I. LCH: overview of the disease. Langerhans cell histiocytosis (LCH) is a rare human disorder characterized by the accumulation of phenotypically abnormal Langerhans cells (LC) within various organs including the skin, bones, lymph nodes, liver, lungs, bone marrow and central nervous system (CNS). Within affected sites, abnormal LC are believed to multiply and secrete proinflammatory mediators that attract and activate additional leukocytes, including T lymphocytes, eosinophils and macrophages. Interestingly, the behavior of LCH varies between patients, with certain lesions (usually those localized to one organ system) disappearing spontaneously or requiring minimal treatment to induce healing, while others (generally disease involving more than one organ system) require treatment with corticosteroids or chemotherapy to induce regression.

To improve LCH treatment and increase the survival and quality of life for affected patients, it is important to understand why LCH lesions develop. Unfortunately, despite many research efforts, the cause of LCH remains unknown. In fact, one of the most fundamental controversies existing in the field of LCH research centers around whether this disease is the result of an intrinsic defect in the Langerhans cells themselves (i.e., is LCH a “neoplastic” disease?) or the result of external “triggers” that drive otherwise normal Langerhans cells to divide in an abnormal manner (i.e., is LCH a “reactive” disease?). This year, the 13th Nikolas Symposium sponsored by Paul and Elizabeth Kontoyonnis, the parents of a child, Nikolas, who has had LCH, brought together
II. The Nikolas symposium – an overview. The Nikolas Symposium is held annually with the primary mission to find a rational cure for LCH. It is sponsored by Paul and Elizabeth Kontoyannis, whose son Nikolas developed LCH in infancy but has survived his battle with this disease. The symposium is an interactive forum during which biologists and clinicians discuss current topics in dendritic cell (DC) biology, and attempt to apply this information towards an improved understanding of LCH. Although LCH is a rare disease, with an estimated incidence in pediatric patients of between 2-4 patients/million children/year, it is anticipated that the research stimulated by this symposium will not only improve our understanding of LCH, but also our understanding of normal human DC, including the molecular aspects governing differentiation, migration and functional activation.

The 13th Nikolas Symposium, held from May 9th through May 12, 2003 focused on whether LCH might be a neoplastic or a reactive disorder. The presentations comprising this year’s symposium covered a variety of topics, including discussions relevant to normal LC and DC development and migration, as well as discussions speculating on how these processes might be perturbed in LCH. This summary will review the presentations given by symposium participants and place them in the context of more general arguments supporting the suggestion that LCH is, on the one hand, a neoplastic disease, or, on the other hand, a reactive disorder.
III. Defining terms: what is a “neoplastic” versus a “reactive” disease? To understand the issue being addressed, it is important to outline the definition of a neoplasm and how a neoplastic process differs from a reactive process. Typically, a neoplasm results from the clonal proliferation of genetically defective precursor cells, which leads to the accumulation of large populations of identical daughter cells that are capable of surviving independently of growth-sustaining signals normally provided by surrounding cells. As the result of uncontrolled division, neoplastic cells accumulate and form tumors, masses of cells that lack the structural organization characteristic of normal tissues. Tumors may be classified as “benign” or “malignant” depending upon their pathological appearance and clinical behavior.

In contrast to neoplastic diseases in which the neoplastic cells are intrinsically defective, reactive disorders are thought to result when “outside” forces, such as environmental pollutants or infectious factors, stimulate the proliferation of genetically normal cells. Interestingly, reactive disorders may share many features with neoplastic diseases, which can make it difficult to distinguish between the two conditions. For example, certain autoimmune diseases (including systemic lupus erythematosis or autoimmune colitis), or diseases associated with an overly robust immune response to infection (such as fulminant mononucleosis) are characterized by the accumulation of activated white blood cells within certain organs. Occasionally, these white blood cells form lesions that resemble neoplastic tumors; however, unlike neoplasms, the structural integrity of affected organs is usually maintained. Despite these similarities, there are features that separate reactive cells from their neoplastic counterparts. First, the accumulation of cells that results as a reaction to an external factor is generally due to a polyclonal expansion of genetically normal precursor cells. This is in contrast to the clonal cell expansion that is
pathognomonic of a neoplastic tumor. Second, reactive cells are unlike neoplastic cells in that they remain dependent upon growth and survival factors produced by neighboring cells, which stimulate proliferation and support their existence in vivo or in vitro.

