FOLIA HISTOCHEMICA
ET CYTOBIOLOGICA
Vol. 45, No. 1, 2007
pp. 21-25

Coexpression of CD1a, langerin and Birbeck's granules in Langerhans cell histiocytoses (LCH) in children: ultrastructural and immunocytochemical studies

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Abstract: Langerhans cell histiocytoses (LCH) represent rare diseases of unclear etiology and pathogenesis. Most of the cases include children, 1 to 15 years of age, and various organs are involved (bones, skin, liver, lymph nodes, bone marrow and other). The diagnosis of LCH used to be established by biopsy of the inflamed tissue and demonstration of expression of markers specific for Langerhans cells: CD1a and langerin. The diagnosis can be ultimately confirmed by demonstration of Birbeck's granules in the electron microscopy. The present study was aimed at immunocytochemical demonstration, in the examined LCH material (skin, bones, lymph nodes), of the specific antigen expression and at comparing it with the presence of Birbeck's granules. In the examined 11 cases co-expression of CD1a with langerin and with the presence of Birbeck's granules was noted. Also in all examined biopsies the expression of S-100 protein on inflammatory cells was found. The results corroborate the usefulness of immunocytochemical studies on CD1a and langerin expression in diagnosis of LCH.

Key words: Langerhans cell histiocytosis (LCH) - Langerin - CD1a - Birbeck's granules

Introduction

The term histiocytosis is used to define a group of diseases from the interface of inflammatory and neoplastic processes, involving cell proliferation within the mononuclear phagocyte system. The proliferation results from an uncontrolled stimulation of immune antigen-presenting cells [7].

Langerhans cell histiocytosis (LCH) represents a type of histiocytosis, relatively seldom with unclear etiology and incompletely recognized pathogenesis, manifesting a broad spectrum of clinical lesions. Even though LCH can develop in all age groups, most of cases concerns children, 1 to 15 years of age. The incidence in this group does not exceed 1/200 000 per year. Variable clinical signs/symptoms of LCH are related to the location of the Langerhans cell infiltrates accompanied by immunoreactive cells. Most frequently, the infiltrate involves bones, skin, gums, ear, lungs, liver, spleen, lymph nodes and bone marrow [1,6].

The clinical pattern allows to distinguish two forms of the disease: the restricted form (mainly in children following the third year of age), in the course of which lesions are noted in bones, skin and lymph nodes, and the generalized form (most frequent in infants), manifesting a multi-organ character [1].

Diagnosis of LCH is based on biopsy of the inflammed tissue and on demonstration of immature Langerhans cells, with a round or oval shape, pink, granular cytoplasm, and without dendritic processes [7]. Using immunocytochemical methods, specific markers can be detected in cells, including CD1a, langerin (CD207) and S-100 protein. The final confirmation of Langerhans cells involves detection of Bir-

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Table 1. Clinical data on examined cases of LCH

No.	Sex	Age	Organs involved						
			skin	lymph nodes	bones	spleen	lungs	liver dysfunction	bone marrow dysfunction
1.	M	18 months	+	+	-	+	-	+	+
2.	F	17 months	+	-	+	-	+	+	+
3.	F	1 month	+	+	-	+	-	+	+
4.	F	1 month	+	+	+	+	-	+	+
5.	M	30 months	+	+	+	+	+	+	+
6.	F	11 months	+	+	-	-	+	+	-
7.	F	29 months	+	+	+	+	+	+	-
8.	M	14 years	+	-	+	-	-	-	-
9.	M	15 years	+	-	+	-	-	-	-
10.	M	14 years	-	-	+	-	-	-	-
11.	M	2 years	-	+	+	-	-	-	-

beck's granules under the electron microscope [4]. Birbeck's granules are typical structures which distinguish Langerhans cells from other immunocompetent cells. Origin and function of Birbeck's granules remain incompletely recognized. They contain langerin (a calcium-dependent lectin), which was detected for the first time in Langerhans cells [8].

