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Elevated Circulating Levels of Interleukin-1 Receptor Antagonist But Not IL-1 Agonists in Hemophagocytic Lymphohistiocytosis

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The familial form of hemophagocytic lymphohistiocytosis (HLH) is an inherited disease with disturbed immunomodulation and characterized by fever, hepatosplenomegaly, cytopenia, hypertriglyceridemia, and coagulopathy, i.e., findings which are similar to many of the reported biological effects of the inflammatory cytokines. Due to the previously shown hypercytokinemia in active HLH with elevated levels of interleukin (IL)-6, tumor necrosis factor-α, and interferon-gamma, it has been suggested that cytokine dysregulation may be of pathophysiological importance. Here we have assayed the serum levels of the members of the IL-1 ligand family, the two agonists IL-1α and IL-1β and the antagonist IL-1 receptor antagonist (IL-1ra), in nine children with HLH and cerebrospinal fluid (CSF) specimens from four children. Serum IL-1ra was elevated in all patients with active disease to a degree which correlated well with disease activity. Furthermore, the levels decreased day by day during treatment of a patient who suffered a relapse. Moreover, high levels of IL-1ra were also detected in CSF during active disease. However, IL-1β levels were all within normal limits and circulating IL-1α levels were normal in all but two patients.

Key words: cytokines, familial hemophagocytic lymphohistiocytosis, hemophagocytic lymphohistiocytosis, interleukin-1, interleukin-1 receptor antagonist

INTRODUCTION

The familial form of hemophagocytic lymphohistiocytosis (HLH) is an inherited disease, also termed familial hemophagocytic lymphohistiocytosis (FHL), with disturbed immunomodulation, characterized clinically by fever, hepatosplenomegaly, cytopenia, hypertriglyceridemia, and coagulopathy [1,2]. The clinical and laboratory findings in HLH are similar to many of the reported biological effects of the inflammatory cytokines, in particular to those of tumor necrosis factor-α (TNF) [3,4].

Important inflammatory cytokines are, in addition to TNF, interleukin (IL)-1 and IL-6 [5–7]. IL-1 is considered to be the prototype of the proinflammatory cytokines and it is rapidly synthesized after appropriate stimuli, primarily by mononuclear phagocytes [7]. The IL-1 family consists of two agonists, IL-1α and IL-1β, and one antagonist, IL-1 receptor antagonist (IL-1ra), which are all structurally related and produced by the same cells [8]. Whereas most IL-1α remains in its precursor form in the cytosol of these cells, a considerable amount of IL-1β is released into the circulation [8]. The presence of IL-1β in the serum has been associated with the sepsis syndrome, rheumatoid arthritis, inflammatory bowel disease, and a number of other inflammatory disorders [8].

Recent studies in various childhood hemophagocytic syndromes have confirmed the presence of elevated circulating levels of not only TNF and IL-6, but also of soluble interleukin-2 receptor (sIL-2R), interferon-gamma (IFN-gamma), and soluble CD8 (sCD8) as well as an increase in urinary levels of neopterin [4,9–13]. Due to the hypercytokinemia observed in HLH, we have previously suggested that this disease may be caused by a genetic disturbance in cytokine regulation [4]. Since HLH is a highly active and even frequently fatal inflammatory disease, we studied the circulating levels of IL-1α and IL-1β as well as those of the recently described IL-1ra in nine children with HLH.

MATERIALS AND METHODS

Patients

The study group consisted of 10 children, 6 boys and 4 girls, with HLH. All but two of these patients had

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TABLE I. Presentation of 10 Children With HLH Included in the Study

Patient no.	Age at diagnosis (months)	Sex	Family history of HLH/ consanguinity[a]	Diagnostic histology[b]
1	12	M	pos/pos	B, L
2	39	M	pos/pos	B, S
3	2	F	pos/pos	S
4	64	M	pos/neg	B, S
5	10	F	neg/neg	B, S
6	74	M	pos/neg	B
7	2	M	pos/pos	B, S
8	0.3	F	neg/pos	S[c]
9	4	M	neg/neg	B, S
10	21	F	neg/pos	B, L

