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MEDICAL PROGRESS

Langerhans cell histiocytosis

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Langerhans cell histiocytosis is a challenging disease and may be manifested in a variety of ways, ranging from a spontaneously regressing solitary lesion of bone to a multisystem, life-threatening disorder. Some forms require little if any treatment, and others need aggressive therapy.[1] During the past decade, major advances have been made in defining the clinical and pathologic criteria needed for diagnosis and treatment.[2, 3] Standardization of nomenclature has made it possible to accumulate and record coherent data, and has cleared the way for a large-scale cooperative international study of the natural evolution of LCH and its response to treatment.[4] These major developments in the clinical arena have been complemented by productive research concerning the basic nature of the histiocyte and its disorders. Various hypotheses have been explored—principally, whether LCH is a clonal disorder,[5, 6] a cytokine-mediated cellular proliferation of Langerhans cells,[7-10] or a reactive process following a viral infection.[11-13]

The definition, natural history, identification, diagnosis, and treatment of Langerhans cell histiocytosis are reviewed below in the light of the newly established clinical and laboratory criteria and the improvements in pathologic and medical imaging techniques of the past few years. The progress made in basic science research will then be presented, together with a discussion of how those findings affect current understanding of the disease.

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CLASSIFICATION AND NOSOLOGY

The term *histiocytosis* identifies a group of disorders that have in common the proliferation of cells of the mononuclear phagocyte system and the dendritic cell system.[14] The individual entities are diagnosed on the basis of certain symptoms, signs, and laboratory information that, when taken together, fulfill generally agreed-on criteria for the diagnosis of that particular disorder. The various histiocytoses of childhood are listed in Table I. Histiocytes (macrophages) and dendritic cells represent two of the major types of nonlymphoid mononuclear cells and are involved in immune and nonimmune inflammatory responses. The cells of both systems are derived from the bone marrow stem cell, the promonocyte. Each of the histiocytoses of childhood is characterized by localized or generalized reactive or neoplastic proliferation of cells similar, if not identical, to one of these cell types. In the case of LCH, the proliferating cell is the Langerhans cell.

DI	Diabetes insipidus
HHV-6	Human herpesvirus type 6
LCH	Langerhans cell histiocytosis
PCR	Polymerase chain reaction

Lichtenstein[15] first recognized the clinical and pathologic characteristics that the various manifestations of the disease had in common, and proposed that they be grouped under the

Table I. Classification of histiocytosis syndromes in children

Class	Syndrome
I	Langerhans cell histiocytosis
II	Histiocytosis of mononuclear phagocytes other than Langerhans cells
	Hemophagocytic lymphohistiocytosis (familial and reactive)
	Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease)
	Juvenile xanthogranuloma
	Reticulohistiocytoma
III	Malignant histiocytic disorders
	Acute monocytic leukemia (FAB M5)
	Malignant histiocytosis
	True histiocytic lymphoma

Data from Writing Group of the Histiocyte Society (Chu T, D'Angio GJ, Favara B, Ladisch S, Nesbit M, Pritchard J). Lancet 1987;1:208-9.

Table II. Histiocytosis X as related manifestations of a single nosologic entity

Eosinophilic granuloma of bone: localized disease in bone
Hand-Schüller-Christian disease: subchronic or chronic disease with mostly the triad of "geographic skull" (from multiple calvarial lesions), exophthalmos, and diabetes insipidus
Letterer-Siwe disease: acute or subacute disseminated disease

Data from Lichtenstein L. A M A Arch Pathol 1953;56:84-102.

term *histiocytosis X* (Table II). The letter *X* was used to underscore the unknowns concerning the nature and cause of the disease. Some of these uncertainties were dispelled by the next advance, which was the description of the Langerhans cell and the identification of the Birbeck granule, an inclusion body visible on electron microscopy in the lesional Langerhans cells.[16] Using the Birbeck granule as a marker, Nezelof et al.[17] in 1973 reported that the lesions of histiocytosis X were the result of the proliferation and dissemination of abnormal histiocytic cells of the Langerhans cell system; consequently the name of the disease was changed to Langerhans cell histiocytosis.[18]

SIGNS AND SYMPTOMS

Langerhans cell histiocytosis can occur in persons ranging in age from newborn to elderly; the peak incidence occurs between 1 and 4 years. Reliable data on the annual incidence are difficult to gather, but the frequency in the pediatric age range has been estimated at 2 to 5 per million per year.[19] In most studies LCH occurs almost equally in male and female subjects, with a small preference for male subjects. The disease is probably underdiagnosed, the bone lesions being symptomless, painful lumps frequently ascribed to trauma. Mild skin disease, often involving the scalp, may be mistaken for seborrheic eczema.[20]

