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Expression of GM-CSF receptor by Langerhans' cell histiocytosis cells

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Abstract Langerhans' cell histiocytosis (LCH) is characterized by the proliferation of large mononucleated cells containing Birbeck granules and expressing CD1a. Recent studies have demonstrated that LCH is a clonal proliferation; however, its aetiology is still unknown. Growth and differentiation of bone-marrow-derived cells are controlled by cytokines. The proliferation, differentiation and activation of normal Langerhans cells are controlled by granulocyte/macrophage colony-stimulating factor (GM-CSF) in vitro. Therefore, GM-CSF could be implicated in the pathogenesis of LCH. Indeed, LCH cells contain GM-CSF, and children with disseminated LCH have an elevated GM-CSF serum level. As a cytokine only acts on cells expressing a specific receptor, we investigated the presence of GM-CSF receptor on LCH cells. Fourteen frozen tissue samples from children with LCH were studied by in situ immunohistochemistry with two mouse monoclonal antibodies specific for the α chain of the GM-CSF receptor (CDw116). LCH cells of all the samples were positively stained with both antibodies. This study suggests that GM-CSF may be a growth factor for LCH cells.

Key words Langerhans cell · Histiocytosis · Human · Immunohistochemistry · GM-CSF receptor · CDw116

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Introduction

The term 'Langerhans' cell histiocytosis' (LCH) embraces several previously used eponyms, including histiocytosis X, Letterer-Siwe disease, Hand-Schüller-Christian disease, eosinophilic granuloma and Hashimoto-Pritzker syndrome [2]. LCH is characterized by the proliferation of large mononucleated cells. The LCH cells belong to the Langerhans cell (LC) lineage, because they contain Birbeck granules within their cytoplasm [13], and express CD1a [4].

Proliferation, differentiation and activation of bone marrow-derived cells are usually controlled by cytokines. Granulocyte/macrophage colony-stimulating factor (GM-CSF) is the one major cytokine implicated in the control of normal LC (for review, see [7]). It induces differentiation and proliferation of human LC from haematopoietic progenitors in vitro [3, 16]. Intradermal injection of GM-CSF to lepromatous patients induces local accumulation of LC [11]. When epidermal LC are activated, they migrate to the draining lymph node, where they differentiate into interdigitating cells (IDC). This physiological activation and maturation of LC can be reproduced in vitro by growing LC in the presence of GM-CSF [15].

The cellular effects of GM-CSF are mediated by a specific membrane receptor. This receptor is a heterodimer with a β-chain common to IL-3 and IL-5, and a specific α-chain [5]. Large amounts of GM-CSF receptor have been detected on cultivated LC and on IDC [10]. Recently a mouse monoclonal antibody specific for GM-CSF receptor α–chain has been produced (hGM-CSF-M1), and this receptor was denoted CDw116 at the 5th International Workshop and Conference on Human Leukocytes Differentiation Antigens (1994).

LCH cells contain GM-CSF [6], and children with disseminated and active LCH have an elevated GM-CSF serum level [8]. LCH cells have also been shown to be activated [9]. Proliferation and activation of LCH cells may be due to GM-CSF, as it is the case for normal LC in vitro. However the presence of GM-CSF receptor on LCH cells has not yet been reported.

Table 1 Clinical characteristics of children with Langerhans' cell histiocytosis (LCH) at the time of biopsy (*LN* lymph node, *BM* bone marrow)

Patient	Sex (years)	Age	Localization of LCH	Tissue sample
1	2.9	M	Skin, LN, BM	Skin
2	8.2	M	Bone	Bone
3	0.3	M	Skin, lung, LN, BM	Skin
4	1.6	M	Skin, bone	Skin
5a[a]	0.3	M	Skin	Skin
5b[a]	0.8	M	Skin, lung, LN	LN
6	2.6	M	Skin, lung, bone	Skin
7	0.6	M	Skin	Skin
8	0.6	M	Skin	Skin
9	0.8	M	Skin, bone, spleen, LN, BM	Skin
10	0.1	F	Skin	Skin
11	0.5	F	Skin	Skin
12	1.1	M	Skin, bone, LN	Bone
13	6	F	Bone	Bone

[a] Same patient

This prompted us to investigate the presence of GM-CSF receptor on LCH cells. Fourteen frozen samples from children with LCH were studied by in situ immunohistochemistry. The staining was compared with that of normal cells on frozen skin, tonsil and lymph node samples. All the LCH samples were stained by the GM-CSF receptor specific monoclonal antibody hGM-CSF-M1 (CDw116). The results were confirmed with a second mouse monoclonal anti-GM-CSF antibody.

