Neurodegeneration in Langerhans cell histiocytosis

Summary of the 30th Nikolas Symposium, Athens, May 12-15, 2022

The Nikolas Symposium

The Nikolas Symposium is an annual meeting hosted by Paul and Elizabeth Kontoyannis, whose son Nikolas developed Langerhans Cell Histiocytosis (LCH) in infancy. Their motivation to initiate the Nikolas Symposium in 1989 was the lack of knowledge on the pathogenesis of this rare disease. The purpose of the meeting is to find a rational cure for LCH through fostering new collaborations between researchers in and outside the LCH field[1]. Accordingly, the symposium is an interactive forum of basic scientists and clinicians who discuss a different topic related to clinical presentation of LCH, the spectrum of its complications (late effects) and the pathological cells involved. The meeting also provides an opportunity for Greek physicians to present difficult cases and to discuss results of their studies with experts in the field. A summary of the 30th meeting is presented here.

Introduction to the 30thsymposium

After a two year delay due to the Covid pandemic, the third anniversary symposium brought together experts in the field of central nervous system (CNS) pathology in relation to two mayor topics that were discussed: myeloid cell involvement and therapeutic targeting of pathologic cells in the brain. Two junior scientists active in the histiocytosis field (Dr. Mathias Wilk and Dr. Egle Kvedaraite) received a Pritchard Fellowship, which provides an opportunity to awardees to present their research at the meeting. Dr. Pieter Leenen, a former Steering Committee member, was honored for his dedicated contribution to the Nikolas Symposium.

Presentation and treatment of CNS histiocytosis

Traditionally, the scientific program was opened by Steering Committee members presenting the concept and history of the meeting (Dr. Maarten Egeler), clinical and pathophysiological features of LCH (Dr. Carl Allen) and the summary of the 29th Nikolas Symposium (Dr. Astrid van Halteren). A fourth member of the Steering Committee, Dr. Eli Diamond, showed classic examples of granulomatous/tumorous LCH lesions, which manifest rather frequently in the brain, spinal cord and at non-parenchymal sites (including base of the skull and dura). These lesions are radiographically, clinically and biologically different from neurodegenerative (ND)-LCH, which is very rare and typically manifests 10-20 years after a LCH diagnosis has been made. Cerebellar atrophy[2] is a typical manifestation of ND-LCH leading to issues with balance, walking and speech. While patients diagnosed with localized or disseminated LCH may present with abnormal CNS imaging results but without clinical symptoms of ND-LCH, only 25% of these patients will develop clinical symptoms associated with CNS dysfunction[3,4.] Data extracted from the French national registry shows that the cumulative incidence of ND-LCH in children ranges from 2-8%[5]. Intriguingly, patients with ND-LCH more often presented with LCH lesion(s) in the pituitary gland, skin, skull base or orbit. The presence of BRAFp.V600E mutation in LCH biopsies derived from these children was associated with the highest prevalence of ND-LCH. Dr.Marita Parthanen summarized the outcomes of a small number of cognitive and behavioral studies performed in children and adolescents with a history of LCH. Particularly patients with CNS presentation seem at risk of diminished verbal and other performance scores[6]. Both Dr. Diamond and Dr. Partanen highlighted that correctly powered

studies addressing neuro-psychological issues in LCH survivors are needed to obtain a comprehensive picture of the impact of LCH on CNS functions

According to a recently published flowchart[2], several treatment options are available for adult patients with clinical symptoms indicative of ND-LCH, including pharmacological inhibitors targeting distinct components of the hyperactive MAP kinase pathway. Intra-arterial melphalan may be a rational alternative for histiocytosis patients with neurological symptoms in whom such inhibitors are contra-indicated[7]. Effectiveness of inhibitors in pediatric ND-LCH cases is currently being explored.

