Langerhans Cell Histiocytosis: Myeloid Cell Programming and Differentiation

Summary of the 28th Nikolas Symposium, Athens, May 10-13, 2018

The 28th Nikolas Symposium organized discussion around the origins of pathogenic cells and mechanisms of myeloid differentiation. A pivotal role for MAPK pathway in LCH is now clear following the seminal discovery of recurrent *BRAF*V600E mutations in LCH lesions and subsequent identification of activating MAPK pathway somatic mutations in almost all cases.[1] *BRAF*V600E has been discovered in hematopoietic progenitor cells, and extent of disease appears to correlate with differentiation state of the original LCH cell.[2] Mechanistic understanding of the role of MAPK hyperactivation in differentiating myeloid cells remains uncertain. The long-standing mission of the Nikolas Symposium is to "search for a rationale cure" for children and adults with LCH. This symposium sought to understand relative contributions of mutation and cell of origin in pathogenesis of LCH in order to identify novel therapeutic opportunities.

Introduction.

The meeting was opened by **Dr. Maarten Egeler** (Toronto), who introduced the history of the Symposium and tasked the participants to commit to present provocative data, share ideas and argue. **Dr. Carl Allen** (Houston) presented the historical evolution of concepts of LCH, clinical overview, and discussed a biological framework form LCH in which the stage of differentiation of a white blood cell that acquires the MAPK mutation may impact extent of disease and response to therapy. According to the "Misguided Myeloid Differentiation" model, the state of differentiation of the myeloid cell in which activating MAPK pathway mutations arise determine the extent of disease.[3] BRAF-V600E$^+$ PBMC in patients with LCH-associated neurodegeneration (LCH-ND) and perivascular BRAF-V600E$^+$ mononuclear cells in brain biopsies support LCH-ND as a tissue-specific manifestation of clonal LCH.[4] Emerging series support high rates of clinical responses in patients with LCH and LCH-ND treated with MAPK pathway inhibitors.[5-7] However, these agents do not appear to kill the pathogenic clone with uncertain potential of MAPK inhibitor monotherapy to cure LCH.[4, 8] **Drs. Matthew Collin** (Newcastle) and **Carl Allen** summarized the 2017 Nikolas meeting and highlighted advances in myeloid cell biology and genomics over the past year that impacted understanding of LCH and framed the 2108 Symposium discussion.

Origins, Differentiation Mechanisms, and Phenotype.

Drs. Jennifer Picarsic (Pittsburgh) and **Jean-Francois Emile** (Paris) presented an overview of histologic features of histiocytic disorders. Historically, these diseases have been characterized by comparisons of cellular features of the lesion cells to those of normal components of the immune system (e.g. "Langerhans" cell histiocytosis shares expression of the cell surface protein CD207 with epidermal *Langerhans* cells). LCH, with a "dendritic cell" phenotype has historically been categorized apart from ECD and JXG, with "macrophage" phenotype. Proposed reclassification of these conditions was discussed. The "L-group" includes LCH, Erdheim-Chester disease (ECD) and systemic juvenile xanthogranuloma (JXG) based on common features of somatic MAPK mutations, presence of circulating precursors, overlap in anatomic distribution of lesions (notably pituitary and LCH-ND), and the phenomenon of mixed histiocytic disease (one lesion with features of both DCs and macrophages or separate lesions with distinct features).[9] Common mutations have also been discovered in adults with myelodysplastic syndrome-related myeloproliferative disorders and histiocytic disorders. [10]At the same time as classification schemes mature, there is increasing recognition of intermediate and mixed phenotypes. Histological appearance, clinical phenotype and potential for response to therapy is likely influenced by MAPK mutations, additional somatic mutations, and differentiation of cell of origin.