Table III. Categorization of LCH patients according to disease extent

1. Restricted Langerhans cell histiocytosis
   a. Biopsy proved skin rash without any other site of involvement
   b. Monostotic lesions, with or without diabetes insipidus, adjacent lymph node involvement, or skin rash
   c. Polyostotic lesions, consisting of lesions in several bones or more than two lesions in one bone, with or without diabetes insipidus, adjacent lymph node involvement, or skin rash
2. Extensive Langerhans cell histiocytosis
   a. Visceral organ involvement, with or without bone lesions, diabetes insipidus, adjacent lymph node involvement, and/or skin rash, but *without* signs of organ dysfunction of any of the following organ systems: lung, liver, or hemopoietic system
   b. Visceral organ involvement, with or without bone lesions, diabetes insipidus, adjacent lymph node involvement, and/or skin rash, but *with* signs of organ dysfunction of any of the following organ systems: lung, liver, or hematopoietic system

The morbidity and prognosis of LCH are strongly dependent on the number of organ systems involved,[21] and on whether normal function of those organs is affected. The disease can be divided into two broad categories with subsets: restricted and extensive (Table III). This working categorization can be used for prognostication and for guidance in patient management. The subsets are ranked in order of increasing chances of that morbidity will develop. Patients with organ dysfunction have a poor prognosis as well, because dysfunction of critical organs, rather than organ involvement per se, is most important in predicting outcome.[22] Besides these initial clinical features of the disease, the outlook for patients with LCH depends on the age of the patient at diagnosis and the rate of disease progression.[21] Imaging abnormalities identified in lung, liver, the hematopoietic system, or other organs are not sufficient evidence of dysfunction. This point is especially important because of the increased sensitivity of modern imaging methods. For example, magnetic resonance imaging of the skull may show extradural compression of the underlying brain from internal protrusion of skull lesions. In the absence of clinical signs, however, this finding does not have implications regarding clinical management beyond those indicated by demonstration of the bony defect by plain X-ray studies. Lahey,[22] in pioneering studies, required objective manifestations, and his criteria still hold. For the lung, they include cough, tachypnea or dyspnea or both, cyanosis, pneumothorax, or pleural effusion attributable to the disease rather than to superimposed infection. Liver dysfunction requires one or more of the following: hypoproteinemia, edema, ascites, and hyperbilirubinemia; and for the hematopoietic system anemia (not caused by iron deficiency or superimposed infec-

tion), leukopenia, neutropenia, or thrombocytopenia. The presence of excessive numbers of Langerhans cells in the marrow aspirate is not by itself considered evidence of dysfunction.

Magnetic resonance imaging provides no great advantage in showing bone lesions. In this respect, the recently described method of immunolocalization of LCH in which CD1a targeting was possible, seems promising. Kelly et al.[23] administered intravenously an indium 111–labeled murine monoclonal antibody against CD1a antigen, which is present on the cell surface of Langerhans cells. They demonstrated that the anti-CD1 monoclonal antibody NA1/34 could be delivered to sites of disease activity in patients with LCH, especially with respect to bony disease. Expansion of this work is awaited with interest.

The involvement of each of the organs will be described separately, but in the extensive forms of LCH, multiple organs may be affected.

Bone lesions. Bone lesions are found in almost all patients with restricted or extensive LCH. Painful swelling is the most common initial sign, the skull being the bone affected most often, followed in frequency by the long bones of the upper extremities and then the flat bones (ribs, pelvis, and vertebrae).[24] There may be adjacent soft tissue swelling. Older children and young adults are more commonly affected. A plain radiograph typically reveals single or multiple irregularly marginated lytic lesions of bone; proptosis from lesions of the orbital wall may also be present. When the mastoid process is involved, the findings can mimic mastoiditis. Extension to the middle ear causes destruction of the ossicles and deafness. LCH of the jaws is often associated with contiguous soft tissue swelling, "floating" teeth, gingival swelling, fractures, or pain. In the spine, the lytic process can result in compression and collapse of the vertebral body, causing vertebra plana.

Skin. Cutaneous lesions are often the first sign of LCH and frequently become manifest as scaly, erythematous, seborrhea-like brown to red papules, especially pronounced in intertriginous zones (behind the ears and in the axillary, inguinal, and perineal areas). Superficial ulceration is a secondary process; weeping lesions similar to eczema are then seen. When the skin is the only organ involved, the patient is usually a male infant less than 1 year of age, and spontaneous regression occurs frequently. Skin lesions may be the sole manifestation of LCH, but careful assessment is needed to ensure that they are not part of more extensive disease. Typical LCH occurs in the skin of adults, but some examples of atypical lesions suggest that the disease may sometimes be different in older patients. Cases of this type (adult type) possibly are examples of large cell lymphoma with a Langerhans cell–like phenotype in some instances.[25]

Lymph nodes. Lymph node involvement in LCH may be seen with restricted bone or cutaneous lesions, or as part of the extensive type. Cervical lymph nodes are affected most often and may reach massive size.

