

PERSPECTIVE

Langerhans cell histiocytosis: A malignant myeloid neoplasm or disorder of immune regulation?

1 | INTRODUCTION

Langerhans cell histiocytosis (LCH) is characterised by lesions containing LCH cells, which are myeloid-derived dendritic cells that co-express CD1a and CD207 (langerin). In addition to LCH cells, these lesions also contain an inflammatory microenvironment of T cells, macrophages, neutrophils, eosinophils, B cells, plasma cells and multinucleated giant cells. Whilst LCH cells are well studied (Figure 1) and important in the pathogenesis of this disease, the inflammatory infiltrate and the corresponding cytokine milieu they produce are central to the resultant organ damage, which is a hallmark of this disease.¹ Although LCH is more common in young children, it occurs at all ages, and the resulting mortality/morbidity varies from lethal to mild despite similar pathology of biopsied lesions.

2 | LCH: MALIGNANT NEOPLASM OR IMMUNE DISORDER?

The question as to whether LCH is a malignancy or a disorder of immune regulation has been debated for many decades.²⁻⁴ This debate has shifted towards this being a malignant disorder, but a number of observations place doubt on this hypothesis; namely, whether LCH cells are indeed clonal⁵; the observation that LCH cells and LCH lesions have not been able to be grown in immunocompromised mice; and that driver mutations, such as BRAF, can occur in normal stem cells and benign lesions⁶ and are not on their own indicative for malignancy.

The clonality studies were performed in 1994 using the X-linked HUMARA assay on tissue sections,⁷ and fluorescence-activated cell sorting (FACS) isolated CD1a cells from 3 female patients with LCH.⁸ The HUMARA assay detects X-chromosome inactivation of the HUMARA gene, which is exclusive to the X chromosome. Using a methylation-sensitive restriction enzyme and polymerase chain reaction, active (unmethylated) and inactive (methylated) HUMARA alleles can be differentiated, and the pattern of X-chromosome