Electron microscopic diagnosis of LCH is frequently difficult, i.a., due to insufficient or poorly fixed material, which affects results of electron-microscopic studies [7]. The immunocytochemical technique is simpler, less complicated and requires only a light microscope to evaluate results of colour reaction. It is also less time-consuming and less expensive. This is of particular significance in diagnosis of LCH when the decision has to be reached and appropriate therapeutic action has to be implemented promptly.

Immature forms of Langerhans cells are assumed to be present in LCH. Such forms after reaching maturity no longer express langerin but expression of CD1a persists [13]. Therefore, full co-expression of langerin and CD1a can not always be observed in Langerhans cells [10]. Also langerin expression in the absence of Birbeck's granules can be very seldom noted in some cases [3,9].

The present study was aimed at demonstrating, by immunocytochemistry, specific markers of Langerhans cells: CD1a, langerin and S-100 protein. Obtained results were compared with the presence of Birbeck's granules, detected under a electron microscope. We hoped to resolve whether in the studied cases of LCH (1) full co-expression of langerin and CD1a can be observed, (2) does maturation of Langerhans cells accompanied by loss in langerin expression occurs and (3) does expression of langerin is always accompanied by the presence of Birbeck's granules in Langerhans cells.

Materials and methods

Material for the studies was obtained in the course of routine diagnosis (biopsy of affected organs: skin, lymph nodes, bones) in 11 patients hospitalized in 1995 to 2003 due to LCH in the Department of Bone Marrow Transplantation, Pediatric Oncology and Hematology, University School of Medicine in Wrocław. In seven cases the disease was generalized (multi-organ) with involvement of skin, lymph nodes, spleen, with dysfunction of liver, lungs and bone marrow. In four cases, the clinically restricted form was diagnosed, with involvement of skin, bones and lymph nodes. Clinical data of the patients are summarized in Table 1.

Biopsies collected for immunocytochemical tests were fixed in 10% buffered formalin and those for electron microscopy in 2.5% glutaraldehyde in 0.1M cacodylate buffer, pH=7.4. The samples were dehydrated, embedded in paraffin for immunocytochemical tests and in Epon 812 for further ultrastructural studies. Sections for ultrastructural studies were routinely contrasted and examined in the JEM-100 electron microscope. Immunocytochemical reactions were performed on paraffin section. For detection of antigen CD1a, S-100 protein and langerin expression the respective antibodies were employed, including mouse monoclonal antibodies against CD1a, clone O10 (DakoCytomation), rabbit monoclonal antibodies against S-100 protein, clone Z0311 (DakoCytomation), and mouse monoclonal antibodies to langerin, clone 12D6 (Novocastra). All the reactions were accompanied by negative controls, in which specific antibodies were substituted by Primary Negative Control (DakoCytomation). The antigens were localized using biotinylated antibodies, streptavidin-biotinylated peroxidase complex (LSAB2 kit) and diaminobenzidine (DAB). All the reagents originated from DakoCytomation.

Results

Immunocytochemical tests and electron microscopic investigations were conducted on a material sampled from lesions detected in the skin, bones and lymph nodes. In all the eleven cases coexpression of CD1a antigen with langerin and the presence of Birbeck's granules was detected in Langerhans cells infiltrating the organs. Also in all the examined cases expression of S-100 protein could be demonstrated in the infiltrate cells.