Table IV. Confidence levels for the diagnosis of class I Langerhans cell histiocytosis

1. Presumptive diagnosis: light morphologic characteristics
2. Designated diagnosis
   a. Light morphologic features plus:
   b. Two or more supplemental positive stains for:
      (1) Adenosenetriphosphatase
      (2) S-100 protein
      (3) α-D-Mannosidase
      (4) Peanut lectin
3. Definitive diagnosis
   a. Light morphologic characteristics plus:
   b. Birbeck granules in the lesional cell with electron microscopy and/or:
   c. Staining positive for CD1a antigen (T6) on the lesional cell

Data from Writing Group of the Histiocyte Society (Chu T, D'Angio GJ, Favara B, Ladisch S, Nesbit M, Pritchard J. Lancet 1987;1:208-9.

Bone marrow. Langerhans cells do not appear to be a normal constituent of the bone marrow, although other dendritic cells can be seen. Pancytopenia caused by bone marrow dysfunction is usually associated with gross hepatosplenomegaly and a poor prognosis.[26]

Liver and spleen. Hepatosplenomegaly in the patient with LCH requires erudite probing. It may herald the presence of organ involvement by LCH, or it may indicate obstructive disease caused by enlarged nodes in the porta hepatis. Both can lead to biliary cirrhosis. Hepatomegaly in a patient with LCH may also reflect Kupffer cell hypertrophy and hyperplasia as an indicator of generalized activation of the cellular immune system, without direct LCH involvement or obstructive hepatopathy.[27] Ascites caused by hypoalbuminemia is a clinical sign of liver dysfunction, which may also be manifested by jaundice and a prolonged prothrombin time. The pathologic patterns range from mild cholestasis to more severe histiocytic infiltration of portal areas with evidence of hepatocellular injury and bile duct involvement, which can finally progress to sclerosing cholangitis, severe fibrosis, biliary cirrhosis, and liver failure. Histologically there is portal infiltration with $CD1a^+$ Langerhans cells, an important LCH marker (Table IV), but Birbeck granules are rarely present. Enlargement of the spleen may be an additional factor responsible for the depression of one or more of the circulating cellular elements of the blood.

Lungs. Pulmonary LCH can occur at any age; it is most common during the third decade. Tachypnea with rib retraction is often the only clinical sign, but fever and weight loss may become prominent. The diagnosis, which is suggested by a diffuse micronodular pattern on chest films, can easily

be confirmed by electron microscopy of the alveolar fluid obtained by bronchoalveolar lavage.[28] The respiratory tract, like the lymph nodes, normally contains low numbers of Langerhans cells, so the cytologic material must be evaluated cautiously. Within an appropriate clinicoradiographic setting, numbers are important: There must be many Langerhans cells to suggest a diagnosis of LCH because the histopathologic presence of Langerhans cells in the bronchoalveolar lavage fluid alone is insufficient evidence to make the diagnosis.[27] Auerswald et al.[29] state that all patients with histologically proven LCH have more than 5% $CD1^+$ cells, whereas normally this amount should be less than 1% $CD1^+$ lavage cells. With time, increasing numbers of cysts form "honeycomb lungs" and, in later stages, large bullae. A spontaneous pneumothorax can result from rupture of an underlying bulla. Emphysematous changes, along with increasing amounts of interstitial fibrosis, may occur in the final phase of pulmonary LCH.[30] There is a strong link between smoking and the primary pulmonary LCH seen in patients.[31] This type of LCH (smoking related) appears to differ distinctly from lung involvement seen in early childhood.

Gastrointestinal tract. Because gastrointestinal involvement by LCH seldom produces prominent clinical manifestations, its frequency is often underestimated. The most common sign is "failure to thrive", which is caused by malabsorption. Other symptoms include vomiting, diarrhea with or without blood, and protein-losing enteropathy. In most cases there is radiographic evidence of alternating dilated and stenotic segments in the small and large bowel, but endoscopic examination with biopsies is needed for diagnosis.[32]

Thymus. The role of the thymus in LCH, if any, is debatable.[33] Thymic enlargement may be obvious on chest x-ray studies. The gland, which may be the only site of disease, is affected prominently in a high percentage of the patients who have died of LCH, as seen at autopsy.[34]

Endocrine glands. Diabetes insipidus, the most common endocrinopathy, can occur before, concurrently with, or subsequent to the development of lesions in extracranial sites. Confirmation of the diagnosis by an appropriate water deprivation test and by measurement of urinary arginine vasopressin is essential because partial defects may occur and may spontaneously remit.[35] DI develops more often in patients with bone disease that affects the skull; furthermore, it is more common among patients with extensive disease than among those with lesions apparently confined to bone.[35] Previously, x-ray and computed tomographic examinations were not rewarding in patients with DI; now, however, gadolinium-enhanced magnetic resonance imaging has proved to be revealing. Thickening of the hypothalamic-pituitary stalk region (>2.5 mm), absence of the posterior pituitary "bright" signal in T1-weighted images, or both, are commonly seen.[36] A word of caution is needed, however, before a cause-and-effect explanation is assigned to these findings. Identical changes have been reported by Imura et al.[37] in adults with idiopathic DI, who do not have and never have had LCH.

