ALK+ histiocytosis: A novel type of systemic histiocytic proliferative disorder of early infancy

John K.C. Chan,1 Laurence Lamant,2 Elizabeth Algar,3,4 Georges Delso,2 William Y.W. Tsang,1 King Chung Lee,1 Karin Tiedemann,4 Chung Wo Chow3,5

1Department of Pathology, Queen Elizabeth Hospital, Hong Kong; 2INSERM, U563, Centre de Physiopathologie de Toulouse Purpan, and Université Toulouse III Paul Sabatier, Toulouse, France; 3Department of Pediatrics, University of Melbourne, Australia; 4Children’s Cancer Center and 5Department of Anatomical Pathology, Royal Children’s Hospital, Melbourne, Australia

Short running title: ALK+ histiocytosis

Address for correspondence: Dr. John K.C. Chan, Department of Pathology, Queen Elizabeth Hospital, Wylie Road, Kowloon, Hong Kong, S.A.R. China. (e-mail: jkcchan@ha.org.hk)
ABSTRACT

We report three cases of a previously uncharacterized form of histiocytosis presenting in early infancy and showing ALK immunoreactivity. The patients presented with pallor, massive hepatosplenomegaly, anemia and thrombocytopenia. Liver biopsy showed infiltration of the sinusoids by large histiocytes with markedly folded nuclei, fine chromatin, small nucleoli, and voluminous lightly eosinophilic cytoplasm that sometimes was vacuolated or contained phagocyted blood cells. One patient developed cutaneous infiltrates that morphologically resembled juvenile xanthogranuloma. The histiocytes were immunoreactive for histiocytic markers (CD68, CD163, lysozyme), S100 protein, ALK (membranous and cytoplasmic pattern), and dendritic cell markers (fascin, factor XIIIa), but not CD1a and langerin. One case successfully analyzed by molecular techniques revealed $TPM3-ALK$ fusion. Thus the spectrum of diseases exhibiting $ALK$ translocation should be expanded to include $ALK+$ histiocytosis. The disease in the three patients (two having been given chemotherapy) resolved slowly over many months.
INTRODUCTION

Histiocytosis in childhood is a heterogeneous group of localized or disseminated diseases. Some forms of histiocytosis result from an underlying genetic defect such as mutation in the perforin gene (familial hemophagocytic lymphohistiocytosis), some are precipitated by an infection such as Epstein-Barr virus-associated hemophagocytic syndrome, some represent monoclonal proliferations such as Langerhans cell histiocytosis, while most do not have an obvious cause. We report three cases of a previously undescribed form of histiocytosis in infants, characterized by distinctive morphology and ALK expression.
MATERIALS AND METHODS

The index case was found to express ALK at diagnosis (Royal Children’s Hospital, Melbourne), and the other two cases (Queen Elizabeth Hospital, Hong Kong) were retrospectively identified based on similarities in clinicopathologic features. Available clinical records and pathologic materials were retrieved for review. Immunohistochemical studies were performed in an automated immunostainer (BOND-MAX) using a Polymer detection system.

RNA was extracted from frozen liver and muscle biopsies of case 1, and paraffin sections of liver biopsy of case 2, using the RNAeasy Midi Kit (Qiagen, Courtaboeuf, France). Synthesis of the first strand cDNA and PCR amplification were performed in a single tube using RT-PCR Access Kit (Promega, Charbonnières, France). The first step consisted of an ALK gene-specific reverse-transcription, followed by 30 cycles of amplification using C-TPM1 (position 97-117 on cDNA TPM3 sequence) and ALK1 (position 4211-4187 on cDNA ALK sequence) primers. The second round was performed on a 1 µl aliquot from the first amplification, using C-TPM3 (position 445-466 on cDNA TPM3 sequence) and ALK2 (position 4171-4148 on cDNA ALK sequence) primers, yielding a 307 bp PCR product.10
RESULTS AND DISCUSSION

Clinical features

The three female infants presented with pallor (Table 1). All had prominent hepatosplenomegaly, but no fever. There was marked anemia and thrombocytopenia, but no leukopenia. Marrow examination showed no obvious abnormal infiltrate or hemophagocytosis. Serologic studies for the common viruses and Toxoplasma were negative. Clinically, storage disease and malignancy were suspected, prompting performance of liver biopsy. Cases 1 and 2 were given dexamethasone and etoposide, while case 3 was not given any specific treatment. The hematological pictures improved slowly over many months, and the hepatosplenomegaly also gradually resolved. The patients were well at 2.5 to 7 years, and showed normal development.

Pathologic findings

The liver biopsies of all three cases showed sinusoidal infiltration by single or small aggregates of histiocytes. The histiocytes were very large, with irregularly folded or lobulated nuclei, fine chromatin and small nucleoli; some contained two to four nuclei. The voluminous eosinophilic cytoplasm often contained small vacuoles, and occasionally phagocytosed lymphocytes, polymorphs, normoblasts, red cells, or hemosiderin (Fig. 1A, 1B, 1C). In case 1, histiocytes also formed aggregates in the portal tracts. Ultrastructural studies were available in cases 1 and 2, and showed cells with short cell processes and abundant cytoplasm containing mitochondria, rough endoplasmic reticulum, ribosomes, lysosomes and phagolysosomes, but not Birbeck granules. There was no ultrastructural evidence of metabolic disease.