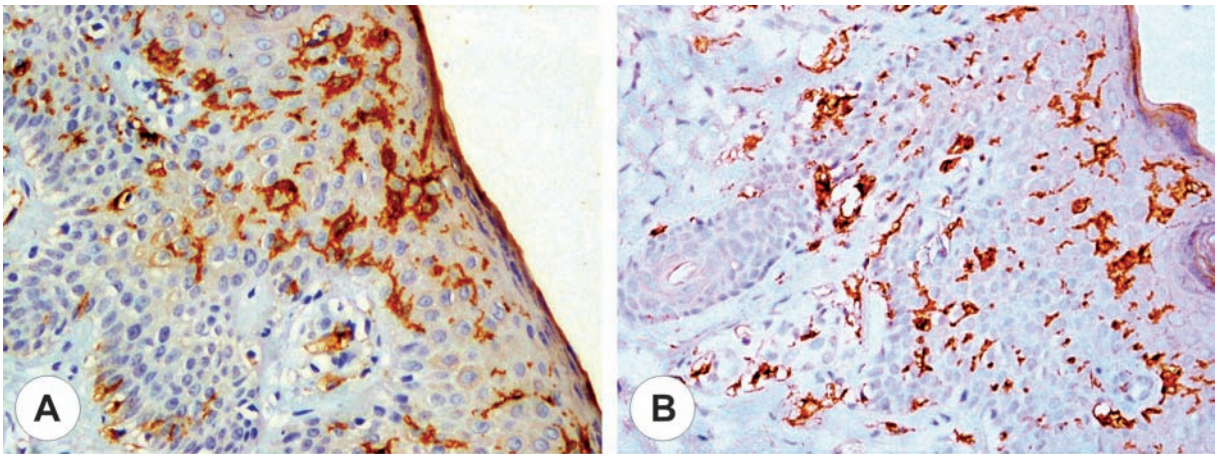

Fig. 1. Expression of CD1a antigen (**A**) and langerin (**B**) in skin biopsies showing Langerhans cells with typical dendritic projections, infiltrating individual epithelial layers. Immunohistochemical reaction, counterstained with hematoxylin. Magn. × 200.

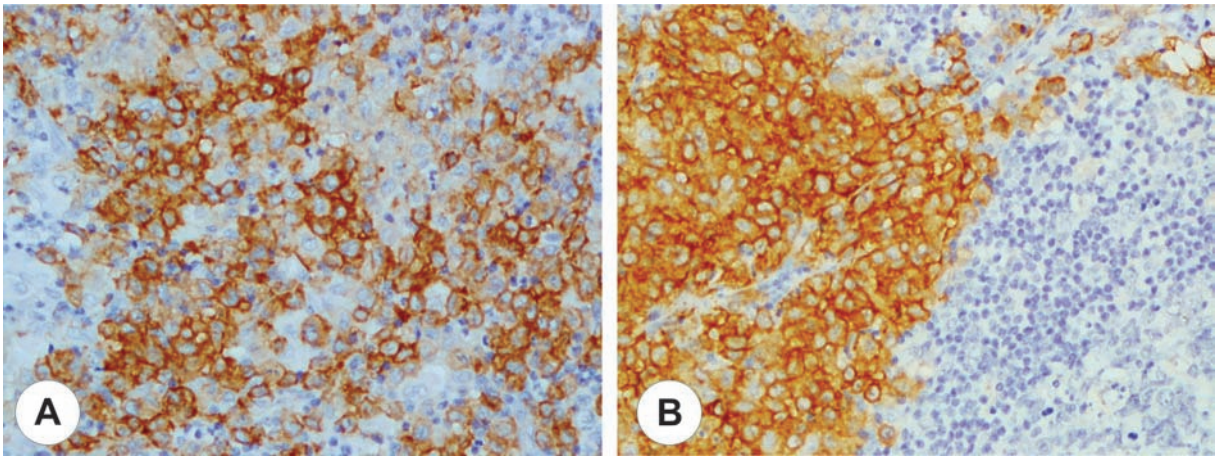

Fig. 2. Expression of CD1a antigen (A) and langerin (B) in lymph nodes demonstrates infiltrating Langerhans cells with the cytoplasmic-membranous expression of both antigens. Immunohistochemical reaction, counterstained with hematoxylin. Magn. × 200.

We were unable to detect differences in expression of individual antigens (langerin, CD1a, S-100) or in presence of Birbeck's granules, which would be related to patient's age or localization of the disease.

Expression of CD1a protein and of langerin in skin biopsies labelled both the typical Langerhans cells with typical dendritic projections and their immature forms, infiltrating individual epithelial layers (Fig. 1A and 1B).

