Molecular Diagnostics and the Histiocytoses

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Abstract: The histiocytoses are a group of rare proliferative disorders derived from dendritic cells and macrophages. The prognosis is variable, ranging from benign lesions though chronic debilitating illness to rare true malignant disorders. Diagnosis is by clinical features, histology including morphology and immunohistochemistry, and recently in haemophagocytic lymphohistiocytosis, using molecular diagnostic techniques. The aetiology of Langerhans cell histiocytosis is still unclear. Recent molecular studies suggest that lesions do not arise from epidermal Langerhans cells, but from accumulation of bone-marrow derived immature myeloid dendritic cells that recruit activated lymphocytes. Macrophage activation syndromes appear to result from lymphocyte/NK cell-driven uncontrolled macrophage activation which leads to proliferation of histiocytes and T cells, and disseminated overactivity of macrophages with cytokine overproduction. Haemophagocytoc lymphohistiocytosis (HLH) refers to a group of recessively inherited disorders that have the clinical and pathological features of an acute and usually fatal form of uncontrolled macrophage activation syndrome. Primary" HLH has been used to describe young children with HLH with proven gene mutations or family history. Evidence of bi-allelic mutations in *PRF1* (encodes perforin), *UNC13D* (encodes MUNC13-4), or *syntaxin 11* are diagnostic of HLH *SH2D1A*, the gene encoding SLAM (signaling lymphocyte activation molecule)-associated protein, SAP, is mutated in X-linked lymphoproliferative disease (XLP) and is associated with HLH in boys with XLP with EBV infections. In patients with Griscelli syndrome type 2, mutations in *RAB27A*, and in patients with Chediak-Higashi syndrome type 1, *LYST* mutations are associated with HLH. In many patients with inherited HLH, the responsible gene or genes remain to be defined.

Keywords: Histiocytoses, lymphoproliferation, immunohistochemistry, molecular diagnostics.

INTRODUCTION

In this review we describe the current classification of the histiocytoses, highlighting new developments and in particular the contribution of molecular diagnostic techniques to improving our understanding of these disorders. We briefly describe the normal function and physiology of the cell types involved, and then detail the histopathological features of the different disorders, emphasising immunohistochemical patterns in distinguishing the entities from reactive histiocytic proliferations.

Histiocytes

The term "histiocyte" is rather loose, and includes both activated dendritic cells and macrophages, with circulating monocytes as intermediate forms. Histiocytic cells are bone marrow-derived and develop from a common CD34-positive stem cell precursor which also gives rise to common lymphoid and myeloid precursors [1]. The only histiocytic cell which is not bone marrow-derived is the follicular dendritic cell which seems to derive from mesenchymal cells [2]. The majority of "histiocytic" proliferations recapitulate the phenotypes of more mature dendritic cells or macrophages normally found in extramedullary tissues.

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Dendritic cells (DCs) are antigen -presenting cells found in tissues that are in contact with the external environment, mainly the skin (Langerhans cells) and the mucosal lining of the respiratory and alimentary tracts. They have branching cytoplasmic processes which increase their surface area and allow them to present antigen to a number of T lymphocytes simultaneously. DCs take up antigen in the periphery and migrate to the paracortex of draining lymph nodes where they present antigen. If the antigen matches a receptor on the T cell, an immune response is initiated. They then become interdigitating DCs. Together with T regulatory cells, DCs are essential for the establishment and maintenance of immune tolerance which can be either "natural" or "self" tolerance where the body does not mount an immune response to self antigens, or "induced" tolerance where tolerance to external antigens can be created by manipulating the immune system. Genetic defects in these processes may lead to autoimmunity [3].

Macrophages are histiocytes within tissues which are derived from circulating monocytes. There are a wide variety of tissue- specific macrophages such as bone osteoclasts, brain microglia, liver Kupffer cells, renal mesangial cells, pulmonary alveolar macrophages, serosal macrophages and the macrophages of the dermis and connective tissues. They are phagocytes which engulf and then digest cellular debris and pathogens, and stimulate lymphocytes and other immune cells to respond to the pathogen. The binding of bacterial molecules to receptors on the surface of a macrophage triggers it to engulf and destroy the bacteria through the

generation of a respiratory burst, causing the release of reactive oxygen species [4]. Pathogens also stimulate macrophages to produce chemokines which summon other cells to the site of the infection. They act in both innate immunity (non-specific defense) and adaptive immunity (initiation of specific defense mechanisms). DCs serve as a link between the innate and adaptive immune systems.

