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Langerhans Cell Histiocytosis in Children: Does Soluble Interleukin-2-Receptor Correlate With Both Disease Extent and Activity?

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Background. Langerhans cell histiocytosis (LCH) is characterized by monoclonal proliferation of activated Langerhans cells. Neither etiology nor pathomechanism of this disorder is presently known. However, despite monoclonality LCH might represent a reactive clonal disorder induced by immune dysfunction rather than a malignant process. To investigate a putative cytokine dysregulation in the pathogenesis of this disorder and searching for parameters of both disease activity and prognosis, serum concentrations of proinflammatory and T-cell derived cytokines were evaluated in LCH patients. ***Materials and Methods.*** Serum levels of IL-1β, IL-2, sIL-2R and TNF-α were determined by ELISA in seven children with different types of LCH: Three children (aged 6, 10 and 14 years, respectively) with single system/single bone disease; one child (11 years) with recurrent single system/multiple bone disease and three children (1, 2 and 2 years, respectively) with multisystem disease. ***Results.*** sIL-2R was elevated at diagnosis in seven children as compared to healthy adults (mean ± SEM: 5,256 ± 3,751 U/ml vs. 73 ± 5.5 U/ml; $P < 0.005$) or healthy children (mean ± SEM: 10,195 ± 2,798 pg/ml vs. 2,638 ± 156 pg/ml; $P < 0.01$). A positive correlation between serum levels of sIL-2R and extent of the disease could be observed. During remission, sIL-2R levels declined. IL-1β, IL-2, and TNF-α remained within the normal range during the study period. ***Conclusions.*** Elevated sIL-2R levels seem to correlate positively with both extent and activity of LCH, thus indicating a pathological T-cell activation as a pathogenetic factor. sIL-2R level is a promising parameter to monitor disease activity in LCH and may also be of prognostic relevance. Med. Pediatr. Oncol. 31:61–65, 1998.

Key words: soluble interleukin-2 receptor; Langerhans cell histiocytosis; histiocytosis X; T-cell activation; prognosis; childhood

INTRODUCTION

Langerhans cell histiocytosis (LCH) encompasses a group of disorders previously known as eosinophilic granuloma, Hand-Schüller-Christian syndrome, Letterer-Siwe disease or histiocytosis X [1]. The clinical varieties of LCH range from single-system disease, which is usually cured by curettage, to multisystem involvement, which can behave like a malignant disease.

While there is general agreement that LCH is a disorder of activated Langerhans cells [2–6], the nature of the disease, whether reactive or neoplastic, remains unknown. Numerous pathogenetic mechanisms have been considered, but none has been proven. The possibility of spontaneous remission [7] and the benign histopathological appearance of lesions in LCH suggest a reactive clonal disorder caused by immune dysregulation rather than a malignant process [6,8].

The interaction of antigen-presenting Langerhans cells with T-lymphocytes results in the production of cytokines, especially of interleukin-2 (IL-2). Cytokines may play an important role in pathogenesis of LCH by attracting inflammatory cells and leading to tissue damage. However, there are only very few data published on different types of cytokines in LCH. We therefore analysed serum samples of children affected by LCH for both proinflammatory and T-cell derived cytokines. In addition, cytokine levels were evaluated for correlation with clinical stages of LCH, disease activity and response to treatment.

MATERIALS AND METHODS

Study Population

Seven children (aged 1–14 years) affected by different clinical stages of LCH were studied (Table I). Treatment was performed according to the LCH-I protocol of the Histiocyte Society [9].

The proinflammatory cytokines interleukin-1β (IL-

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TABLE I. Clinical Characteristics of Patients With LCH

Patient no.	Age	Staging[a]	Organ involvement
1	14.5 yr	1	Bone
2	6.3 yr	1	Bone
3	5 yr	2	Bone
4	25 mon	3	Bone, bone marrow, hypophyseal gland, skin
5	11 mon	3	Lung, skin, lymph node
6	30 mon	3	Bone, lung, skin, liver
7	11 yr	1	Bone

[a]1 = single system, single site; 2 = single system, multiple sites; 3 = multisystem disease.