Origin of macrophages in the CNS

Acute inflammation is associated with a temporary increase in cytokines and growth factors, which may affect hematopoietic output of myeloid cells as well as neuro-endocrine function[8]. Dr. Pieter Leenen discussed various clinical conditions wherein inflammation leads to altered levels of cortisol and glucocorticoids. This affects CD163 expression and tissue entry by blood-derived monocytes as well as their maturation into tissue resident macrophages (RTM). Whether this concept also applies to CNS-associated macrophages is unknown. RTM in the brain, including microglia cells, are thought to be seeded by waves of cells produced during embryonic hematopoiesis occurring in respectively the yolk sac and fetal liver. These TRM are long-lived and have self-renewal potential, which would render their replenishment independent of blood-derived monocytes. Dr. Florent Ginhoux and his team identified Ms4a3 as a specific gene expressed by murine granulocyte-monocyte progenitors (GMP) in the bone marrow of adult mice[9]. Based on this information, they designed mouse models wherein only the progeny of GMP (blood monocytes and granulocytes) express a red dye. Using this fate-mapping model, they studied the composition of TRM isolated from various tissues including the brain. Over time, and in the absence of inflammation, epidermal Langerhans cells isolated from the skin and various types of macrophages present in the liver and brain (microglia cells, meningeal macrophages and perivascular macrophages) contained no monocyte-derived cells. Choroid plexus macrophages seem the only CNS-associated macrophage subtype that is replenished, in part, by GMP-derived monocytes[9]. These monocytes can be derived either from the blood or from bone marrow niches adjacent to the brain and spinal cord, which serve as a reservoir of monocytes under conditions of neuro inflammation[10]. The absence of the blood brain barrier in the choroid plexus and pituitary gland respectively probably facilitates entry of mutation-carrying monocytes under inflammatory conditions.

Myeloid cells in blood and brain specimen of LCH patients

Although limited autopsy material of LCH patients had thus far been investigated[11,12], the current hypothesis is that ND-LCH is caused by myeloid cells expressing similar MAP kinase pathway-activating somatic mutations as found in LCH lesions manifesting in other tissues. Dr. Egle Kvedaraite presented data on LCH precursor cells identified in the blood of patients with active disease[13]. Combining single cell index data (FACS protein) and single cell RNA seq (smartseq) technology, she discussed core genes and signaling pathways which have been identified. Her data suggest that NOTCH dependent cross-talk between DC2 and DC3/monocyte lineages promotes a pathognomonic LCH program, which is in line with earlier observations by Hutter et al[14,15]. Interestingly, DC3 express CD1c, CD14 and CD163 which may link these cells to the VE-1^{+} (identifies BRAFp.V600E protein) CD33^{+} CD14^{+} CD163^{+} monocytic cells found around blood vessels in the white matter of autopsied brain tissue as discussed by Dr. Jennifer Picarsic[12]. Based on the lack of P2RY12 expression by these monocytic cells (a marker identifying tissue resident microglia cells) combined with MCP-1

expression by blood vessels (chemokine attracting monocytes from the blood into the tissue), the authors concluded that *BRAF* mutated cells in the CNS most likely originate from a progenitor cell present in the bone marrow of this patient. This assumption is strengthened by the presence of *BRAF* mutated cells in the blood of patients who developed ND-LCH later on. The common presence of blood DC3 cells expressing BRAFp.V600E, a mutation found also in non-Langerhans cell histiocytosis or mixed histiocytosis, fits with the concept that histiocytic neoplasms involve an overlapping spectrum of myeloid cells all derived from a progenitor active during definitive hematopoiesis as proposed by Dr. Picarsic.

Mutation-carrying myeloid cells in murine disease models

An alternative hypothesis for the presence of *BRAF* mutated cells in the brain has been put forward by Dr. Frederic Geissman et al. His team published data showing that *BRAF* mutated cells in the brain originate from a yolk sac progenitor cell, which seeds offspring cells, including microglia cells, during a certain stage of fetal development[16]. Mice generated by this research group spontaneously develop a severe neurodegenerative disorder over time, characterized by an accumulation of ERK-activated microglia cells, abnormal behavior, cerebellar ataxia, astrogliosis and neuronal death. Dr. Matthias Wilk discussed his research into the presence of BRAFp.V600E expressing cells in the brain of mice wherein the mutant BRAF protein is artificially overexpressed either under the CD11c[17] or Scl[18] promotor. In both settings, expression of the *BRAF* mutation is strictly confined to cells of the dendritic cell-macrophage lineage leading to disseminated LCH-like disease. He discovered that the pons, cerebellum, medulla and cerebral cortex of these mice often contain *BRAF* mutated cells. These mice also developed aberrant behavior and muscle weakness over time, a situation comparable to ND-LCH where *BRAF* mutated cells are most frequently found in the brain stem, pons and cerebellum.