In the material obtained from lymph nodes the infiltrate of Langerhans cells was more massive, which was reflected by more pronounced expression of CD1a antigen and langerin. The immunocytochemical reaction manifested preferentially membranous character of CD1a and a cytoplasmic-membranous character of langerin (Fig. 2A and 2B). Similar results of the immunocytochemical studies were observed in infiltrates of Langerhans cells in bones, but the colour reaction was less intense.

In the same biopsies examined in an electron microscope, typical Birbeck's granules were observed. They were characterized by elongated club or tennis racket resembling shape and a laminar structure (Fig. 3A and 3B).

Discussion

The interesting investigative problem, directly linked to diagnosis of LCH, involves clarification of origin of the pathognomic Birbeck's granules in Langerhans cells. Birbeck's granules are typical elements which distinguish Langerhans cells from other antigen-presenting cells [12]. Most probably they are functionally associated with the cell membrane and the new reports indicate that they are related to endosomal compartments. They do not seem to represent special cytoplasmic organelles but represent invaginations of cell membrane to the cellular interior [12]. Mc Dermott *et*

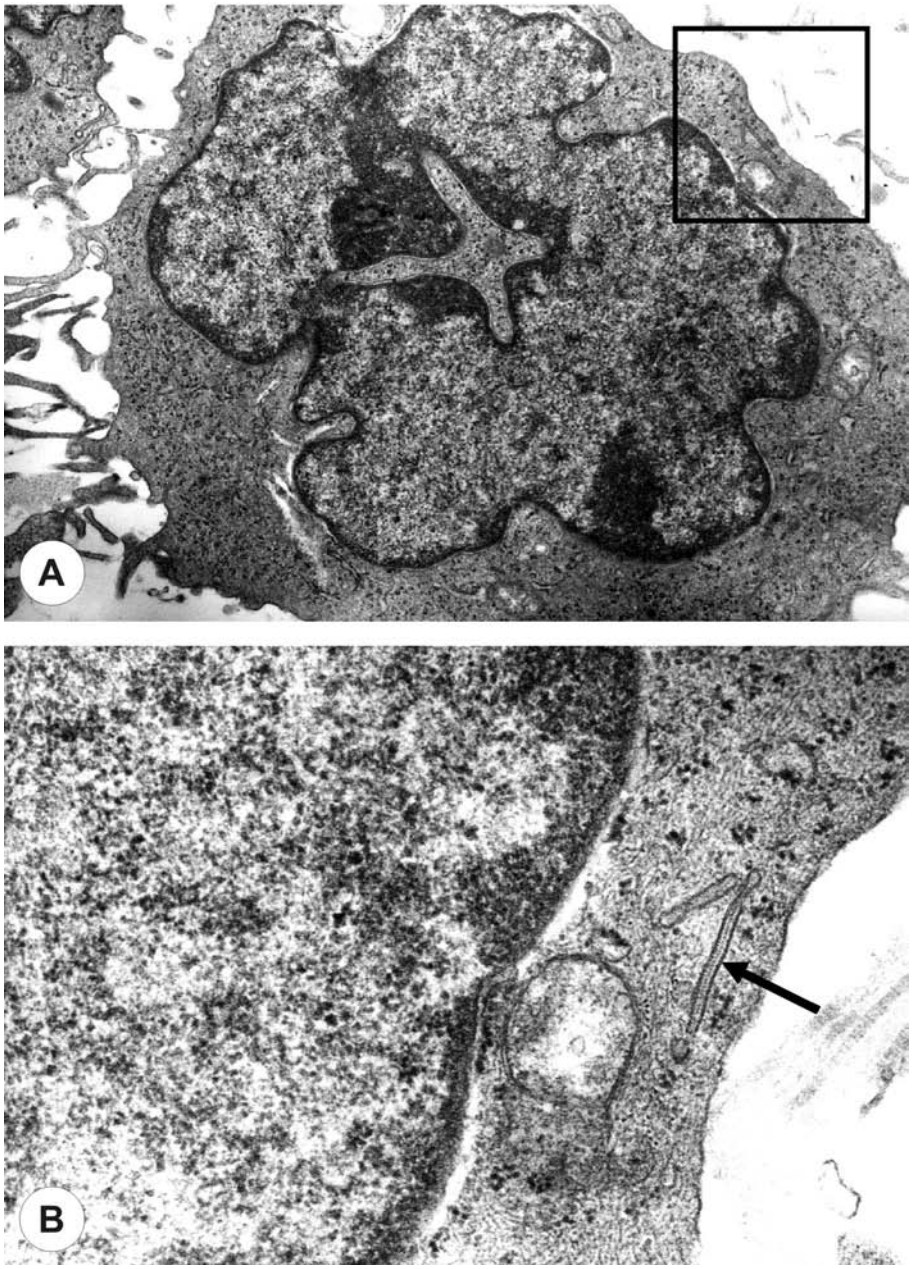

Fig. 3. Langerhans cell of skin (**A**), examined in an electron microscope. Magn. × 15 000. Framed area of the same cell showing typical Birbeck's granules (**B**). Magn.× 62 500.

al. in studies conducted on human Langerhans cells showed that Birbeck's granules were not formed by endocytosis but represented an endogenous compartment of cell membranes, which stored langerin, a protein typical of Langerhans cells [8].

Langerin is assumed to be present in immature Langerhans cells in LCH while loss in expression of the protein is related to transformation of the cells to their mature forms [13]. The hypothesis has been confirmed by studies demonstrating parallel presence of langerin and of CD1a in Langerhans cells in LCH [10]. Cases in which Birbeck's granules and langerin expression didn't co-exist in the cells were very rare [3,9].