1β), tumor-necrosis-factor-α (TNF-α) and the T-cell derived cytokine interleukin-2 (IL-2) and its soluble receptor (sIL-2R) were quantified at diagnosis (patients 2–7), during treatment (patients 3 and 4) and 1–19 months after treatment (patients 1, 2, 3, 4 and 7) (Table II). First, serum levels of the cytokines were determined by enzyme-immunoassay kits from R&D Systems in four patients (patients 1–4) and in 46 healthy adults (method 1). Due to limited supplies, three patients (patients 5–7) and 48 healthy children were then studied using enzyme-immunoassay kits from Immunotech (method 2). For sIL-2R the measuring unit changed from U/ml (method 1) to pg/ml (method 2). Therefore the results of patients 1–4 were compared with 46 healthy adults and the results of patients 5–7 were compared with 48 healthy children analysed by the appropriate enzyme-linked immunosorbent assay (ELISA) system.

Serum samples of healthy children were obtained during routine blood sampling prior to elective surgery after informed consent by the parents.

Serum Acquisition

Blood was drawn in Lithium-Heparin tubes (R&D Systems) or EDTA tubes (Immunotech), immediately centrifugated at 600*g* for 10 min and frozen at −80°C until use. Aliquots were used only once after thawing.

Cytokine Assays

To measure serum levels of IL-1β, TNF-α, IL-2 and sIL-2R, ELISA was utilized according to the manufacturer's procedures (R&D Systems, distributed by Biermann GmbH, Bad Nauheim, Germany; Immunotech, France, distributed by Dianova-Immunotech GmbH, Hamburg, Germany). All samples were tested in duplicate. The optical density of the samples was determined photometrically at 405 nm using the ELISA reader Spectra Classic II (SLT Labinstruments GmbH, Crailsheim, Germany) and plotted against a standard curve. The detection limits were 5 pg/ml for IL-1β, IL-2 and TNF-α (R&D, Immunotech), 5 U/ml (R&D, method 1) and 200 pg/ml (Immunotech, method 2) for sIL-2R, respectively. The standard was calibrated by reference to WHO standard.

Statistical Methods

Analysing the results obtained by the two kits separately, Mann-Whitney U-test was used for intergroup comparisons and a $P < 0.05$ was considered significant. The results were expressed as mean ± standard error of the mean.

RESULTS

sIL-2R was significantly elevated at diagnosis in patients 1–4 with LCH in comparison with healthy adults (method 1: 5,256 ± 3,751 U/ml vs. 73 ± 5.5 U/ml; $P < 0.005$) and in patients 5–7 in comparison with healthy children (method 2: 10,195 ± 2,798 pg/ml vs. 2,638 ± 156 pg/ml; $P < 0.01$) (Fig. 1). A positive correlation could be observed between serum levels of sIL-2R and extent of the disease. Despite the small number of patients this correlation is seen in both groups analysed by different ELISA systems (Fig. 2). For statistical correlation analysis the number of patients was not sufficient.

Three boys with single-system, single-site bone lesion (patients 1, 2 and 7) had moderately increased serum levels of sIL-2R. Higher levels were found in serum samples of children with multifocal or multisystem disease (patients 3, 4, 5 and 6; Fig. 2). However, regressing signs of disease activity until the end of treatment were accompanied by a substantial reduction of sIL-2R serum levels (patients 2, 3 and 4; Fig. 3).

In patient 3 a steady state of sIL-2R was followed by a local recurrence of the disease. After 3 and 9 months still elevated serum levels of sIL-2R normalized in patient 4 and 3, respectively.

IL-2 at diagnosis was detectable only in one girl with multisystem disease (patient 4). Other types of cytokines remained within the normal range or were undetectable by the method used.