Growth retardation resulting from anterior pituitary involvement and growth hormone deficiency is seen in fewer than 1% of patients.[38] Growth failure in children with LCH is nonetheless considered common,[39] which suggests a multifactorial basis in most patients. Occult gut involvement, which causes malabsorption, and corticosteroid treatment are contributory causes. Most patients have catch-up growth once the disease remits. A long-term follow-up study in Rotterdam found no differences in height compared with that of control children.[40]

Central nervous system.* Acute signs of central nervous system involvement, such as intracranial hypertension or seizures, are rare. Progressive ataxia, dysarthria, nystagmus, hyperreflexia, dysdiadochokinesia, dysphagia, blurred vision, or cranial nerve deficits can develop, at times years after the original diagnosis of LCH,[41] but they are not common. Though LCH can affect the brain,[42] early data were derived from static evidence obtained from biopsy or autopsy specimens. The advent of magnetic resonance imaging has added immeasurably to our understanding of the process; affected areas appear as bright signals on T1-weighted images.[43] Thus the areas of early involvement can be identified clearly, whereas the findings on computed tomography scans were often ambiguous. Moreover, the pattern of spread can be followed in patients with progressive disease. These lesions are largely symmetric and most often are first seen in the cerebellum, after which they move anteriorly to affect the paraventricular cerebral white matter. Biopsy specimens of such areas show histiocytic infiltrates with xanthomatous changes. Most of these histiocytes were phenotypically akin to ordinary macrophages; however, a few histiocytes had the typical phenotype of Langerhans cells. Birbeck granules were seen by electron microscopy in only 1 of 13 patients[43]; unfortunately there was no information on the presence or absence of the CD1a antigen. Brain involvement of this type is found more commonly in patients with DI and skull defects, which may precede the CNS manifestations.

DIAGNOSTIC STEPS

The Writing Group of the Histiocytic Society, an international body, outlined in 1987 the morphologic, immunohistochemical, and clinical criteria required for the diagnosis of LCH and the other histiocytic disorders in children.[2] The various entities were grouped as follows: LCH as class I, the histiocytoses of mononuclear phagocytes other than Langerhans cells as class II, and malignant histiocytic disorders as class III. The Histiocyte Society[2] also published confidence

*See also the discussion of DI, in the section on endocrine glands, above.

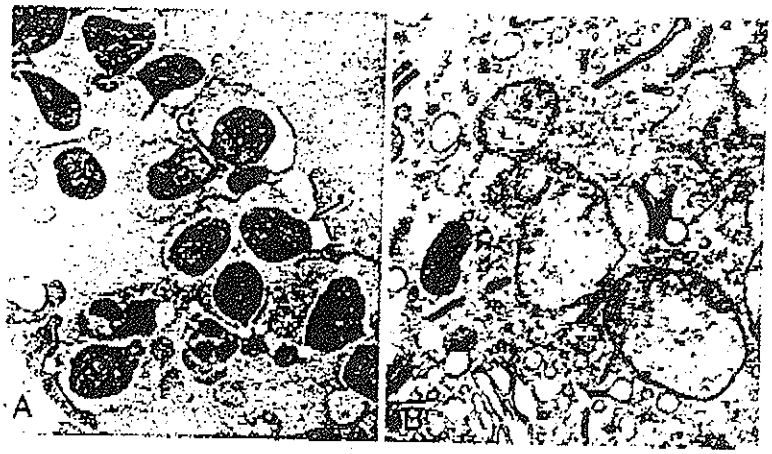

Figure. A, Nucleus *(arrow)* of Langerhans cell is often folded, indented, or polylobulated and has an irregular granular chromatin pattern.[45] B, Precise identification requires cytochemical features or electron microscopic demonstration of Birbeck granules *(arrow)*. These organelles are cytoplasmic lamellar plates with a central striated line and an occasional terminal vesicular dilation, which gives it the so-called tennis racket appearance.

levels for the diagnosis of LCH (Table IV). These standards have been widely adopted for diagnosis, patient management, and research.