Dendritic cell maturation from monocytes is regulated in part by IL-4, granulocyte-macrophage colony stimulation factor and tumour necrosis factor -α. Macrophage production from myeloid precursors is driven by IL-1, IL-3, macrophage colony-stimulation factor and granulocyte-macrophage colony- stimulating factor. More recently the boundaries between DCs and macrophages have become blurred with some authors suggesting a continuum between macrophages and DCs with considerable phenotypic plasticity. Both macrophages and dendritic cells can be driven in the direction of the other by various stimuli e.g. cells with dendritic cell phenotype can be converted to macrophage phenotype by IL-10 or macrophage colony -stimulating factor [5]. A recent concept is that of different subsets of macrophages, namely M1, the short-lived inflammatory macrophage, and M2, the so-called tissue macrophage [6]. The M2 macrophage is likely to be closely related to, or identical to the interstitial DC. The practical implication of this plasticity for the histopathologist is that the distinction of macrophages from dendritic cells is not absolute, and histiocytic lesions commonly contain a mixture of monocytes, macrophages and dendritic cells, even if one cell type dominates. Macrophage fusion is a common phenomenon, resulting in multinucleated giant cells. Incubation with IL-4 encourages macrophages to assume an epithelioid appearance.

Classification of the Histiocytoses

The classification of histiocytoses in this review is based on the Histiocyte Society classification of the histiocytic disorders that primarily affect children (Table **1**). The classification does not include granulomatous disorders or lysosomal storage diseases, although these disorders should be included in the histopathological differential diagnosis of the histiocytoses.

In the Histiocyte Society classification nosology, based on the lineage of lesional cells and biological behaviour, is related to the ontogeny of histiocytes. DC-related disorders of varied biological behaviour are dominated by Langerhans cell histiocytosis. Juvenile xanthogranuloma is considered to represent a disorder of dermal dendrocytes, while Rosai-Dorfman syndrome (also known as sinus histiocytosis with massive lymphadenopathy) is a disorder of an unknown cell, intermediate between an interdigitating dendritic cell and a macrophage. Reticulohistiocytoma is the best-defined example of the solitary histiocytoma. The hemophagocytic syndromes are the most common of the macrophage-related disorders. All the histiocytic malignancies are rare tumours of adults as well as children. There is some overlap of the Histiocyte Society classification with the 2008 WHO classification of "histiocytic and dendritic neoplasms" which encompasses Langerhans cell histiocytosis, histiocytic sarcoma and the dendritic cell sarcomas.

Table 1.   Histiocyte Society Classification of the Histiocytic Disorders (1997)

| **Dendritic Cell Related** |
| Langerhans cell histiocytosiss |
| Juvenile xamthogranuloma and related processes |
| Solitary histiocytoma of various dendritic cell phenotypes |
| **Macrophage Related** |
| Hemophagocytic syndromes |
| Primary hemophagocytic lymphohisitocytosis (familial and sporadic) |
| Secondary hemophagocytic syndromes |
| Rosai -Dorfman disease |
| Solitary histiocytoma with macrophage phenotype |
| **Malignant Diseases** |
| Monocyte related |
| Leukaemias |
| Monocytic leukaemia (M5A and B) |
| Acute myelomonocytic leukasmia (M4) |
| Chronic myelomonocytic leukaemia |
| Extramedullary monocytic tumour or sarcoma |
| Dendritic cell -relaed histiocytic sarcoma |
| Specifiy phenoptype (follicular dendritic cell, interdigitating dendritic cell etc.) |

Recognition of the different histiocyte subsets in day-to-day diagnostic histopathology practice depends on the expression of a panel of antigens detected by antibodies which work on formalin-fixed paraffin-embedded tissue (Table **2**). Of interest is the fact that in rare cases, there may be overlap of different histiocytic syndromes in the individual patient. For example, juvenile xanthogranuloma may occur in a patient with Langerhans cell histiocytosis (LCH), LCH may be complicated by macrophage activation syndrome, and a lymph node from a patient with LCH may show focal Rosai-Dorfman changes.

Table 2.   Immunophenotype of Histiocytes Encountered in the Histiocytoses

	Langerhans Cell	**Dermal Dendritic Cell**	**Interdigitating Dendritic Cell**	**Macrophage**
HLA -DR	++	+	++	+/-
CD1a	++	-	-	-
CD14	-	-	-	+
CD68	+/-	+	+/-	++
CD163	-	-	-	+
Factor XIIIa	-	+	-	-
Fascin	-	+	+/-	-
Langerin (CD207)	++	-	-	-
Lysozyme	-	-	-	++
S 100	++	-	++	+/-

Fascin indicates an activated and mature dendritic cell phenotype.
From the point of view of characterising the dendritic saromas, CD21 stains the folllicular dendritic cell and CD123 the plasmacytoid dendritic cell.

DENDRITIC CELL-RELATED HISTIOCYTOSES

Langerhans Cell Histiocytosis (LCH)

Clinical Features

LCH is a rare monoclonal disease of the dendritic cell system which affects 2-5 children per million annually [7, 8]. It most frequently affects children aged 1-4 years but can occur at any age. Histologically it is characterised by the 'LCH cell', which is a histiocytic cell with a large amount of eosinophilic cytoplasm and kidney-shaped nucleus. LCH cells are positive on immunostaining for S100 protein, CD1a and langerin (CD207). Fascin staining is negative indicating these are immature dendritic cells. Birbeck granules are identified on electron microscopy. The lesions may form granulomas and may be associated with a characteristic mixed infiltrate of eosinophils, neutrophils, lymphocytes, macrophages and multinucleated giant cells. Long-standing lesions may burn out and be replaced by fibrosis.

