Langerhans cell histiocytosis: a cytokine/chemokine-mediated disorder?

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ABSTRACT. Langerhans cell histiocytosis (LCH) is a rare disorder characterized by an abnormal accumulation and/or proliferation of cells with a Langerhans cell phenotype. Although no clear cause of LCH has been identified, it has been postulated that LCH might be the consequence of an immune dysregulation, causing Langerhans cells to migrate to and accumulate at various sites. Production of cytokines and chemokines is a central feature of immune regulation. Cytokines are abundantly present within LCH lesions. We review here the potential role of cytokines and chemokines in the pathogenesis of LCH. The type, distribution, and number of different cytokines released within lesions can provide clues to the possible aetiology of LCH and, ultimately, might offer therapeutic possibilities using recombinant cytokines or antagonists for this disorder.

Key words: Langerhans cell histiocytosis, cytokines, chemokines, dendritic cells

Langerhans cell histiocytosis (LCH) is characterized by an abnormal accumulation of cells with the same phenotype as normal Langerhans cells, together with inflammatory cells such as eosinophils, lymphocytes, neutrophils, macrophages and mast cells [1]. Lesions can occur in various sites and organs. The symptoms and prognosis of LCH depend on the number of lesions, whether a single or multiple organs are affected, (skeleton, lymph nodes, skin, liver, bone marrow, ears, lungs are most frequently involved), and on the presence or absence of organ dysfunction (liver, lungs and bone marrow are the major organs at risk). Reactivation of the disease is not uncommon. Diabetes insipidus can be present at diagnosis, but can occur also much later, after disappearance of symptoms, but it is seen mostly in patients diagnosed with multisystem disease [2]. The severity in symptoms at presentation and the clinical evolution is related to age. The most extensive presentation of LCH causing life-threatening symptoms was previously named Letterer-Siwe disease [3], which occurs during the neonatal stage, whereas a solitary bone lesion, previously named eosinophilic granuloma, is more frequently seen in older children and adults [4]. In between is a broad spectrum of symptoms, such as the combination of diabetes insipidus, exophthalmos and skeletal lesions typical of what was called Hand-Schüller-Christian disease [5]. In 1953, Lichtenstein correlated these three different clinical patterns and was the first to refer to these diseases as “histiocytosis X” [6]. Two decades later the histiocytes present in the lesions as described by Lichenstein were shown to share their phenotype with the Langerhans cells of the normal dermis [7], so Langerhans cell histiocytosis is the correct term to use now [8]. The rarity of LCH necessitates the registration and inclusion of patients in international protocols with well-defined therapeutic strategies in which combinations of corticosteroids and chemotherapy are used according to the extent of the disease [9].

The hallmark for diagnosis is the histological examination of the lesion demonstrating LCH cells (expressing CD1a and S-100) plus inflammatory cells: eosinophils, lymphocytes, neutrophils, monocytes, etc. [10]. Ultrastructural...
examination can demonstrate the presence of Birbeck granules in the LCH cells, however, this examination needs to be performed using immediately-fixed frozen tissue, which is not always available. It was described recently that immunohistochemistry for a new marker, Langerin (CD207), correlates with the presence of Birbeck granules [11], and can substitute for the more difficult electron microscopy examination. Langerin and CD1a are both now accepted as diagnostic criteria by the Histiocyte Society [8, 12].

As there are no clear, etiological factors and because of the peculiar and sometimes dramatic course of LCH, it has been long debated whether or not LCH is a malignant disease or an immune dysregulation [2, 10, 12]. Cytokines can contribute to the development of such clinico-pathological abnormalities if local action and transient production of a particular cytokine becomes out of control [1]. Here we review the literature concerning LCH and the cytokine/chemokine network that might support the hypothesis that LCH is an immunological disorder.

On the basis of the number and arrangement of conserved cysteines, chemokines are subdivided in two, multi-number families, namely the CXC and CC chemokines. The CC chemokines possess four cysteines, of which the first two are adjacent, whereas in CXC chemokines these are separated by one other amino acid [13, 14]. In contrast to cytokines, which signal through multi-chain, high affinity receptor complexes composed of cytokine binding and signal transducing subunits, chemokines bind to glycosaminoglycans, and activate cells via G protein-coupled, seven-transmembrane receptors [13, 14].

FUNCTION OF LANGERHANS CELLS

Langerhans cell histiocytosis is associated with unexplained, aberrant behaviour of Langerhans cells (LCs). Langerhans cells are immature dendritic cells, which are responsible for specific, first-line immunological defence in the skin. Dendritic cells (DCs) are potent and unique antigen-presenting cells referring to their ability to induce T and B cell responses, as well as immune tolerance. They reside in the peripheral tissues in an immature state where they exert a sentinel function for incoming antigens. On contact with foreign material (antigens), these cells take up and process the antigen, and then migrate to draining lymph nodes, where they present the antigen to the T cells of the immune system for an appropriate response. This function is a complex cascade of events involving antigen recognition, uptake and degradation, differentiation, migration, cell contact, T-cell signalling and activation. Migration of DC into tissue depends on a cascade of discrete events, including chemokine production and regulation of chemokine receptor expression [15].