DISCUSSION

LCH is a disorder of unknown etiology, characterized by an accumulation of cells with Langerhans cell (LC) phenotype, together with different types of inflammatory cells [5,10]. LCH cells resemble LCs at an early stage of cell activation [3,4]. Although LCH cells express more costimulatory molecules than LCs, they are functionally defective in antigen presentation [3,11]. Moreover, LCH cells produce a wider range of cytokines as compared to normal epidermal LC [12,13]. In addition, the cytokines GM-CSF and TNF-α are able to generate mature interdigitating dendritic cells from haematopoietic progenitors, positive for CD 34 [14]. So, the uncontrolled proliferation of cells in LCH lesions could be induced by a

TABLE II. Serum Levels of sIL-2R in LCH

Patient no.	Serum level: At diagnosis	Under treatment	After treatment	Outcome	Normal range	*P* value
Method 1[a]						<0.005
1	—	—	190	No active disease	<150	
2	393	—	291	No active disease	<150	
3	2,740	1,624	460	No active disease	<150	
4	12,635	9,850	943	No active disease	<150	
Method 2[b]						<0.01
5	19,133	—	—	Lost to follow up	<4,500	
6	8,450	—	—	Died	<4,500	
7	4,711	—	3,664	No active disease	<4,500	

[a]R&D systems, U/ml.
[b]Immunotech, pg/ml.

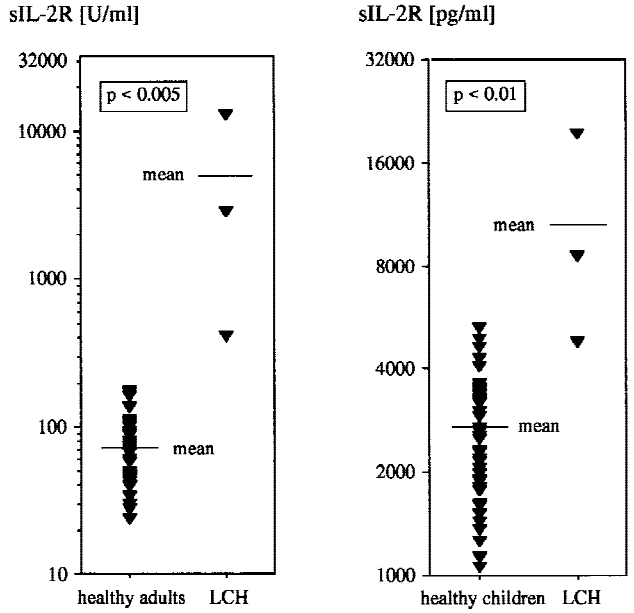

Fig. 1. Serum levels of sIL-2R are elevated in children with Langerhans cell histiocytosis at diagnosis compared to healthy adults (n = 46; method 1) and healthy children (n = 48; method 2). For methods 1 and 2, see text.

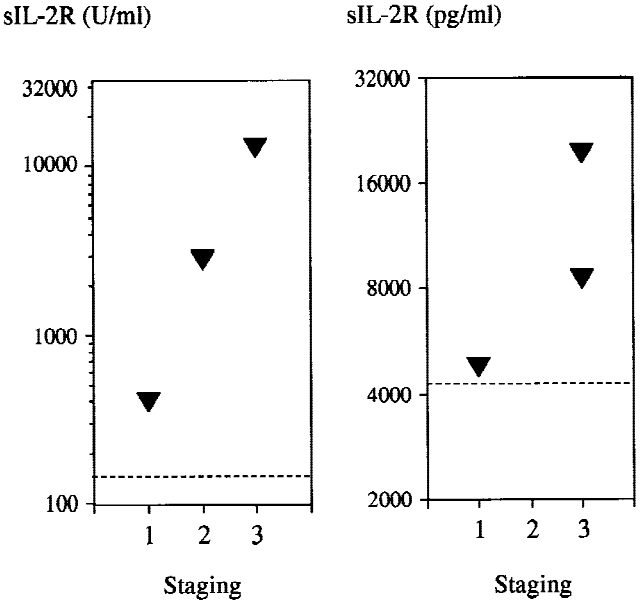

Fig. 2. Serum levels of sIL-2R correlate with extent of the disease at diagnosis in both groups analysed by different ELISA systems. For statistical correlation analysis the number of patients was not sufficient. Staging: 1 = single system, single site; 2 = single system, multiple sites; 3 = multisystem disease. Broken line indicates normal range.

pathological secretion of these cytokines. Also, clinical symptoms such as fever, failure to thrive and bone resorption may be cytokine dependent.

