Langerhans cell histiocytosis: malignancy or inflammatory disorder doing a great job of imitating one?

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Langerhans cell histiocytosis (LCH) is the unifying designation for a rare proliferative disorder that occurs predominantly in childhood and involves the main antigen-presenting cell of the epidermis. LCH can present in a multitude of ways, from a self-limited rash that resolves spontaneously to a systemic multi-organ disease with a 20% mortality rate. Because some forms behave in a relatively benign manner and are associated with an inflammatory cell infiltrate, it has been proposed that LCH might be a reactive disease. However, its neoplastic nature is suggested by the fact that the proliferating cells in LCH are clonal and overexpress p53. Nonetheless, no recurrent genomic, genetic or epigenetic abnormalities have been identified. Instead, a variety of molecular abnormalities that are consistent with disordered Langerhans cell maturation have been described. A faithful small animal model would aid our understanding of the pathophysiology of LCH but, to date, none exists. Challenges to the creation of a model include the lack of characteristically recurrent genetic abnormalities and the absence of a truly tissue-specific promoter to drive expression of genetic elements solely in Langerhans cells. Still, some of the phenotypic abnormalities in adhesion molecule or chemokine receptor expression might be modeled with sufficient precision to allow the testing of novel therapies.

The disease

Langerhans cell histiocytosis (LCH) is a clonal proliferative disease of Langerhans cells (LCs), the primary antigen-presenting cells of the skin. It occurs predominantly, but not exclusively, in children and is quite rare. Recent estimates infer an incidence of 2-9 cases per million children under the age of 15 (Guyot-Goubin et al., 2008). The clinical manifestations of LCH are highly variable and depend to a large extent on the number of body sites infiltrated by the pathologic LCs, the specific organs involved, and the precise anatomical location of the cellular infiltration (Degar et al., 2009). Limited disease involving a single infiltration site can present as a skin rash, bone pain or soft tissue swelling. More extensive disease can present with symptoms of specific organ failure, such as diabetes insipidus which results from invasion of the pituitary stalk or respiratory insufficiency which results from pulmonary involvement; an example of the former is described in the accompanying case study. Extensive disease may also be associated with systemic signs of illness including fever or lymphadenopathy, as well as multiple organ failure. Pulmonary LCH is non-clonal and, in many cases, appears to be a reactive inflammatory disease that is primarily seen in adult smokers: this disease is probably distinct from the childhood form of LCH and will not be considered here further.

Historically, distinct disease entities were identified and named based on specific patterns of disease. Eosinophilic granuloma was applied to histiocytic infiltration that produced one (mono-ostotic) or more (polyostotic) lytic bone lesions. Hand-Schüller-Christian disease identified the clinical triad of bone lesions, exophthalmos and polyuria that resulted from diabetes insipidus. Letterer-Siwe disease described a fulminant course characterized by hepatosplenomegaly, lymphadenopathy, skin rash, bone lesions, anemia and a bleeding diathesis. A gradual recognition that all three disorders involved the same histiocyte-like cell led to their unification under the heading of histiocytosis X. When that common cell was shown definitively to be derived from a LC, this complicated nosology was simplified by naming the disorder LCH (Nezelof et al., 1973).

Of course, merely applying a single name to a disease cannot simplify it. LCH remains a complex disease with a wide array of presentations and clinical courses. Approximately two-thirds of children with LCH have single-system disease that most commonly affects bone, but that can also involve skin, lymph nodes or the central nervous system (CNS). The remaining children have multisystem disease, which tends to be present in younger children. Even within the multisystem group, the involvement of so-called 'risk organs' portends a worse outcome and occurs in about half of these children. These risk organs are the bone marrow, liver and lungs [although it is not clear whether lung involvement is an independent predictor of adverse outcome, as opposed to a concomitant of disseminated disease (Ha et al., 1992; Braier et al., 2004; Odame et al., 2006)]. Even in the absence of any pathologically documented involvement of the CNS by pathologic LCs, it should be appreciated that a debilitating neurodegenerative syndrome has been

