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Pathological Consequence of Misguided Dendritic Cell Differentiation in Histiocytic Diseases

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Abstract

Histiocytic disorders represent a group of complex pathologies characterized by the accumulation of histiocytes, an old term for tissue-resident macrophages and dendritic cells. Langerhans cell histiocytosis is the most frequent of histiocytosis in humans and has been thought to arise from the abnormal accumulation of epidermal dendritic cells called Langerhans cells. In this chapter, we discuss the origin and differentiation of Langerhans cells and dendritic cells and present accumulated evidence that suggests that Langerhans cell histiocytosis does not result from abnormal Langerhans cell homeostasis but rather is a consequence of misguided differentiation programs of myeloid dendritic cell precursors. We propose reclassification of Langerhans cell histiocytosis, juvenile xanthogranuloma, and Erdheim–Chester disease as inflammatory myeloid neoplasias.

1. THE MONONUCLEAR PHAGOCYTE SYSTEM

The term mononuclear phagocyte systems (MPSs) was first proposed by Ralph van Furth to describe hematopoietic cells determined by a mononuclear morphology (as opposed to polymorphonuclear granulocytes) and high phagocytic activity (Linz et al., 2007). The MPS comprises circulating monocytes and tissue-resident macrophages and dendritic cells (DCs) that populate all tissues. Besides their crucial role in tissue homeostasis, they are critically implicated in inflammation, autoimmunity, and cancer. Most cell types within the MPS share a common origin with a commitment to the MPS lineage at the stage of the macrophage and DC progenitor (MDP) (Fogg et al., 2006), yet their defined phenotype and function at the mature stage is highly distinctive.

Within this chapter, we will specifically discuss the differentiation program of the DC lineage. Moreover, we will provide evidence that human histiocytoses—a complex of disorders with a broad manifestation spectrum and potential fatal outcome—are the pathophysiological consequence of a dysregulated differentiation of the MPS emphasizing the importance of tight regulation of this process *in vivo*.

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2. THE DC LINEAGE

DCs are a heterogeneous group of hematopoietic cells that reside in all tissues but are particularly enriched in interface tissues and lymphoid organs. They were first described in the early 1970s by Ralph Steinman and Zanvil Cohn and distinguished from macrophages by their explicit morphology and superior capacity to activate T cells in an antigen-specific manner (Steinman & Cohn, 1973, 1974). DCs can be classified into two main subsets that comprise plasmacytoid DCs (pDCs) and classical DCs. pDCs exert only limited phagocytic and antigen-presenting activity and are, in the absence of inflammation, mostly restricted to lymphoid tissue. Upon activation, pDCs produce high levels of type 1 interferon and are closely implicated during viral infections and autoimmune diseases. This chapter will mainly discuss the development and differentiation of classical DCs. The origin and development of pDCs is comprehensively discussed in a recent review by Reizis (2010).

In contrast to pDCs, classical DCs can be found in steady-state conditions in all lymphoid, connective, and interface tissues. With respect to their primary localization, they can generally be divided into lymphoid organ-resident and nonlymphoid tissue-resident DCs. In addition to lymphoid-resident DCs, peripheral lymph nodes (LNs) also harbor tissue-resident migratory DCs that have migrated to the LN via the lymphatic vessels and are characterized by high expression of MHCII but intermediate CD11c expression. Migration of tissue-resident DCs to the LN is constitutive and occurs at slow rate in the steady state, dramatically increases during inflammatory conditions, and depends on the expression of the chemokine receptor CCR7 and its ligands (Ohl et al., 2004). During inflammation, an additional DC population deriving from blood monocytes and called inflammatory DC, which includes a subpopulation termed TNF–iNOS-expressing DCs (Tip DC), transiently infiltrates the injured tissue and dissipates when the injury resolves (Merad, Sathe, Helft, Miller, & Mortha, 2013).

Due to their potent endocytosis activity and migratory capacity, DCs are dedicated to sample peripheral tissue antigens, process and present antigens, migrate to the LNs, and initiate a specific T-cell-mediated immune response against invading pathogens while maintaining tolerance to self-antigens (Idoyaga et al., 2013; Mellman & Steinman, 2001; Steinman, Hawiger, & Nussenzweig, 2003).

In addition to their specific morphology, DCs are phenotypically defined by the expression of CD45, high amounts of major histocompatibility class II antigens (MHCII), and the integrin CD11c while lacking other hematopoietic lineage markers. This definition is limited by the promiscuous expression of the markers MHCII and CD11c within the hematopoietic lineages. High MHCII expression levels are shared by B cells and activated macrophages, while CD11c is expressed on activated T cells, B cells, NK cells, and distinct macrophage populations. Additional DC marker, for example, the tyrosine kinase receptor fms-like tyrosine kinase 3 (Flt3, also expressed on hematopoietic stem cells and DC precursor), is useful to distinguish DC from tissue macrophages; however, their applicability is limited due to sensitivity to collagenase digestion, a technique that is often required for the isolation of nonlymphoid tissue DCs (Miller et al., 2012). The very recent identification that the zinc finger transcription factor zbtb46 is a marker specific for the DC lineage within the MPS represents an exciting prospect in terms of DC identification and manipulation using zbtb49 DTR mouse models (Meredith et al., 2012; Satpathy et al., 2012).

3. ORIGIN AND REGULATION OF DC DIFFERENTIATION

Similar to other hematopoietic cells, the homeostasis of DCs in lymphoid and nonlymphoid tissue depends on the dynamic balance between local division of terminally differentiated cells, cell survival, and replenishment by circulating precursors. In addition, tissue-resident

DCs contribute to the DC pool in the LNs where they present antigen to T cells (Randolph, Angeli, & Swartz, 2005).

Differentiated DCs display only very low proliferation rates within the tissue (Ginhoux et al., 2009; Kabashima et al., 2005; Waskow et al., 2008), which is tightly regulated by Flt3 and the cytokine lymphotoxin-β receptor (Ginhoux et al., 2009; Kabashima et al., 2005; Waskow et al., 2008). Additionally, Flt3 could be implicated in cell survival (Singh et al., 2012) and local Csf-2, which seems to exert antiapoptotic effects in nonlymphoid tissue (Greter, Helft, et al., 2012; Hirata, Egea, Dann, Eckmann, & Kagnoff, 2010).

Parabiotic mouse models demonstrated that DCs, with the exception of Langerhans cells (LCs), are rapidly replaced in the tissue by cells derived of the parabiont partner supporting the hypothesis of a rapid turnover of differentiated DCs even in the steady state with a constant reconstitution mediated by circulating precursors (Ginhoux et al., 2009; Liu et al., 2007; Merad et al., 2002). In the past decade, several studies have greatly contributed to the understanding of the origin and regulation of these specific precursors.