The skin biopsy of case 1 showed a non-circumscribed dermal lesion resembling early juvenile xanthogranuloma predominated by non-lipidized cells. The lesional cells were
similar to those seen in the liver, but were admixed with occasional multinucleated giant cells with wreath-like nuclei but which lacked the peripherally-located vacuoles characteristic of Touton giant cells (Fig. 1D).

In all three cases, the lesional cells in the liver showed strong positive staining for CD68 (PGM1, Dako), CD163 (10D6, Novocastra) and lysozyme (antiserum, Dako) (Fig. 1E), heterogeneous staining for S100 protein (antiserum, Dako) and factor XIIIa (NCL_FXIIIa, Novocastra), and uniform staining for ALK (ALK1, Dako) and ALKc (from Dr. B. Falini, Italy) in a membranous and weak cytoplasmic pattern (Fig. 1D, 1F). Immunostaining with anti-phospho-ALK (antiserum, Cell Signaling Technology) showed granular cytoplasmic labeling suggesting ALK fusion protein phosphorylated on tyrosine 664. There was focal staining for CD45RB (PD7/26, Dako) and fascin (55K-2 Dako), CD1a (O10, Novocastra), langerin (12D6, Novocastra), CD20 (L26, Dako), CD3 (PS1, Novocastra) and CD30 (BerH2, Dako) were negative. The Ki67 (antiserum, Dako) proliferative index was low (<2%).

Marrow biopsies were available for ALK immunostaining in cases 2 and 3; these biopsies were originally interpreted on morphologic grounds as negative. Surprisingly scanty ALK positive cells, solitary or in tiny aggregates, were demonstrated. On retrospective comparison with corresponding fields in the hematoxylin-eosin-stained sections, the abnormal histiocytes could be identified.

RT-PCR demonstrated chimeric TPM3-ALK transcripts in the liver and muscle biopsies of case 1, suggesting presence of t(1;2)(q25;p23). The test was unsuccessful in case 2, presumably due to RNA degradation, and there was insufficient material in case 3 for the study.

**Occurrence of ALK translocation**
Primary systemic anaplastic large cell lymphoma commonly exhibits a unique t(2;5) or variant chromosomal translocation, with ALK gene being fused with a housekeeping gene, such as NPM, TPM3 and TFG, resulting in expression of ALK protein.\textsuperscript{12-15} Currently, only three other tumor types are known to exhibit ALK translocation and ALK expression – ALK+ large B-cell lymphomas, inflammatory myofibroblastic tumors, and <5% of non-small cell lung carcinomas.\textsuperscript{16-23} This report expands the spectrum to include ALK+ histiocytosis. In all these proliferations, the formation of X-ALK homodimers/polymers using dimerisation sites at the N-terminus of ALK partners mimics ligand binding, and is responsible for activation of the ALK catalytic domain and oncogenic properties of the fusion protein.\textsuperscript{12}

**ALK+ histiocytosis as a distinctive disease**

We propose the designation “ALK+ histiocytosis” for this distinctive entity presenting in early infancy, characterized by proliferation of morphologically distinctive histiocytes with unique expression of ALK. The presence of liver and subtle bone marrow indicates that the disease is systemic. Features suggesting that the histiocytes are macrophages include: presence of phagocytosis; immunoreactivity for CD163, a histiocytic marker not expressed on dendritic cells; and ultrastructural features of phagocytic cells. On the other hand, positive staining for factor XIIIa and fascin suggests a relationship with dendritic cells. Thus the macrophage versus dendritic cell lineage of ALK+ histiocytosis remains to be clarified.

ALK expression is a unique feature of this form of histiocytosis, since it is absent in other histiocytic proliferations that we have studied, including Langerhans cell histiocytosis (10 cases), juvenile xanthogranuloma (8 cases solitary, 1 case systemic), familial hemophagocytic lymphohistiocytosis (3 cases) and Rosai-Dorfman disease (6 cases). Although presence of ALK gene translocation may suggest that ALK+ histiocytosis is a neoplastic disorder, we are cautious on this interpretation because of the favorable outcome in
case 3 even without cytotoxic therapy, and in case 2, even though no further cytotoxic therapy was given for residual disease. Furthermore, the delayed clinical response to cytotoxic therapy in cases 1 and 2 renders it difficult to ascribe the response to chemotherapy versus natural resolution of disease.

In summary, ALK+ histiocytosis is a distinct form of histiocytic proliferative disorder that clinically may suggest a storage disorder. Based on this limited series, the disease tends to resolve slowly, but can be life-threatening during the active phase.
Authorship

Contribution: J.K.C.C. and C.W.C. conceived the study, collected and analyzed the data, and drafted the manuscript. E.A., W.Y.W.T., K.C.L. and K.T. contributed patient materials and analyzed the data. L.L. and G.D. performed special studies and analyzed the data.

The authors declare no conflict of interest.

