Blood spotlight on Langerhans Cell Histiocytosis

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ABSTRACT

Langerhans cell histiocytosis (LCH) is a rare disease affecting subjects of any age, with widely variable clinical manifestations and different outcomes. The precise chain of events driving lesional granuloma formation has remained elusive for many years. There is evidence for inherited predisposition and derangement of apoptosis and inflammation in lesional dendritic cells. Recently somatic V600E BRAF mutation in myeloid precursor dendritic cells was associated with the more aggressive form of the disease, while the same mutation in a more differentiated dendritic cell might drive a less aggressive disease. Whether this picture convincingly put LCH in the field of myeloid neoplasm remains to be assessed. Altogether, these findings suggest that future therapeutic strategy might incorporate a screening of this genetic mutation for high risk patients, potentially suitable for target therapy.
Introduction

Langerhans cell histiocytosis (LCH) is a rare disease mainly seen in children, but it can present at any age.\textsuperscript{1,2} The clinical manifestations range from a solitary, asymptomatic osteolytic lesion, called “eosinophilic granuloma”, which tends to heal spontaneously, to multiple osteolytic lesions of the skull, intriguingly associated with exophthalmos and diabetes insipidus, formerly called the “Hand-Schuller-Christian” disease. In addition, in a small proportion of patients, especially toddlers, an ominous form of LCH (once described as the “Abt-Letterer-Siwe” disease) may run an acute course, with dissemination to various organs other than bones (particularly liver and spleen), shaping a multi-system disease as life-threatening as childhood acute lymphoblastic leukemia. Based on solid pathology findings and on a bit of intuition, in 1953 Lichtenstein suggested that these three conditions were part of a common disease spectrum and he coined the term Histiocytosis X: conveying the message that dysregulated proliferation of a histiocytic cell was at the heart of the problem, but that the trigger for this was unknown.\textsuperscript{3}

The diagnosis of LCH is based on finding in a biopsy, usually of skin or bone, a granuloma consisting of pale histiocytic cells with infolded nuclei, eosinophils and multinucleated giant cells. Staining of the histiocytic cells for CD1a (and CD207 langerin) has become part of the diagnosis (the demonstration by electron microscopy of Birbeck granules is no longer required).\textsuperscript{1,3} The morphology of individual lesions is so uniform as to make it impossible for the pathologist to know whether a biopsy was from a patient with unifocal or with multifocal disease, from a child or from an adult, and whether clinically the disease was indolent or life-threatening: thus after more than half a century, Lichtenstein’s notion is fully vindicated.

Systemic manifestations of LCH

Skin and bone lesions are the most frequent but fortunately the least threatening manifestations of LCH. At the other end of the spectrum, massive liver involvement and dysfunction may lead to rapidly fatal outcome or, sometimes, to late post-inflammatory fibrosis (sclerosing cholangitis). In a minority of these patients liver transplantation may prove curative; but in many the disease reactivates in the transplanted organ.\textsuperscript{4} Pulmonary involvement is one of the most puzzling features of LCH. Children may have disseminated interstitial pulmonary nodules and cysts, responsive to systemic therapy.\textsuperscript{5} On the other hand, isolated pulmonary LCH is
by far the most frequent manifestation in adults. A correlation with cigarette smoking has been clearly documented. Yet, in most cases smoking cessation is not sufficient to stop the inflammatory process, and these young adults may show progression to multi-cystic metaplasia and become oxygen-dependent. In such patients the role of LCH-directed chemotherapy remains unclear, because existing reports are on small series, and no prospective studies have been conducted. End-stage organ failure often requires lung transplantation, and here too disease recurrence may take place.

Diabetes insipidus (DI), resulting from destruction of the supraoptic-paraventricular nuclei in which vasopressin is produced, occurs in about 12-15% of patients with disseminated LCH. Osteolytic lesions of the skull base and facial bones predispose to DI, likely due to local vascular dissemination. Once DI is established, reversal may be exceptional. Thus, DI can be a disabling sequela and it may progress to multiple anterior pituitary hormone deficiency. A small number of patients with LCH, especially among those with DI, may develop bilateral symmetric alterations in the cerebellar grey matter, in the basal ganglia and in the brainstem. Histopathology from cerebellar biopsies and from autopsies revealed neuronal loss, axonal degeneration and a profound T-cell inflammation. Its pathogenesis remains unsolved, and propagation from long-standing granulomatous lesions of the craniofacial bones to the intracranial space, with stimulation of chemokine/cytokine tissue damage or initiation of an autoimmune response to brain components have been suggested. Neurodegenerative-LCH may be devastating and unfortunately no effective therapy is available so far.

