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CASE REPORT

Differential Expression of Markers in Extensive and Restricted Langerhans Cell Histiocytosis (LCH)

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Langerhans cell histiocytosis (LCH) represents a poorly defined pathologic entity characterized by diverse clinical appearence and falling into two major categories namely a restricted and an extensive disease. Since the outcome and the course of the disease is variable, we postulated that this might be reflected by the phenotype of the Langerhans cells. We have selected 11 adult restricted cases and 10 extensive childhood cases and compared the phenotype of LCH cells by immunohistochemistry on paraffin sections. Morphometric analysis indicated a significantly higher expression of histiocytic (CD68, S-100, lysozyme) markers in the adult restricted cases compared to the extensive form of the disease. Both groups were equally positive for LCH marker CD1a and negative for T cell marker CD4. On the other hand, HLA-DR expression was significantly higher in LCH cells of the extensive childhood cases suggesting higher activation. These data suggest that LCH cells have a different phenotype in the extensive childhood and restricted adult LCH where the latter is characterized by a more differentiated histiocytic phenotype. (Pathology Oncology Research Vol 2, No3, 184–187, 1996)

Key words: Langerhans cell histiocytosis; restricted and extensive variants; markers; immunohistochemistry

Introduction

The term Langerhans cell histiocytosis (LCH) has been accepted by the Writing Group for the Histiocyte Society instead of the mysterious term Histiocytosis X.[1] However, the "X" may be still appropriate because of the fragmentary knowledge of the etiology, pathobiology, prognosis and treatment of the disease. The tumor-like behaviour of LCH is supported by the finding that LCH is a clonal proliferation of Langerhans cells.[2,3] LCH clinically represents at least two entities according to the extent of the disease; the restricted and the extensive variants where the restricted form is more common in a dults.[4] The normal Langerhans cells are characterized by the expression of MHC antigens, lymphoid markers like CD4,11,14,29,45 and histiocytic markers such as lysozyme or S-100 protein.[5,6] Meanwhile, the most specific markers of Langerhans cells are CD1a expression and the presence of Birbeck granules.[5] The phenotype of LCH cells is considerably different from their normal counterpart; characterized by the loss of alloantigen-presenting potential and by the appearance of PNA binding, IL2R and IFNγR.[5] We have suggested that the clinically different LCH subgroups may be characterized by altered LCH cell phenotype therefore we have analyzed the expression of selected LCH markers (CD1a, CD4, CD68, HLA-DR, lysozyme and S-100) on paraffin embedded LCH samples from disseminated childhood and localized adult cases using immunohistochemistry.

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Materials and methods

Biopsy specimens from 11 adult restricted LCH and from 10 childhood extensive LCH cases were selected from the files of the National Korányi Institute and from the 1st Institute of Pathology & Experimental Cancer Research. The disease in the adult LCH cases was uniformly in the lung, whereas in the childhood cases the samples were collected from the skin, lymph nodes or bones all representing extensive LCH disease (*Table 1 and 2*).

Table 1. **Clinical data of the adult restricted LCH cases**

	Age/Sex (year)	*Localization*	*Clinical diagnosis*	*TEM*
1	25/male	lung	silicofibrosis	–
2	20/female	lung	rib tumor	–
3	41/female	lung	histiocytosis	–
4	31/male	lung	disseminated tumor	–
5	20/female	lung	disseminated tumor	–
6	22/female	lung	disseminated tumor	–
7	29/female	lung	sarcoidosis	–
8	18/male	lung	sarcoidosis	–
9	24/male	lung	sarcoidosis	–
10	17/female	lung	disseminated tumor	–
11	28/female	lung	sarcoidosis	–

After paraformaldehyde fixation and paraffin-embedding serial histologic sections were cut and mounted on glass slides. Some samples of childhood LCH were fixed in 1% glutaraldehyde-paraformaldehyde mixture, post-fixed in osmium and embedded in EPON resin for routine transmission electron microscopic (TEM) examination.