IV. Mechanisms regulating normal dendritic cell development and function. Dr. Jonathan Austyn (Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Headington Oxford, UK) provided a comprehensive overview of DC development. Although there are areas of uncertainty, it is now generally accepted that DC comprise different subsets with distinct developmental origins. Moreover, it appears likely that myeloid DC and Langerhans (and related) cells derive from myeloid progenitor(s), whereas plasmacytoid DC originate from the common lymphoid progenitor. Progenitors to the myeloid DC and LC, respectively, seed various tissues (such as the heart and kidney) as well as epithelia (including the skin), where they sample, internalize and process antigens, and are responsive to a variety of maturation stimuli including endogenous (TNFα, IL-1) and exogenous (microbial products) components. In response to these signals, maturing DC migrate via blood and/or lymph to secondary lymphoid tissues where they deliver activation signals for T cells, in part due to their acquisition of costimulatory molecules. Hence, both myeloid DC and LC play a pivotal role in the induction of immune responses to foreign antigens. In contrast, it has been speculated that a progenitor(s) to plasmacytoid DC gains direct access to lymphoid tissues from the blood. Although their function is not fully understood, plasmacytoid DC have been proposed to play regulatory roles in immune responses and/or to contribute to the maintenance of tolerance to self-antigens.
In addition to developing from hematopoietic precursor cells, DC can also originate directly from blood monocytes. For example, monocytes develop into DC when cultured in the presence of the cytokines GM-CSF +/- IL-4. More recently, it has been shown that subcutaneous injection of latex microspheres in mice leads to recruitment of monocytes that phagocytose the particles and develop into DC capable of homing to regional lymph nodes. In an in vitro model, it has also been shown that monocytes can traverse endothelial cell monolayers into a supporting collagen gel, where a large proportion develop into DC that subsequently migrate out of the matrix via reverse transendothelial migration. Monocytes are phenotypically heterogeneous, and additional evidence has been provided that CD16+, but not CD14+ cells, give rise to DC that preferentially undergo this process of reverse transendothelial migration across resting endothelial cells. Dr. Autsyn speculates that CD16+ monocytes may represent a pool of DC precursors that can seed normal tissues, whereas CD14+ cells may migrate preferentially into inflamed sites and develop into DC. It is possible that this dual mode of DC recruitment and maturation allows for the rapid expansion of the DC pool during induction of innate and adaptive immune responses.

Focusing primarily on human cells, Dr. Derek Hart (Mater Medical Research Institute, South Brisbane, Australia) presented work from his laboratory investigating human DC derived from peripheral or umbilical cord blood. Using flow cytometry and a panel of monoclonal antibodies, Dr. Hart’s group identified 5 non-overlapping subsets of CD16+ DC precursors within human peripheral blood. When used as stimulators in allogeneic mixed leukocyte reactions (MLR), these subsets functioned heterogeneously, suggesting that they can differentially generate T cell responses. Dr. Hart’s laboratory has also characterized how peripheral blood DC numbers vary with age, and has shown that distinct DC populations differentially mobilize into the peripheral
blood following various stimuli, such as treatment with G-CSF, surgery or allogeneic bone marrow transplantation.

Dr. Hart’s laboratory has identified and characterized many surface molecules on resting or activated DC, which may provide new targets by which the immune response may be either augmented or suppressed. Among the molecules that he discussed was CRMF-44, a DC-associated differentiation antigen whose expression is upregulated on activated cells. To investigate the therapeutic utility of a monoclonal antibody directed against CRMF-44, activated human DC were treated in vitro with anti-CRMF-44 antibody and complement, which resulted in significant depletion of myeloid DC. Depletion of DC via this treatment reduced the ability of peripheral blood cells to stimulate allogeneic T lymphocyte responses in MLR, as well as T-cell proliferative responses to specific antigens. In related experiments, depletion of DC in a mouse model of graft-versus-host disease was found to minimize the morbidity and mortality associated with this condition. Taken together, these studies suggest that the anti-CRMF-44 antibody may work as an immunosuppressive mediation by selectively targeting activated DC. Because CMRF-44 is also expressed at low levels on LC, it is possible that similar treatment with CMRF44-specific antibody may be of benefit to LCH patients. In a similar manner, antibodies against some of the other DC-associated surface markers under investigation in Dr. Hart’s laboratory may provide targets for novel LCH therapies.