The detection of clonality in LCH cells [15,16] indicates that LC proliferation is probably the consequence of genetic mutation rather than a reactive phenomenon. However, the occurrence of spontaneous remissions [7] and the failure to detect genetic abnormalities or abnormal DNA content of the LCH cells [17] do not support interpreting the pathological process as a malignant neoplastic one. Moreover, monoclonal expansion seen in LCH could also represent a host response to chronic antigenic stimulation, as to some type of B-lymphocyte activation after antigen contact. Under conditions of chronic stimulation dominant clones of dendritic cells might emerge.

The appearance of IL-2R on LCH cells also suggests that in these pathological conditions LCs are activated and promoted to proliferate [2,4]. Elevated levels of sIL-2R therefore might be caused by Langerhans cell proliferation in a host uncontrolled fashion. Another explanation for both the elevated sIL-2R level in serum and the expression of IL-2R on LCH cells could be a deeply disturbed cooperation between LCs as antigen presenting cells and T cells as competent effector cells.

However, until now neither consistent immune function abnormality nor elevated levels of sIL-2R have been

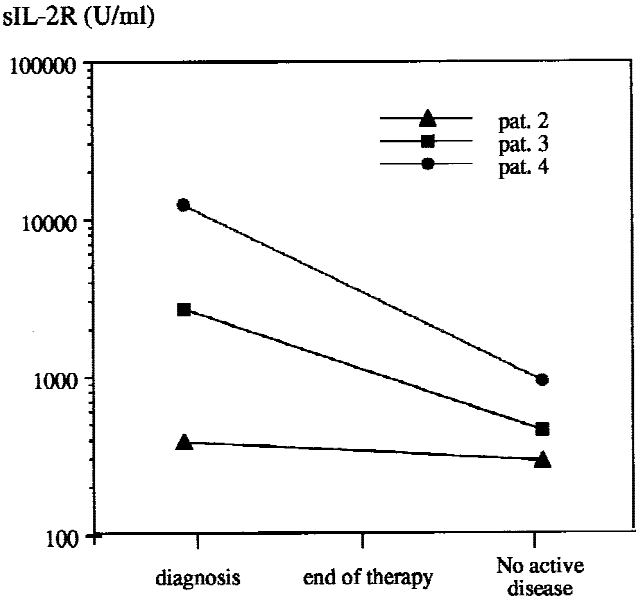

Fig. 3. Serum levels of sIL-2R decline during the course of Langerhans cell histiocytosis (semilogarithmic scale).

detected in patients with LCH [12,18,19]. On the contrary our study revealed significant elevated sIL-2R levels in sera of children with different types of the disease at diagnosis [20]. Shed IL-2R in the serum is a marker of strong antigenic stimulation and has been found in diseases characterized by T-cell activation, e.g. graft vs. host disease, T-cell leukemia, nephrotic syndrome, and in some types of autoimmune diseases [21–23]. sIL-2R was also raised in sera of children with hemophagocytic lymphohistiocytosis [18]. In our study seven children with various forms of LCH showed a positive correlation of sIL-2R levels with both disease activity and extent of LCH. Decreasing sIL-2R levels, observed during treatment, may indicate a role for IL-2/sIL-2R-complex in the pathomechanism of this intriguing disease. The slow decrease of sIL-2R in two patients is not clear, but may be an indication for a still active disease without clinical symptoms.

Because sIL-2R is capable of binding to IL-2, it may inhibit the normal immune response by occupying the attachment region of this T-cell-derived cytokine [24]. It is known that IL-2 plays an important role in T-cell activation, but also in programmed cell death induction for already activated T-cells [25]. Disturbances of this finely tuned system might allow an uncontrolled proliferation of T-cells and Langerhans cells which also express the IL-2 receptor [2,4].

Although our study cannot exactly show the underlying pathogenetic mechanism in LCH, it emphasizes the role of T-lymphocytes and their interaction with interdigitating dendritic cells and/or LCs in the pathogenesis of LCH.

Further studies should confirm these positive correlations observed in our study. If so, sIL-2R may be a good parameter for monitoring of the disease activity and could probably serve as an important prognostic factor.

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