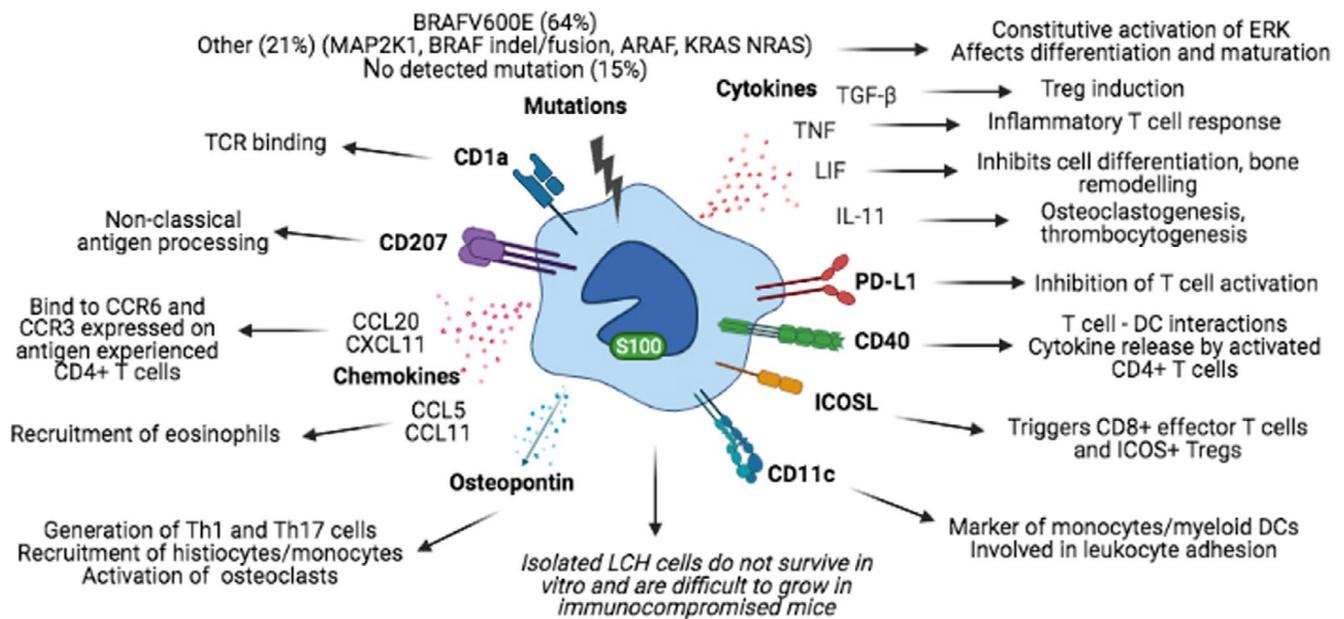
inactivation determined. Thus, if specific cell populations are of clonal origin, the assay should recognise this. Since the work on clonality in LCH specimens, studies have highlighted the shortcomings of using the HUMARA assay as an approach to detect clonality,^{9,10} with a number of haemopoietic cell lineages (including myeloid dendritic cells) demonstrating non-clonal skewing of the HUMARA alleles. Furthermore, the FACS sorted CD1a cells from the 3 patients with LCH demonstrated skewing of the HUMARA alleles, which was interpreted as clonal at the time, could also be challenged on another front in that CD1a-positive cells comprise both a significant population of polyclonal CD3 cells expressing CD1a and LCH cells expressing CD1a.⁵ FACS sorted CD3-positive T cells from LCH lesions expressed CD1a in 10.2 to 80 per cent (mean 45 per cent) of the total CD1a cell population (all samples of LCH lesions studied to date have demonstrated CD3 cells expressing CD1a), and as these were demonstrated to be polyclonal by T-cell receptor rearrangement studies.⁵ Similarly, work published by Allen et al¹¹ in the supplementary data of their publication also reported that FACS sorted CD3 cells from LCH lesions contained high levels of CD1A mRNA expression. These findings cast significant doubt on the clonality of LCH cells, and it is now timely to reconsider the original clonality studies as not being conclusive. Mutations of the MAPK/ERK cell-signalling pathway have been found in 70 to 75 per cent of LCH lesions,^{12,13} and all LCH lesions are thought to have activation of the ERK pathway.¹⁴ The inability to find mutations in all LCH lesions indicates that the cytokine milieu may play an important part in activation of the ERK pathway. Some patients have responded to BRAF inhibitors but these effects are abolished on cessation of therapy.¹⁵ MAPK pathway mutations have been demonstrated in non-malignant cells including nevi¹⁶ and benign thyroid lesions¹⁷ and, therefore, are insufficient alone for tumorigenesis. Although a number of studies have suggested that LCH cells may be derived from early myeloid precursors (immature CD1a-positive dendritic cells), these data are not sufficient to conclude that LCH is a malignancy. Additionally, BRAF mutations have recently been identified in many cell lineages in patients with LCH,

Abbreviations: BRAF, Serine/threonine-protein kinase B-Raf; ERK, Extracellular signal-regulated kinase; FACS, Fluorescence-activated cell sorting; Foxp3, Forkhead box P3; HLA-DR, Human leukocyte antigen-DR isotype; HUMARA, Human androgen receptor gene; LCH, Langerhans cell histiocytosis; MAIT cell, Mucosal-associated invariant T cell; MAPK, Mitogen-activated protein kinase; TGF β , Transforming growth factor beta; Treg, Regulatory T cell.