**Treatment of LCH**

LCH tends to run a favorable course in the large majority of patients: indeed, most patients with solitary lesions do not need treatment as long as the lesion remains solitary (although it must be acknowledged that even biopsy itself may exert some therapeutic effect and speed-up the healing process). However, in a minority of cases the disease may be aggressive and even life-threatening. For a long time the management of patients was conducted on an empirical basis, reflecting the different views and the uncertainty that have prevailed regarding the nature of the disease. Leukemia-oriented chemotherapy was used in children with disseminated disease by the Austrian-German group during the 80s, with favorable response. On the other
hand, based on the concept of an inflammatory disease, anti-inflammatory agents, especially steroids, were used by the British group; although this achieved disease control in most cases, children with chronic/reactivating LCH developed unacceptable side effects of long-lasting steroid exposure. Since the early ‘90s an international cooperative group of pediatric hematology-oncology specialists met the challenge of conducting three consecutive randomized trials in this “orphan disease”. Overall, these studies provided meaningful lessons: 1) In LCH-I comparison of 6-month therapy of vinblastine or etoposide, together with an initial 3-day pulse of prednisone, in all patients with MS-LCH were equivalent with respect to response, survival, disease reactivation, permanent consequences and toxicity. However, early response and prevention of disease reactivation were inferior to results of the more aggressive (five drug combination) and longer (12 months) DAL-HX83/90 protocols, suggesting a need to intensify treatment. 2) In LCH-II, MS-LCH patients were stratified by risk (high risk being those with age <2 years and/or involvement of “risk organ”, i.e. liver, spleen, hematopoietic system, and lung) and randomized to the standard combination of prednisone and vinblastine vs. additional etoposide. In both treatment arms, these patients showed faster disease resolution and a higher survival rate than those in LCH-I, although the 44% reactivation rate was still high. Lack of advantage, together with its reported leukemogenic potential, caused the discontinuation of study of etoposide in MS-LCH. 3) In the successor study, LCH-III, the efficacy of increasing intensity by adding methotrexate in risk organ patients (treated for 12 months) and prolonged initial intense therapy if only partially responding by 6 weeks, was tested but did not provide a better outcome; otherwise, extending the duration of treatment to 12 months proved superior to 6 months in reducing the rate of disease reactivation in children who had achieved disease control. As a result of these trials, the former intuition that patients with LCH should be stratified according to their very different risk of progression and failure, has been validated. Patients with localized disease should be treated conservatively with very limited exceptions; patients with multisystem disease without involvement of vital organs should be treated with the combination of vinblastine and prednisone for a total duration of at least 12 months, which represents the standard of care in pediatric LCH, aiming at limitation of the disease course and thus also of permanent consequences. Yet, additional unmet clinical needs stand in front of us: about one third of patients who achieve complete control of the disease will suffer relapse during the following months, most often within the same tissue/organ(s) type(s) involved at presentation. Fortunately,
whereas in childhood leukemia relapse is usually associated with very unfavorable prognosis, relapsed LCH is usually amenable to treatment with the same agents used before, or even with anti-inflammatory agents: for this reason the term “reactivation”, already proposed since 1982,\(^\text{16}\) is now preferred to the term relapse.\(^\text{17}\) Beyond disease reactivation, a number of permanent consequences\(^\text{16}\) including DI, neurodegeneration, sclerosing cholangitis, pulmonary failure, reduced linear growth and other endocrine dysfunctions, and bone deformities, represent quite a heavy burden for the quality of life of patients cured from LCH.\(^\text{17,18}\) But even more compelling is the remaining minority (20\%) of MS-LCH patients -- with involvement of liver, spleen, bone marrow -- who fail to respond within 2-3 weeks, who still face an unacceptably high risk of early mortality, in the range of 40\%.\(^\text{12-15}\) In these patients very intensive chemotherapy, similar to that used for acute myeloid leukemia, turned out to be useful,\(^\text{19}\) whereas the role of hematopoietic stem cell transplantation remains uncertain.\(^\text{20}\) But definitely this is the subset of patients for whom novel experimental therapies, derived from improved knowledge of LCH pathogenesis, appear warranted.