Isolated pulmonary LCH is an interstitial lung disease occurring predominantly in adult cigarette smokers that is generally thought to be a non-clonal reactive condition. Similarly the LCH described in lymph nodes adjacent to some epithelial cancers and Hodgkin's lymphoma appears to be polyclonal [9].

The disease may be single system (in bone, skin, lymph node, pituitary, thymus, mucosa, soft tissue or lungs in adults) or multisystem. Lesions involving spleen, liver, bone marrow and lung, in the context of multisystem disease, are associated with high -risk disease. Cutaneous lesions are common (40% of patients) and are often the presenting manifestation. Characteristically lesions are found on the trunk, scalp, napkin area and intertriginous folds, typically consisting of erythematous pink or brown papules which become scaly, crusted or purpuric. The disease has a variable prognosis depending on the extent of organ involvement, ranging from a complete cure to fatal. The congenital self-healing type (Hashimoto-Pritzker) usually only shows cutaneous involvement with multiple nodular umbilicated skin lesions [10]. However, this diagnosis can be made only retrospectively as nearly 60% of patients with neonatal skin lesions go on to develop multisystem disease [11].

Histopathological Features (Fig. 1)

In the skin, lesions show clusters and sheets of LCH cells immediately beneath the epidermis and focally invading the epidermis with small aggregates in the upper epidermis. There is a variable admixture of inflammatory cells. There is immunophenotypic heterogeneity and not all cells stain with a particular marker in an individual case. Rarely, lesional cells are langerin-positive CD1a-negative which suggests a slightly more mature phenotype. Lesions with this phenotype are more likely to involve the CNS or liver, and local recurrence is more common [12]. CD68 is expressed in LCH cells as small paranuclear intracytoplasmic accumulations, but shows coarse cytoplasmic granular expression in larger macrophages. CD68 is especially prominent in the reactive CD1a-positive multinucleated giant cells that are occasionally observed in bony lesions. Fibrosis is a characteristic of long-standing "burnt out" lesions. In lymph nodes the infiltrate is characterisitically sinusoidal. In liver
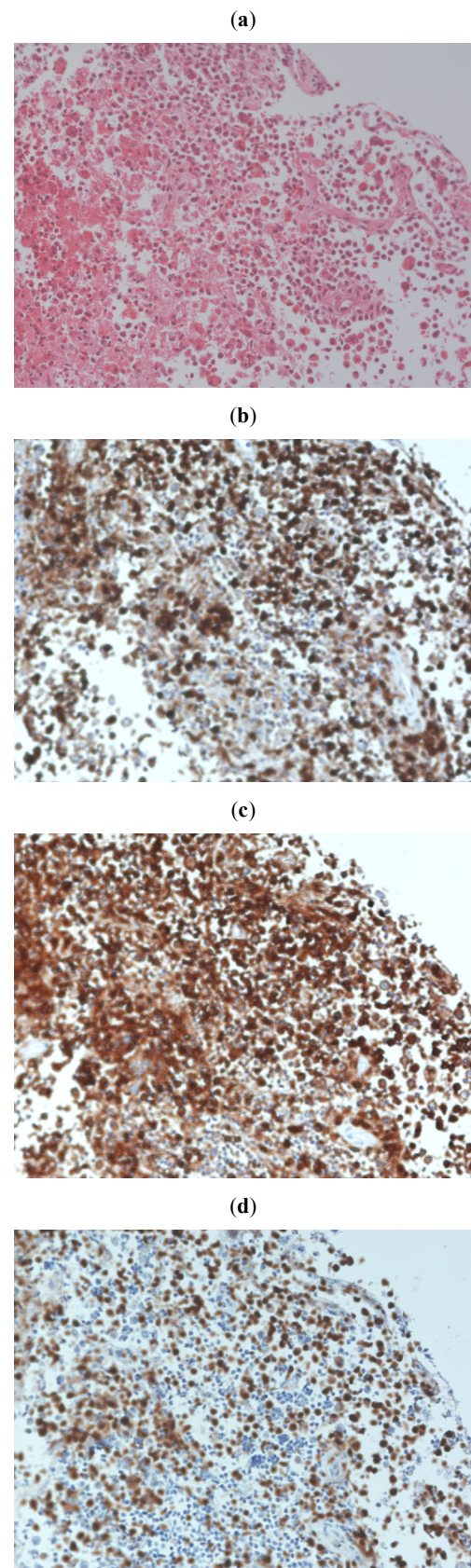
(a)

(b)

(c)

(d)

Fig. (1). Langerhans cell histiocytosis. Needle biopsy of scalp lesion in a 2 year old boy who also had perineal rash, polydipsia and headache. H&E stain (**a**) shows LCH cells with convoluted nuclei and ample eosinophilic cytoplasm. Immunostaining shows langerin (**b**), CD1a (**c**) and S 100 (**d**) positivity.

the infiltrate homes to bile duct epithelium, resulting in sclerosing cholangitis. In the lung the infiltrate homes to bronchial epithelium, while fibrosis results in honeycomb lung. Interestingly the pattern of infiltration in the skin is different in the self-healing form from the usual form with mid and deep dermal nodules of LCH cells. Extension into the subcutis may occur but there is no epidermotropism.