In humans and mice, monocytes/macrophages and DCs share a common precursor named macrophage–dendritic cell progenitor (MDP; Fig. 5.1), which has lost granulocytic potential and marks the stage of commitment to the MPS during development (Fogg et al., 2006). MDPs give rise to monocytes and a DC-restricted precursor termed common DC precursor or CDP. CDPs have lost monocyte–macrophage differential potential and give rise exclusively to pDCs and circulating precursors of classical DCs—the pre-DC (Liu et al., 2009; Naik et al., 2007; Onai et al., 2007)—which migrate to the tissue and differentiate into lymphoid tissue $CD4^{-}CD8\alpha^{+}$ and $CD4^{+}CD8\alpha^{-}$ DCs (Liu et al., 2009) and nonlymphoid tissue $CD103^{+}CD11b^{-}$ and $CD103^{+}CD11b^{+}$ DC (Bogunovic et al., 2009; Ginhoux et al., 2009). The commitment of hematopoietic progenitors to the DC lineage is strongly dependent on the receptor fms-like tyrosine kinase 3 (Flt3) and its ligand Flt3L as loss of Flt3 expression in these progenitors results in the loss of DC differentiation potential, whereas enforcement of its expression in Flt3-negative progenitors restores their DC potential (Merad & Manz, 2009). As depicted in the preceding text, Flt3 and its ligand also contribute to the homeostasis of peripheral DC by regulating their proliferation and survival in tissues. Mice lacking either Flt3 or its ligand have strongly reduced numbers of pDCs and DCs in lymphoid organs and peripheral tissues (Ginhoux et al., 2009; Waskow et al., 2008), whereas injection of Flt3L in mice and humans leads to substantial increase of these cells in the blood and different tissues (Fong et al., 2001; Manfra et al., 2003).

4. DC HETEROGENEITY

Due to their differential functional properties and their requirement of distinct transcription factors during development, lymphoid-resident and tissue-resident DCs can further be classified into two major subpopulations commonly distinguished based on the expression of different antigen or integrin on the cell surface (Miller et al., 2012). One subset of DC present in lymphoid and nonlymphoid tissue is dependent on the transcription factor inhibitor of DNA binding 2 (ID2), interferon-regulatory factor 8 (IRF8), and basic leucine zipper transcriptional factor ATF-like (BATF), and the second subpopulation differentiates independently of these factors in lymphoid and nonlymphoid tissue.

5. BATF-IRF8-ID2-DEPENDENT DC LINEAGE

$Batf3^{-/-}$ mice entirely lack a distinct population of DCs in LNs and spleen in steady state that express the CD8 antigen but not the CD11b integrin, or other macrophage markers, while $CD8^{-}$ DCs in lymphoid tissues are unaffected (Hildner et al., 2008). $CD8^{+}$ DCs account for 20–40% of total DC in lymphoid organs and are phenotypically immature in the

steady state (Shortman & Heath, 2010). Besides Batf3, their differentiation is strongly dependent on Flt3l (Ginhoux et al., 2009), IRF8 (Schiavoni et al., 2002), and ID2 (Hacker et al., 2003). In addition, the mammalian target of rapamycin promotes the differentiation of $CD8^+$ DC; while the loss of its negative regulator, the tumor suppressor gene PTEN leads to expansion of $CD8^+$ cells in murine lymphoid tissue (Sathaliyawala et al., 2010).

In nonlymphoid tissues, the correlate to the lymphoid-resident $CD8^+$ DCs with regard to origin and function is represented by DCs expressing the integrin CD103 but lacking CD8 markers (Helft, Ginhoux, Bogunovic, & Merad, 2010). $CD103^+$ DCs populate most connective tissues and express low levels of CD11b or other macrophage marker. CD103 expression on DC is modulated by the tissue environment especially by local production of the cytokine Csf-2 (Edelson et al., 2011; Greter, Helft, et al., 2012; Zhan et al., 2011). Moreover, most $CD103^+$ cells express the C-type lectin receptor langerin with exception of intestinal $CD103^+$ cells (Merad, Ginhoux, & Collin, 2008).

$CD103^+CD11b^-$ nonlymphoid tissue DCs share the same differentiation requirements in terms of transcription factors and regulators as lymphoid-resident $CD8^+$ DCs (Edelson et al., 2010; Ginhoux et al., 2009). Their dependence on Flt3 during differentiation is even more pronounced as they are completely absent in Flt3-deficient bone marrow chimeras, while $CD8^+$ DCs are only reduced by one-third in lymphoid tissues (Ginhoux et al., 2009). It is important to note that in infected mice, tissue-resident $CD103^+$ DCs and $CD8^+$ lymphoid-resident DCs can still form in the absence of Batf3. The development of $Batf3^{-/-}$ $CD103^+$ and $CD8^+$ DC is dependent on the related transcription factors Batf and Batf2 that compensate for the lack of Batf3 in a cytokine-driven manner and mediate the physiological expansion of the DC subpopulations based on their common capacity to interact with other transcription factors such as IRF8 (Tussiwand et al., 2012).

In the intestine, an additional $CD103^+$ DC population resides in the lamina propria and displays high expression levels of CD11b. Lamina propria $CD103^+CD11b^+$ DCs develop independently of Batf3, IRF8, or ID2 and must therefore be regarded as a separate entity (M. Bogunovic and M. Merad (unpublished data); Edelson et al., 2010).

In addition to sharing the same precursor and differentiation program, lymphoid-resident $CD8^+$ DCs and tissue-resident $CD103^+CD11b^-$ DC share a similar transcriptome in the steady state (Miller et al., 2012). They express similar levels of pattern recognition receptors (e.g., high levels of the toll-like receptor TLR3) to sense double-stranded viral RNA and exhibit comparable repertoires of lectin-like receptors and distinct chemokine receptors (e.g., XCR1) (Hashimoto, Miller, & Merad, 2011).

Their phenotypical resemblance is mirrored by their common functional specialization. Batf3-dependent DCs excel in presenting microbial antigen and cell-associated antigen to $CD8^+$ T cells (Bedoui et al., 2009; den Haan, Lehar, & Bevan, 2000; Helft et al., 2012; Hildner et al., 2008). Their critical and nonredundant role in the induction of a specific $CD8^+$ T-cell immune response is primarily based on their superior ability to cross present exogenously acquired antigen on MHCI molecules. $CD8^+$ DCs express, for instance, the GTPase Rac2 that restricts phagosomal protease activity, thus allowing the transfer of intact peptide to the cytosol for sufficient presentation on MHCI molecules (Savina et al., 2009). Besides, Batf3-dependent DCs are very potent producers of IL-12 and IL-15 (Mattei, Schiavoni, Belardelli, & Tough, 2001; Sung et al., 2006), two cytokines involved in the differentiation of cytotoxic $CD8^+$ T cells. Of note, Batf3-dependent DCs also exclusively express the chemokine receptor XCR1, which ligand XCL1 is produced by naive $CD8^+$ T cell establishing an additional link between these DC subpopulation and $CD8^+$ T cells (Dorner et al., 2009).