Immunohistochemical (IHC) labeling was carried out for the histiocyte markers, CD68, lysozyme, S100 and lymphoid markers, CD4, CD1a and HLA-DR on all specimens. 4 mm-thick paraffin sections were mounted on poly-L-lysin-coated or Superfrost+ (Shandon) glass slides, and dried overnight at 37°C. Sections were deparaffinized in xylene and hydrated through graded alcohol. Endogenous peroxidase was blocked with 3% H_2O_2 in methyl alcohol, applied for 20 minutes at room temperature. In case of the CD68 antibody, after rinsing with 0.1 M phosphate-buffered saline (PBS, pH 7.4) the sections were digested with trypsin (0.025%) at room temperature for ten minutes and washed thoroughly with tap water. The detection of HLA-DR and the CD1a markers required microwave antigen retrieval after the deparaffination (3x5 min., 750 W, pH 6, in 0.05 mM citrate-buffer).

The technique of streptavidin-biotin complex was used for IHC when 3% BSA normal serum served as blocking at room temperature for 30 minutes. The primary layer was anti-S100 protein (rabbit IgG, without dilution, DAKO), anti-CD68 (KP1, mouse monoclonal IgG, 1:10, DAKO), anti-lysozyme (rabbit IgG, 1:100, DAKO), anti HLA-DR (mouse monoclonal IgG, without dilution, Amersham), anti-CD4 (OPD4, mouse monoclonal IgG,

Table 2. **Clinical data of the extensive childhood LCH cases.**

	Age/Sex (year)	*Localization (biopsy)*	*Clinical diagnosis*	*EM*
1	13/male	skull	NHL	–
2	1.5/female	mastoid processs	?	+
3	2.0/female	lymph node	histiocytosis	–
4	4.0/male	skull	tu. capitis	+
5	1.5/female	phalanx	enchondroma	–
6	2.0/male	frontal bone	?	+
7	1.0/female	skin	histiocytosis	–
8	1.0/female	vulva	?	+
9	3.0/female	skin	cyst	–
10	15/female	lymph node	NHL	+

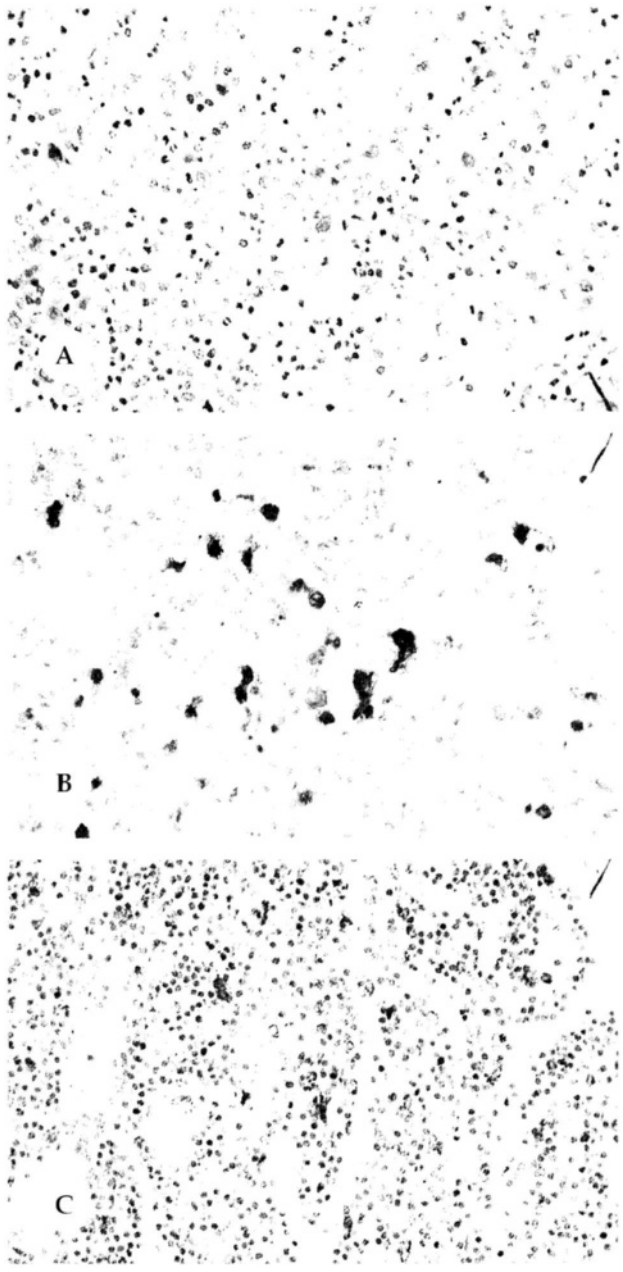

Figure 1. *Expression of histiocytic markers in LCH cells of childhood and adult cases. Immunohistochemistry. A. childhood case; S-100 staining. Note the rare positivity of LCH cells (DAB reaction). High power view. B. childhood case; Lysozyme staining. Note the rare positivity of LCH cells (DAB reaction). C. CD68 labeling of childhood LCH. Note the frequent strong positivity of LCH cells. (DAB reaction).*