The data prompted us to examine reciprocal correlation between the presence of langerin and Birbeck's granules in cells of LCH infiltrate. We have also evaluated the expression of CD1a antigen, equally selective in labelling of Langerhans cells. S-100 protein expression facilitated determination of the scope of Langerhans cell infiltrate. In every of the 11 cases, coexpression of langerin and CD1a antigen was observed along with presence of Birbeck's granules in infiltrates in the skin, lymph nodes and bones. Other authors obtained similar results even if they did not always use in parallel all the diagnostic techniques (electron microscopy, immunocytochemistry) and all characteristic markers. Chikwava and Jaffe [2] demonstrated coexpression of langerin and CD1a antigen in 24 cases of LCH using an archival material (paraffin blocks with autopsy material). In all the examined

samples of bones, skin, lymph nodes they observed expression of CD1a and langerin, using the same antibody clones which were used in our study. Geissmann *et al.* [5] studied differentiation of Langerhans cells in LCH and immunocytochemically demonstrated co-expression of CD1a and langerin in 25 cases of LCH, in the material resembling the presently employed one. However, authors did not use electron microscope to examine the presence of Birbeck's granules in Langerhans cells. In turn, Smetana *et al.* in their immunofluorescent studies, revealed strong reciprocal correlation (r=0.976) between CD1a antigen and langerin expression in cells obtained from bronchopulmonary lavage fluids in 8 patients with pulmonary form of LCH [11]. Such data clearly indicate high specificity and efficacy of both markers (CD1a, langerin) in diagnosis of various forms of LCH, in various material. It has also been found that co-expression of the markers can be noted not only in Langerhans cells present in the inflammatory infiltrate in LCH. Séguier *et al.* obtained results similar to ours and to the other quoted authors in the material sampled from gingiva of operated patients [10]. In our study the specific expression of CD1a antigen and langerin in Langerhans cells has been additionally confirmed by detection of Birbeck's granules in cells.

Results of our studies, performed on 11 cases of LCH in children have failed to document maturation of Langerhans cells, which at least in some cases of the disease would be associated with loss of langerin expression. Most probably we have examined such cases of LCH, in which the infiltrate manifested prevalence of only immature forms of Langerhans cells, which co-expressed langerin and CD1a. Also in the case of Birbeck's granules, which are pathognomic for LCH, they were always present in parallel with langerin expression, thus confirming their co-existence.

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Received: July 5, 2006
Accepted after revision: August 1, 2006