In addition to efficiently mounting a cytotoxic $CD8^+$ T-cell response to pathogens and tumor antigens, Batf3-dependent DCs have also been implicated in peripheral tolerance in the steady state by promoting deletional tolerance of antigen-specific CD8+ T cells (Qiu et al., 2009) and inducing antigen-specific regulatory T cells (Idoyaga et al., 2013).

6. BATF3–ID2–IRF8-INDEPENDENT DC LINEAGE

Although DCs of the Batf3–ID2–IRF8-independent lineage share high CD11b expression while lacking CD8 expression, they are phenotypically highly heterogeneous both in lymphoid tissue and in nonlymphoid tissue with regard to the expression of markers like CD103, CD172a, F4/80, and CX3CR1 (Helft et al., 2010). This heterogeneity is also reflected by a differential dependence on specific transcription factors, for example, IRF4, IFR 2, and RBPJ, and cytokines during differentiation. A significant portion of lymphoid $CD11b^+$ DCs, for instance, develop independently of Flt3 and Flt3L (McKenna et al., 2000; Waskow et al., 2008) and require the macrophage cytokine Csf-1 suggesting that $CD11b^+$ $MHCII^+$ $CD11c^+$ cells refer to mixed cell populations that likely contain macrophages and DCs (Bogunovic et al., 2009).

Given the diverse origins of $CD11b^+$ DCs, significant effort has recently been invested in further refining the phenotypical and functional subclassifications of this lineage. The expression of the high-affinity IgG receptor FcγRI, also termed CD64, and the macrophage marker Mertk differentiates $CD11b^+$ DCs from macrophages (Cerovic et al., 2013; Gautier et al., 2012; Langlet et al., 2012; Tamoutounour et al., 2012).

$CD11b^+$ DCs have been mainly implicated in MHCII presentation to $CD4^+$ T cells, which is in line with their enhanced expression of MHCII-antigenic pathway-associated proteins (Merad, Sathe, Helft, Miller, & Mortha, 2013). In an influenza infection model, $CD11b^+$ predominantly presents antigen to $CD4^+$ T cells *ex vivo* (Kim & Braciale, 2009). Moreover, antigen delivery specifically to $CD11b^+$ DCs induces a more efficient $CD4^+$ T-cell response *in vivo* in steady state than a CD8 T cell response. (Dudziak et al., 2007; Idoyaga et al., 2013).

7. MONOCYTE-DERIVED DCs AND INFLAMMATORY DCs

In vitro, human monocytes efficiently differentiate into DCs in the presence of Csf-2 (Sallusto & Lanzavecchia, 1994) and had therefore originally been considered to be the immediate precursor of all DC before this hypothesis was challenged by the discovery of the common DC progenitors, the CDPs (Onai et al., 2007).

During inflammation, however, monocytes do give rise to cells expressing intermediate levels of MHCII and CD11c (Dominguez & Ardavin, 2010). However, it remains unclear whether these cells represent bona fide DCs or rather constitute inflammatory antigen-presenting cells. In addition, evidence has accumulated over the last years that some inflammatory DCs might also directly derive from tissue-resident hematopoietic progenitors (Takizawa, Boettcher, & Manz, 2012).

8. THE LC EXCEPTION

LCs are DCs that reside in the outer layer of the epidermis of the skin. Phenotypically, LCs are characterized by lower MHCII expression levels, intermediate CD11c levels, and high expression of langerin (CD207), which is associated with the formation of characteristic intracytoplasmatic Birbeck granules. Moreover, LCs express the markers CD11b and F4/80, the sialoglycoprotein CD24, the adhesion molecule E-cadherin, and the epithelial cell adhesion molecule EpCAM and lack the chemokine receptor CX3CR1 (Borkowski, Nelson,

Farr, & Udey, 1996; Merad et al., 2008; Stutte, Jux, Esser, & Forster, 2008; Tang, Amagai, Granger, Stanley, & Udey, 1993).

In contrast to all other DC populations, LCs do not arise from adult bone marrow-resident myeloid progenitor cell, but from embryonic precursors—mostly fetal liver-derived monocytes (Hoeffel et al., 2012) that populate the skin prior to birth and maintain themselves locally throughout life in steady-state conditions (Merad et al., 2002). However, upon severe inflammatory injury such as UV light exposure, LCs are replaced by circulating Gr-1^{hi} blood monocytes (Ginhoux et al., 2006). Nevertheless, it remains elusive if these blood-borne monocyte-derived LCs during inflammation are equivalent to steady-state LCs in terms of function and turnover in the tissue.

In line with their distinctive ontogeny compared to other DCs, LCs are independent of Flt3 and develop normally in Flt3 and Flt3L-deficient mice (Ginhoux et al., 2009) but require Csf-1 receptor (Csf-1R) for their development (Ginhoux et al., 2006). Strikingly, LC can still form in the absence of Csf-1 but fail to develop in the absence of IL-34, a recently identified cytokine in humans, mice, and birds shown to bind the Csf-1R with higher affinity than Csf-1 (Greter, Lelios, et al., 2012; Wang et al., 2012). These results are consistent with recent data showing that IL-34 is the only Csf-1R ligand expressed in the epidermis of mice and humans, whereas both Csf-1 and IL-34 are produced in mouse and human dermis (Greter, Lelios, et al., 2012). Interestingly, we found that IL-34 was not required for the recruitment and differentiation of monocytes into LCs in skin exposed to UV light but was required to maintain monocyte-derived LC in the epidermis once the inflammation resolves (Greter, Lelios, et al., 2012). In addition to IL-34, autocrine production of TGF-β controls LC homeostasis and is essential for the retention of LCs in the epidermal tissue (Bobr et al., 2012; Borkowski, Letterio, Farr, & Udey, 1996; Kaplan et al., 2007; Zahner et al., 2011).

The functional role of epidermal LCs during immune responses to pathogens and peripheral tolerance varies with regard to the type of inflammatory signal and its localization. One additional confounder hereby is certainly, especially in older studies, the insufficient discrimination of LC from other skin DCs, especially the langerin-expressing dermal $CD103^+$ DCs.

Upon *Candida albicans* infection, epidermal LCs fail to induce Th1 or $CD8^+$T-cell response in *C. albicans* infection model but promote strong Th17 differentiation (Igyarto et al., 2011). However, migratory LCs were also shown to mount a $CD4^+$T-cell response after immunization with recombinant hen egg lysozyme–MCC protein, although less efficient when dermal DCs were absent (Shklovskaya, Roediger, & Fazekas de St Groth, 2008). Additionally, our group showed that host LCs are sufficient to prime allogeneic T-cell responses to induce skin graft-versus-host disease (Merad et al., 2004).