Dr. Ronald Germain (Bethesda) described imaging approaches to investigate complex immune interactions. Immune responses involve cell-cell interactions within lymphoid tissues, trafficking of activated cells to sites of effector function, and the migration of innate and adaptive effector cells within peripheral tissues. To gain a more detailed appreciation of the relationships among cell movement, tissue architecture, and immune function, his group used intravital multiphoton microscopy and novel multiplex immunohistochemical

methods called Histo-cytometry and Ce3D to analyze immune cell dynamics and tissue micro-anatomy.[11, 12] Innate immunity involves many myeloid cell types, and dynamic intravital imaging was used to reveal the neutrophil swarming response to sterile tissue injury, decipher the molecular signals involved, and in a new peritoneal wall imaging model, uncover the unexpected role of fixed tissue macrophages in 'cloaking' of individual dead cells to prevent neutrophil swarming responses and concomitant tissue damage. The sequential recruitment of neutrophils and then monocytes to sites of tissue injury was described and this cascade recruitment process following injury was suggested as one possible contributor to the formation of LCH lesions. The role of cell localization in both innate and adaptive immunity was addressed using Histo-cytometry that reveals at high resolution the spatial positioning and activation state of cells with complex phenotypes in tissues, especially of dendritic cell and T cell subsets. The technology involved in these localization studies was highlighted - with an ability to use as many as 8-12 different colors and antibodies not only to surface markers but to phospho-proteins and cytokines in a sequential manner to achieve marker analysis at a multiplex of > 30 parameters, and also to conduct imaging in large 3D volumes in an quantitative manner, our quantitative imaging technology is ideally suited to studies of tissue samples from animal models and human patients with respect to the phenotype, number, location, signaling state, and function of immune cells and stromal elements in tissue sites. This talk illustrated the power of *in situ* imaging for the acquisition of a more accurate picture of the molecular, cellular, spatial, and temporal aspects of cell function and signaling events in host immune responses. It also highlighted new exploratory strategies for deeper analysis of nature of LCH lesions, with the possibility of revealing potential checkpoints susceptible to clinical intervention.

Dr. Christopher Glass (San Diego) presented the questions of fixed identity (*nature*) versus plasticity in response to environmental input (*nurture*) that determine macrophage identity and function. Tissue resident macrophages seed tissues during embryonic development, then are joined or replaced by hematopoietic-derived macrophages later in life depending on tissue-specific homeostasis and extrinsic factors such as inflammation and injury.[13] Enhancers play a role in determining cellular identity, with transcription factors responding to environmental stimuli with the ability to recruit epigenetic regulators to activate or repress gene expression. For example, loss of NCoR in macrophages results in impaired responses to TLR agonists.[14] SETD5, which contains 2 ERK1 phosphorylation sites, interacts with NCoR and exhibits H3K4 methltransferase activity. A model is proposed in which MAPK pathway mutations may have cell-specific consequences based on targeting of ERK1/2 to cell-specific enhancers.[15] The role of epigenetic regulation in physiologic macrophage differentiation and function suggests similar mechanisms which remain largely unexplored are likely to underlie differentiation of myeloid precursors in histiocytic disorders.

Dr. Chris Bock (Vienna) discussed the importance of analyzing heterogeneity within biological systems. Many fundamental cellular features are diluted with analysis of bulk tissue. Single-cell "multi-omics" (assays of genome, epigenome, transcriptome, proteome and metabolome) can provide comprehensive profiles of the same cell. more accurately characterize cellular differentiation landscapes through can be used to predict mechanisms of transcriptional regulation.[16] Multi-omics profiling may be used to create maps to identify regional subdivisions of tumors with biological features that vary in potential for drug resistance, relapse and metastasis. Large-scale analysis of single-cell methylation data can further reveal differentiation hierarchy patterns that may underlie clinical variability in tumors. Further investigations in this area to understand pathogenic mechanisms and predict disease risk in patients with LCH are warranted.

Dr. Steffen Jung (Rehovot) developed fate-mapping models to analyze the functional capabilities of tissue macrophages derived from different origins. Using CX_3CR1 promoter-driven Cre recombinase expression, he demonstrated that major tissue-resident macrophage populations, including liver Kupffer cells and lung alveolar, splenic and peritoneal macrophages, are established prior to birth and maintain themselves subsequently during adulthood. Further, he demonstrated that short-lived $LY6C^+$ monocytes (mouse correlate of human $CD14^+16^+$ and $CD14^+CD16^-$ monocytes) are precursors of blood-resident $LY6C^-$ cells (correlate of human $CD14^{lo}CD16^+$).[17] $LY6C^+$ monocytes are rapidly recruited to sites of inflammation and tissue remodeling, where they extravasate and can give rise to monocyte-derived DCs and macrophages. By contrast macrophage-like $LY6C^-$ cells in blood vessels patrol endothelial surface and coordinate repair by