Hematoxylin-eosin–stained Langerhans cells typically have a moderate amount of homogeneous, pink, granular cytoplasm and distinct cell margins[44] (Figure), but precise identification requires more detail (Table IV). Definitive diagnosis rests on demonstration of the immunocytochemical features of the Langerhans cell or on electron microscopic demonstration of Birbeck granules in the cytoplasm[16] (Figure). Besides the presence of Birbeck granules, CD1a positivity is the other requirement for definitive diagnosis. It must be recognized, however, that because LCH may represent a spectrum of Langerhans cell maturation, all markers may not be present in every case.

The finding of conventionally processed tissue that reveals lesions consistent with those defined in the literature is now designated a "presumptive diagnosis." The benefits of accurate communication among physicians and of the comparison of results obtained by various treatment centers are self-evident. An additional important consequence of this international "histiocytic language" has been to facilitate large-scale cooperative studies of the natural evolution of LCH and its response to treatment.

THERAPY

Treatment of patients with LCH depends on the extent of disease. The number of organs involved and the implications of that involvement are of governing importance. When patients are assigned to one of the two categories described above (Table III), the prognosis is excellent for those with restricted presentations. It is guarded to ominous for those with extensive LCH, especially for patients with organ dysfunction.

Restricted Langerhans cell histiocytosis. Patients with apparently restricted LCH need careful staging of their disease to ensure that the lesions are not part of a more extensive process. The clinical course is generally benign and spontaneous remissions are common.

The treatment of patients with LCH apparently localized to the skin is patient dependent. Initially, treatment may be unnecessary because in many cases, mainly in infants, the lesions regress spontaneously. The first step in treatment is the application of topical steroids; if the lesions are unresponsive, resolution usually can be obtained with mild systemic chemotherapy. Topical nitrogen mustard[46] or irradiation[19] also has been used with success in patients who disease is resistant to other forms of therapy.

A single bone lesion tends to resolve spontaneously during a period of months to years. Biopsy of the lesion, necessary to confirm the diagnosis, may initiate healing with or without curettage. Criteria for additional treatment include intense pain and the threat of unacceptable deformity or disability, such as bone growth impairment, fracture, loss of hearing (from temporal bone involvement), or loss of permanent teeth (from maxillary or mandibular disease). Intralesional infiltration of corticosteroids, either as adjunctive therapy for extensive LCH with bone involvement or as primary therapy for the monostotic form, has been reported to be effective, convenient, and safe.[47] Radiation therapy in low doses (150 cGy/day for 4 days) has also been used for this purpose, usually when other measures have failed.[19]

When only lymph nodes are involved, the prognosis is favorable and most patients recover, usually without therapy.

One of the challenges in LCH management arises when a patient who was seen because of localized pain or a lump is then found to have multiple bone lesions. Some physicians

prefer curettage of one or more lesions; others instill corticosteroids. In Rotterdam, The Netherlands, after the diagnosis has been established by biopsy of the largest lesion, the patient is seen frequently in the outpatient clinic; half of the patients are found not to need systemic therapy because the lesions heal spontaneously.

Extensive Langerhans cell histiocytosis. The approaches to the treatment of extensive LCH with or without organ dysfunction have been almost as varied as the clinical manifestations of the disease. Most patients have been treated with systemic chemotherapeutic agents because of the progressive nature of generalized LCH. An interesting and conservative approach was reported by McLelland et al.,[48] who reserved systemic treatment for overtly ill patients, to whom a short course of prednisolone was given. Thus 25 of 44 patients with extensive disease were not treated with cytotoxic drugs; 8 of the 25 did not require systemic therapy, and the remaining 17 patients were given only prednisolone. These results compare favorably with those reported after more aggressive approaches.[24] Patients with extensive disease but without organ dysfunction will especially benefit from this approach, because their outlook is favorable.

Studies have demonstrated the efficacy of a variety of chemotherapeutic agents, either as monotherapy or in combination. These agents include (in alphabetic order) chlorambucil, cyclophosphamide, cytarabine, daunomycin, etoposide, mercaptopurine, methotrexate, mechlorethamine (nitrogen mustard), procarbazine, vinblastine, and vincristine, all with or without the use of corticosteroids.* These drugs have been shown to be effective, at least temporarily, in 50% to 60% of the patients treated. The validity of some older reports has been questioned; most if not all of these early studies reported only initial responses and did not give the follow-up information necessary to evaluate the impact of therapy on survival. There are also considerable doubts inherent in the review of clinical trials that were conducted for more than half a century in a variety of clinical settings and that were based on less than strict clinical and pathologic diagnostic criteria. Furthermore, comparisons of results obtained by drugs not assessed by the randomized trial mechanism are open to many uncertainties. For these reasons, an international randomized clinical study, organized through the auspices of the Histiocyte Society, was initiated in April 1991.[4] It is designed to examine the risks and benefits of vinblastine versus etoposide, two agents widely used in moderate- to high-risk patient populations. The two groups will be compared with respect to rates of disease response and recurrence, morbidity, and early and late toxic effects, including possible oncogenesis.