In the context of contact hypersensitivity, specific deletion of LCs resulted in enhanced immune responses (Kaplan, Jenison, Saeland, Shlomchik, & Shlomchik, 2005). These results are in line with human studies revealing a fundamental role of LCs in the homeostasis of skin-resident regulatory T cells (Seneschal, Clark, Gehad, Baecher-Allan, & Kupper, 2012),

9. THE HUMAN DC LINEAGE

The differentiation program and functional specializations of human DCs and their subpopulation are significantly less understood than those of their murine counterparts due to the limited accessibility to human tissue. It is mainly based on studies on skin and blood DCs or DC derived from tissue explants, obtained in the setting of specific pathologies like

malignant transformation and/or chronic tissue injury that likely affect DC composition and biology.

Analogous to murine DCs, human DCs are defined as hematopoietic cells expressing high levels of MHCII and CD11c while lacking other specific lineage markers. In humans, however, CD11c seems to be more promiscuous as it is also expressed on most monocytes and macrophages (O'Doherty et al., 1994).

In the human blood, the two DC subsets are defined by either expression of the marker CD1c (also termed BDCA1) or CD141 (or BDCA3) (Dzionek et al., 2000; MacDonald et al., 2002). $CD141^+$ DCs only account for a minor population of DCs in the blood. CD1c+ and CD141 + DCs have also been identified not only in the human spleen and LN (McIlroy et al., 2001; Poulin et al., 2012; Segura et al., 2012; Velasquez-Lopera, Correa, & Garcia, 2008) but also in the bronchoalveolar lavage fluid, lung, tonsils, decidua, and kidney (Ban, Kong, Qu, Yang, & Ma, 2008; Demedts, Brusselle, Vermaelen, & Pauwels, 2005; Fiore et al., 2008; Haniffa et al., 2012; Jongbloed et al., 2010; Lindstedt, Lundberg, & Borrebaeck, 2005; Tsoumakidou, Tzanakis, Papadaki, Koutala, & Siafakas, 2006; Yu et al., 2013).

In human skin, DCs are present throughout the epidermal ad dermal layers. LCs in the human epidermis not only share the expression of CD45, MHCII, EpCAM, and langerin with their murine counterparts but also express high levels of CD1a, which is absent from murine LCs (Hunger et al., 2004; Valladeau et al., 1999). Earlier studies have showed that the dermis contains $CD1a^+$ $CD14^-$DCs and the $CD1a^-CD14^+$ DCs (Nestle, Zheng, Thompson, Turka, & Nickoloff, 1993). Accumulated evidence suggests that the $CD1a^-CD14^+$ DCs do not represent a homogeneous DC population, but are contaminated by dermal macrophages that express CD163 in addition to CD14 (Zaba, Fuentes-Duculan, Steinman, Krueger, & Lowes, 2007). More recent studies revealed that the dermis also contains $CD141^+$ DCs that are related to $CD141^+$ DC identified in the blood, whereas CD1a +$CD14^-$ DCs also express CD1c and are related to circulating CD1c+ DCs the present suggest (Haniffa et al., 2012).

Interestingly, $CD141^+$ DCs uniquely express the markers XCR1 and Clec9A as well as high levels of TLR3, excel in the production of IL-12 and type 1 and type 3 interferon upon stimulation with a TLR3 agonist, and efficiently cross present cell-associated and soluble antigen to $CD8^+$ T cells, implying a phenotypical and functional proximity to murine CD8+ DCs (Bachem et al., 2010; Haniffa et al., 2012; Harman et al., 2013; Huysamen, Willment, Dennehy, & Brown, 2008; Lauterbach et al., 2010). This conclusion was supported by a recent gene chip meta-analysis of the transcriptome of human and murine DC subsets, which revealed a close relationship between human $CD141^+$ DC and murine $CD8^+$ DCs, while the transcriptome of human $CD1c^+$ DCs is more related to mouse $CD11b^+$ DCs (Robbins et al., 2008).

$CD141^+$ DCs also express Batf3 and IRF8, while lacking IRF4 (Robbins et al., 2008), and inhibition of Batf3 in cultures of human hematopoietic progenitors abolishes the differentiation of $CD141^+$ DCs but not $CD1c^+$ DCs (Poulin et al., 2012), implying also a similar regulation of human and murine DC subset differentiation. In contrast, specific IRF8 mutations in immunodeficient patients are associated either with a total absence of DCs and monocytes or with a depletion of blood $CD1c^+$ rather than $CD141^+$ DCs (Hambleton et al., 2011). Additionally, $CD1c^+$ DCs also seem to be able to produce high amount of IL-12 and to cross prime $CD8^+$ T cells (Mittag et al., 2011).

Taken together, these findings elucidate fundamental differences between human and murine DC subsets despite significant overlaps emphasizing the need of further detailed

characterization of DC subsets and their biology in the human setting during steady state and pathology.

10. HUMAN HISTIOCYTOSES AS A RESULT OF DYSREGULATED DIFFERENTIATION OF THE HUMAN MYELOID COMPARTMENT

Histiocytic disorders are diseases arising from abnormal development or function of "histiocytes." Literal translation of *histiocyte* is "tissue cell." Although histiocyte refers only to tissue-resident DCs and macrophages, classification committees remain loyal to the archaic nomenclature for "histiocytosis" that is meant to encompass all cells of the mononuclear phagocytic system (Chu et al., 1987; Favara et al., 1997). Histiocytic diseases are characterized by presumed lineage and biological behavior into DC disorders, macrophage-related disorders, and malignant histiocytic disorders (Favara et al., 1997; Jaffe, Weiss, & Facchetti, 2005; Table 5.1). With the caveat that the phenotype of mononuclear phagocytes may be dynamic and influenced by environmental stimuli, the cellular origins of pathological histiocytes may be inferred according to cell-surface markers and histologic characteristics (Table 5.2; Fig. 5.1). The "histiocytic" umbrella therefore covers a wide range of disorders, from physiological reaction to pathological inflammatory stimuli to bona fide malignancy, with the common link of abnormal phagocytes. Understanding of the pathogenesis of histiocytic disorders and the identities of the myeloid culprits is advancing rapidly along with improved experimental technologies and molecular characterization of normal and pathological myelomonocytic populations.