Materials and methods

Tissue

Diagnosis of LCH was established according to the criteria of the Writing Group of the Histiocytosis Society [20]. The patients were treated in our institution from September 1991 to November 1994. Clinical data are shown in Table 1. Skin, bone and lymph node biopsies were performed for diagnostic purpose. Biopsy specimens were divided in two parts. The first was immediately frozen in liquid nitrogen for immunohistochemistry. The second part was placed in buffered formalin for histopathological observation. As controls, three frozen normal skin biopsies, two frozen tonsils and two dermatopathic lymphadenopathies were analysed.

Immunohistochemistry

Immunohistochemistry was performed on frozen sections according to an indirect three-stage immunoperoxidase protocol. For negative controls, the first antibody was replaced by an irrelevant monoclonal antibody of the same isotype. Monoclonal antibody OKT6 anti-CD1a was obtained from Ortho-Diagnostic Systems (Roissy, France).

The mouse monoclonal antibody hGM-CSFR-M1 was kindly provided by Immunex (Seattle, USA). This antibody was generated against COS cells transfected with the human GM-CSF receptor α–chain. Its specificity has been demonstrated by dot blot, immunoprecipitation, and neutralization of GM-CSF bioactivity. It was ascribed to the human CDw116 (GM-CSF receptor) cluster at the 5th International Workshop and Conference on Human Leukocytes Differentiation Antigens. It is of IgG1 isotype, and was used at a final concentration of 2.6 μg/ml. The second anti-GM-CSF receptor α–chain mouse monoclonal IgG [GM-CSF (R) α (S-50)] was purchased from Santa Cruz Biotechnology (Santa Cruz, USA). Its specificity has been demonstrated by Western blotting and immunoprecipitation. It was used at a final concentration of 1 μg/ml.

Results

Histological examination of the 14 skin, bone and lymph node biopsies of LCH showed accumulation of large round cells without dendritic expansions. They had an abundant homogeneous pink cytoplasm and a lobulated nucleus, often with 'coffee bean' appearance. These cells were often associated with eosinophils, lymphocytes and macrophages. LCH cells of all samples expressed CD1a on frozen sections.

LCH cells in the 14 frozen biopsies were stained by hGM-CSFR-M1. The majority of large mononucleated cells was stained (Fig 1). The staining of LCH cells was bright. Lymphocytes, fibroblasts, endothelial cells and keratinocytes were not stained. Replacement of the primary antibody by an irrelevant mouse monoclonal antibody of the same isotype yielded negative results. Polymorphonuclear eosinophilic cells, which showed intense cytoplasmic reactivity due to endogenous peroxidase, were ignored. At a final concentration of 2.6 μg/ml, no background was observed with hGM-CSF-M1. Similar results were obtained with the second anti-GM-CSF a-chain monoclonal antibody [GM-CSF (R) α (S-50)], but the background staining was more significant. No differences of staining of the LCH cells was observed between localized and diffuse forms of the disease.

Serial frozen sections of three normal skin biopsies were stained with CD1a and CDw116. The majority of LC (CD1a-positive epidermal dendritic cells) was usually negative with CDw116. Few dendritic expansions within the epidermis were weakly stained with CDw116, whereas few mononucleated CD1a negative cells of the dermis were stained. Higher concentrations of hGM-CSFR-M1 (up to 50 μg/ml) did not enhance the staining of the epidermal Langerhans' cells. In the two control tonsils, germinal centre macrophages and some non-lymphoid cells of the paracortical zone, consistent with interdigitating