*LCH: inflammatory or neoplastic?*

Although in most cases it has only localized manifestations, LCH must be considered a systemic disease. Unlike leukemia, LCH does not seem to arise from the bone marrow and to spread elsewhere. The basic lesion, the granuloma - the morphology of which was exhaustively described already by 19\(^\text{th}\) century pathologists - is characteristically enriched in dendritic cells (DC): this is reminiscent of the typical pattern of tissue reaction to an intracellular pathogen, of which the tuberculous granuloma is the prototype. Again it is since the 19\(^\text{th}\) century that an infectious origin of LCH had been surmised but never proven; and extensive epidemiological studies have failed to identify significant infectious associations.\(^\text{19}\) On the other hand, since the early nineties it has been claimed that clonal cell populations are present based on the analysis through an X-linked marker (e.g. the human androgen receptor assay, Humara) of lesional tissue from females with LCH.\(^\text{22}\) This finding is per se non conclusive of cancer, since although cancer must be clonal, not every clonal population is cancer: indeed, clonal populations have been observed in benign disorders. Unexplained remission can occur in neoplasm, exceptionally even in widely metastatic cases; but spontaneous healing in LCH is not exceptional: on top of what is frequently seen in skin and bone lesion of children, the provocative observation that some young adult patients with pulmonary LCH remit after smoking cessation is one of the
fascinating and incompletely understood features of LCH, suggesting that there may be many pathobiological contributions to the disease process.

The fact that an animal model of LCH has been lacking for a long time has been a forceful stimulus to investigating pathogenesis by ex vivo studies and by looking for bio-markers. Clinical manifestations of LCH – such as aggressive chronic granuloma formation, bone resorption, and soft tissue lesions with occasional neuro-degeneration – show similarities with those observed in other IL-17A-related human diseases (Mycobacterium infection, Crohn’s disease, rheumatoid arthritis and multiple sclerosis). In one study peripheral blood samples from patients with LCH were put in culture: the resulting monocyte derived-DC showed extended life-span and propensity to undergo cell fusion leading to the formation of multinucleated giant cells. These features make them excellent candidates to be at the origin of the characteristic granuloma. Remarkably, both of these properties - extended life-span and propensity to fuse - were reproduced in culture by exposing normal control DCs to interleukin 17A (IL-17A); this phenomenon could be suppressed by exposure to anti-IL17 antibodies, and restored by re-exposure to IL-17A, suggesting an autocrine role for this cytokine with respect to granuloma formation. In the same study, patients with LCH were found to have high levels of IL-17A. However, a second group was unable to detect IL-17A messenger RNA or protein in samples from LCH patients. The authors suggested that the initial finding could be attributed to lack of specificity of the anti-IL17A antibody. Subsequently, this laboratory failed to identify any cells in LCH lesions with IL-17A gene expression, concluding that evidence for IL-17A as a significant factor in LCH had remained inadequate, thus setting a “IL-17 controversy” in LCH. Yet, the DC fusion activity of IL-17A in vitro was not dependent on an IL-17A antibody and has not been challenged, suggesting that IL-17A secreted by other cells might play a pathogenic role in LCH to foster tissue-aggressive giant myeloid inflammatory cell formation (Figure A). Recently, Murakami et al. confirmed higher levels of IL-17A in 38 patients with LCH compared to controls. Furthermore, Lourda et al. found an increased frequency of IL-17A+ mononuclear cells in the bloodstream of LCH patients by using intracellular cytokine staining followed by flow cytometry analysis. The majority of the IL-17A+ cells were monocytes, which by RT-PCR showed higher levels of both IL-17A and Retinoic acid orphan receptor C (RORC) mRNA, associated with higher
disease activity.\textsuperscript{27} The finding that monocytes play a central role in this process appears to reconcile the controversy and agrees with the recent report of an immature marrow-based dendritic cell of origin in LCH.\textsuperscript{28}

In spite of much recent progress on LCH that has just been reviewed, there remain three major questions to which we have only partial answers.