10.1. Langerhans cell histiocytosis

10.1.1 Clinical overview—Langerhans cell histiocytosis (LCH), the most common histiocytic disease in humans, is estimated to occur in 5 children per million, which is similar incidence as acute myelogenous leukemia or Hodgkin lymphoma (Guyot-Goubin et al., 2008; Salotti et al., 2009; Stalemark et al., 2008). The majority of cases are in children, but LCH does occur *de novo* in approximately 1 adult per million (Baumgartner, von Hochstetter, Baumert, Luetolf, & Follath, 1997). The clinical presentations of LCH are highly variable, from a trivial self-resolving lesion to aggressive, life-threatening multisystem disease. Infrequent occurrence and overlapping presenting symptoms with more common pediatric conditions make LCH a challenging diagnosis to consider. However, once biopsy is performed, diagnosis is generally straightforward as inflammatory lesions with infiltrating CD1a+/CD207+ histiocytes are specific to LCH (Table 5.2; Fig. 5.2).

LCH is generally categorized according to extent of disease (single lesion vs. multifocal) and organ systems involved ("nonrisk" vs. lung, liver, spleen, or bone marrow "risk"). Lesions in the skull merit special consideration: Lesions of the facial bones and skull base are associated with increased risk of subsequent development of diabetes insipidus and other pituitary endocrinopathies as well as LCH-associated neurodegenerative disease (Grois et al., 2010). Overall survival was nearly 100% in patients with lesions limited to nonrisk organs, where 5-year survival was 74% for patients with risk organ involvement on the Histiocyte Society LCH-II trial (Gadner et al., 2008).

Due to incomplete understanding of the pathogenesis of LCH, treatment strategies have relied on empiric observation and cooperative clinical trials. Treatment for most isolated bony lesions is surgical resection or curettage. The current frontline standard of care for most children with multifocal, multisystem LCH is vinblastine and prednisone, though this strategy is poorly tolerated in adults (Cantu et al., 2012). Recurrent or refractory disease is relatively common, with one registry study reporting 46% reactivation rate within five years of initial response to therapy in patients with multisystem LCH (Minkov et al., 2008). Many

patients who fail to achieve a durable response to vinblastine/prednisone do respond to salvage therapies that include agents with efficacy in treatment of acute myelogenous leukemia, including cytarabine, cladribine, and clofarabine (Cantu et al., 2012; Egeler, de Kraker, & Voute, 1993; Rodriguez-Galindo, Jeng, Khuu, McCarville, & Jeha, 2008; Weitzman et al., 2009). Some patients with progressive disease despite chemotherapy have been cured with allogeneic hematopoietic stem cell transplant (Akkari et al., 2003; Cooper et al., 2008; Steiner et al., 2005).

10.2. LCH pathogenesis

10.2.1 A brief history—LCH has a complicated history and remains an enigmatic disorder. What has come to be known as LCH first appeared in medical literature around the turn of the 1900s with reports of children with combinations of lytic bone lesions, mucosal lesions, and diabetes insipidus. Other case reports followed describing infants with hepatosplenomegaly, lymphadenopathy, and anemia along with lytic granulomatous bone lesions (reviewed in Arceci, 1999). Hand–Christian–Schüller disease came to describe patients with multifocal eosinophilic granulomas, and Letterer–Siwe disease described a fulminant, lethal disorder including histiocytic infiltration of bone marrow, liver, and spleen. In 1953, Dr. Lichtenstein observed common histology of the many eponymous classifications of what we now recognize as LCH and hypothesized that they represent a spectrum of a common disorder, which he proposed to be collectively named histiocytosis X (Lichtenstein, 1953). The “X” indicated incomplete knowledge of pathogenesis and cell of origin. With the advent of electron microscopy, Drs. Nezelof and Basset recognized a common cytoplasmic structure, the pentilaminar Birbeck granule, in both epidermal LCs and histiocytes in histiocytosis X lesions (Nezelof, Basset, & Rousseau, 1973). This disorder was then rebranded “Langerhans cell histiocytosis.”

10.2.2 Genetic inheritance and LCH—One family registry study indicated that rare LCH patients may have first-degree relatives with LCH, though it is not clear if this is significantly greater than one would expect by chance. Interestingly, monozygotic twins were observed to share a higher risk of multisystem risk LCH than presumed fraternal twins (92% vs. 10%), which was interpreted as evidence for inherited risk factors (Arico, Scappatici, & Danesino, 2005). Common precursor cells in shared fetal circulation of monozygotic twins may also explain the observation. Of over 900 patients seen at the Texas Children’s Histiocytosis Clinic, there have been only one family with nontwin sibling LCH patients and one with a parent and child both having LCH. While it remains plausible that potential risk alleles such as tumor necrosis factor gene promoter polymorphisms (McClain, Laud, Wu, & Pollack, 2003) or exposures such as parental occupational exposure to metal (Jubran, Marachelian, Dorey, & Malogolowkin, 2005) may be shared by siblings, Mendelian inheritance of penetrant LCH “genes” in the majority of cases seems unlikely. Additional reported associations from family studies that may inform LCH pathogenesis include increased risk of other cancers in LCH patients (Egeler et al., 1998) and increased risk of family history of thyroid disease (Bhatia et al., 1997).

10.3. Immunological disorder or cancer?

The designation “Langerhans cell” histiocytosis assumes a model in which LCH arises from aberrant epidermal LCs (Nezelof et al., 1973). Two possibilities for pathogenesis that have been considered and debated over the past decades include immune dysregulation versus malignant transformation of epidermal LCs.

10.3.1 Immune dysregulation in LCH—Examination of LCH biopsy under the microscope reveals a complex inflammatory process, and LCH lesions are often described as granulomas. In addition to the pathognomonic “Langerhans” cells, inflammatory infiltrate

typically includes lymphocytes, macrophages, and eosinophils (Fig. 5.1). Despite early suggestions of a viral etiology for LCH, more recent investigations fail to identify LCH-associated pathogens (Jeziorski et al., 2008), and LCH patients have not been reported to have clinically evident immunodeficiency or specific susceptibility to infectious agents (Nezelof & Basset, 2004).

The lesional LCH cells have some features of immature epidermal LCs, including expression of high levels of the lectin langerin (CD207) and the antigen CD1a and lack of migration to draining LNs. However, like activated DCs, CD207+ cells that accumulate in LCH lesions express high levels of T-cell costimulatory molecules and proinflammatory cytokines (Allen et al., 2010; Geissmann et al., 2001; Laman, Leenen, Annels, Hogendoorn, & Egeler, 2003). Dozens of cytokines, chemokines, and cytokine and chemokine receptors have been hypothesized to play roles in LCH pathogenesis, though the mechanisms by which the pathological LCs orchestrate inflammation within the lesions remain to be fully defined (reviewed in Allen et al., 2010; Geissmann et al., 2001; Laman et al., 2003). In addition to the local “cytokine storm” within LCH lesions, many studies also report increased levels of plasma and serum proinflammatory cytokines and chemokines in patients with active LCH, including tumor necrosis factor alpha, soluble interleukin-2 receptor alpha, RANK ligand, and osteoprotegerin (Coury et al., 2008; Rosso et al., 2003; Rosso, Roy, Zelazko, & Braier, 2002). One study reported interleukin-17A (IL17A) production by LCH cells resulting in elevated systemic levels of IL17A, though this observation has not been reproduced (Allen & McClain, 2009; Coury et al., 2008; Makras et al., 2012; Peters, McClain, & Allen, 2011). Interestingly, regulatory $CD4^+CD25^+$ T cells (Tregs) are enriched in LCH lesions, and patients with LCH may have impaired skin delayed-type hypersensitivity responses (Senechal et al., 2007).