The differential diagnosis depends on the site of involvement. Skin lesions can mimic mastocytosis, juvenile xamnthogranulma and nevi. Many chronic dermatoses show an increase in perivascular spindle-shaped CD1a-positive Langerhans cells. Bony lesions are most likely to be confused with culture-negative chronic recurrent multifocal osteomyelitis *(*CRMO*)* which is characterised by plasma cell infiltration. A good rule of thumb is the absence of plasma cells within the lesions of LCH although they may be present in the surrounding inflammatory reaction. Fibrotic lung lesions with few LCH cells may look like interstitial fibrosing lung disease. Within the bone marrow, stromal cells are S 100-positive as may be activated macrophages. Only large CD1a-positive and langerin-positive cells can be considered diagnostic. In lymph nodes, dermatopathic lymphadenopathy, which may or may not be associated with a skin rash, shows paracortical replacement by interdigitating DCs and Langerhans cells, in contrast to the sinusoidal involvement of LCH. Fascin staining is strongly positive indicating their maturity.

Molecular Genetics

After decades of research, the causes of LCH remain speculative. The disease is hypothesised to originate from an unregulated proliferation of immature Langerhans cells. However, the aetiology remains unclear. Current models support either a malignant proliferation of a dendritic cell subset or a chronic inflammatory disease involving dendritic and other immune cells [13-16]. However, there are no definitive studies to prove either an infectious or oncologic etiology for LCH. While some earlier studies suggested LCH might arise from herpes virus infections, this hypothesis has not been supported [17-19]. The pro-inflammatory cytokine interleukin 17 (IL17) was recently suggested to play a role in lesion formation in LCH. However a follow-up study failed to find evidence of *IL17A* expression in any cells in LCH lesions or detectable IL17 protein in plasma of patients with active LCH [20, 21]. Functional epidermal Langerhans cells are thought to present antigen to naïve T-cells. However, in LCH the langerin-positive Langerhans cells are phenotypically immature and do not efficiently stimulate a primary T-cell response [22, 23]. Interestingly, enrichment of CD4+CD25highFoxP3high regulatory T cells has been described in LCH lesions, though the etiology and implications for this phenomenon remain to be defined [24].

Additionally, extensive studies have evaluated gene mutations and neoplastic transformation as a factor in LCH. The strongest support for LCH as a neoplastic process is clonality of the CD1a-positive cells from LCH lesions [25, 26]. However, further evidence for a genetic cause of LCH is lacking. There are case reports of LCH in siblings and some twins, though the incidence may not be higher than would be expected by chance [27]. Earlier studies cited cytogenetic abnormalities in cells from LCH lesions, and even in mononuclear cells from peripheral blood from patients with active LCH [28-30]. However, we have not observed cytogenetic abnormalities on over 20 LCH samples at Texas Children's Hospital, and a more recent study using array CGH failed to identify any mutations in langerin-positive LCH cells [31].

In order to further define pathogenesis of LCH, we have performed cell-specific gene expression studies. Langerin-positive cells were isolated from LCH lesions and compared to control epidermal Langerhans cells. Surprisingly expression of many genes previously associated with LCH including cell-cycle regulators, pro-inflammatory cytokines and chemokines previously reported as abnormal in LCH were not significantly different from control Langerhans cells in our study. However, several novel genes whose products activate and recruit T cells to sites of inflammation including *SPP1* (*osteopontin*) were highly over-expressed in Langerin-positive LCH cells. Furthermore, several genes associated with immature myeloid dendritic cells were over-expressed in Langerin-positive LCH cells. In a parallel series of experiments, we compared gene expression in CD3-positive cells to expression in peripheral CD3-positive cells from the same LCH patients. The expression profile suggested that the LCH lesions are enriched with activated regulatory T cell phenotype with increased expression of *FOXP3, CTLA4* as well as *SPP1*. These results lead us to propose a model of LCH pathogenesis in which lesions do not arise from epidermal Langerhans cells, but from accumulation of bone-marrow derived immature myeloid dendritic cells that recruit activated lymphocytes. This concept is supported by recent data showing that dendritic cells other than classic Langerhans cells can express langerin. (Allen and McClain, Presented at Histiocyte Society 2009, Nikolas Symposium 2009).

Juvenile Xanthogranuloma

Clinical Features

This is a group of disorders with many similarities to LCH characterised by the juvenile xanthogranuloma cell (JXG cell) which has a typical immunophenotype consistent with derivation from the dermal dendrocyte [32]. The most common presentation is with a small solitary cutaneous or ocular lesion, though multiple lesions may also occur. A small subpopulation of children under the age of 3 months may have widespread systemic involvement. Benign cephalic histiocytosis is a localised variant in young adults in which clusters of lesions on the head, face or upper body are progressive [33]. Xanthoma disseminatum is characterised by numerous skin and mucosal lesions in adolescents and young adults in which there may also be visceral involvement. Erdheim-Chester disease is an aggressive variant in adults with symmetrical bone sclerosis and systemic involvement, mostly involving lung, kidney, retroperitoneum or heart [34].