1. Why does a monocyte-derived DC become an LCH cell? The long-lasting question of a triggering pathogen in LCH has been revitalized by Murakami et al., who recently reported elevated amounts of Merkel cell polyomavirus DNA in the peripheral blood cells of 2 of 3 LCH patients with high-risk organ involvement, but not in the blood cells of 12/12 patients with “low risk” LCH. Yet, with lower viral loads, an elevated number of Merkel cell polyomavirus DNA sequences was detected in 12 LCH tissues, in comparison with controls other than dermatopathic lymphadenopathy.\textsuperscript{29} Previous studies on peripheral blood chromosomes had showed that patients with LCH display an excess of spontaneous chromosomal breaks, more evident during the acute phase of the disease;\textsuperscript{30} these findings resemble the genomic instability induced by RSV, HCV, or EBV.\textsuperscript{30,31}

2. What are the key signals that drive monocyte derived-DCs to form a granuloma? Hutter et al. showed that JAG2-mediated Notch activation confers phenotypic and functional aspects of LCH to DCs.\textsuperscript{32} Olsson et al. found that monocyte-derived DC treated with IL-17A express BCL2A1/BFL1, a pro-survival member of the Bcl-2 family.\textsuperscript{33} They also proposed that exposure to anti-IL-17A may decrease BFL1 and synergized with chemotherapy to eradicate LCH-DC.\textsuperscript{34} Identification of BRAF\textsuperscript{V600E} mutation in 35 of 61 (57%) archived specimens of LCH tissue opened a novel research avenue in LCH.\textsuperscript{35} This finding was confirmed by a French group,\textsuperscript{36} who yet was unable to document \textsuperscript{V600E}BRAF in peripheral blood cells of patients with LCH, confirming that it is a somatic mutation within the lesional cells.\textsuperscript{37} In both studies \textsuperscript{V600E}BRAF did not seem to correlate with age, clinical presentation, or outcome. Yet, those data were not conclusive: (i) only about 40% of LCH patients have somatic mutations of BRAF; (ii) retroviral transduction of \textsuperscript{V600E}BRAF in myeloid cells resulted in growth arrest and cell death; (iii) in genetically engineered mouse models, \textsuperscript{V600E}BRAF mutation in mature Langerhans cells was insufficient to develop LCH.\textsuperscript{38} In a very recent study of 100 LCH lesions, of which 64 carried the \textsuperscript{V600E}BRAF mutation within infiltrating CD207+ DCs, patients with active, high-risk
LCH were found to carry \(^{V600E}\)BRAF in circulating CD11c+ and CD14+ fractions and in bone marrow CD34+ hematopoietic cell progenitors, while the mutation was restricted to lesional CD207+ DC in low-risk patients. In a new mouse model, the expression of conditional \(^{V600E}\)BRAF enforced under the langerin promoter was sufficient to drive LCH-like disease. In summary, while expression of \(^{V600E}\)BRAF in marrow DC progenitors was found to recapitulate the human high-risk LCH, \(^{V600E}\)BRAF expression in differentiated DCs resembled low-risk LCH.\(^{39}\) Based on these findings, the authors propose classification of LCH as a myeloid neoplasia. Taking into account this bunch of data, it is tempting to propose a working model for the impairment of transduction pathways driving LCH pathogenic DC development (Figure 2).

3. Why do some subjects develop LCH? Studies on familial clustering of the disease had documented that about 1% of patients with LCH have another affected member in the family. When focusing on twin pairs, the rate of concordance is as high as 10% in non-identical twins, and an impressive 92% in identical twins.\(^{40,41}\) These data strongly point to a genetic component predisposing to LCH,\(^{40,44}\) even though efforts by Egeler et al. to identify an LCH-associated locus through a genome-wide screening have not been successful.\(^{45}\) Interestingly, in this study all patients had diploid genomes, which, if anything, casts additional doubt on the neoplastic origin of LCH.

In conclusion, from the clinical point of view LCH is a rare disorder with widely variable manifestations. After decades in which its pathogenesis remained elusive, recent findings suggest that somatic mutation occurring in a bone marrow myeloid progenitor drive a neoplastic process, while the same somatic mutation occurring at a more differentiated stage drives a self-limiting, inflammatory disorder. The finding of \(^{V600E}\)BRAF mutation in all concurrent pulmonary nodules\(^{46}\) suggests either multiple independent events, or a true spread within the affected lungs. The monocyte/DC plays a pivotal role, and might have been genetically predisposed\(^{40,44}\) to becoming over-stimulated by an infectious trigger.

Until now improved outcomes for patients with LCH have accrued from empirically adopted chemotherapeutic regimes: recent exciting advances in the pathogenesis of LCH may suggest to incorporate in therapeutic trial prospective validation of the association of \(^{V600E}\)BRAF mutation with multisystem, aggressive LCH and higher risk of treatment failure, and also to explore the use of frontline target therapy with vemurafenib\(^{47}\) in accurately defined subgroups of patients with LCH.
Acknowledgement. The authors are grateful to Prof. Lucio Luzzatto (I.T.T., Florence) for fruitful discussion and contribution to manuscript writing, and to Associazione Italiana Ricerca Isteocitosi (AIRI) O.N.L.U.S. for supporting research.