While immunological trigger for LCH remains to be proven, the immune function in patients with LCH is not normal, and inflammation accounts for many of the clinical manifestations of the disease. The clinical efficacy of thalidomide in some patients demonstrates the therapeutic potential for therapies targeting the inflammatory cascades set in motion by pathological DCs in LCH (McClain & Kozinetz, 2007).

10.3.2 LCH as a neoplastic disorder—In parallel with a search for an inflammatory etiology for LCH, investigators have examined the possibility that LCH may arise from malignant transformation of the epidermal LC. A major finding supporting the neoplastic hypothesis is the observation that CD1a+ cells from LCH lesions are clonal (Yu et al., 1994; Willman et al., 1994). An exception to this observation may be smoking-associated isolated pulmonary LCH in adults, where infiltrating histiocytes may be polyclonal (Yousem, Colby, Chen, Chen, & Weiss, 2001). Despite clonality, LCH is not a classic malignancy. The pathological DCs do not appear dysplastic. Mitoses are rarely seen in LCH lesions, with Ki67 positive by immunohistochemistry in fewer than 2% of cells (Senechal et al., 2007). It is also possible that clonality represents reactive proliferation of a local progenitor, as regional clonality has been shown in normal epidermal LCs under physiological conditions (Merad et al., 2002; Waskow et al., 2008). Cells from LCH lesions are not viable without growth factor *in vitro*, and no successful xenograft models have been reported (Nezelof & Basset, 2004). Karyotypes from LCH biopsies are typically normal. While genetic lesions have been reported in some cases of LCH, a study using array CGH and single-nucleotide polymorphism arrays with DNA from purified CD1a+ LCH lesion cells failed to identify gross chromosomal abnormalities (da Costa et al., 2009). While no recurrent genetic deletions or translocations have been reported, the activating *BRAFV600E* point mutation was discovered in the majority of a series of LCH lesions, further supporting classification of LCH as a myeloid neoplastic disorder (Badalian-Very et al., 2010).

Adding to the complexity of deciphering the pathogenesis of LCH, as biological understanding has LCH evolved, so have the definitions of "cancer" (Hanahan & Weinberg, 2011). Some "hallmarks of cancer" observed in LCH include mutated *BRAF*, (Badalian-Very et al., 2010), avoiding immune destruction through increased Treg infiltrate and TGF production (Allen et al., 2010; Egeler, Favara, van Meurs, Laman, & Claassen, 1999; Favara & Steele, 1997; Senechal et al., 2007), tumor-promoting inflammation through local and systemic cytokine storm (Allen et al., 2010; Laman et al., 2003), activating invasion and metastasis through expression of metalloproteases (Allen et al., 2010; da Costa et al., 2005; Hayashi et al., 1997), and resisting cell death through overexpression of *BCL21* and *BAX* by CD207+ cells (Allen et al., 2010; Amir & Weintraub, 2008; Schouten et al., 2002).

10.4. Cousins of LCH: juvenile xanthogranuloma and Erdheim–Chester Disease

10.4.1 Juvenile xanthogranuloma—Juvenile xanthogranuloma (JXG) is a histiocytic disorder, most common in early childhood, characterized by lesions composed of cells phenotypically similar to the dermal dendrocyte: $CD14^+$, $CD68^+$, $fascin^+$, and factor $XIIIa^+$ (Figure 5.2, Table 5.2). The lipid-laden multinucleated "Touton" giant cells have a characteristic macrophage morphology (Fig. 5.2). The incidence is not proven, but may be estimated at 1 case per million children (Janssen & Harms, 2005). JXG typically manifests as self-limited skin lesions in the neck, trunk, and head. However, almost any organ system can be involved, including orbit and brain (Dehner, 2003). Skin lesions in children generally resolve spontaneously over time. However, large retroperitoneal masses, liver lesions, bone marrow involvement, or brain tumors may be progressive and potentially life-threatening. LCH-based chemotherapy strategies have been effective in some patients with systemic JXG (Stover, Alapati, Regueira, Turner, & Whitlock, 2008).

The etiology of JXG and basis for prevalence in children and tissue distribution is not known. In a single patient, HUMARA assay analysis of a single lesion suggested the cells (90% histiocytes) were clonal (Janssen, Folster-Holst, Harms, & Klapper, 2007). Interesting associations have been reported between patients with JXG, neurofibromatosis (types 1 and 2), and juvenile chronic myelogenous leukemia that potentially implicates Ras pathway hyperactivity as shared pathogenesis among these hematologic disorders (Iyengar, Golomb, & Schachner, 1998; Tan & Tay, 1998; Winh & Sweet, 2002).

10.4.2 Erdheim–Chester disease—Erdheim–Chester disease (ECD) is an extremely rare histiocytic disorder of adults, with fewer than 500 cases reported in the literature. Histologically, ECD can be indistinguishable from the foamy macrophages of JXG: CD14+, CD68+, CD1a−, and CD207− (Figure 5.2, Table 5.2) (Winh & Sweet, 2002). Patients with ECD develop Table characteristic multifocal osteosclerotic lesions of long bones and may also develop extraskeletal histiocytic infiltrates at other sites. Like LCH, patients may develop pituitary involvement with diabetes insipidus. Retroperitoneal, renal, and cardiac involvement are unique features of ECD. Mean survival from time of diagnosis is 3 years (Veyssier-Belot et al., 1996).

As with LCH and JXG, the etiology of ECD remains elusive. Lesions have characteristic inflammatory infiltrate. Proinflammatory cytokines are expressed within ECD lesions, and increased expression of IFN-α, interleukin-6, interleukin-12, and monocyte chemotactic protein-1 has been observed in serum from ECD patients, interpreted as a systemic Th1 response (Arnaud et al., 2011; Stoppacciaro et al., 2006). Therapy with interferon-alpha (IFN-α) or recombinant interleukin-1 receptor-α (IL-1Rα) has been effective in some patients. Like LCH, significant cellular proliferation is not observed within ECD lesions (Stoppacciaro et al., 2006). Results from clonality studies are mixed with reports of monoclonal and polyclonal pathological histiocytes within lesions (Al-Quran, Reith,

Bradley, & Rimsza, 2002; Chetritt et al., 1999; Vencio et al., 2007). Differences may be due to technical issues or possibly disease subgroups. The recent finding of frequent *BRAFV600E* mutations in the majority of ECD patients is consistent with a common precursor cell in patients with the somatic mutation (Haroche et al., 2012). *BRAFV600E* in ECD may also indicate common mechanisms of pathogenesis with LCH.