Histopathological Features (Fig. 2)

Early lesions have small histiocytes with folded bland nuclei and a moderate amount of vacuolated cytoplasm. Touton giant cells with a wreath of nuclei around a central eosinophilic core and a xanthomatous periphery are seen in about 85% of cases, and are pathognomonic but not necessary for the diagnosis. As lesions age, xanthomatous

cells increase and spindle cells also increase in number. Lymphocytes are interspersed and there may be occasional eosinophils. The immunophenotype is characteristic. Cells show membrane staining with the monocyte-macrophage marker CD14 and coarse granular cytoplasmic staining with CD68. CD163 shows strong surface staining. In addition to these macrophage markers, cells also stain with the dendritic cell markers Factor XIIIa and fascin. CD1a and Langerin are negative. There may be weak S100 staining in less than 30% of cases. The major differential diagnosis is LCH but histology and immunostaining (CD1a-negative, S100-negative) should discriminate. Older lesions which contain more spindle cells simulate benign fibrous histiocytoma but the JXG immunophenotype is characteristic, although dermal fibrous histiocytomas may have large numbers of factor XIIIa-positive cells interspersed.
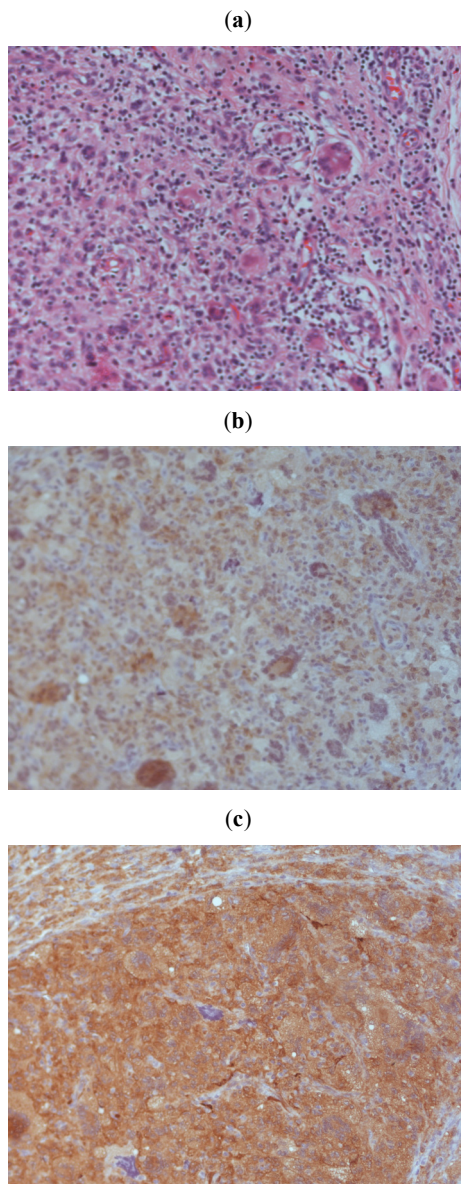
(a)

(b)

(c)

Fig. (2). Juvenile xanthogranuloma. Biopsy of skin lesion from an 18 month-old boy with an extensive xanthomatous eruption. H&E stain (**a**) shows Touton giant cells and admixed histiocytic cells. Immunostaining demonstrates Factor XIIIa (**b**) and fascin (**c**) positivity.

No consistent abnormalities have been found, nor is there information on clonality of lesions. However, patients with JXG, neurofibromatosis, and juvenile chronic myelogenous leukemia have been reported [35-37].

Reticulohistiocytoma

Clinical Features

The solitary reticulohistiocytoma is a cutaneous lesion seen in neonates and young adult males. Multicentric reticulohistiocytosis is a cutaneous and arthropathic condition [38]. Most children have self-limiting disease with non-deforming arthritis, while 50% of adults progress to a deforming polyosteoarthropathy.

Histopathological Features

Lesions contain large histiocytic cells with one to three eccentric oval or grooved nuclei and abundant deeply eosinophilic glassy cytoplasm with intervening inflammatory cells. The histiocytic cells stain for CD68, and may show light staining for factor XIIIa and muscle- specific actin, but are negative for S 100, fascin and CD1a. The differential diagnosis includes Rosai-Dorfman disease, juvenile xanthogranuloma and melanoma

Molecular Genetics

There is no information on cytogenetic abnormalities or clonality of lesions

MACROPHAGE-RELATED HISTIOCYTOSES

Macrophage Activation Syndromes

Macrophages are constantly changing their function and phenotype in response to a wide variety of microenvironmental factors. These changes are modulated by activating cytokines like interferon-γ, tumour necrosis factor, IL-1, IL-2 and macrophage inhibitory factor, and inhibitory mediators like IL-4, IL-10, IL-13 and transforming growth factor -β.