To understand how DC instruct and regulate immune responses, Dr. Paula Ricciardi-Castagnoli (University of Milano-Bicocca, Department of Biotechnology and Biosciences, Milano, Italy) has performed a series of elegant gene expression profiling experiments. Using an in vitro system in
which mouse bone marrow-derived DC are exposed to various proinflammatory (lipopolysaccharide/LPS, schistosome eggs, leishmania) or anti-inflammatory stimuli (shistosomula, dexamethasone), Dr. Ricciardi-Castagnoli’s laboratory prepares fluorescently labeled cDNA probes that are subsequently hybridized with commercially available gene microarrays. Patterns of gene expression are analyzed using computational methods. Among several very interesting findings, one of Dr. Ricciardi-Castagnoli’s most striking observations is the identification that IL-2 mRNA is transiently upregulated at very early time points following the treatment of DC with activating stimuli. The production of IL-2 by activated DC stimulates Natural Killer (NK) cell cytokine production and allogeneic T cell responses in MLR. These data suggest that by producing IL-2, DC play an integral role in priming both innate (NK, and possibly NKT cell-dependent) and adaptive (T, B lymphocyte-dependent) immune responses.

V. LCH – what we are learning from research on LCH and related disorders. Dr Ronald Jaffe (Department of Pathology, Children’s Hospital, Pittsburgh PA) presented an overview of LCH pathology. LCH lesional cells, like normal LC, express CD1a, S100, Langerin, Class II and CD68(k) molecules and they contain Birbeck granules. Unlike their normal counterparts, however, lesional cells are morphologically abnormal in that they are oval in shape and not dendritiform. Moreover, lesional LCH cells appear to have their MHC class II molecules stored in vesicles and not on the cell surface, suggesting perturbations in the molecular transport of these molecules. Pathology studies of LCH have led to major improvements in the classification of lesions; however, many questions remain unanswered. For example, are LCH cells “immature” and arrested in development, as their abnormal morphology and surface marker expression suggest? Furthermore, what leads to the accumulation of LCH cells in affected
tissues? Do these cells accumulate in tissues because they are recruited to inflamed sites, are they inhibited from leaving, or both?

One of the most notable features of LCH is the propensity for lesional cells to accumulate not only in the skin, but also in other sites where one would not normally find LC. To better understand the mechanisms underlying the abnormal migration of LCH cells, Dr Nicola Annels (Leiden University Medical Center, Leiden, The Netherlands), presented elegant work investigating the expression of chemokines and their cognate receptors within LCH tumors. Using immunohistochemical, immunofluorescent and confocal analyses, Dr. Annels has shown that lesional LCH cells express the immature DC marker CCR6 and lack expression of the mature DC marker CCR7, supporting the observation that they might be arrested in development. Remarkably, LCH lesional cells also express CCL20/MIP-3alpha, the ligand for CCR6, indicating that by expressing both the receptor and its cognate ligand, LCH lesional cells may stimulate their own retention within affected tissues. LCH cells express other inflammatory chemokines, including CCL5/RANTES and CXCL11/I-TAC, which might contribute to the recruitment of eosinophils and T cells, respectively.

Along with CD1a⁺ lesional cells, other cell types are found in LCH lesions, including multinucleated giant cells (MGC). It is thought that MGC are generated locally at sites of tissue injury by the fusion of highly stimulated macrophages. However, MGC resemble osteoclasts, cells involved in bone remodeling via matrix degradation. Thus, in an LCH bone lesion, the most likely identity of an MGC is an osteoclast. Dr. Annels has begun to investigate this possibility by examining the expression of osteoclast-specific markers on the MGCs within LCH lesions. She
has found that LCH-associated MGC express the osteoclast-specific enzymes, TRAP (tartrate-resistant acid phosphatase) and Cathepsin K, a cysteine protease with high matrix degrading potential. These MGC also expressed CD68, providing further evidence that MGC are derived from the macrophage lineage. Surprisingly, MGC found within LCH lesions obtained from sites other than bone (such as skin and lymph node) express TRAP and Cathepsin K. Moreover, they express CD68 and CD1a, which raises the interesting question as to whether lesional LC can directly give rise to MGCs and subsequently become osteoclast-like cells.