10.4.3 *BRAF* in histiocytic diseases—The activating mutation *BRAFV600E* had first been described in 57% of LCH lesions in cohort of patients with different manifestations of LCH (Badalian-Very et al., 2010) Subsequently, these findings were confirmed in a second cohort showing an enrichment of the mutation enriched in the $CD1a^+$ fraction of the lesions and extended by the identification of two additional potential activating *BRAF* mutations (Kansal et al., 2013; Satoh et al., 2012) BRAF is a central kinase of the RAS/RAF/MEK pathway, which is essentially involved in numerous cell functions including cell proliferation and migration and is frequently mutated in cancer cells (Davies et al., 2002). The *BRAFV600E* mutation leads to a constitutive activation of the downstream kinases extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinase/ERK kinase (MEK) (Maurer, Tarkowski, & Baccarini, 2011). Both downstream kinases are highly activated in LCH cells as shown by immunofluorescence staining supporting the functional relevance of the mutation in LCH cells (Badalian-Very et al., 2010). Besides LCH, *BRAF* mutations are common in lesions of patients with the ECD (54% of all lesions analyzed), but have not yet been reported in other histiocytoses (Haroche et al., 2012). We have identified *BRAFV600E* in lesions from two patients with combined LCH and JXG (unpublished). However, further studies with extended samples sizes are required to establish the role of specific *BRAF* mutations or other oncogenic mutations in the spectrum of histiocytic disorders.

Not only does the identification of *BRAF* mutations in LCH and ECD support a neoplastic origin of these histiocytoses and further understanding of their pathophysiology, it also sets the stage for new therapeutic strategies. Specific BRAF inhibition has been shown to be highly efficient in the treatment of BRAFV600E-expressing malignancies, for example, melanoma and hairy-cell leukemia (Bollag et al., 2010; Dietrich et al., 2012). A very recent case report on the treatment of three patients with advanced multisystemic ECD with concurrent LCH with the BRAF inhibitor vemurafenib documented dramatic therapeutic response rate within the first months of treatment (Haroche et al., 2013). However, it remains to be proven if these responses are sustained or only transient, as frequently observed in the treatment of melanoma with BRAF inhibitors (Poulikakos & Rosen, 2011). Moreover, although no major adverse events occurred during the brief courses of the treatment in this small study (Haroche et al., 2013), large-scale trials in melanoma with prolonged treatment protocols report complex side effect profile including minor grade events (e.g., arthralgia, fatigue, and pruritus) and severe reactions (e.g., rashes and the development of cutaneous squamous cell carcinoma) (da Rocha Dias et al., 2013). A detailed risk–benefit calculation will be essential to determine LCH patients for whom BRAF inhibition would be an appropriate therapy, especially considering the young age of most patients, minimal toxicity of current chemotherapy, and relatively low mortality from LCH. Strategies to specifically target downstream pathways, such as MEK inhibition, may decrease the overall risks for these patients. We advocate the use of targeted inhibition in the context of clinical trials to prove efficacy and safety of BRAF inhibitory approaches in the treatment of advanced ECD and LCH.

10.5. Inflammatory myeloid neoplasms: histiocytic diseases arise from misguided myeloid precursors

The cell of origin of LCH has been assumed to be the epidermal LC, and models for pathogenesis have focused on maturation, activation, or transformation of this cell. However, the transcriptome of $CD207^+$ cells from LCH lesions is most consistent with an immature myeloid DC phenotype relative to the control epidermal LC ($CD207^+$) transcriptome (Allen et al., 2010). Furthermore, expression of CD207 in mice and humans is more promiscuous than initially anticipated (Chikwava & Jaffe, 2004; Ginhoux et al., 2007; Helft et al., 2010; Poulin et al., 2007; Segerer et al., 2008), opening up the possibility that LCH could arise from a number of lineages. Intriguingly, $CD207^+$ DCs are detected in the steady state within the same lymphoid and nonlymphoid tissues where LCH lesions arise, in contrast to the restricted tropism for epidermis and LN of the LC. Furthermore, DC maturation may be heterogeneous within lesions, with variable CD1a+/CD207 – subpopulations (Chikwava & Jaffe, 2004; Coury et al., 2008; Peters et al., 2011). Extended immunofluorescence staining with BRAFV600E-specific antibodies also revealed that the mutations are not entirely limited to $CD207^+$ cells within the lesions, but could be detected in $CD207^-$ cells (Sahm et al., 2012). These observations are essential in the understanding of the origin of LCH cells, as epidermal LCs and other $CD207^+$ DCs do not share a common ontogenetic program. Reports of patients with concurrent or serial histiocytic lesions of more than one phenotype also support the potential for a common precursor for LCH, JXG, and ECD (Haroche et al., 2013; Hoeger, Diaz, Malone, Pritchard, & Harper, 2001; Patrizi et al., 2004; Tsai, Tsou, Hung, Wu, & Chang, 2010).

As discussed in the preceding text, LCs derive from fetal liver monocytes (Hoeffel et al., 2012) populating the skin prior to birth and maintain themselves locally throughout life in steady-state conditions (Merad et al., 2002), whereas $CD207^+$ DCs outside the epidermis arise from circulating precursors known as pre-DCs (Naik et al., 2007). Together, these observations support a model of LCH pathogenesis in which accumulation of $langerin^+$ cells in the lesions arises from dysregulated differentiation and/or recruitment of precursor cells of the DC lineage than proliferation or accumulation of dysregulated mature epidermal LCs.

Given new discoveries in DC biology and pathogenesis of histiocytic disorders, we propose that the “inflammation versus malignancy” debate for LCH may be misguided. LCH and JXG and ECD have both inflammatory and neoplastic characteristics. We hypothesize that *BRAFV600E* or other stimuli may influence lineage commitment, migration, and proliferation of myeloid DC precursors. Once precursors migrate to sites of lesions, they recruit and activate “bystander” cells in the inflammatory lesions that define the diseases. We therefore propose that LCH, JXG, and ECD be classified as *inflammatory myeloid neoplasms*.

11. CONCLUSION AND PERSPECTIVE

In mice and humans, DCs comprise a heterogeneous group of phagocytic active, antigen-presenting cells with distinct origin, differentiation, and functional specialization in steady state and inflammation. In the recent decade, evidence has accumulated strongly supporting the hypothesis that the disease complex of human histiocytosis represents the pathophysiological consequence of a dysregulated differentiation of the myeloid lineage greatly contributing to the understanding of the pathophysiology and diversity of histiocytosis.