Macrophage activation syndromes appear to result from lymphocyte/NK cell-driven uncontrolled macrophage activation which leads to proliferation of histiocytes and T cells, and disseminated overactivity of macrophages throughout the body with cytokine overproduction. It is possibly mediated by tumour necrosis factor-α with ineffective deactivation through depressed NK and cytotoxic CD8+ T cell activity. Systemic macrophage activation syndrome can develop in response to a number of different stimuli of which viral infections are the best known. Other causes include bacterial infections, parasitic infections, rheumatological disorders, cancers, lymphoproliferative disorders, LCH, parenteral nutrition and multi-organ failure. An increase in the number and size of activated macrophages, with or without haemophagocytosis is a feature of the condition, best seen in the bone marrow but also in the spleen, liver and lymph nodes. The cytokine storm may cause bone marrow depression, hepatomegaly with increased heptocellular enzymes and effects on the clotting cascade. The condition abates when the initiating condition is treated or disappears, but in severe instances it can be fatal.

Haemophagocytic Lymphohistiocytosis (HLH)

Clinical Features

Haemophagocytoc lymphohisitocytosis (HLH) or familial haemophagocytic lymphohistiocytosis refers to a group of recessively inherited disorders that have the clinical and pathological features of an acute and usually fatal form of uncontrolled macrophage activation syndrome. Treatment is by stabilisation with immunosuppression and/or chemotherapy followed by stem cell or bone marrow transplantation.

Diagnosis may be the biggest challenge in treatment of HLH. Initial signs and symptoms are difficult to distinguish from more common conditions such as sepsis. Furthermore, concurrent viral infections, bacterial infections, malignancy or auto-immune disease do not exclude a diagnosis of HLH. Prominent early clinical signs may include fever, hepatomegaly, splenomegaly, neurological abnormalities, rash, and lymphadenopathy [39]. There may be a history of consanguininty or previous sibling death. The clinical features are dominated by the effects of the cytokine storm. Infants present with prolonged fever and cytopenias within the first 2 years of life, neurological and meningeal signs and symptoms, hepatomegaly with abnormal liver function tests, splenomegaly and sometimes a skin rash. Laboratory features include hypofibrinogenaemia, hyperferritinaemia, high levels of circulating CD25 (IL-2 receptor), soluble CD163 and hypertriglyceridaemia. NK cell function is low or absent. "Primary" HLH has been used to describe young children with HLH with proven gene mutations or family history. Older children are sometimes described as having "secondary" or acquired HLH with the assumption that the condition is due to antigen challenge rather than genetic predisposition. However, inherited familial HLH is not limited to infants. Several case reports, series and reviews cite inherited HLH in older children, adolescents and adults with defects in HLH-associated genes. Furthermore, the distinction between "primary" and "acquired" HLH is not useful in the acute clinical setting. Without therapy, all patients meeting criteria for HLH would be expected to have 10% survival without therapy [39]. If treated with therapy according to the Histiocyte Society protocol HLH-1994, approximately 55% of patients survive, and there is no significant difference in outcomes between patients presumed to be "primary" versus "acquired" [40, 41].

Histopathological Features (Fig. 3)

An excess of activated macrophages, usually with haemophagocytosis is the hallmark. These cells can be found in the bone marrow, spleen and lymph nodes. In the liver, activated Kupffer cells showing haemophagocytosis are accompanied by a marked portal tract infiltrate of activated CD8-positive T cells. The CSF may show haemophagocytic macrophages. However, it should be emphasized that hemophagocytosis is neither necessary nor sufficient for a diagnosis of HLH. Bone marrow biopsies fail to demonstrate hemophagocytosis in 20% of patients with active HLH [42].

Molecular Diagnostics

The diagnostic criteria for HLH have been defined by the collective experiences of the members of Histiocyte Society
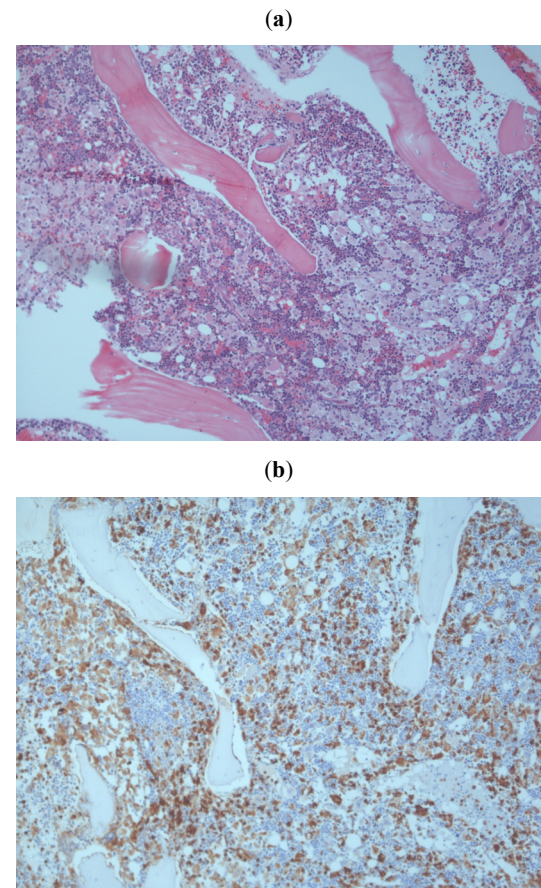
(a)