DC progenitors are generated in the bone marrow giving rise to circulating DC precursors that enter non-lymphoid organs as immature DCs, called interstitial DCs for most organs, but Langerhans cells when localized in the skin. In a resting, so-called immature state, DCs express a variety of inflammatory chemokine receptors, such as CCR1, CCR2, CCR4, CCR5, CCR6, CCR8 and CXCR4, which participate in their recruitment into inflamed tissue and/or allow their residency in non-lymphoid tissue [15]. Furthermore, immature DCs produce high levels of inflammatory chemokines, such as MIP-3α/CCL20, MCP-1/CCL2 and RANTES/CCL5, which help in recruiting circulating DCs and other immune cell types to the inflamed tissue [16].

In the absence of ongoing inflammatory and immune responses, DCs constantly migrate at low rates to draining lymph nodes. In inflammatory conditions, after antigen uptake, (maturing) DCs shut off antigen capture and class II MHC molecules synthesis (changing from the immature to the mature state), and migrate to draining lymph nodes into the T cell area of lymphoid organs where they prime the rare, circulating, naïve antigen-specific lymphocytes. The maturation of DCs is associated with the downregulation of CCR6 and the upregulation of receptors for constitutive chemokines such as CXCR4 and CCR7, which allow these cells to respond to lymphoid chemokines [15]. SLC (secondary lymphoid tissue chemokine)CCL21 is a ligand for CCR7 and is expressed at high levels by high endothelial venules (HEVs) in lymph nodes and by stromal cells in T cell areas of many secondary lymphoid organs. Epstein-Barr virus–induced molecule 1 ligand chemokine (ELC)/CCL19, another ligand of CCR7 is present in T cell areas of lymphoid tissue. In the secondary lymph tissue, DCs are called interdigitating DCs. LCH might be caused by a deregulation in this cascade of events regulated to a great extent by cytokines and chemokines, resulting in abnormal accumulation and/or proliferation of LCs [12, 17]. Indeed, LCs in LCH lesions display an intermediate activation status combining both immature and mature features [18, 19], which may cause their aberrant behaviour.

LANGERHANS CELLS AND CHEMOKINES

Chemokines have already emerged as major regulators of DC migration. DC subsets express a distinct pattern of functional chemokine receptors at different stages of their maturation. Immature DCs express receptors for inflammatory chemokines, including CCR1, CCR2, CCR4, CCR5, CCR6, CCR8 and CXCR4, which enable the recruitment of immature DCs to sites of inflammation where cognate ligands are produced [15]. For example, the CCR1 ligand macrophage inflammatory protein-1α (MIP-1α)/CCL3 chemotactacts blood mononuclear cell-derived immature DCs in a dose-dependent manner (figure 1). Similarly, the MIP-1α variant LD78β/CCL3L1 was found to be a potent chemoattractant for immature DCs. Despite the fact that post-translational modifications of LD78β/CCL3L1 by proteolytic cleavage further increase the monocyte chemotactic activity of this MIP-1α variant, these do not affect its DC chemotactic potency (figure 1). In addition, immature DCs express G protein-coupled receptors for chemotactic factors other than chemokines, such as platelet-activating factor, anaphylatoxin and chemerin [15].

LCs present in normal dermis express the chemokine receptor CCR6 (among others) [15, 20]. Similarly to these immature, resting LCs, LCs in LCH lesions express CCR6 [17, 21]. Because the CCR6 ligand, MIP3α/CCL20 is expressed by cutaneous keratinocytes [22], expression of CCR6 may be one of the mechanisms that keep pathological LCs anchored in the epidermis. After activation, LCs downregulate CCR6, which allows them to leave the skin, and upregulate a distinct chemokine receptor, CCR7, the ligands for which are expressed by cells in regional lymph
of LCs showed that GM-CSF and TNF-α are important for the maturation and trafficking of LCs [23].

A study of growth factors that cooperate in the generation of LCs from hematopoietic stem cells (CD34+ precursors) also demonstrated that mature LCs co-expressed CCR6 and CCR7, consistent with the patterns of chemokine receptor expression and found that they all co-expressed CCR6 and CCR7, consistent with the presence of IL-1, GM-CSF and TNF-α in LCH lesions [2].