recruiting neutrophils.[18] In the case of intestinal CX_3CR1-expressing macrophages, macrophage-derived IL-10 (anti-inflammatory cytokine) is dispensable for gut homeostasis and maintenance of colonic T regulatory cells. However, loss of IL-10 receptor expression impaired conditioning of monocyte-derived macrophages, resulting in spontaneous development of severe colitis.[19] Despite phenotypic similarity of macrophages, these studies highlight the complexity that underlies cellular function, with critical contributions from ontogeny, intrinsic hard-wiring, and extrinsic factors.

In patients with high-risk LCH, *BRAF*V600E (or another MAPK mutation) is identified in 100% of $CD207^+$ LCH lesions cells, but in very few (<1%) hematopoietic stem cells. Mutation frequency in peripheral blood mononuclear cells (PBMC) is variable from patient to patient and may be identified in myeloid as well as lymphoid lineages.[2, 20, 21] Pathways from BRAF-$V600E^+$ pluripotent HSC to $CD207^+$ LCH lesion cell remains uncertain. **Dr. Venetia Bigley** (Newcastle) described early steps of DC differentiation. Distinct subsets are responsible for the multifaceted functions of DC in immunity and tolerance. This heterogeneity is initiated during ontogeny where DC potential has been observed both within lymphoid primed (LMPP) and myeloid (GMP) progenitors. Through phenotypic analysis, single cell transcriptomics and in vitro culture of healthy controls, we can identify two pathways of DC development with differential dependence on transcription factor IRF8. Their differential requirement for IRF8 was interrogated through analysis of a unique population of humans with *IRF8* mutations, conferring a range of IRF8 functional activities from partial to complete loss. The classical IRF8-high pathway is exquisitely sensitive to IRF8 deficiency. Its terminal branches are attenuated by IRF8 haploinsufficiency and the entire structure is selectively ablated in patients with heterozygous dominant negative mutation of *IRF8*, leading to a selective deficiency of pDC, cDC1 and $CD5^+$cDC2. The alternative pathway is less sensitive to IRF8 deficiency with $CD5^-$cDC2 and monocytes preserved in heterozygous *IRF8* mutation but abrogated by biallelic mutations, leading to absolute DC deficiency and monocytopenia.[22, 23] **Dr. Caroline Hutter** (Vienna) presented data supporting the potential for $CD14^+$ monocytes and $CD1c^+$ (cDC1) to differentiate into $CD207^+$ cells *in vitro*, with distinct transcriptional profiles and cell surface markers depending of these $CD207^+$ cells depending on lineage of origin. Extrinsic as well as intrinsic features have potential to impact LCH $CD207^+$ cell phenotype: Dr. Hutter identified a critical role for Notch signaling through JAG2 for differentiation of $CD14^+$ monocytes into CD207+ cells.[24, 25] ***Pritchard Fellow*** **Karen Phaik Har Lim** (Houston) described her work aiming to define the cell of origin of LCH using LCH lesions, patient PBMC specimens and BRAF-V600E as a molecular tag. In addition to identifying *BRAF*V600E in $CD207^+/CD1a^+$ and $CD207^-/CD1a^+$ lesion cells, she found *BRAF*V600E in $CD1c^+$ lesion cells, but not in $CD14^+$ monocytes. Additionally, she identified HLA-DQB2, a cell-surface molecule previously localized only to physiologic epidermal Langerhans cells, on LCH lesion $CD207^+$ and $CD1a^+$ cells as well as $CD1c^+$ cells in blood and lesions. She hypothesizes blood $CD1c^+$ cells to be the origin for LCH lesion DCs. **Dr. Janie Borst** (Amsterdam) reviewed models of myeloid differentiation from precursor cells to osteoclasts. Under homeostatic conditions, macrophages, osteoclasts and DCs may arise from a tripotent progenitor described as the MODP, with DC differentiation driven by GM-CSF.[26] Activated MAPK from somatic mutations could conceivably bypass the need for GM-CSF (which signals through MAPK) to drive DC differentiation. Such a progenitor may be particularly relevant for LCH where multinucleated giant cells may arise in LCH as well as in juvenile xanthogranuloma lesions.