Therapeutic approaches to restore microglia and neuronal function

Neurodegenerative diseases involving dysfunctional microglia cells (Alzheimer's disease, AD) and neuronal loss are currently untreatable. Dr. Kate Monroe presented data demonstrating the therapeutic potential of a TREM2-targeting monoclonal antibody[19]. TREM2 is expressed by homeostatic microglia cells in the CNS and plays a key role in their function. Her research team generated a monoclonal antibody that increases membrane expression of TREM2 through its binding to the cleavage site at the stalk of the molecule, thereby preventing its shedding from the membrane. In vitro exposure of microglia cells to this agonistic 4D9 antibody also induced activation of intracellular Syk signaling and increased the number of cells with Aβ42 (major component of amyloid plaques in AD brains) uptake. In vivo exposure to 4D9 antibody resulted in increased TREM2 expression by microglial cells without affecting the total number of microglia cells. These data suggest that 4D9 antibody treatment promotes a shift from homeostatic microglial cells to plaque-reducing cells, as evidenced by a reduced amyloid plaque load throughout the cortex of mice prone to develop AD-like disease. Introduction of a protein sequence in the Fc part of the 4D9 antibody that targets binding to transferrin receptors which are expressed at the blood-brain barrier, significantly increased CNS penetrance upon intravenous injection of the engineered 4D9 antibody. Dr. Mathew Blurton-Jones shared a different approach to increase the number of functional microglial cells in the brain. His team generates viable and functional microglia cells from induced pluripotent stem cells (iPSC) generated skin or blood samples derived from AD patients. These so called iMGL express P2RY12, PU.1 (myeloid-associated transcription factor) and TREM2 and their transcriptomic profile significantly overlaps with the transcriptome of human adult- or fetus-derived microglia

cells, but not of other myeloid cell types including CD14+ CD16$^-$ monocytes[19]. Interestingly, iMGL engraft in MITRG mice expressing hCSF1, hCSF2 and hTPO) and also repopulate the brains of FIRE-hCSF1 mice that naturally lack microglia cells and Langerhans cells. Finally, Dr. Don Cleveland presented his extensive research on antisense oligonucleotide (ASO) therapy. Both antisense oligonucleotides and short hairpin interfering RNA molecules bind to newly synthesized mRNA molecules and thereby prevent their translation into proteins. Successful ASO delivery to neurons and glia cells has been shown in rodent and nonhuman primate disease models. Moreover, intrathecal ASO therapy is currently being explored in several clinical trials performed in patients with distinct forms of amyotrophic lateral sclerosis (ALS). Polypyrimidine tract binding protein 1 (PTB) is a key factor for neurogenesis. Chronic reduction of PTB expressed by different murine brain cells including astrocytes, glial cells and oligodendrocytes promotes their conversion into neurons. In line with this approach, Dr. Cleveland discussed how a single intra-cerebroventricular injection of a selective AOS transiently suppresses the levels of PTB mRNA and of its neuronal analogue nPTB resulting in the generation of mature and functionally active neurons in the cortex and dentate gyrus of aged mice[21]. Using a new spatial transcriptomic approach (MERFISH), current studies now focus on the identification of other genes involved in the generation of new neurons.

Concluding remarks

Neurodegeneration is a rare, but physically devastating, late effect of LCH potentially driven by neuro-inflammation induced by somatic mutation-expressing myeloid cells of potentially different origin (monocytes versus homeostatic microglia cells). Treatment of ND-LCH is notoriously difficult given that conventional therapies generally yield limited results[23]. Oral administration of a BRAF inhibitor (PLX4720) delayed the onset and mitigated neurological symptoms in the murine model wherein the BRAF mutation is confined to yolk sac erythro-myeloid progenitor cells[16]. A similar approach induced clinical and radiological improvement in several patients with ND-LCH[12]. It is conceivable that BRAF and MEK inhibitors will gain popularity as first line therapy for patients presenting with BRAFp.V600E$^+$ LCH lesions that are associated with increased risk of ND-LCH[5]. Novel therapeutic strategies, which are currently being explored in other disorders confined to the CNS, show that functional reversal of malfunctioning microglia cells and even replenishment of lost neurons may be feasible. This may provide new options for patients with progressive ND-LCH refractory to currently available (targeted) therapies.