The symptoms of diabetes insipidus can be treated with desmopressin (1-desamino-8-D-arginine vasopressin). The effect of LCH systemic therapy on the appearance or progression of DI has not yet been established (see below).

Several advances in the management of LCH with the above-mentioned conservative therapies have been made. Improved treatment, however, is required, and several new strategies for either immunoregulatory or cytotoxic therapy are under study. The recently described therapeutic effect of cyclosporine is among the more promising findings in patients with extensive LCH and organ dysfunction not responsive to conventional therapy.[49] The rationale for using cyclosporine arises from its immunosuppressive and immunomodulatory effects, by selective inhibition of the cellular immune response and cytokine-mediated cellular activation. 2-Chlorodeoxyadenosine, which is potently toxic to monocytes, both in vitro and in vivo,[50] has been used successfully in the treatment of a patient with LCH refractory to conventional chemotherapy.[51] This agent might provide both a specific cytotoxic effect and a means of controlling immune-mediated cytokine release. Monoclonal antibody therapy, reported by Kelly and Pritchard,[52] also is of great interest both as a cytotoxin and as an immunoregulator. These authors have used the specificity of a monoclonal antibody directed against the CD1a antigen on Langerhans cells. These new approaches serve as excellent starting points for future development of effective therapies.

FOLLOW-UP AND SEQUELAE

The sequelae and late effects of treatment are dependent on the varied "natural history" of LCH itself and on the original signs and symptoms that dictate the intensity of treatment. Comparing morbidity among series has always been hampered by the lack of uniformly accepted schedules and methods of evaluation. This problem has largely been overcome by the standardized criteria that make accurate comparisons possible[3] (Tables VA and VB).

Late sequelae of the disease and its treatment seem to be more frequent than originally expected. The few studies focused on late-effects report rates ranging from 33% to 50%.[48, 53-55] The untoward sequelae include intellectual problems, neurologic symptoms, endocrine abnormalities (such as DI and growth failure), and orthopedic disabilities. There is no evidence that prolonged treatment prevents these sequelae, with the possible exception of diabetes insipidus. There was a 36% incidence of DI in patients treated conservatively (i.e., symptomatically) by McLelland et al.[48] This can be compared with the 15% overall frequency in the 106 children reported by Gadner et al.,[56] who treated all patients with multiple-agent chemotherapy and continued "maintenance" treatment for 2 years. With the exclusion of six patients who had DI at diagnosis, only 10 (10%) of the remaining 100 children given prolonged therapy subsequently had that complication.

There is a need to find the most effective and least toxic

*These agents have been reviewed by Egeler and Neshit,[24] and the interested reader is referred thereto. A detailed reference list is also available on request.

Table VA. Laboratory and radiographic investigations to be carried out when LCH is suspected

Investigation	Follow-up test interval when organ system is:		
	Involved	Not involved	Monostotic lesion
Laboratory evaluation			
Hemoglobin and/or hematocrit	Monthly	Every 6 mo	None
Leukocyte count and differential cell count	Monthly	Every 6 mo	None
Platelet count	Monthly	Every 6 mo	None
Liver-function tests (ALT, AST, ALP, bilirubin, total protein and albumin)	Monthly	Every 6 mo	None
Coagulation studies (PT, PTT, fibrinogen)	Monthly	Every 6 mo	None
Urine osmolality measurement after overnight water deprivation	Every 6 mo	Every 6 mo	None
Radiographic evaluation			
Chest radiographs, posteroanterior and lateral	Monthly	Every 6 mo	None
Skeletal radiograph survey	Every 6 mo	None	Once at 6 mo

Data from Clinical Writing Group of the Histiocyte Society (Broadbent V, Gadner H, Komp DM, Ladisch S. Med Pediatr Oncol 1989;17:492-5).
ALT, Alanine Transaminase; *AST*, aspartate transaminase; *ALP*, alkaline phosphatase; *PT*, prothrombin time; *PTT*, partial thromboplastin time.