Authorship Contributions. MA designed and wrote the manuscript. CD contributed to data interpretation, drew the figures, and contributed to writing the manuscript.

Conflict of Interest Disclosure. The authors take the full responsibility of the manuscript and have no conflict of interest to disclose.
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Legend to figures

**Figure 1 - Major cell, cytokine and protease players in Langerhans cell histiocytosis (LCH) lesion:** This simplified overview indicates the main cytokines, chemokines and proteases found in LCH lesion which may play a major role in cell recruitment, survival, fusion and in inflammatory and tissue-aggressive activities, supported by pro-inflammatory cytokines and MMPs, respectively. The origin of the pathogenic DC in LCH lesion is still a matter of debate. Recent results argue that pathogenic DC do not arise from Langerhans cell but from accumulation of bone-marrow-derived immature myeloid cells able to differentiate into DC such as Monocytes, MDP or CD135+ LMP. Abbreviations: CCL, chemokine (C-C motif) ligand; DC, dendritic cell; GM-CSF, granulocyte-macrophage-colony stimulating factor; IFN, interferon; IL-, interleukin; LMP, lympho-myeloid progenitor; MDP, macrophage and DC precursor; MMP, matrix metalloproteinase; RANKL, receptor activator of nuclear factor κ-B ligand; TNF, tumor necrosis factor; Treg, regulatory T cell.

**Figure 2 – Mechanisms of accumulation of LCH pathogenic DC through proliferation and prolonged survival:** (A) DC survival is basically regulated by exogenous concentration of GM-CSF on CSF2R. Low GM-CSF concentration (black) stimulates the selective activation of Ser^{585}/14-3-3/PI-3 kinase, the “Survival Only” pathway, while high GM-CSF concentration (red) can give rise to assembling of CSF2R chains in dodecamer, thus involving the Jak/STAT, Ras/mitogen-activated protein kinase and PI-3 kinase pathways downstream of Ser^{585} and Tyr^{577} phosphorylation (red bullet). In IL-17A inflammatory microenvironment (green), Monocyte-derived DC survival can be prolonged for weeks by IL-17A treatment.\(^{33}\) The TRAF6-dependent IL-17R transduction is able to activate both PI3K / Akt and NF-κB pathways.\(^{48}\) (B) In LCH patients, while low concentration of GM-CSF will normally induce PI3K / Akt (black), other molecular specificities turn DC survival and phenotype into aggressive myeloid cells: the presence of IL-17A activates both PI3K / Akt and NF-κB pathways (green), leading to increased survival, inflammation and DC fusion. In addition, pathogenic LCH DC express JAG2 and activate transduction downstream of its receptor Notch (brown),\(^{32}\) which may account for tissue destruction via MMP expression.\(^{V600EBRAF}\) mutation will ensure constitutive activation of MAPK pathway (red). It would be important in the future to explore the Jak2 / Stat5 pathway which may be absent in LCH DC. From this recent knowledge, new therapeutic interventions in LCH may (i) neutralize IL-17A, (ii) inhibit NOTCH and (iii)\(^{V600EBRAF}\) / MAPK pathways and possibly (iv) activate the Jak2 / Stat5 pathway to reverse LCH DC phenotype towards normal DC phenotype as long as we don’t understand further the origin of this impairment of DC differentiation.
Normal DC progenitors during IL-17A inflammation

A

Low [GM-CSF] High + IL-17A

GM-CSF
CSF2R
Jak2 Jak2 Jak2 Jak2

14-3-3
Stat5

Ras
PI3K
Akt

Raf
MAPK
c-fos
c-jun

IKKαβ
NF-κB

IL-17A
IL-17R

Act1
TRAF6
Tak1

14-3-3
Shc

BCL2A1 induction

constitutive MCL1

Survival Only
Survival & Proliferation

Long-term survival, Inflammation & Fusion

B

DC progenitors in LCH

Ligand Receptor
low [GM-CSF] IL-17A JAG2
CSFR2 IL-17R NOTCH

Transduction
PI3K Akt
NF-κB
MAML
V600E BRAF MAPK

Biological Effect
Survival
Survival Inflammation
DC fusion
Abnormal Phenotype
MMPs
Proliferation

Figure 2
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