Finally, it is thought that GM-CSF and IL-1 are essential for LCs to acquire a full antigen-presenting phenotype. The presence of IL-1, GM-CSF and TNF-α in LCH lesions suggests that LCs may provide an optimal microenvironment to generate the specific, pathological LC phenotype, and to prolong their viability, possibly by creating autocrine loops [30-32].

Upregulation of the following cytokines in LCH lesions has been shown: IL-1, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-17A, IFN-γ, GM-CSF, TNF-α, transforming growth factor-β (TGF-β) and leukemia inhibitory factor (LIF) [31-35]. As mentioned above, this cytokine mixture may cause systemic and local signs, and symptoms of LCH. Predominant sources of cytokines involved in LCH are CD4+ T-helper (Th) cells and macrophages, but also the pathological LCs and eosinophils [31]. LCH lesions can be seen as inflammatory ‘niches’, which not only facilitate recruitment and retention of LCs, but also attract other types of inflammatory cells [31]. Another important cytokine-producing cell in LCH lesions are the multinucleated giant cells (MNGC) [12, 36]. These cells are found in osteitic as well as non-osteitic LCH lesions and express the characteristic osteoclast markers, tartrate-resistant acid phosphatase and vitronectin receptor, as well as the enzymes cathepsin K and matrix metalloproteinase-9 [36]. Two cytokines abundantly expressed in LCH lesions, macrophage colony-stimulating factor (M-CSF) and receptor activator NF-κB ligand (RANKL), stimulate the fusion of normal DCs to form MNGCs [12].

LCH lesions in bones are often characterized by osteolysis. In LCH cells, IL-1α and IL-1β, and other cytokines related to bone resorption, such as TNF-α, are abundantly present and may activate osteoclastic bone resorption, providing an explanation for the development of osteolytic lesions. In addition, interferon-γ (IFN-γ) stimulation has been reported to enhance the IL-1 secretion by LCH cells.

PRESENCE OF CYTOKINES IN LCH LESIONS

Studies of the characteristics of LCs indicate that granulocyte/macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-1 and tumor necrosis factor-α (TNF-α) are important for the maturation and trafficking of LCs [23]. A study of growth factors that cooperate in the generation of LCs showed that GM-CSF and TNF-α act together to generate LCs from hematopoietic stem cells (CD34+ precursor cells). The differentiation of dermal LCs to lymph node interdigitating dendritic cells is enhanced by GM-CSF and IL-1 [24, 25]. GM-CSF and IL-1 also have a role in the recruitment of LCs to various tissue sites [26]. Indeed, IL-1β is a potent inducer of monocyte chemotactic protein-1 (MCP-1)/CCL2 [27], MCP-3/CCL7 [28] and MIP-3α/CCL20 [29], ligands for CCR2, CCR1 and CCR6, respectively, which are expressed on immature DCs [15]. On the basis of these data, one can envision the proliferation and migration of Langerhans cells in LCH when the production of growth factors and the chemokine-inducing cytokines IL-1 and TNF-α is stimulated [2, 12, 29].

Normal skin dendritic cells or Langerhans cells express specific chemokine receptors. The chemokine receptor expression is altered upon cell activation and the maturation leads to preferential expression of CCR7. This receptor binds SLC/CCL21 expressed by high endothelial venules (HEV) and ELC/CCL19 produced in paracortical T cell areas. In LCH the aberrant expression of chemokines, e.g. in bone tissue, may lead to recruitment of Langerhans cells to ectopic sites where growth stimulation leads to granulomatous reactions.

Figure 1

Chemotaxis of immature, monocyte-derived dendritic cells in response to the MIP-1α variants LD78α/CCL3, LD78β/CCL3L1 and NH2-terminally truncated LD78β/CCL3L1(-2), missing the two NH2-terminal amino acids. Cells were generated as described [44].

Figure 2

Normal skin dendritic cells or Langerhans cells express specific chemokine receptors. The chemokine receptor expression is altered upon cell activation and the maturation leads to preferential expression of CCR7. This receptor binds SLC/CCL21 expressed by high endothelial venules (HEV) and ELC/CCL19 produced in paracortical T cell areas. In LCH the aberrant expression of chemokines, e.g. in bone tissue, may lead to recruitment of Langerhans cells to ectopic sites where growth stimulation leads to granulomatous reactions.
Cytokines and chemokines in Langerhans cell histiocytosis

LCH lesions generate a local ‘cytokine storm’, a term referring to both the high level and the diversity of cytokines and chemokines produced in situ. The major cellular sources of these cytokines are the LCH cells and T cells. Other producers of cytokines and chemokines might be the stromal cells of the lesion microenvironment, eosinophils and macrophages [43]. The pattern of chemokine and cytokine expression favors recruitment of Langerhans cell progenitors as well as their maturation and rescue from apoptosis, thereby explaining the pathological accumulation of LCH cells. In addition, the pleiotropic effects of the cytokines support local amplification cascades of cellular proliferation and activation, involving autocrine and paracrine stimulatory loops. Finally, several of the cytokines produced in LCH lesions are accountable for systemic symptoms such as fever, failure-to-thrive, and for the well-known sequelae such as osteolysis and fibrosis leading to organ dysfunction [17].