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Case study

An 18-month-old boy came to medical attention because of polyuria and polydipsia, and was diagnosed with diabetes insipidus. The patient had been the product of an uncomplicated full-term gestation and delivery. During infancy, he had severe 'cradle cap', intractable diaper rashes and multiple episodes of otitis media. At the time of his presentation with diabetes insipidus, a 'lump' was noted on his right cheek that corresponded to a temporal bone lesion on imaging. Biopsy of the mass confirmed LCH. He was treated with corticosteroid and vinblastine chemotherapy for 6 months, with improvement in the temporal bone lesion but no change in the diabetes insipidus. Approximately 7 months after the patient had finished therapy, his temporal bone lesion increased in size and a new lytic lesion was discovered in his right femur on imaging studies. The patient received an additional year of chemotherapy (vinblastine, prednisone and mercatopurine). Panhypopituitarism developed during this period. He continued to experience occasional episodes of otitis media and mastoiditis and was found to have a mild hearing impairment. At the age of 6 years, learning difficulties were noted. An MRI scan showed a 'nonspecific' signal abnormality in the brainstem and cerebellum. On neurological examination, the patient demonstrated mild tremor, dysdiadochokinesia and poor balance.

associated with some cases of LCH (Grois et al., 2005). A diagnosis of LCH depends on pathological identification of the characteristic, cytologically benign lesional LCs, with their 'coffee bean'-shaped cleaved nuclei, accompanied by an inflammatory infiltrate. Bone lesions can contain a considerable number of associated non-neoplastic osteoclasts. Pathologic LCs express CD1a and Langerin (CD207), and positive immunohistochemical staining of cells that have the appropriate histological appearance is required for diagnostic confidence.

Delineating therapy for such a protean disease can be challenging. How does one develop a unified approach to a disease that, in its limited form, can occasionally undergo spontaneous remission, whereas, in its most aggressive form, is associated with a significant mortality rate? This variability plus the rarity of the illness make the performance of informative clinical trials extremely challenging. Thus, the literature is filled with anecdotal reports of more or less successful treatments of LCH.

Nonetheless, some general treatment rules have emerged and some trials in advanced disease have provided guidance. Mono-ostotic lesions can resolve after biopsy (even if the excision is incomplete) or after instillation of methylprednisolone into the lesion site (Cohen et al., 1980; Capanna et al., 1985). Radiation therapy is also effective. Polyostotic disease can be treated with systemic chemotherapy. This is, of course, also the approach to the treatment of multisystem disease. A handful of large, international multicenter trials have been performed over the last two decades that have tested conventional cytotoxic chemotherapy (Gadner et al., 1994; Minkov

et al., 2000; Gadner et al., 2008). In general, responses are good and can be durable in patients without organ failure. However, there remains a core cohort of 20% of patients with multisystem disease whose disease course is very aggressive and who die of their disease. Even when chemotherapy is effective, many survivors live with permanent sequelae of the disease.

Etiology and pathophysiology

One of the enduring questions about the pathophysiology of LCH is whether or not it is a malignancy. At some point, it becomes meaningless to have a semantic argument about a proliferative disease that kills a significant proportion of its victims and that responds, in some cases, to cytotoxic chemotherapy. But, because this is apparently the same disease that can regress spontaneously or disappear after biopsy, the question has some legitimacy. Could LCH be a reactive disease of activated LCs that is analogous, perhaps, to the secondary histiocytoses that arise in response to viral infections (Janka et al., 1998)? This seems unlikely because, at least so far, no microbial genomes have been identified in pathologic LCs and no epidemiological studies have suggested an infectious or environmental cause. Herpesviruses had been associated anecdotally with LCH but a recent study showed that the prevalence of antibodies against these viruses, as well as their titers, were the same in LCH patients and age-matched controls (Jeziorski et al., 2008).