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LCH cell properties



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FIGURE 1 Characteristics of LCH cells. The diagram summarises what we currently know about expression of cell-surface molecules, the production of cell-signalling molecules, mutations harboured by LCH cells and the possible consequences of these. Created in BioRender.com

including lymphoid populations such as B cells, T cells and natural killer cells.¹⁸

3 | LCH CELLS AND THE ASSOCIATED CYTOKINE MILIEU

Langerhans cell histiocytosis cells make up 4–70 per cent of cells within lesions. Although they share common features with epidermal Langerhans cells and activated Langerhans cells, they differ by the low cell-surface expression and intracytoplasmic localisation of HLA-DR,^{19,20} and their inability to respond to allogeneic T-cell stimulation.²¹ Additionally, intralesional LCH cells have recently been shown to be heterogeneous at the cellular, transcriptomic and epigenomic level, with a shared developmental hierarchy across all lesions studied.²² The LCH microenvironment contains proinflammatory cytokines consisting of high levels of tumour necrosis factor, interferon gamma, granulocyte-macrophage colony-stimulating factor, interleukin 1 β and interleukin 2.^{1,23,24} Kannourakis and Abbas reported in 1994 that local T-cell activation within LCH lesions led to the production of cytokines, including transforming growth factor beta (TGF β) and leukaemia inhibitory factor.¹ Egeler and colleagues confirmed these findings and demonstrated that both T cells and

LCH cells produced high levels of cytokines.²³ Collectively, these studies confirmed that both T cells and LCH cells contributed to the cytokine milieu within LCH lesions. Interestingly, the addition of a T-cell co-stimulatory molecule, CD40 ligand, to LCH cells induced upregulation of HLA-DR on LCH cells and improved the allogeneic response to T cells.²⁰ This indicates that the LCH microenvironment may be inhibitory to T-cell immune responses.

4 | SYSTEMIC EFFECTS OF LCH

The lesion microenvironment is not the only place where the inflammatory effects of LCH are observed. Plasma and serum levels of various cytokines have been reported in patients with active LCH. Of note, the interleukin 17A/23 signalling pathway has been suggested to play a role in LCH pathogenesis,^{25,26} with plasma interleukin 17A being associated with severe neurological sequelae.²⁶

5 | THE ROLE OF T CELLS IN LCH

T cells are involved in immune surveillance, and it is known that LCH cells have low expression of HLA-DR, CD83, CD86 and CD208, which

play an important role in antigen-dependent T-cell activation.^{19,20} An interesting clinical observation is the regression of LCH lesions following biopsy or injection of low-dose steroids into the lesion, indicating that the possible disruption of the LCH microenvironment leads to immune responses that lead to resolution of the lesion. LCH lesions have been shown to have high numbers of Foxp3 Tregs, which are a regulatory T-cell subset that expresses the forkhead box P3 transcription factor.²⁷ Tregs help to maintain immunological tolerance by suppressing T-cell activation by secreting inhibitory cytokines TGF β and interleukin 10 and by direct cell to cell contact and hence promote the inhibition of anti-tumour responses. LCH cells are considered to be immature dendritic cells, which have been shown to promote Treg production and hence immune tolerance within LCH lesions. Our group have demonstrated that cytotoxic T-cell subsets, including mucosal-associated invariant T (MAIT) cells, are significantly reduced within LCH lesions and in the peripheral blood of patients with LCH.^{28,29} The reduced numbers of MAIT cells may impair their ability to upregulate CD40 ligand, which is required for the maturation of LCH cells. MAIT cells or other cytotoxic T-cell subsets may be required for the resolution of the inflammatory milieu in LCH lesions, and hence the lesions themselves. The presence of elevated Tregs and reduced cytotoxic T-cell subsets within LCH patients indicates that an immunological approach to the treatment of this disorder is warranted. More research is required to determine the exact immune-modulating approach to LCH. The role of the abundant CD3 cells expressing CD1a within LCH lesions is yet to be determined.

6 | CONCLUSION

The past studies on LCH biology have failed to conclusively make the case that LCH is a malignant neoplasm. Whilst MAPK pathway mutations provide evidence that LCH may be neoplastic, there are also abundant data to indicate that LCH is a disorder of immune regulation and could be treated immunologically. T cells have a central role in cell-mediated immunity and are highlighted to have many potential roles in LCH pathogenesis, and thus, they are exciting targets for new immunotherapeutic treatment options for patients with LCH.

CONFLICT OF INTEREST

No funding or conflict of interest to declare.

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