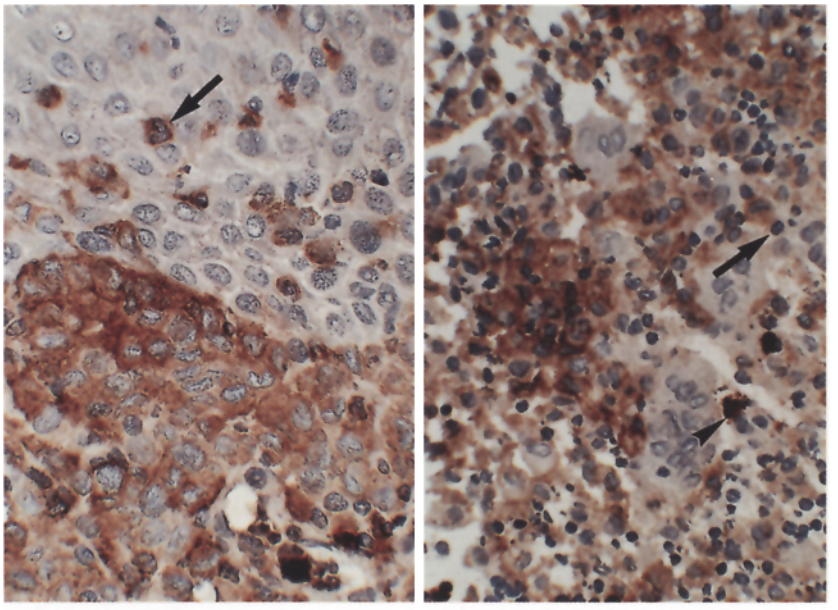

Fig. 1 Immunohistochemical staining of frozen samples of Langerhans' cell histiocytosis (LCH) with anti-GM-CSF receptor (hGM-CSFR-M1, CDw116). *Left:* skin sample of patient 8. LCH cells are located within the superficial dermis (*lower part*), and some of them (*arrow*) infiltrate the epidermis (*upper part*). All LCH cells are CDw116 positive. ×400. *Right:* lymph node sample from patient 5. LCH cells are CDw116 positive, while multinucleated giant cells and lymphocytes (*arrows*) are negative. Some eosinophils (*arrowheads*) are present. ×400

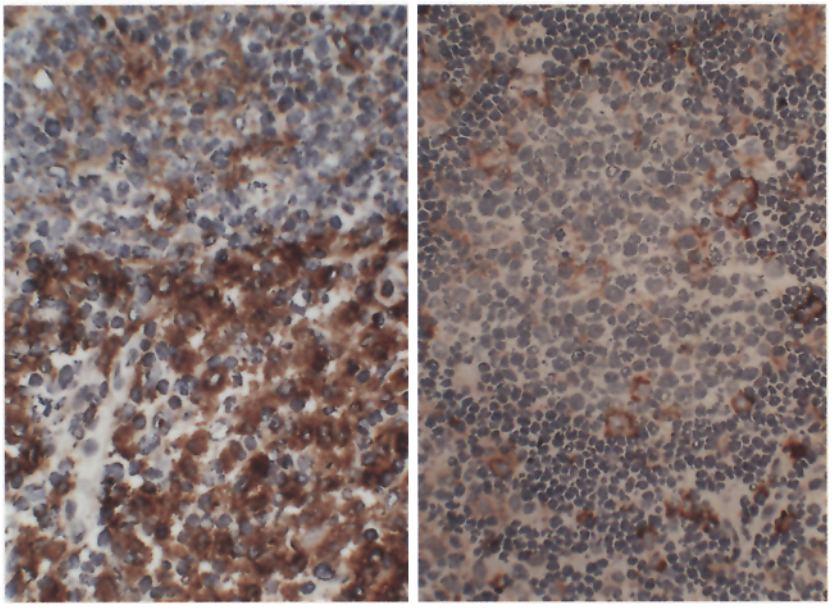

Fig. 2 Immunohistochemical staining of frozen samples with anti-GM-CSF receptor (hGM-CSFR-M1, CDw116). *Left:* dermatopathic lymphadenopathy. Interdigitating dendritic cells of the interfollicular area (*lower part*) are CDw116 positive, while B lymphocytes of the folicle (*upper part*) and T lymphocytes of the interfollicular area are negative. ×200. *Right:* dermatopathic lymphadenopathy. Germinal center macrophages are CDw116 positive, while centro-follicular and mantle zone lymphocytes are negative. ×200

dendritic cells (IDC), were stained. In the two dermatopathic lymphadenopathies, the paracortical zones were hyperplastic and contained a very high number of CD1a+ interdigitating cells (IDC). Serial sections (4 μm thick) disclosed a positive staining of the majority of IDC with hGM-CSFR-M1 (Fig 2). The macrophages of the germinal centres were also CDw116-positive (Fig 2).