[a]pos = positive; neg = negative.
[b]B = bone marrow; L = lymph node; S = spleen.
[c]The hemophagocytosis in the splenic aspiration biopsy was only discrete and not diagnostic. The diagnosis was based instead on findings of hepatosplenomegaly, anemia, thrombocytopenia, hypertriglyceridemia, decreased high-density lipoprotein (HDL)-cholesterol, hyponatremia, hypoalbuminemia, hyperbilirubinemia, elevation of serum transaminases, pleocytosis in the CSF with protein elevation, consanguinity, and partial response to steroid and etoposide treatment.

siblings with HLH and/or parental consanguinity (Table I). All had a clinical picture compatible with HLH and all also fulfilled the diagnostic criteria for HLH, except for one girl (no. 8) with neonatal onset and severe encephalopathy, for whom no complete autopsy was allowed (Table I) [1,14]. Remission was defined as a lack of fever, splenomegaly, cytopenia, and hypertriglyceridemia during at least 1 month, whether treatment was given or not.

One child (no. 2) was assayed daily during initial treatment of a relapse (Table II). Four years after initial diagnosis, as this patient had been treated regularly with intravenous (IV) teniposide (100 mg/m^2) once every second week for 3 months, the interval between the IV infusions was prolonged to 3 weeks. However, during the second of these prolonged intervals a moderate relapse occurred with fever, splenomegaly, anemia, thrombocytopenia, and elevation of the serum transaminases. The boy was then treated with etoposide (100 mg/m^2 IV) on days 0 (the day of the relapse), 3, and 7 [15]. He received hydrocortisone (200 mg IV) on days 0 and 1, followed by prednisolone (60 mg perorally) daily in combination with indomethacin (50 mg daily per rectum) starting on day 1.

Cytokine Assays

Serum samples from 9 of the 10 children (no. 1–9) had been taken during active disease at onset (no. 1, 7, and 9) or relapses and from three children during remission. In one child (no. 10) only the cerebrospinal fluid (CSF) was studied. In two children (no. 1 and 2), samples from two bouts were available and analyzed. The samples had been stored frozen, but some of them had been previously thawed for a short time in order to enable other serum analyses. From four children (no. 3–5, 10), samples from the CSF were also analyzed.

IL-1α, IL-1β, and IL-1ra were all determined with enzyme immunoassay (EIA) procedures (Quantikine; Research and Diagnostic Systems, Minneapolis, MN). The immunoassay was performed as described by the manufacturer except the last step, where the result was read with chemoluminescence as previously described [4]. Normal serum values (mean + 1 SD) were as follows, determined in sera from 30 normal blood donors: IL-1α, <3.9 pg/ml; IL-1β, <3 pg/ml; and IL-1ra, <1,670 pg/ml. In addition, IL-1β was determined with IL-1β-EASIA (Medgenix; Fleurus, France) to ascertain detection of both free and receptor or carrier protein-bound cytokine. The IL-1β-EASIA test is composed of several monoclonal antibodies detected against different epitopes on the cytokine molecule.

Moreover, serum samples were also analyzed for IL-1 bioactivity on a murine thymocyte proliferation assay, utilizing responder cells from IL-1-responsive and unresponsive NMRI mice as previously described [16]. The tests were made with serial dilutions of serum samples and calibrated with known amounts of IL-1 as a standard. This bioassay does not discriminate between IL-1α and IL-1β and has a sensitivity of 1.0 pg/ml and 0.5 pg/ml of the recombinant human IL-1α and IL-1β, respectively [16].

RESULTS

The circulating levels of IL-1ra were increased (range 7,000–>30,000 pg/ml) in all of the children with active HLH (Fig. 1). Also, the levels studied at later bouts in two children (no. 1 and 2) were elevated. The elevation was reversible and the values observed during HLH in clinical remission were within normal limits (median 280, range 160–1,600 pg/ml). The IL-1β levels, as determined by the Quantikine enzyme-linked immunosorbent assay (ELISA), were all within normal limits (i.e., <3 pg/ml) during active disease and remission. Slightly elevated IL-1α levels were detected in 2 of 10 samples obtained during active disease, whereas all other samples contained normal amounts.