Table VB. Laboratory and radiographic investigations to be carried out on specific indication of LCH

Test	Indication	Follow-up test
Bone marrow aspirate and trephine biopsy	Anemia, leukopenia, or thrombocytopenia	At 6 mo
Pulmonary function tests	Abnormal chest, radiograph, tachypnea, intercostal retractions	Every 6 months
Lung biopsy: preceded by bronchoalveolar lavage, when available; when diagnostic obviates the lung biopsy	Patients with abnormal chest radiographs in whom chemotherapy is being considered; to exclude opportunistic infection	None
Small bowel series and biopsy	Unexplained chronic diarrhea or failure to thrive, evidence of malabsorption	None
Liver biopsy	Liver dysfunction, including hypoproteinemia not caused by protein-losing enteropathy, to differentiate active LCH of the liver from cirrhosis	To be performed if and when all other disease has resolved, but liver dysfunction persists, to distinguish cirrhosis from continuing LCH
CT scan of brain/hypothalamic-pituitary axis, with intravenous contrast enhancement (MRI preferable)	Hormonal, visual, or neurologic abnormalities	Every 6 mo
Panoramic dental radiography of mandible and maxilla; oral surgery consultation	Oral involvement	Every 6 mo
Endocrine evaluation	Short stature, growth failure, diabetes insipidus, hypothalamic syndromes, galactorrhea, precocious or delayed puberty; CT or MRI abnormality of hypothalamus/pituitary	None
Otolaryngology consultation and audiogram	Aural discharge, deafness	Every 6 mo

Data from Clinical Writing Group of the Histiocyte Society (Broadbent V, Gadner H, Komp DM, Ladisch S. Med Pediatr Oncol 1989;17:492-5).
CT, Computed tomography; *MRI*, magnetic resonance imaging.

therapy for LCH in each of its several manifestations. Evidence implicating antineoplastic therapy as a cause of secondary malignancies has recently been recognized[57] and includes the possible leukemogenesis associated with type II topoisomerase inhibitors such as etoposide.[58] The risk/benefit ratio in the use of chemotherapy or radiotherapy or both, and the manner of their use, need to be weighed carefully. Patients in whom the mortality rate can reach 50%[59] should

not be denied treatment for fear of a less than 5% incidence of therapy-related second malignancies; the basic disease without effective therapy poses a much greater risk. Even so, studies are under way to minimize the leukemogenic risk of effective drugs such as etoposide through changes in dosage and scheduling.

PROGRESS IN BASIC RESEARCH

Although the cause is still unknown, investigative research in three major fields has advanced the understanding of LCH and has provided new insights to stimulate further biologic and clinical investigations of the disease.

LCH: a neoplastic or a reactive clonal disorder? For decades it has been thought that LCH is a reactive proliferation rather then a neoplastic process (e.g., because mitotic figures are sparse or absent[45]). In an attempt to quantify the percentage of cytologic atypia and mitotic activity in LCH, Risdall et al.[18] reviewed 51 cases and found mild cytologic atypia in half. The mitotic rates were low, ranging up to 23 per 10 high-power fields, with a median of 2. The DNA content analysis of LCH cells by flow cytometry has not shown aneuploid subpopulations in most of the patients studied.[60] However, DNA aneuploidy can appear sporadically in the lesions of a particular patient.[61, 62] It remains to be seen whether sporadic aneuploidy is of any value in indicating the course and outcome of the disease.

With progress in exploring the basic nature of the histiocyte and its disorders, recent laboratory studies have demonstrated that the cells in some forms of LCH are clonal expansions. Willman et al.[5] studied the clonality of lesions from 10 female subjects with the use of four different X-linked polymorphic probes. Clonal histiocytes were detected in the lesions of 9 of the 10 patients, 3 of whom had restricted disease of bone and 6 a form of extensive disease with or without organ dysfunction. In the tenth patient the clonality could not be interpreted with confidence. The clonality of cells from LCH lesions, first identified by Willman et al.,[5] was later verified by other investigators in three additional patients with extensive disease. Yu et al.[6] used isolated CD1a$^+$ LCH cells from the lesional tissues of three previously untreated female subjects affected with generalized LCH, and in each case the sorted "LCH" cells were purely clonal. The number of patients studied is still small, and these early observations need to be substantiated in patients with LCH across the spectrum of disease. The results so far are compatible with the hypothesis that LCH is a clonal neoplastic disorder arising from somatic mutations, and that these mutations cause the clonal expansion of Langerhans cells or their precursors in the bone marrow and other organs. However, clonal proliferation of rare progenitor cells resident in or attracted to lesions in response to cytokines may produce a *nonneoplastic* clonal proliferation of histiocytes.[10] "Clonality" therefore does not necessarily indicate a malignant process; clonal cells have been detected in several disorders that are not malignant.[63] A key question that remains is whether "clonality" has therapeutic implications.