Rather, the preponderance of evidence suggests that LCH is neoplastic. Most compelling are two independent reports demonstrating clonality of pathologic LCs (Willman et al., 1994; Yu et al., 1994). Consistent

with a genetic basis for LCH, there are reports of familial clustering and a high degree of concordance among presumed monozygotic twins (Arico et al., 1999). Of course, the presence of recurrent genetic abnormalities in LCH would provide much stronger support for this idea but, because of its rarity, only a handful of surveys have been published and none of these is quite definitive. For example, a study of five patients identified non-recurrent cytogenetic abnormalities (Betts et al., 1998), but a later analysis detected no such abnormalities in 31 cases of LCH (da Costa et al., 2009). Array comparative genomic hybridization (CGH) analysis of a small sample of patients appeared to show widespread copy number variation and recurrent loss of heterozygosity (LOH) involving chromosome 4 (Murakami et al., 2002). A PCR-based LOH evaluation identified an increase in fractional allelic loss in multisystem and high-risk disease when compared with single-system and low-risk disease (Chikwava et al., 2007). Again, however, a well-performed study involving a larger number of patients failed to detect significant copy number variation (da Costa et al., 2009), although this study did confirm consistent p53 expression by pathologic LCs (Weintraub et al., 1998; da Costa et al., 2009). The basis of this p53 expression is unclear since, in seven samples, no mutations were found in exons 5-8, but p53 overexpression may explain the sensitivity of some cases to chemotherapy or radiotherapy.

Another generally accepted feature of pathologic LCs is their abnormal maturation. The phenotype of these cells mixes characteristics of resting and activated LCs. Pathologic LCs express some co-stimulatory and adhesion molecules that are associated with activated LCs (Emile et al., 1994; Tazi et al., 1999). For example, they lose expression of E-cadherin, which may be required for the migratory phenotype of activated LCs (Geissmann et al., 1997). However, Birbeck granules, which are absolutely characteristic of pathologic LCs, are present in resting LCs and disappear after activation (Stossel et al., 1990).

Along the same lines, there is some evidence for abnormal expression of chemokine receptors. Ordinarily, resting LCs express the chemokine receptor CCR6 (among others) (Dieu et al., 1998; Sallusto et al., 1998; Sozzani et al., 1998). Because

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Clinical terms

Diabetes insipidus – excessive production of dilute urine as a consequence of reduced or absent secretion of antidiuretic hormone (ADH) by the pituitary gland, or insensitivity of the kidney to ADH

Dysdiadochokinesia – inability to perform rapid alternating movements; usually a consequence of cerebellar dysfunction

Exophthalmos – protrusion of one or both eyes

Histiocytosis – a proliferative disorder of inflammatory cells derived from the macrophage or dendritic cell lineage

Langerhans cell – the antigen-presenting dendritic cell of the epidermis

Lymphadenopathy – swelling of lymph nodes usually owing to cellular infiltration

Mastoiditis – infection of the mastoid bone, which lies behind the ear; often a result of untreated or aggressive otitis media

Otitis media – middle ear infection

Panhypopituitarism – a constellation of clinical signs and symptoms resulting from a global decrease, or loss, of pituitary hormone secretion

the CCR6 ligand, CCL20, is expressed by cutaneous keratinocytes, expression of CCR6 may be one of the mechanisms that keeps pathologic 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 (CCL19 and CCL21) are expressed by cells in regional lymph nodes. This is thought to be one of the reasons for the directional migration of activated LCs to lymph nodes. Our group examined 24 cases of LCH for patterns of chemokine receptor expression and found that they all co-expressed CCR6 and CCR7, consistent with abnormalities in maturation (Fleming et al., 2003). It should be noted, however, that another research group detected only CCR6 expression by pathologic LCs; the reason for this discrepancy is not known (Annels et al., 2003).