The same negative findings for IL-1α and IL-1β were found when serum samples were analyzed by a murine thymocyte IL-1 bioassay. Neither were elevated levels of IL-1β detected by an IL-1β-EASIA test.

The IL-1ra levels seemed to reflect well the severity of the disease. Thus, the three specimens with ≥25,000 pg/ml were all taken during severe disease. Moreover, the levels in the child studied during initial treatment of a relapse decreased day by day (Table II; Fig. 2).

TABLE II. Levels of IL-1α, IL-1β, and IL-1ra as Correlated to Clinical and Laboratory Findings During a Moderate Relapse of HLH and Its Initial Treatment in Patient No. 2

	Earlier value	Days after onset of a treatment					
		0	1	2	3	6	7
Clinical and laboratory features							
Fever (°C)	none	40.0	39.2	36.8	none	none	none
Hemoglobin (g/L)	115	102	87	84	81	86	84
Platelets ($\times 10^9$/L)	252	37	50	50	69	99	109
Leukocytes ($\times 10^9$/L)	5.8	7.3	2.2	2.3	2.6	3.2	4.9
Neutrophils ($\times 10^9$/L)	3.0	1.7	1.0	1.4	1.8	1.8	2.3
Triglycerides (mmol/L)	1.5	2.4	2.7	3.8	4.6	4.6	3.5
Aspartate aminotransferase (U/L)	22	60	—[a]	18	—	—	—
Alanine aminotransferase (U/L)	11	102	—	60	—	—	—
Sodium (mmol/L)	141	129	130	138	138	—	150
Interleukins (pg/ml)							
IL-1α (ref < 3.9)	—	2.2	<1	<1	<1	<1	<1
IL-1β (ref < 3.0)[b]	—	<1	1.7	<1	1	1.1	1.2
IL-1β (ref < 3.0)[c]	—	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
IL-1ra (ref < 1,670)	—	5,000	3,000	2,800	1,100	480	280

[a]Not determined (all dashes).
[b]Quantikine method.
[c]EASIA method.

The CSF of four HLH patients, sampled during active disease, were also examined. Two children (no. 10 at onset and no. 5 at relapse) had markedly elevated levels of IL-1ra, >30,000 and 25,000 pg/ml, respectively. The values in the other children (no. 3 and 4) were <1,000 and 4,200, respectively. The amount of IL-1α was moderately increased (7.5 pg/ml) in one of the four CSF samples. The IL-1β levels were <3 pg/ml, i.e., within normal limits, in all of these specimens.

DISCUSSION

HLH is characterized by fever, hepatosplenomegaly, cytopenia, hypertriglyceridemia, hypofibrinogenemia, and active hemophagocytosis by macrophages in the mononuclear phagocyte system (MPS) [14]. FHL is an inherited disorder of immunomodulation, most probably autosomal recessive [17]. However, hemophagocytic syndromes may also be found secondarily to other conditions, seen also in adults, including malignancy-associated (MAHS) and infection-associated hemophagocytic syndrome (IAHS) [14]. The clinical picture in FHL and other hemophagocytic states is dominated by symptoms and laboratory changes which are characteristic effects of the inflammatory cytokines, such as fever, cytopenia, elevation of serum transaminases, hypoalbuminemia, coagulopathy, and hypertriglyceridemia due to suppression of lipoprotein lipase activity [1–7,10–12,18].