LCH: a cytokine-mediated disorder? Cytokines, soluble secretory products of lymphocytes and monocytes, regulate cell growth and differentiation of immunocompetent and hematopoietic stem cells by binding to specific receptors on target cells. Activators can cause these cells to release some cytokines or to cease secreting others, which leads to cell transformation, proliferation, phagocytosis, and other functions. This complex pattern is referred to as activation.[7] Histiocytes, including dendritic cells, are subject to "down" or "up" regulation by activators—hence the hypothesis that LCH may be cytokine related. The disease is associated with unexplained, aberrant behavior of Langerhans cells, which are normally responsible for first-line specific immunologic defense in the skin. On contact with foreign material (antigens), they will take up and process the antigen and migrate (as so-called veiled cells) to the draining lymph nodes, where they can present the antigen to the immune system (usually to T cells) for an appropriate response. This normal function is a complex cascade of events involving antigen recognition, uptake, degradation, migration, differentiation, cellular activation, contact, signaling, and differentiation. Somewhere along this line of defense, which is regulated to a great extent by cytokines, a signal is possibly not correctly given or interpreted. An exaggerated activation of cytokines or a loss of control of cytokine activation, resulting in LCH, might be the consequence. The specific route of the cytokine cascade involved in LCH has not yet been established, but many of the systemic and local signs and symptoms of LCH may be caused by one or more of the cytokines; it is known that certain cytokine levels are increased in the affected tissues of patients with LCH.[8-10] A recent study of growth factors that cooperate in the generation of Langerhans cells showed that granulocyte-macrophage colony-stimulating factor and tumor necrosis factor-α can work together to produce Langerhans cells (20% contained Birbeck granules) from hematopoietic stem cells (CD34$^+$ precursor cells).[64] On the basis of these data, one can envision a proliferation of Langerhans cells in LCH when these two factors are stimulated. Both in vivo and in situ studies are needed to clarify the possible regulatory role of cytokines, T cells, their receptors, and their inhibitors in the pathogenesis of LCH. Such research needs to be conducted in the context of the various clinical forms of LCH, because the cytokine profile of a single LCH bone lesion may differ significantly from that of a soft-tissue lesion in a patient with extensive disease.

LCH: a viral disease? Because the cells of the mononuclear phagocyte system have important functions in immunoregulation, much effort has been expended in defining possible subtle aberrations in the immune system of patients

with LCH. One or more immunologic mechanisms could be involved, as indicated by abnormalities of immunoglobulin levels, mitogen responses, and T-suppressor cells in patients with LCH.[65, 66] No consistent defect has, however, been demonstrated. With or without such an immunologic irregularity, it has been postulated that LCH may be the reactive result of a viral infection that causes the Langerhans cells to proliferate. Pursuing this idea, one can postulate that the proliferation of Langerhans cells in LCH may be a physiologically appropriate but clinically pathologic response to a viral infection, with or without cytokine involvement. A specific virus might activate histiocytes but also impair immune regulation of subsequent histiocytic proliferation. By disabling the suppressor T cell, it would amplify the host immune response to the primary infection. Polymerase chain reaction techniques can be applied for the extraction of DNA in the search for specific viral codes, and can be used in stored fixed tissue.

Recently, three comprehensive studies of possible viral etiology have been reported. McClain et al.[11] presented the results of in situ hybridization and PCR studies of 56 patients. No positive results were obtained when probes were used against viral DNAs (human T-cell viruses types I, II, and III [human immunodeficiency virus], adenovirus, cytomegalovirus, Epstein-Barr virus, parvovirus, herpes simplex virus, and human herpesvirus type 6). These negative findings were confirmed by ultrastructural studies.[12] The HHV-6 was also suspected of playing a role in the pathogenesis of LCH by Leahy et al.[13] This recently described member of the human herpesvirus family has been associated with atypical or malignant lymphocytic processes and immune complex disorders. Lesional tissue of 30 patients with LCH was retrospectively examined by PCR for the presence of HHV-6. In contrast to the findings by McClain et al.,[11] HHV-6 DNA was detected in lesions from 14 (47%) of 30 patients with LCH. On clinical subgroup analysis, HHV-6 DNA was found in 10 (63%) of 16 patients with extraosseous disease and in 4 (29%) of 14 patients with disease limited to bone. Herpes simplex virus DNA was not found in any of the LCH specimens.[13] Additional in situ hybridization studies of LCH in more samples, with a view to localizing the HHV-6 DNA or other viral DNA to the Langerhans cell, are needed. This finding would help to clarify the potential pathogenic role of the virus in this disease and would possibly lead to more specific understanding and treatment of LCH.

SUMMARY AND CONCLUSIONS

The first major stride toward understanding LCH was taken when ultrastructural studies identified the proliferating cells as part of the Langerhans (dendritic) cell system.[16, 17] Another step forward was the definition of the morphologic, immunohistochemical, and clinical criteria needed for the diagnosis of LCH.[2, 3] Meanwhile, modern imaging studies have disclosed lesions that were not previously visible, especially those in the brain and the pituitary gland. These advantages have had a major impact on clinical management by making it possible to compare data from different institutions and to centralize coherent clinical and therapeutic data.[4] Moreover, the agreement concerning diagnostic criteria provides a solid foundation for current clinical trials and for laboratory research regarding the possible roles of the immune system, clonality, and cytokines in the etiology of LCH.

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