Dr. Paul Seldon (Dermatology Section, Faculty of Medicine, Imperial College, London England), this year’s recipient of the Artemis Fellowship, a travel award sponsored by the Nikolas Symposium, has used a method known as suppression subtractive hybridization (SSH) with which to generate an LCH-specific cDNA library from laser microdissected CD1a+ lesional cells. SSH is particularly suited for LCH research because this method requires minimal quantities of starting material for the generation and evaluation of expressed transcripts. Using this technique, Dr. Seldon has identified several differentially expressed genes, which can be broadly divided into migration-associated factors, receptors, drug resistance factors and intracellular signaling proteins. The identification and characterization of novel LCH-specific genes may explain the development of lesions and identify possible targets for therapeutic intervention.

One of the most poorly understood areas within the field of LCH involves the etiology of lesions involving the CNS. To better understand the mechanisms contributing to CNS LCH, Dr. Hans Lassman (Division of Neuroimmunology, Brain Research Institute, University of Vienna,
Kim E. Nichols, MD  
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Austria) has analyzed brain biopsies and autopsy samples from patients with CNS involvement. Consistent with previous reports, he finds that there are two types of CNS LCH lesions. First, there are “classical” LCH granulomas, which are primarily located in the circumventricular organs and the meninges. Although there is heterogeneity among specimens, LCH granulomas have relatively small proportions of CD1a+ lesional cells that are admixed with larger numbers of T and B lymphocytes and macrophages. Second, there exist what are known as “neurodegenerative” lesions, which predominately involve the cerebellum and brain stem. These lesions occur in the setting of a profound inflammatory reaction, which is dominated by Class I MHC-restricted cytotoxic T cells. In some specimens, there is additional deposition of immunoglobulin and activated complement. Dr. Lassman suggests that the pathology of neurodegenerative LCH lesions closely reflects that seen in paraneoplastic encephalitis, namely the presence of CD8+ T cells that mediate disease, the activation of microglial cells and the absence of monocyte recruitment. To gain further insight into neurodegenerative lesions, Dr. Lassman has screened for the expression of DC-associated antigens in LC present within the skin or in granulomas, and their expression in normal and diseased CNS tissue. He finds that many DC antigens appear both on normal LC and on microglia-like cells within inflamed brain LCH lesions. Based on these observations, is conceivable that specific DC-associated antigens serve as targets against which an autoimmune response develops in patients with CNS LCH.

Well-characterized animal models for LCH have not been described. However, the availability of an animal model would facilitate investigations concerning the pathogenesis and therapy of LCH. Based on the hypothesis that LCH cells are intrinsically disturbed in cell proliferation and/or survival (see below, Dr. Pancras Hogendoorn), Dr. Pieter Leenen (Department of Immunology,
Erasmus Medical College, Rotterdam, The Netherlands) has established a model in which recipient mice are injected with different murine DC cell lines and are observed for the development of LCH-like lesions. His most promising results were obtained using DC cell lines derived from mice infected with the transforming Malignant Histiocytosis Sarcoma Virus (MHSV). In vitro characterization of these MHSV-transformed cell lines revealed remarkable differences in their phenotype, reflecting an arrest at different stages of maturation. Some of these lines could be assigned to the LC lineage, as indicated by their expression of Langerin, an LC-specific surface lectin. Upon transfer of the MHSV-transformed cell lines into recipient mice, different outgrowth profiles were obtained in various organs, suggesting differential homing capacities. In addition to potential differences in homing, transplanted DC demonstrated organ-specific phenotypic adaptations, such as the induction of MHC class II or CD8 expression by certain DC lines in the spleen, but not in other organs. Like human LCH, the lesions generated by the transplanted DC contained not only tumor cells, but also T cells, macrophages and mature DC. In contrast, eosinophils were not found in the murine lesions.