Previous studies on children with FHL have revealed increases in serum levels of TNF, IFN-gamma, neopterin sIL-2r, IL-6, and sCD8 [4,9,10]. In the present study we have demonstrated that, in addition to the cytokines previously studied, circulating IL-1ra is also elevated in active disease and that this alteration is reversible, i.e., patients in remission have values within normal limits (Fig. 1). The degree of this elevation is correlated to the severity of the disease, and declines day by day following initiation of therapy (Table II, Fig. 2). Whether this phenomenon merely reflects a disturbed MPS homeostasis or is of etiological importance remains to be elucidated. However, the present study demonstrates that the inflammatory reaction in HLH is not caused by a deficiency in IL-1ra production but further search for deficiencies in cytokine inhibition in HLH is still warranted.

We were unable to show increased circulating levels of IL-1β, which is the major proinflammatory IL-1 agonist, in our patients. This was surprising, since the biological effects of IL-1β include fever, thrombocytopenia, leukopenia (at high doses), hypoalbuminemia, and mononuclear cell activation [7], all features typically seen in active HLH. In addition, elevated levels of circulating IL-1 have been detected in a number of conditions which, clinically, are not nearly as inflammatory in nature as HLH [7]. Moreover, IL-1 decreases transcription of mRNA encoding lipoprotein lipase [7], the key enzyme of triglyceride removal, which is compatible with the typical hypertriglyceridemia in HLH [18]. Similarly, elevated circulating levels of IL-1α were found only in a minority of the patients and these elevations were small. These negative results with IL-1 agonists could not be explained by technical difficulties since two different EIA methods, claimed to detect both free and receptor or carrier-bound IL-1β as well as a bioassay, gave the same negative findings. However, the previously shown elevation of IFN-gamma, IL-6, and TNF could well explain the

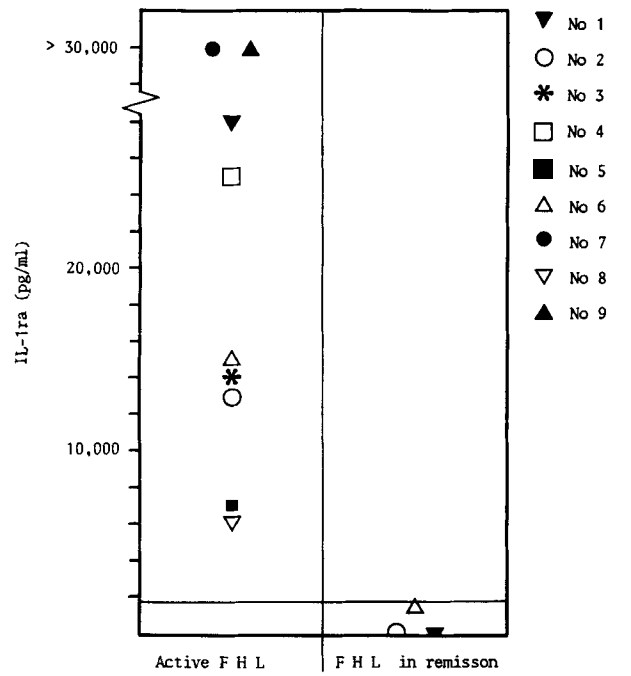

Fig. 1. Serum IL-1ra levels in nine children with HLH during active disease and in remission. The horizontal lines indicate the reference level.