Arrows and Targets for LCH.

Prichard Fellow **Roei Mazor** (Tel-Aviv) presented challenging cases of adults with Langerhans Cell Histiocytosis and Erdheim-Chester Disease highlighting the difficulty of managing heterogeneity among patients with a rare diagnosis. He shared his experiences creating a comprehensive multi-disciplinary referral center to gain expertise and facilitate research studies to improve outcomes of patients. Drs. **Adreakos** (Athens), **Kattamis** (Athens), **Kottaridis** (London), **Moschovi** (Athens), and **Polychronopoulouo** (Athens) led discussion of challenging cases of LCH. While there is evidence for front-line therapy for children with LCH,[27] data are lacking to guide therapy for children with fail to be cured with vinblastine/prednisone and for adults with LCH, or for LCH-associated neurodegeneration. **Dr. Milen Minkov** (Vienna) led a panel

discussion of strategies to organize efforts to define optimal therapies through cooperative clinical research groups, collaboration and advocacy.

Universal MAPK pathway activation in myeloid precursors has been established as a driver of lesion formation in LCH. While MAPK pathway inhibition induces high response rates, BRAF-V600E and/or MEK inhibition may not cure LCH and toxicity profiles make life-long therapy suboptimal. Alternative rationale therapies are therefore needed. **Dr. Andy Clark** (Birmingham) discussed insights into function of tristetraprolin (TTP) a novel target for suppression of macrophage-mediated inflammation. TTP (encoded by ZFp36) is a critical negative regulator of innate immunity. The p38 MAPK pathway stabilizes mRNA by inactivating TTP. Intriguingly, inactivation of TTP protects against LPS-induced organ damage, experimental sepsis and experimental arthritis.[28-30] Could manipulation of MAPK-induced TTP quench the cytokine storm in LCH?

Dr. Catherine Bollard (Washington D.C.) discussed potential of applying immunotherapy to LCH. Immune-based treatment strategies, such as checkpoint inhibitors (e.g. PD1 blockers) and chimeric antigen receptor (CAR) T cells, have started a new frontier for treatment in non-Hodgkin lymphoma (NHL). CAR T-cell therapies that target CD19 are a promising and attractive therapy for B-cell NHLs, with a product approved by the US Food and Drug Administration in 2017. Changes in the target, hinge, or costimulatory domain can dramatically alter the persistence and efficacy of the CAR T cells. The ZUMA trials from Kite used CD19-(CD28z) CAR T cells, whereas the TRANSCEND studies from Juno and the JULIET studies from Novartis used CD19-(4-1BBz) CARs. Despite the recent successes with CAR T-cell clinical trials, major concerns associated with this therapy include cytokine release syndrome, potential neurotoxicities, B-cell aplasia, loss of tumor antigen leading to relapse, and cost and accessibility of the treatment. Antigen specific T cells targeting EBV and other non-viral tumor associate antigens, by contrast, have shown promise with appreciably less toxicity than CD19-CAR T cells.[31-33] However, so far, such products have been limited to small studies and have not garnered widespread enthusiasm yet. Overall, immune-based treatment strategies have given oncologists and patients hope when there used to be none, as well as a new basket of tools yet to come with further research and development and offer potential applicability to patients with LCH. However, critical questions will need to be considered before broadening applicability to LCH such as whether precursor or differentiated CD1a/CD207+ LCH cells express PD1 and tumor associated antigens (e.g. PRAME)? And if so, would the use of tumor antigen specific T cells with a checkpoint inhibitor such as nivolumab be a safe, feasible and effective combination therapy for patients with LCH?

Summation

Drs. Matthew Collin (Newcastle) and **Peter Beverley** (Oxford) reviewed the presentations and discussions of the 28th Nikolas Symposium. Broad strokes of pathogenesis of LCH have been identified: activating MAPK pathway mutation + myeloid precursor. However, more details of mechanisms regulating differentiation, tissue specificity, inflammation, and responses to therapy remain blurred. Increasing experience with MAPK inhibitors and advances in more granular understanding of pathogenic mechanisms in LCH are beginning to point therapeutic strategies beyond vinblastine/prednisone.

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May 1, 2019

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