Dr. Leenen’s studies show that the transplantation of mouse LC cell lines into recipient animals models some of the important features of human LCH. These features include the differential homing capacities of MHSV-transformed cells, variations in their cellular phenotype depending on the specific tissue environment, and their capacity to attract different inflammatory cell types, in particular T cells and macrophages, to the lesions initiated by proliferating LC. Although the mouse cell lines used in these experiments represent transformed, and therefore malignant DC, which are potentially different from human LCH cells, these cell lines provide valuable tools with which to study fundamental aspects of LCH in vivo.
VI. The fundamental question: is LCH a neoplastic disease? Over the years, various investigators have suspected that LCH may be a neoplastic disease, but interest in this possibility heightened when, in 1994, it was demonstrated by two separate groups that the Langerhans cells comprising LCH lesions were clonal in nature. Using molecular methods, both groups showed that CD1a+ LCH cells demonstrated a clonal pattern of X-chromosome inactivation, indicating that they might be daughter cells derived from the same original precursor cell.

More recent investigations, summarized nicely at this year’s symposium by Dr. Pancras Hogendoorn (Department of Pathology, Leiden University Medical Center, the Netherlands) and Dr. Jean Gogusev (Institut National de la Sante et de la Recherche Medicale, Paris, France), have provided additional support for the possibility that LCH might be a disorder of neoplastic origin. First, Dr. Hogendoorn’s laboratory has examined LCH lesions for their expression of proteins that regulate cell division and survival. In all specimens examined, lesional LC express Ki-67, a marker of cellular proliferation, indicating that they are continuously dividing. In addition, most samples concurrently express gene products that inhibit cell division, such as the tumor suppressor proteins p53 and pRB, the cell cycle inhibitors p21 and p16, and the growth suppressive receptors TGF-B types I and II. Dr. Hogendoorn hypothesizes that despite the expression of these inhibitory gene products, signals favoring cell division must predominate in LCH tumors. This may be due to abnormal environmental factors such as upregulated secretion of growth and/or survival factors, or to genetic alterations within lesional cells that allow them to escape from normal growth inhibitory pathways. Further supporting the outgrowth of lesional
cells is their increased expression of the BCL-2 protein, which would favor cell survival over programmed cell death.

To identify genomic alterations contributing to LCH, Dr. Gogusev’s group has performed Comparative Genomic Hybridization (CGH) and Loss of Heterozygosity (LOH) analyses on a series of LCH specimens and on a novel cell line (DOR-1), established from an LCH granuloma. Recurrent genetic abnormalities were found in all primary lesions and in the DOR-1 cell line, including loss of genetic material from regions of chromosomes 1p, 5, 6, 7, 9, 16, 17 and 22q, and gain of genetic material on chromosomes 2q, 4q and 12. Dr. Gogusev’s findings suggest that genomic instability and recurrent chromosomal abnormalities might affect the expression of oncogenes and/or tumor suppressor genes, which could contribute to the development and progression of LCH lesions.

Work presented by Dr. Maurizio Arico (Director, Onco-Ematologia, Ospedale dei Bambini “G. Di Cristina”, Palermo Italy) demonstrates that there are also rare families in which more than one member is affected by LCH, a finding that suggests a possible genetic or “hereditary” cause in these cases. When taken in combination with other factors, such as the clinically aggressive behavior of certain forms of LCH, the absence of a defined external factor driving the proliferation of LC in most childhood forms of LCH, and pathology studies demonstrating that lesional cells are arrested in differentiation, the combined genetic data from these and other investigators provide strong support for the argument that LCH might be a neoplastic disease.
VII. The fundamental question: is LCH a reactive disorder? As pointed out by Dr. Anthony Chu (Consultant Dermatologist, Imperial College School of Medicine, London), certain data also exist to support the notion that LCH might be, at least in a subset of cases, a reactive disease. These data are strongest for adolescent or adult patients with isolated pulmonary histiocytosis (i.e. absence of involvement of other organs). In this group of patients there is a direct relationship between disease activity and cigarette smoking. For example, exposure to cigarette smoke initiates disease, while cessation of smoking generally results in disease regression. Recent genetic studies have also shown that LC obtained from patients with isolated pulmonary histiocytosis are not genetically clonal. Currently, it is not known which chemical factors in cigarette smoke initiate pulmonary histiocytosis or why certain people are susceptible to developing this form of the disease.

