immunotherapy and/or vinblastine. Patients that did not respond to the first treatment sequentially received doxorubicin and etoposide. Patients in the poor prognosis group received 4 week cycles of vincristine, doxorubicin, cyclophosphamide, and prednisone for 9 courses (*45*). The DAL-HX 83/90 stratified

patients in 3 groups; multifocal bone disease, soft tissue involvement without organ dysfunction, and with organ dysfunction. All patients received etoposide, prednisone, and vinblastine for 6 weeks, followed by 1 year of prednisone, vinblastine, and 6-mercaptopurine. Patients with soft tissue involvement without organ dysfunction and with organ dysfunction received etoposide or etoposide and methotrexate additionally, respectively (*46*). The outcome in terms of disease-related permanent consequences for patients with organ involvement was better in the DAL-HX 83/90 study compared to the AIEOP-CNR-HX 83 study.

The LCH-I trial was initiated to study the effects of mono-chemotherapy in patients with multisystem LCH (*47*). Patients received either vinblastine or etoposide for 6 months in combination with an initial single high-dose methylprednisolone. Although the overall mortality rate in this study was comparable to that of the DAL-HX 83/90 study, the combination therapy proved superiority in terms of reactivation rate, reactivation free interval and development of permanent disabilities (*48*).

In the LCH II study, the value of intensification of treatment for patients with multisystem LCH with risk organs involved and patients younger than 2 years of age was investigated. The treatment consisted of continuous prednison in combination with vinblastine (arm A) or vinblastine and etoposide (arm B) for 6 weeks. Continuation therapy included 6-mercaptopurine, prednisone, and vinblastine (*49*). The results showed that involvement of risk organs and a rapid response to initial therapy are important predictors for outcome. Furthermore, comparison to the LCH-I study showed that intensification of treatment was associated with higher rapid response and survival rates. Another important observation of this study is that the age at diagnosis of younger than 2 years is not a significant prognostic factor.

The LCH III study was based on 3 patient groups, namely multisystem risk, multisystem low risk, and single system multifocal bone disease or localized special site involvement. For the multisystem risk group, the effect of the addition of methotrexate to prednisolone and vinblastine during induction (arm A of the LCH-II study) as well as to the continuation therapy of prednisolone, vinblastine, and 6-mercaptopurine was tested. Results showed that addition of methotrexate resulted in an improved survival of patients compared to previous studies. Patients in the low risk group were treated with prednisolone and vinblastine for 6 weeks. Responders were additionally treated with continuation therapy for either 6 or 12 months to evaluate the effect of extended treatment on the prevention of reactivation and

permanent sequelae. Patients in the last group received prednisone and vinblastine for 24 weeks.

LCH-S-98 evaluated the efficacy of 2-6 courses of 2-chlorodeoxyadenosine (2-CdA) monotherapy as salvage therapy for patients with risk LCH not responding to initial therapy and for patients with chronic recurrent low-risk LCH (*50*). Patients with low-risk multisystem or multifocal bone disease had a better response to this treatment compared to those with risk organ involvement. The conclusion of this study was that the effect of 2-CdA therapy depends on the age of the patient and the length of time from diagnosis (*50*). Preliminary results of a combination of 2-CdA and cytarabine as therapy for patients with severe multisystem disease, resistant to conventional therapy, are promising (*51*).

The LCH-CNS study was initiated in 2000. The main goal of this study was to standardize the diagnostic and follow up programs used by clinicians in order to obtain a better understanding of the natural course and the pathological mechanisms of CNS disease as well as a better view on the outcomes of different therapies used. Although a substantial amount of data has been published so far on different aspects of CNS involvement in LCH, the disease process has not been completely elucidated and the most beneficial therapy has still to be found.

Upon specific indications, patients with refractory LCH are treated with bone-marrow transplantation (*52*). In case of liver or lung failure, organ transplantation is needed as well. A couple of agents have been given to low-risk LCH patients which have proven to be successful, at least short-term, such as thalidomide (*53*), IFN-gamma (*54*), TNF-α-blocker (*55*), and cyclosporine-A (*56*). Larger studies will have to be done in order to determine the long-term efficacy of these agents.

Based on the results of the above discussed studies, the LCH-IV study has been designed and will be initiated in the near future. The main goals of this new study will be 1) to investigate whether mortality in patients with MS-LCH with risk organ involvement can be further decreased by an early switch to a defined Salvage protocol (2-CdA/Ara-C or RIC-HSCT), 2) to determine whether reduction of the reactivation rate and permanent sequelae in MS-LCH can be achieved by further prolongation (12 vs. 24 months) and intensification (+/- 6MP) of continuation therapy, 3) to determine whether prolongation (6 vs. 12 months) of continuation therapy will reduce the reactivation rate and permanent sequelae in a subset of SS-LCH (multifocal bone disease, isolated tumorous CNS lesions, or isolated “CNS-Risk”

lesions), 4) to study whether patients with non-risk MS-LCH, multifocal bone disease, isolated tumorous “CNS-Risk” or “CNS-Risk” lesion, who do not respond to first-line therapy or patients who have disease progression or reactivation in non-risk organs, can benefit from second-line therapy with PRED/ARA-C/VCR followed by continuation therapy (24 months of Indomethacin vs. 6MP/MTX) in terms of achieve disease resolution, prevent further reactivations and permanent sequelae, 5) to investigate whether systemic therapy with low dose cytarabine can achieve improvement of the neuro-psychological symptoms in patients with clinically manifest neurodegenerative CNS-LCH.