(b)

Fig. (3). Macrophage activation syndrome. Bone marrow biopsy from a 2 year old boy with biopsy-proven multisystem Langerhans cell histiocytosis and bone marrow failure. H&E stain (**a**) shows a diffuse infiltrate of large pale macrophages which show positive immunostaining for CD 68 (**b**).

as well as accumulation of case reports, series and studies of patients with pathologic inflammation defined as HLH [40]. In addition to clinical findings (fever and splenomegaly), laboratory studies (blood counts, triglycerides, fibrinogen, ferritin, soluble IL-2 receptor), histologic findings (hemophagocytosis) and immune function studies (natural killer cell function) constitute the current criteria that define HLH [40]. While a ferritin level over 500 µg/liter is the range deemed as abnormal in the current HLH-2004 treatment protocol, a retrospective study of patients with elevated ferritin over a two year period at Texas Children's Hospital found ferritin concentrations over 10,000 µg/liter to be 90 percent sensitive and 96 percent specific for HLH. Evidence of bi-allelic mutations in *PRF1* (encodes perforin), *UNC13D* (encodes MUNC13-4), or *syntaxin 11* are also diagnostic of HLH [43-45]. *SH2D1A*, the gene encoding SLAM (signaling lymphocyte activation molecule)-associated protein, SAP, is mutated in X-linked lymphoproliferative disease (XLP) and is associated with HLH in boys with XLP with EBV infections [46]. In patients with Griscelli syndrome type 2, mutations in *RAB27A*, and in patients with Chediak-Higashi syndrome type 1, *LYST* mutations are associated with HLH [47, 48]. In many patients with inherited HLH, the responsible gene or genes remain to be defined.

Macrophage Activation Syndrome (MAS)

MAS describe patients with symptoms of HLH in the setting of active autoimmune diseases, including idiopathic rheumatoid arthritis or systemic lupus erythematosus [49]. MAS are differentiated from HLH when there is clinical suspicion for an autoimmune disease accompanying clinical and laboratory evidence for HLH. Patients with MAS may be successfully treated with steroids or steroids and cyclosporin [49, 50]. However, many patients with HLH triggered by an auto-immune disease also have mutations in HLH-associated genes. When immune suppression alone is unable to control pathologic inflammation, patients with "MAS" may require chemotherapy as well as immune suppression, and possibly hematopoietic stem cell transplant.

HISTIOCYTOSIS OF CELL INTERMEDIATE BETWEEN INTERDIGITATING DENDRITIC CELL AND MACROPHAGE

Rosai-Dorfman Disease

Clinical Features

Also known as sinus histiocytosis with massive lympadenopathy, the disease usually presents with very large painless cervical lymphadenopathy associated with fever, raised ESR and a poyclonal hyperglobulinaemia. The disease can also occur extranodally, with or without cervical node involvement, the most frequent sites being skin and subcutaneous tissue, the orbit, bone and meninges, although any organ can be involved. Most cases occur in adolescents or young adult males. Some large nodes regress spontaneously without treatment. Soft tissue lesions can be indolent but may respond to chemotherapy.

Histopathological Features (Fig. 4)

In lymph nodes the infiltrate is sinusoidal, often causing so much distension and distortion of sinuses that the sinusoidal pattern is not recognisable. The lesional cells are very large, with a central large hyperchromatic nucleus set in abundant pale cytoplasm. Nucleoli are single, moderately large and distinct. Mature lymphocytes, many plasma cells and neutrophils are often present between the histiocytes and within the cytoplasm. The presence of intact cells within the cytoplasm, as many as 5-10 lymphocytes within the pathologic histiocytes, is the characteristic emperipolesis, although it may be less prominent in extranodal sites. Late or involuting lesions have less Rosai-Dorfman cells, and more xanthoma cells and spindle fibroblasts, and late lesions can simulate inflammatory myofibroblastic tumour. Immunohistochamically, the cells mark as a hybrid of macrophages and dendritic cells. They express surface CD14, CD15, CD25, CD163, granular cytoplasmic lysozyme and CD68. In addition, they show strong cytoplasmic expression of S-100 and fascin. However, the cells are negative for CD1a and langerin.

Molecular Diagnostics

There are no known molecular or cytogenetic features, and the large Rosai-Dorfman cells are not clonal [51].

MALIGNANT HISTIOCYTOSES

Histiocytic Sarcoma

Clinical Features

This is a disease of adults with a mean age of 45-55 years and equal sex ratio. It may also occur as a second malignancy in young males with mediastinal germ cell tumour. Presentation is with a mass involving lymph nodes, soft tissue, and gastrointestinal tract.