In regards to the other forms of childhood or adult LCH (for example, bone lesions or LCH lesions involving multiple organs), there exist several unrelated observations to suggest they might be the result of a reactive expansion of Langerhans cells, rather than a neoplastic, cancer-like process. First, as noted by Dr. Ron Jaffe (see above), there exist other human non-malignant diseases such as the chronic dermatides (including dermatopathic lymphadenitis, DL), in which phenotypically immature LC accumulate in the lymph nodes that drain chronically inflamed skin. In patients with DL, it is believed that local perturbations in cytokine and/or chemokine levels interfere with normal DC maturation and migration. Therefore, phenotypically immature, but functionally active DC, may persist in involved lymph nodes and amplify an ongoing inflammatory process. Second, LCH is known to undergo spontaneous remissions in a subset of cases. While this property is not generally characteristic of neoplastic disorders, examples of
spontaneous cancer remissions do exist. Third, it has been incidentally noticed that LCH can “flare” or re-occur when a patient develops a cold or other infectious process, suggesting that LCH cells may be reactivated by the same external stimuli that activate normal immune cells. Conversely, milder forms of LCH may respond favorably to treatment with antibiotics, indicating that lesional cells are capable of “switching off” their activity in a manner similar to normal white bloods cell that are no longer needed. Fourth, it is has been extremely difficult to grow LCH lesional cells in the laboratory, suggesting that these cells require essential growth and survival factors that are only present within the body, but not outside of it. In contrast, it is usually, but not always, possible to grow neoplastic cells for long periods of time in the laboratory. Fifth, LCH lesional cells have never been shown to harbor mutations in genes that regulate cell division or survival. This property is quite different from neoplastic cells, which almost always have mutations in one or more of these categories of genes. It should be noted, however, that detection of mutations can be difficult, and it is possible that this area has not yet been adequately investigated.

**VIII. Summary and conclusions: does it really matter whether one considers LCH a neoplastic or reactive disease?** In an effort to address one of the most fundamental questions regarding LCH, this year’s Nikolas Symposium provided indirect evidence to support the possible classification of LCH as both a reactive and a neoplastic disorder. Is such a dual classification for LCH possible? As pointed out by Dr. Robert Arceci (Pediatric Oncology, Johns Hopkins Oncology Center, Baltimore, USA) during his introduction to the symposium and by Dr. Jon Pritchard (Department of Haematology/Oncology, Royal Hospital for Sick Children, Edinburgh, UK), who led the patient-parent session, LCH is an extremely heterogeneous disease that is
characterized by a spectrum of clinical and pathological features. It may not be possible to generate one unifying model that sufficiently explains such a variable disease. Rather, the microscopic and clinical diagnosis of LCH may represent a continuum of disorders, with some patients developing lesions that are appear reactive in nature, while others develop lesions that are more neoplastic. A similar model was proposed by Dr. Dorothy Crawford (School of Biomedical and Clinical Laboratory Sciences, University of Edinburgh, UK) who used post-transplantation lymphoproliferative disease (PTLD) as an example of a human disease that, like LCH, has features of both immune dysregulation and cancer. PTLD is a disease that occurs in patients with suppressed immune systems and is associated with an abnormal expansion of white blood cells following infection with Epstein-Barr virus (EBV), a virus which also causes infectious mononucleosis (“Glandular Fever”). As in LCH, some patients with PTLD have a milder form of the disease that appears to be due to the increased proliferation of otherwise normal white blood cells. In contrast, other patients develop more aggressive disease, in which the white blood cells have acquired genetic abnormalities and neoplastic features.

Does it really matter whether one classifies LCH as a neoplastic or a reactive disease? In reality, the lack of an answer does not significantly change how we treat LCH patients at this time, given the limited range of currently effective therapies. Ultimately, however, the answer to this question might provide additional insight into LCH, which could facilitate the development of newer and more effective treatments for this disease. It is the hope of Paul and Elizabeth Kontoyonnis, along with all who participate in the Nikolas Symposium, that the use of these therapies will cure future LCH patients and prevent the long-term side effects that are associated with this disease. We also hope that solving the puzzle of LCH will
lead to treatments for patients who suffer from other diseases caused by abnormal LC migration and function, and possibly for patients with cancer. In regards to the latter possibility, a growing area of research involves the development of vaccines designed to boost the normal immune response against common and rarer forms of cancer, such as lymphoma and melanoma. Given their important role during the initiation of immune responses, DC and LC are being intensively investigated in laboratories throughout the world. Thus, progress in LCH research might facilitate the development of anti-tumor vaccines, which could more effectively treat cancer.