The heterogeneity of the clinical features in combination with the rarity of the disease often leads to a delay in diagnosing LCH. This especially holds true for adults. Often, they visit a variety of clinics and are subsequently treated by medical specialists other than hemato-oncologists, depending on the symptoms. Treatment protocols for adults with LCH are not readily available. Their treatment is mostly based on experience in children. The International registry of the Histiocyte Society reported that, of a cohort of 274 adults from 13 countries, 29.9% was initially treated with vinblastine with or without steroids (*28*). Of this group 3% additionally received etoposide. Almost 7% was treated with etoposide alone. Interestingly, in approximately 40% of the adult patients a wait and see approach was chosen, even in cases of multifocal LCH.

Pulmonary LCH can go into spontaneous remission when the patient stops smoking. In case needed, corticosteroids can be given (*37*). For bone lesion in adults, Cantu et al. reported in a retrospective study that cytosine arabinoside was effective and minimally toxic compared to a combination of vinblastine and prednisone (*57*). A randomized therapeutic trial could lead to a standard approach for adults with LCH.

Scope of this thesis

As this introduction has pointed out, there are still some critical questions that need to be answered regarding the etiology and pathogenesis of LCH. Also, research on finding targeted therapies for LCH should be taken a step further, as patients are often treated with chemotherapeutic agents that cause serious side effects. With the studies described in this thesis, we aimed to unravel some aspects of DC and LC biology and linked these to LCH pathology. Furthermore, we have initiated the evaluation of a human monoclonal antibody for its potential use as a diagnostic and therapeutic tool in LCH.

To start, **Chapter 3** focuses on the Wnt/beta-catenin signaling pathway in human monocyte-derived DCs and LCH, in the continuous search for the etiology of LCH. LCH cells appear to be arrested in an immature activated stage of differentiation, which could be caused by an endogenous maturation block. Beta-catenin is, as transcriptional co-activator in the Wnt signaling pathway, involved in the maturation of mouse bone marrow-derived DCs (*58*) as well as human mouse DCs from hematopoietic progenitor cells (*59*). Initial experiments showed that the transcriptionally active form of beta-catenin is not present in LCH cells. To give strength to the hypothesis that altered signaling of beta-catenin in LCH cells could play a role their inability to mature, Real-time Qualitative-PCR and inhibition assays with a beta-catenin/TCF complex inhibitor were performed to determine whether beta-catenin is involved in the maturation of human monocyte-derived DCs. The lack of LCH animal models has impeded research on its etiology and pathogenesis. A mouse model of malignant histiocytosis was created in the 1980s. This model is based on infection of mice with Malignant Histiocytosis Sarcoma Virus (MHSV). Initial assessment of infected mice showed transformation and accumulation of precursor cells and macrophages, causing symptoms resembling the human disease involving malignant histiocytes. In the following years after the establishment of the MHSV mouse model, the knowledge on the heterogeneity of macrophages and DCs as well as their overlapping origins, phenotypes and functions increased. Also, improved molecular techniques have indicated that many cases previously diagnosed as malignant histiocytosis were actually Anaplastic Large Cell Lymphoma or DC malignancies. To this end, in **Chapter 4**, this mouse model is re-evaluated. Extensive analysis on the phenotype of the transformed cells and derived

cell lines, as well as their adaptive capacity upon injection in BALB/c mice is described.

In **Chapter 5**, steps towards a rational therapy for LCH, with potentially minimal side effects, are made. A fully human anti-CD1a monoclonal antibody, named CR2113, is evaluated for its functional properties, which include specificity, affinity, and cell-specific cytotoxicity. The murine anti-CD1a monoclonal antibody NA1/34 has served as a starting point for development of antibody-mediated therapy for LCH (*60*). Toxic effects, due to its murine origin made further clinical evaluation, unfortunately, less attractive. Being completely human, CR2113 may, therefore, provide a useful agent in the treatment of LCH.

The debate on the etiology of LCH is ongoing. Previous research, based on the hypothesis that LCH is a neoplastic disease, has shown that LCH cells in single system skin and bone LCH as well as in multisystem disease have an active telomere maintenance system in terms of telomerase activity (*61*). In a small number of samples, telomere length was determined and correlated to telomerase activity. In **Chapter 6**, telomere length analysis in LCH, by dual detection of CD1a expression by immunofluorescence and telomere length by fluorescence in situ hybridization, is extended with 30 patients, suffering from local, multisystem or systemic disease. The results are compared to telomere lengths in LCs found in reactive lymph nodes and in healthy skin.

Chapter 7 provides a discussion on the findings of the above mentioned studies. Furthermore, suggestions for further research are provided.

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