The identification of the oncogenic mutation *BRAFV600E* in LCH and ECD, along with increasing evidence for a clonal DC precursor, prompts us to propose a reclassification of these diseases as *inflammatory myeloid neoplasms* and opens new perspectives for

functional studies and therapeutic strategies. Future studies of histiocytic disorders will dissect the functional consequences of the mutations for DC differentiation, maturation, and effector function that will inform our understanding of physiological DC biology and also elucidate novel diagnostic and therapeutic strategies for patients.

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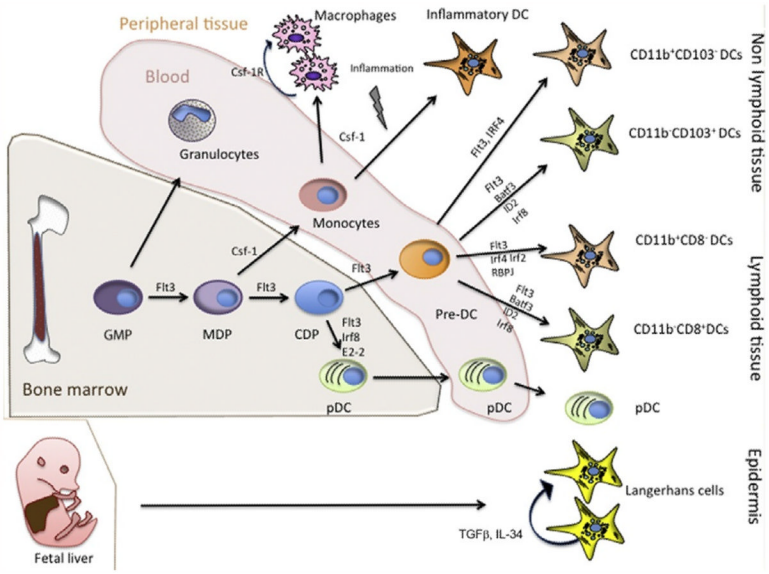

Figure 5.1.

Origin and developmental regulation of the mononuclear phagocyte system (MPS) in mice: With the exemption of Langerhans cells, which derive from early embryonic progenitors in the fetal liver and maintain themselves locally throughout life, monocyte and dendritic cell subpopulations in lymphoid and nonlymphoid tissues share a common developmental origin arising from bone marrow-resident hematopoietic precursors. The lineage commitment to the MPS is thereby marked by the differentiation of the granulocyte and macrophage progenitors (GMP) to macrophage DC progenitors (MDP), which have lost granulocyte potential and exclusively give rise to monocytes and dendritic cells. MDPs differentiate into common DC progenitors, which give rise to pre-DCs and plasmacytoid DC (pDC) that egress the bone marrow after differentiation and mainly repopulate lymphoid tissues in the steady state. Circulating pre-DCs represent the common precursor also for all DC subpopulations within the lymphoid and nonlymphoid tissue. Transcription factors and cytokines involved in the regulation of specific differentiation pathways are indicated.

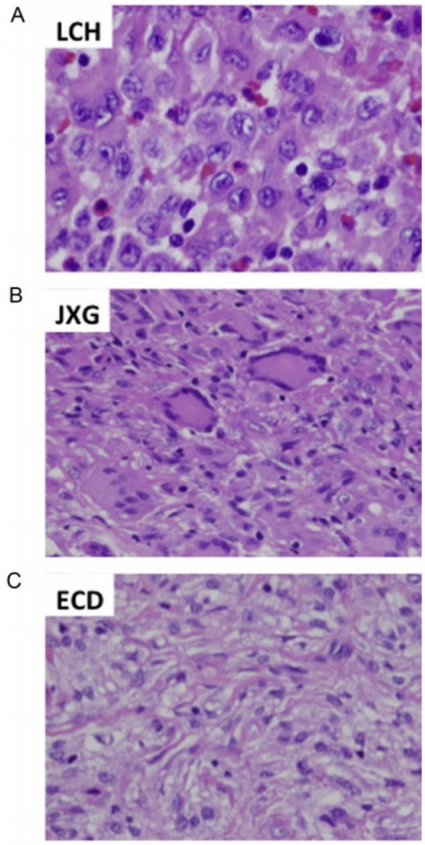

Figure 5.2.

Histology of Langerhans cell histiocytosis, juvenile xanthogranuloma, and Erdheim–Chester lesions. (A) LCH: H&E stain demonstrates classic clusters of histiocytes with reniform nuclei with a background of mixed inflammatory cells, including lymphocytes, eosinophils, and macrophages. (B) JXG: Lesions are composed of smaller histiocytes with folded bland nuclei and vacuolated cytoplasm. Xanthomatous Touton giant cells (arrow) with a wreath of nuclei are observed in a majority of cases. An inflammatory infiltrate is also present. (C) ECD: ECD lesions may be histologically indistinguishable from JXG. Lesions are interogeneous with foamy histiocytes and an inflammatory infiltrate. *Images courtesy of Dr. John Hicks, Baylor College of Medicine*.

Table 5.1

Classification of histiocytic disorders

Dendritic cell related
Langerhans cell histiocytosis
Juvenile xanthogranuloma/Erdheim–Chester disease
Macrophage related
Hemophagocytic syndromes
Primary hemophagocytic lymphohistiocytosis
Secondary hemophagocytic syndromes
Rosai–Dorfman disease
Malignant diseases
Monocyte-related leukemias
Extramedullary monocytic tumor (myeloid sarcoma)
Macrophage-related histiocytic sarcoma
Dendritic cell neoplasms (malignant histiocytosis)

For detailed review on the classification of histiocytosis, see references (Favara et al., 1997; Jaffe et al., 2005; Pileri et al., 2002).

Table 5.2

Phagocyte characteristics among histiocytic disorders

	Langerhans cell histiocytosis	Juvenile xanthogranuloma	Erdheim-Chester disease	Rosai-Dorfman disease	Hemophagocytic lymphohistiocytosis	Malignant histiocytosis
HLA-DR	++	−	−	+	+	+
CD1a	++	−	−	−	−	−
CD207 (langerin)	+++	−	−	−	−	−
Birbeck granules	+	−	−	−	−	−
CD14	+/−	++	++	++	++	−
CD68	+/−	++	++	++	++	+/−
CD163	−	−	−	++	++	−
Factor XIIIa	−	++	++	−	−	−
Fascin	−	++	++	+	+/−	++
Hemophagocytosis	+/−	−	−	−	+/−	−
Clonality	+ (−) in some adult pulmonary LCH	+	+/−	−	– reactive macrophages	+
BRAFV600E	57%[a]	+[b]	54%[c]	0%[a,c]		

[a] Badalian-Very et al. (2010).

[b] Unpublished observation.

[c] Haroche et al. (2012).