Dr. Astrid van Halteren,
Department of Internal Medicine / Clinical Immunology
NFU Expertise Center for Histiocytic Disorders
Erasmus Medical Center, Rotterdam, The Netherlands

References

1. Allen C et al. The coming of age of Langerhans cell histiocytosis. Nat Immunol 2020;21:17 (PMID 31831887);
2. Cohen Aubart F et al. Histiocytosis and the nervous system: from diagnosis to targeted therapies. Neuro Oncol 2021;23:1433-1446 (PMID 33993305);
3. Grois N et al. Course and clinical impact of magnetic resonance imaging findings in diabetes insipidus associated with Langerhans cell histiocytosis. Pediatr Blood Cancer 2004:43:59-65 (PMID 15170891);

4. Wnorowski M et al. Pattern and course of neurodegeneration in Langerhans cell histiocytosis. J Pediatr 2008;153:127-132 (PMID 18571550);
5. Héritier S et al. Incidence and risk factors for clinical neurodegenerative Langerhans cell histiocytosis: a longitudinal cohort study. Br J Hematol 2018;183:608-617 (PMID 30421536);
6. Nanduri VR et al. Cognitive outcome of long-term survivors of multisystem Langerhans cell histiocytosis: a single-institution, cross-sectional study. J Clin Oncol 2003;21:2961-2967 (PMID 12885816);
7. Francis JH et al. Intra-arterial Melphalan for neurological non-Langerhans cell histiocytosis. Neurology 2021;96:1091-1093 (PMID 33980709);
8. Esquifino AI et al. Neuroendocrine-immune correlates of circadian physiology: studies in experimental models of arthritis, ethanol feeding, aging, social isolation and calorie restriction. Endocrine 2007;32:1-19 (PMID 17992597);
9. Liu Z et al. Fate Mapping via Ms4a3-expression history traces monocyte-derived cells. Cell 2019;178:1509-1525 (PMID 31491389);
10. Cugarra A et al. Skull and vertebral bone marrow are myeloid cell reservoirs for the meninges and CNS parenchyma. Science 2021;373:eabf7844 (PMID 34083447);
11. Grois N et al. Neuropathology of CNS disease in Langerhans cell histiocytosis. Brain 2005:128:829-838 (PMID 15705614);
12. McClain KL et al. CNS Langerhans cell histiocytosis: common hematopoietic origin for LCH-associated neurodegeneration and mass lesions. Cancer 2018:124:2607-2620 (PMID 29624648);
13. Kvedaraite E et al. Human dendritic cells in cancer. Sci Immunol 2022;70:eabm9409 (PMID 35363544);
14. Hutter C. et al. Notch is active in Langerhans cell histiocytosis and confers pathognomonic features on dendritic cells. Blood 2012;120:5199-5208 (PMID 23074278);
15. Schwentner R et al. JAG2 signaling induces differentiation of CD14+ monocytes into Langerhans cell histiocytosis-like cells. J Leukoc Biol 2019;105:101-111 (PMID 30296338);
16. Mass E et al. A somatic mutation in erythro-myeloid progenitors causes neurodegenerative disease. Nature 2017;549:389-393 (PMID 28854169);
17. Berres ML et al. BRAF-V600E expression in precursor versus differentiated dendritic cells defines clinically distinct LCH risk groups. J Exp Med 2014:211:669-683 (PMID 24638167);
18. Bigenwald C et al. BRAFV600E-induced senescence drives Langerhans cell histiocytosis pathophysiology. Nat Med 2021;27:851-861 (PMID 33958797);
19. Schlepckow K et al. Enhancing protective microglial activities with a dual function TREM2 antibody to the stalk region. EMBO Mol Med 2020;12:e11227 (PMID 32154671);
20. Abud EM et al. iPSC-derived human microglia-like cells to study neurological diseases. Neuron 2017;94:278-293 (PMID 28426964);
21. Qian H et al. Reversing a model of Parkinson's disease with in situ converted nigral neurons. Nature 2020;582:550-556 (PMID 32581380);
22. Maimon R et al. Therapeutically viable generation of neurons with antisense oligonucleotide suppression of PTB. Nat Neurosci 2021;24:1089-1099 (PMID 34083786);
23. Allen CE et al. Neurodegenerative central nervous system Langerhans cell histiocytosis and coincident hydrocephalus treated with vincristine/cytosine arabinoside. Pediatr Blood Cancer 2010;53:416-423 (PMID 19908293);