Histopathological Features

The infiltrate is composed of large epithelioid cells with abundant eosinophilic cytoplasm. Nuclei are oval and variable in size with a large amphophilic nucleolus. There may be focal pleomorphism with giant cells. There may be an admixture of lymphocytes and eoinsophils [52].
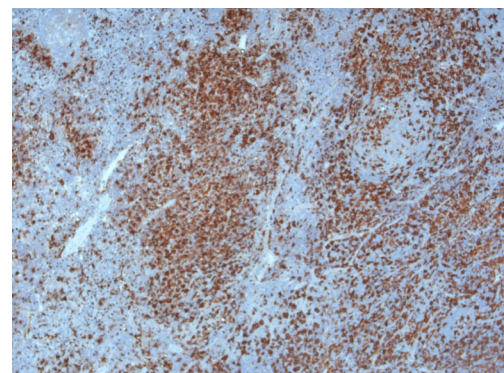
(a)
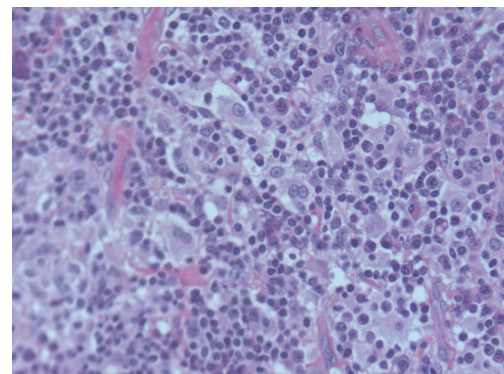
(b)

Fig. (4). Rosai-Dorfman disease. Lymph node biopsy from a 12 year old boy with massive cervical lymphadenopathy. H&E stain (**a**) shows large sinusoidal macrophages demonstrating emperipolesis of lymphocytes. Immunostaining shows S 100 positivity (**b**).

Immunohistochemically, the cells show intense granular cytoplasmic positivity for CD68 and both membrane and cytoplasmic staining for CD163. CD14 is strongly expressed on the cell membrane. Lysozyme is present as granular cytoplasmic staining. S100 is variable. CD1a is rarely seen. Focal Langerin immunoreactivity is rarely seen. Follicular dendritic cell markers CD21 and CD35 are negative, and CD 30 is not expressed [52]. The differential diagnosis is wide,

generally that of other large cell lymphomas with epithelioid features

Molecular Diagnostics

A few cases have occurred following previous leukaemias, and the histiocytic sarcoma generally has the same clonal markers as the leukaemia [53].

Langerhans Cell Sarcoma

Clinical Features

This is usually a tumour of adults that presents as a soft tissue mass, a nodal tumour or as multiorgan involvement with spleen, liver, lung and bone marrow disease.

Histopathological Features

The infiltrate is composed of large, oval to spindle shaped cells with anaplastic features, nuclear variation and atypical mitoses. The nuclei may be grooved or folded. Mitoses are common. There is ample eosinophilic cytoplasm. Birbeck granules are usually demonstrated electron microscopicially. Immunohistochemistry reveals CD1a and S-100 positivity, although expression may be variable. Langerin staining is positive in some but not all cells. Paranuclear CD68 is present, and fascin, CD21 and CD35 are absent.

Molecular Genetics

A series of dendritic cell sarcomas, including Langerhans cell sarcomas, in patients with follicular lymphomas showed a clonal progression from the B cell lymphoma to the dendritic cell sarcomas [53].

Interdigitating Dendritic Cell Sarcoma

Clinical Features

These are rare tumours of adulthood that present as nodal or extranodal masses, the latter involving sites such as nasopharynx, retroperitoneum, mesentery and testis.

Histopathological Features

This is a spindle cell neoplasm which in lymph nodes is paracortical, a useful distinction from follicular dendritic cell sarcoma. The spindle cells form fascicles and whorls. Nuclei are oval to elongated, and have a distinct small nucleolus. There are usually many small T lymphocytes between the spindle cells. Immunohistochemically, the cells express vimentin, S-100, HLA-DR and fascin. Small amounts of CD68 have been reported in some, but CD163 is absent, as are CD1a, Langerin, CD21 and CD35.

Molecular Genetics

As with Langerhans cell sarcomas, follicular lymphomas and myeloid dendritic cell sarcomas with the same translocations have been observed [53].

CONCLUSION

The histiocytoses are a complex group of disorders, not least because the histological differential diagnosis is wide, and necessarily includes many benign reactive histiocytic proliferations. As in all areas of pathology, a careful clinical history, taking into account family history, age, sex and presence or absence of systemic symptoms as well as careful clinical examination and evaluation of ancillary investigations can often direct the pathologist to the correct category. Meticulous microscopic examination, in particular careful evaluation of a panel of immunostains, in most cases will yield the correct diagnosis. At present, the main contribution of cytogenetic and molecular diagnostic techniques is in the area of the familial haemophagocytic syndromes where rapid advances have occurred in recent years. We seem to be tantalisingly close to solving the complex area of Langerhans cell histiocytosis, and it is highly likely that molecular diagnostic techniques will solve the conundrum of this rare and elusive disease within the next few years. It is to be hoped that the information thus gained will direct therapy and inform the development of a cure for this disease which in a proportion of patients is severely debilitating and sometimes fatal. That will still leave the challenge of the hybrid dendritic cell/macrophage diseases of juvenile xanthogranuloma and Rosai-Dorfman disease to tackle.

ACKNOWLEDGEMENTS

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