Models

There is no acceptable and faithful animal model for LCH. Attempts have been made to culture pathologic LCs with the intention of using them in a xenotransplantation model. However, the one report of a cell line that grew from a CD1a$^+$ LCH bone lesion describes the cells as CD1a$^-$ and Langerin$^-$, indicating that they are not

pathologic LCs (Gogusev et al., 2005). Another group used HeLa cells that were engineered to express CD1a as a model for testing the diagnostic and therapeutic efficacy of derivatized anti-CD1a antibodies (Murray et al., 2000). This is obviously not a disease model. Yet another group isolated an autonomously proliferative cell line from the spleen of an IFN$\gamma^{-/-}$ mouse that had been treated with the tumor-initiating and -promoting agents DMBA and TPA, respectively (Kammertoens et al., 2005). Although capable of forming tumors in syngeneic mice, the phenotype of these cells marks them clearly as immature dendritic cells rather than LCs.

The Bernese mountain dog suffers from a distressingly frequent systemic histiocytosis that bears some resemblance to LCH. Interestingly, the disease varies in frequency among pedigrees suggesting vertical transmission, which would be consistent with a genetic predisposition (Padgett et al., 1995; Affolter and Moore, 2000). Although the dog is not really a tractable model for breeding-based analysis over the short term, it could be exploited for a reverse genetic approach to identifying genes that contribute to the condition.

A recent report described the 'Mushi' (multisystem histiocytosis) mouse (Steiner et al., 2008). This is a transgenic model in which the CD11c promoter drives the simian virus 40 (SV40) large T antigen. The phenotype of this model is a full-penetrance, systemic proliferative disease with some pathological features of LCH, but showing more frequent mitoses and no skin or bone involvement. The tumor cells are Langerin$^+$ but they are also CD8α^+. Thus, the abnormal cell is a conventional dendritic cell (which is known to express Langerin) and not an LC (which is CD8α^-) and, therefore, this is not an LCH model. In retrospect, it may not be surprising that a large T antigen-driven system did not faithfully model LCH since there is no evidence yet for inactivation of the retinoblastoma pathway in pathologic LCs.

There is no question that a rodent model of LCH is needed desperately. Genetically defined rodent models of malignancy have been invaluable resources for therapeutic target validation and for preclinical testing of novel therapies that are directed toward those targets. These models have all been constructed based on knowledge of recurrent genetic abnormalities in human can-

Clinical and basic research opportunities

● Defining recurring genetic or epigenetic abnormalities in LCH
● Defining the molecular basis for tissue distribution of pathologic LCs
● Understanding the cytokine and signaling networks that are responsible for the inflammatory and osteolytic complications of LCH
● Developing a rodent model that faithfully recapitulates LCH pathology
● Developing and testing novel therapeutic agents for LCH

cers. Until the same knowledge base can be built for LCH, it will be very unlikely that a truly faithful rodent model will be developed.

Another problem is the absence of uniquely tissue-specific promoters for LCs. For example, the CD11c promoter that was used to develop the 'Mushi' mouse, cited above, is active in LCs, but it is also active in many other myelomonocytic cell types. The Langerin promoter is somewhat more selective for LCs but, as noted above, it is also active in CD8α^+ conventional dendritic cells. The Dec2FR (dectin-2) promoter has also been reported to be highly specific for LCs (Bonkobara et al., 2004). Even in this case, transgenic mice that were engineered to express luciferase under the control of the Dec2FR promoter showed some luciferase expression in activated T cells. Nonetheless, although these promoters are not cleanly LC-specific, they are all capable of driving expression of heterologous genes in LCs. So long as caveats about expression in other cells types are considered, they could be used to interrogate LC function.

Although recurrent genetic abnormalities have not been identified in LCH, it may be reasonable to use rodent models to test the relevance of other molecular abnormalities that have not necessarily been demonstrated to be pathogenetic. For example, disordered expression of chemokine receptors was described above and it has been suggested that this may account partly for the patterns of widespread invasion in multisystem disease. This hypothesis could be tested by engineering gratuitous expression of CCR6 and/or CCR7 in LCs using transgenic technology. A phenotype of LC infiltration in target

organs might justify a trial of chemokine receptor antagonists as symptomatic therapies.

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