1:20, DAKO) and anti-CD1a (MoAbO10, mouse monoclonal IgG, without dilution, Immunotech[7]), in all cases for 40 min at 37°C. A biotinylated second layer (anti-mouse, 1:200, Amersham or anti-rabbit, 1:200, Amersham, depending on the type of the primary antibody, for 30 min at 37°C) and a streptavidin-biotin horseradish peroxidase complex (1:200, Amersham, 30 minutes, 37°C) as a tertiary layer. Between the various layers double washings with PBS were performed and the layers were covered with PARAFILM to achieve better antibody dispersion.

Peroxidase activity was visualized with diaminobenzidine (DAB, DAKO) or 3-amino-9-ethylcarbazole (AEC, Shandon) and finally the section was counterstained with hematoxylin. Negative controls were samples, in which the primary antibody was replaced by 3% BSA. The positive controls were always paraffin-embedded, Birbeck granule-positive LCH cases.

Immunohistochemical reactions were evaluated by morphometric analysis, counting 100 LCH histiocytes selected from three random fields. Statistical evaluation of the data was performed by using ANOVA single factor analysis.

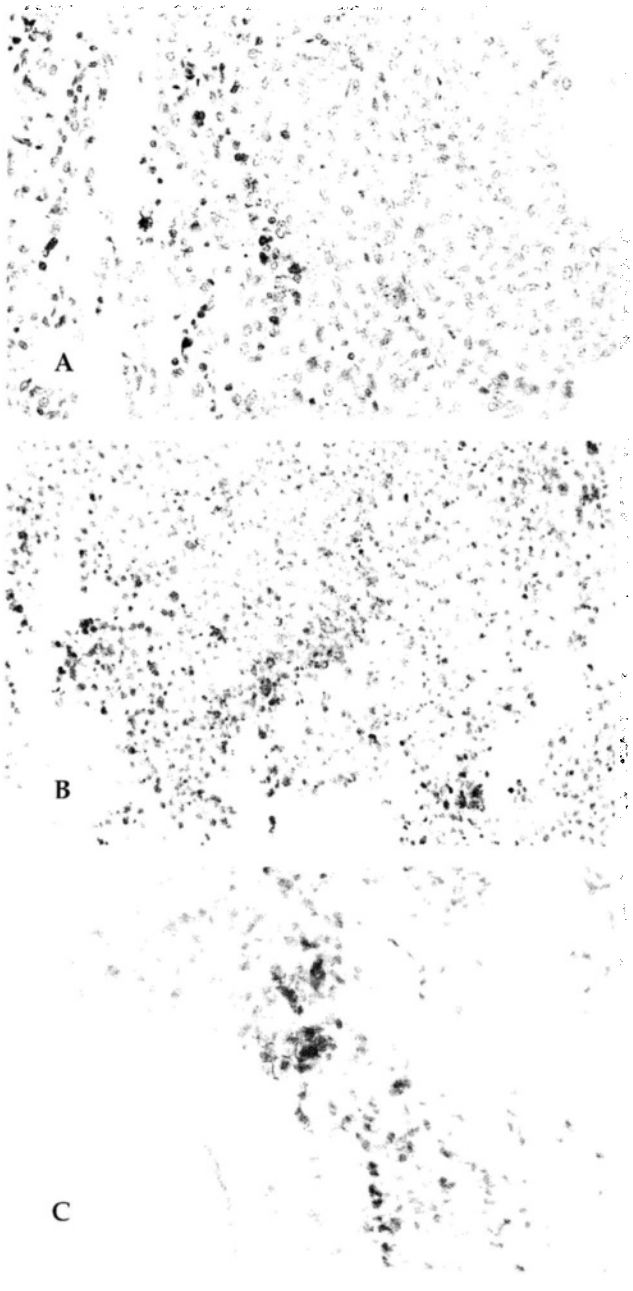

Figure 2. Expression of lymphocytic markers in LCH cells of childhood and adult cases. Immunohistochemistry. A. CD4 labeling; adult case. Positive LCH cells could not be observed. B. HLA-DR; adult case. Positivity of LCH cells is rare. C. CD1a labeling; childhood case (AEC reaction). Note the frequent positivity of LCH cells.