Discussion

Using in situ immunohistochemistry with two different monoclonal antibodies, we demonstrated the presence of GM-CSF receptor on the majority of LCH cells, in all the LCH samples that we tested. The GM-CSF receptor was also present on macrophages and IDC in non-tumour samples.

The culture of human cord blood haematopoietic progenitors in the presence of GM-CSF and tumour necrosis factor α induces differentiation and proliferation of LC [3]. Culture of human peripheral blood cells in the presence of GM-CSF and IL-4 induces differentiation and proliferation of LC [16]; GM-CSF also induces activation of LC in vitro [19], and intradermal injection of GM-CSF to lepromatous patients induces local accumulation of LC [11].

The cellular effects of GM-CSF are mediated by a specific membrane receptor. Using competition labelling

with ^{135}I-stained GM-CSF, Kämpgen et al. demonstrated that human activated LC and dendritic cells express a high-affinity GM-CSF receptor [10]. Furthermore, the density of this receptor at the surface of activated LC is comparable to that of haematopoietic progenitors. However, these authors failed to detect GM-CSF receptor at the surface of freshly extracted LC, which could be due either to a low expression of GM-CSF receptor on non-activated LC, or to the destruction of this receptor during trypsin extraction. In the present study, we failed to detect the expression of CDw116 on normal intraepidermal LC, whereas the staining was bright on IDC. Our in situ results suggest that the expression of CDw116 is much lower on resting LC than on IDC.

GM-CSF receptor is a heterodimer *trans*-membrane protein. The β–chain is common to IL-3 and IL-5 receptor, and is necessary for transmission of the signal [5]. The α-chain is GM-CSF specific. Monoclonal antibodies directed against the α-chain of the GM-CSF receptor have been recently produced, and this protein was designated CDw116 at the 5th International Workshop and Conference on Human Leukocytes Differentiation Antigens. CDw116 is expressed on haematopoietic progenitors. It has been detected on cells of the monocyte/macrophage lineage but not on blood lymphocytes [1, 22]. These data are consistent with the positive staining of macrophages and the negative staining of lymphocytes, as well as fibroblasts, keratinocytes and endothelial cells in the present study. The mRNA of the α-chain of the GM-CSF receptor has recently been detected by in situ hybridization on bone marrow trephine biopsies [17]. However, few data have been published concerning the regulation of the expression of the α-chain, and we still do not know whether this regulation is mainly pre- or post-transcriptional, as is the case for GM-CSF. Therefore, detection of the protein is more informative physiologically.

Because of its effects on normal human LC, GM-CSF may be implicated in the pathogenesis of LCH. Indeed, LCH cells contain GM-CSF [6], and children with disseminated and active LCH have an elevated GM-CSF serum level [8]. Furthermore, the LCH cells are activated LC [9], as is the case for normal LC cultivated in the presence of GM-CSF [19]. The present study demonstrates that LCH cells express GM-CSF receptor. Therefore, proliferation, accumulation and activation of LCH cells may be due to the GM-CSF, as demonstrated in vitro for normal LC.

LCH is a clonal proliferation [20, 21], implying that the proliferating cells have a genomic mutation,which may be responsible for the uncontrolled proliferation of the clone. As GM-CSF is one of the main LC growth and differentiation factors, the mutation may involve the GM-CSF production or the GM-CSF receptor function. However, this remains unconfirmed or otherwise, and the presence of GM-CSF and GM-CSF receptor may simply be related to the activation state of LCH cells.

The prognosis of diffuse LCH is uncertain [12]. Some of the children have to be treated with high-dose chemotherapy and bone marrow transplantation [14]. GM-CSF is currently used to accelerate leucocyte recovery after bone marrow transplantation. As GM-CSF is LC proliferation factor, and LCH cells express GM-CSF receptor, the use of GM-CSF should be avoided during the treatment of LCH.

We have demonstrated the expression of GM-CSF receptor by LCH cells. GM-CSF is a growth factor of normal LC and is present within LCH cells; our results suggest that GM-CSF may be implicated in the pathogenesis of LCH.

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