Correspondence: Dr. John K.C. Chan, Department of Pathology, Queen Elizabeth Hospital, Wylie Road, Kowloon, Hong Kong, S.A.R. China; e-mail: jkcchan@ha.org.hk.
References


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<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
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<tr>
<td><strong>Sex and age</strong></td>
<td>F/neonate</td>
<td>F/3 months</td>
<td>F/3 months</td>
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<tr>
<td><strong>Presentation</strong></td>
<td>Pallor; failure to thrive;</td>
<td>Pallor; mild jaundice</td>
<td>Noted to have persistent pallor on regular check-up</td>
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<tr>
<td></td>
<td>abdominal distension; ascites;</td>
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<td></td>
<td>lower limb edema</td>
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<tr>
<td><strong>Fever</strong></td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
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<tr>
<td><strong>Hepatosplenomegaly</strong></td>
<td>Gross (liver 11 cm and spleen 9 cm below costal margin)</td>
<td>Gross (liver 6 cm and spleen 4 cm below costal margin)</td>
<td>Demonstrated on ultrasound examination; a 2 cm hypodense area in dome of liver on CT scan</td>
</tr>
<tr>
<td><strong>Blood counts</strong></td>
<td>Hb 4.3 g/dL</td>
<td>Hb 3.4 g/dL</td>
<td>Hb 6.3 g/dL</td>
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<tr>
<td></td>
<td>Platelet 3 x 10^9/L</td>
<td>Platelet 12 x 10^9/L</td>
<td>Platelet 57 x 10^9/L</td>
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<tr>
<td><strong>Serum albumin level</strong></td>
<td>15 g/L</td>
<td>19 g/L</td>
<td>NA</td>
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<td>(N: 29-45 g/L)</td>
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<tr>
<td><strong>Serum ferritin level</strong></td>
<td>361 µg/L</td>
<td>1060 µg/L</td>
<td>NA</td>
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<tr>
<td>(N: 8-135 µg/L)</td>
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<td><strong>Serum triglycerides</strong></td>
<td>1.7 mmol/L</td>
<td>1.9 mmol/L</td>
<td>NA</td>
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<td>(N: 0.9-2 mmol/L)</td>
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<td><strong>Plasma fibrinogen</strong></td>
<td>3.3 g/L</td>
<td>3.93 g/L</td>
<td>NA</td>
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<tr>
<td>(N: 0.8-3.8 g/L)</td>
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<td><strong>Treatment and</strong></td>
<td>Mechanical ventilation for increased ascites impairing breathing. Given dexamethasone and etoposide. Gradual improvement, with reduction of ascites but persistence of hepatosplenomegaly. Successfully extubated after 16 days. Then developed multiple</td>
<td>Given dexamethasone and etoposide for about 8 months. Anemia gradually improved, platelet count rose, and biochemical profile normalized. Second biopsy was taken at 1 year (reported then as normal, but abnormal histiocytes were identified retrospectively on ALK immunostaining), and no further</td>
<td>Empirically treated with antibiotics. The hemoglobin gradually dropped further, and platelet count remained on the low side. Progressive increase in size of liver (12 cm) and spleen (3 cm). No specific treatment given. Then condition gradually improved, hepatosplenomegaly regressed,</td>
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<td>brownish to purplish macules, over scalp and trunk, which gradually regressed. At 2.5 years, the child was growing well, with normal blood counts and liver function tests; 3 cm hepatomegaly persisted.</td>
<td>treatment was given. Well, with normal growth at 5 years.</td>
<td>and blood counts normalized. Well at 7 years.</td>
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NA = Not available
Legends for figures

Figure 1

Liver and skin biopsies. (A) Liver biopsy of case 2 shows infiltration of sinusoids by large histiocytes which on causal examination are difficult to distinguish from the hepatocytes. (B) Liver biopsy of case 1 shows aggregates of histiocytes (arrows) in the sinusoids. The histiocytes have irregularly folded nuclei and abundant lightly eosinophilic cytoplasm. (C) Morphologic spectrum of proliferated histiocytes in the liver sinusoids. The histiocytes have one or two nuclei, which often show marked irregular foldings and small nucleoli. Some contain phagocytosed blood cells, brown pigment or fine vacuoles. (D) Skin lesion of case 1 shows dermal infiltrate of mononuclear cells and multinucleated giant cells; the latter showed wreath-like nuclei (upper panel). These cells are immunoreactive for ALK (lower panel). (E) The histiocytes in the sinusoids show positive immunostaining for CD163. (F) Immunostaining for ALK highlights the sinusoidal distribution of the histiocytes (left panel). Higher magnification shows cell membrane and weak cytoplasmic staining (right panel).

Images were captured with Olympus DP71 camera mounted on an Olympus microscope model BX61. The objectives used for capturing the images were as follow: Panel A, x20 objective; Panel B, x100 objective; Panel C, x100 objective; Panel D, x40 objective; Panel E, x60 objective; Panel F left, x10 objective; Panel F right, x60 objective. Images were acquired using Olympus DP Controller, and whitening of the background and cropping of the images were performed using Adobe Photoshop CS.
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