It has been shown that the CD1a+ cells in LCH are in an arrested state of activation and thus act like immature DCs, despite an abundant presence of TNF-α and IL-1. This ambiguous phenotype is also reflected at the level of chemokine receptor expression, although some conflicting results have been published with regard to the chemokine receptor CCR7, which is upregulated upon maturation of normal interstitial DC. However, there is firm evidence for the expression of CCR6, explaining the retention of these LC-like cells in LCH lesions in response to local overexpression of MIP-3α/CCL20, the ligand for CCR6. Furthermore, dysregulated production of chemokines by the CD1a+ LCH cells probably induces the accumulation of various other inflammatory cell types in these lesions. Thus, chemokine and chemokine receptor patterns probably explain LCH predilection sites and lesion composition. Recent advances in LCH immunology suggest that clonal changes in DCs might underlie the aberrant immune interaction with T cells, leading to a unique pathological picture [43]. Although altered immune responses may play a role in the pathophysiology of the disease, there is no evidence that LCH arises from a primary defect in the immune system [38]. Both in vivo and in situ studies are needed to clarify further the possible regulatory role of cytokines and chemokines, their receptors and inhibitors, and of the lesional inflammatory cells in the pathogenesis of LCH.

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CONCLUSION

LCH is caused by an uncontrolled clonal proliferation of DCs with characteristics of immature, although partially activated LCS. Their aberrant interactions with the lesional microenvironment and T cells present therein lead to high production levels of diverse cytokines and chemokines [43]. Signs and symptoms of LCH can be explained by the presence of granulomatous lesions, not only in skin or lymph nodes, where LCS normally reside, but also in many other sites such as bone marrow, lung and liver. Other inflammatory cells may also accumulate within these lesions, such as eosinophils, T cells and macrophages.

further contributing to the osteolytic capacity of LCH cells [35]. Furthermore, IL-1, TNF-α and IFN-γ can synergize to enhance chemokine production in various cell types such as endothelial cells, fibroblasts, keratinocytes and tumor cells [37]. In particular, chemokines activating receptors on immature DCs such as MCP-2/CCL8, MIP-1α/CCL3 and MIP-3α/CCL20 are synergistically upregulated by these cytokines and hence potentially favor further DC accumulation [37]. IL-1 and TNF-α are not just primary cytokines regulating the growth and maturation of histiocytes including Langerhans cells. The increase in TNF-α and IL-1 levels in lesional tissues of LCH patients suggests that they could also play a role in the pathophysiology of the disease, by causing the symptoms of fever, osteolysis, and hematologic and hepatic dysfunction [38]. However, despite abundant TNF-α, lesional LCH cells remain immature and produce chemokines that are responsible for the retention of these cells in LCH lesions. IL-4 (predominantly produced by lymphocytes in LCH lesions) and IL-10 (detected in macrophages in eosinophilic granulomas), suppress cell-mediated immunity, and may contribute to increased susceptibility of LCH patients to infections [1, 32]. The presence of TGF-β in LCH lesions may have important implications for the development of fibrosis [39], which is often present in end-stage LCH lesions. Pulmonary lesions are often fibrotic and the development of diabetes insipidus in LCH of the central nervous system has also been related to fibrosis [35]. T cells are an important part of the accumulation, proliferation and differentiation of cells in LCH lesions. Their presence within the lesion can be a consequence of locally produced CCL2, CCL5, CCL20 and CXCL11 [17]. The juxtaposition of T cells and LCH cells in all tissues suggests the intimate interactions of these two cell types, contributing to cytokine production. Pathogenic effects of these cytokines are likely to include chemotaxis of inflammatory cells, overexpression of adhesion molecules, and fibrosis, necrosis and osteolysis [31]. Cytokines, such as TNF-α and IL-1 enhance the expression of adhesion molecules on vascular endothelium and promote the local accumulation of leukocytes. IL-1β and TNF-α induce MCP-1/CCL2 and eotaxin/CCL11, which could explain lymphocyte and eosinophil influx [1, 40]. Alternatively, immature DCs constitutively secrete MIP-4/CCL18 and, after activation, produce the inflammatory chemokine MIP-3α/CCL20 [41]. In particular, LCH lesions overexpress the chemokine MIP-3α/CCL20, which chemoattracts immature DCs via CCR6 [42].
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