Results

The expression of the S100 protein and lysozyme in restricted (adult) or extensive (childhood) LCHs was extremely variable, it was present in about 80% of the LCH cells in adults (data not shown), but only 40% in children (*Fig.1a,b*). We found higher expression of the third macrophage marker, CD68 in both groups of patients (about 60% in childhood (*Fig.1c*) and about 90% in adults), indicating that in childhood extensive disease CD68 is more frequently expressed than other macrophage markers. According to morphometry there was no statistical difference between the frequency of macrophage markers in LCH cells of the adult restricted disease (data not shown). However, statistical analysis indicated significantly lower expression of macrophage markers in childhood extensive LCH compared to the restricted adult ones (*Fig.3*).

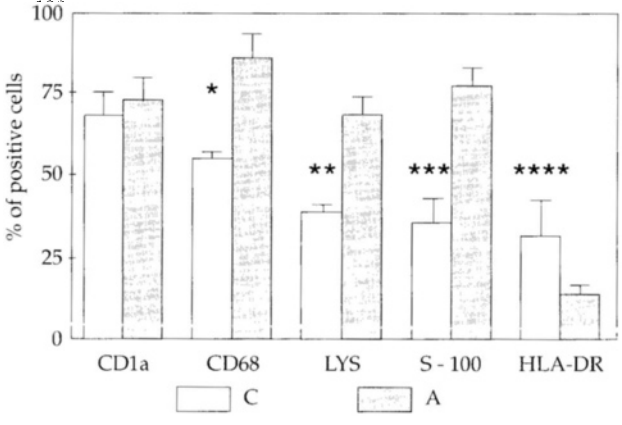

*Figure 3. Morphometric measurement of the expression of markers on LCH cells in childhood and adult cases. Minimum 100 LCH cells were evaluated at three randomly selected fields/sample and the % of positive cells was counted. Data were evaluated by single factor ANOVA test. C=childhood cases; A=adult cases. P < *= ; **=; ***= ; ****=*

The expression of the lymphoid markers indicated further differences in the LCH phenotype of extensive (childhood) and restricted (adult) LCH. The CD4 expression was found to be negative on LCH cells of both types of diseases *(Fig.2a)*, although the reactive lymphoid population around the lesion showed clear positive staining. The expression of the HLA-DR on LCH cells was significantly higher in children *(Figs 2b and 3)* than in adults. Finally, the most specific LCH cell marker, CD1a, was expressed at similar high frequency (about 70%) both in children and adult LCH cases *(Fig.2c and 3)*.

Discussion

The clinical presentation and outcome of LCH clearly suggest that this disease has at least two variants; the restricted and the extensive forms and each may have further subtypes.[1] This difference in the clinical course may be determined by the different phenotypes of the LCH cells. LCH cells express several normal and activation LC markers besides some others such as IL2R, IFNγR, PNA.[5,8,9] There are indications during routine diagnostic activities that some frequently used LCH markers such as S-100, can not be used reliably in childhood (extensive) LCH unlike in adults restricted, therefore we have selected cases from the extensive and restricted LCH group to compare the expression of some macrophage (S-100, lysozyme) and lymphoid markers (CD1a, CD4, HLA-DR) applicable to routinely fixed and paraffin embedded tissues. CD1a, the most selective LCH marker was uniformly expressed in both types of LCH disease supporting previous data that the mAb010 is a reliable marker for LCH.[7] CD4 proved to be an unreliable marker in our hands (by using mAb OPD4) since both LCH groups gave completely negative results. The positivity of the lymphoid population excluded the technical problem. HLA-DR expression was previously analyzed in LCH and found to be expressed similarly to normal LC cells.[5,6,10] Our quantitative data indicate that the HLA-DR expression was significantly higher in the extensive diseases suggesting higher level of activation. The similarity of LCH cells to activated Langerhans cells was previously suggested.[6] This possibility is further supported by our findings that the expression of macrophage markers in extensive LCH is lower than on the restricted form suggesting that the later may represent a more differentiated but less activated form.

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