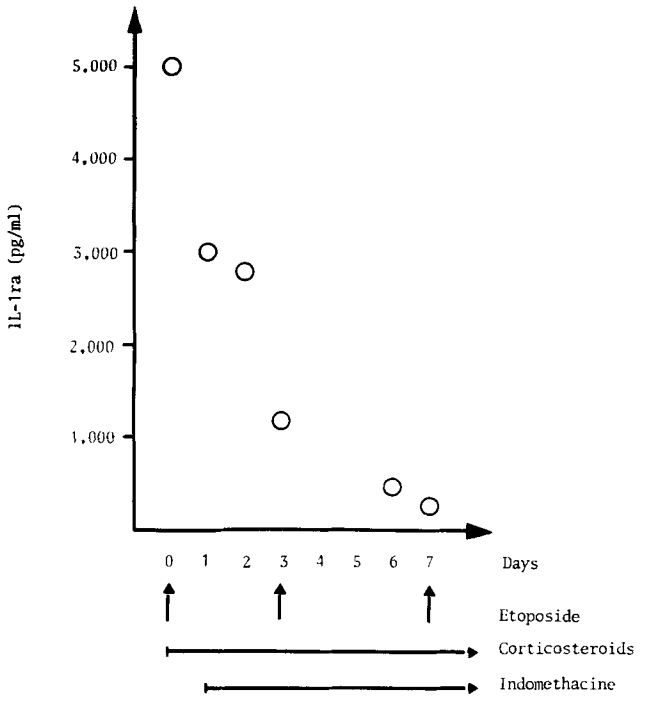

Fig. 2. Day-by-day serum IL-1ra during the initial treatment of a moderate relapse of FHL of patient 2 (see Table I). Each dose of etoposide was 100 mg/m^2 IV. Hydrocortisone was administered (200 mg IV) on days 0 and 1, followed by 60 mg prednisolone perorally each day. The indomethacin dose was 50 mg daily per rectum. Day 0 = first day of treatment.

above inflammatory activities despite the normal levels of IL-1 [4].

Our results with respect to IL-1 are in accordance with recent findings by Fujiwara et al. [13], who demonstrated elevated concentrations of IL-1β in only 5 of 27 patients with hemophagocytic syndromes and of Ishii et al. [11], who observed such elevation in 5 of 12 children with IAHS. It is noteworthy that IL-1 has a role in boosting natural host defense mechanisms and not only are high levels of IL-1 lethal, but failure to produce IL-1 is also associated with mortalities, as seen in patients with sepsis [19].

The interesting but puzzling discrepancy with elevation in the level of an antagonist (IL-1ra) without concomitant increases in the agonists (IL-1α and IL-1β) cannot be explained by this study. It may be speculated that IL-1 activity was exerted mainly locally, serving as an autocrine and paracrine messenger, whereas the responding antagonist activity, produced merely as a consequence of the inflammation, also reached the systemic circulation. Another factor which may contribute to the discrepancy observed is the recently discovered difference in the intracellular secretory pathways between IL-1ra and IL-1, even though the same cell may be synthesizing both cytokines [20]. Also, the possibility of an underlying defect of IL-1β converting enzyme (ICE) can be considered since such a defect would hamper IL-1β production and, interestingly, since an ICE defect would affect apoptosis which might have pathophysiological importance in HLH. However, the present findings could also reflect a very rapid but transient elevation of the IL-1 agonists, which escaped the analysis protocol, followed by a more prolonged elevation of the IL-1ra. It should be noted that Munoz et al. [19] only found elevated IL-1β in 57% of patients with sepsis when over 80% of those patients had elevated IL-6 and TNF levels, indicating that serum levels for the IL-1 agonists may be less informative.

Further, the production of the different members of the IL-1 cytokine family in humans is also dependent upon the mode of induction. Following endotoxin injection, levels of IL-1ra are 100-fold greater than those of IL-1β; in contrast, other bacterial inducers (e.g., *Borrelia burgdorferi*) promote more IL-1β than IL-1ra [21]. It is of particular interest that triggering of monocytes via the immunoglobulin Fc receptor stimulates the production of IL-1ra, but not of IL-1 [21]. In addition, other cytokines such as IL-4, IL-10, IL-13, and transforming growth factor beta (TGF-β) may also regulate the balance between IL-1 and IL-1ra production during disease [21].

Therapeutically, the T-lymphocyte activation in HLH justifies the use of immunomodulators, such as the T-lymphocyte inhibitor cyclosporine A. Furthermore, it can be speculated that direct cytokine-inhibiting factors, such as cytokine receptor antagonist binding proteins and

cytokine antibodies, might be used in HLH therapy in the future, since cytokines are the mediators of inflammation. However, at present, bone marrow transplantation is still the treatment of choice for children affected by FHL if an appropriate donor is available [22].

CONCLUSIONS

Although HLH is characterized by signs associated with inflammatory cytokines, circulating IL-1α and IL-1β were not elevated during active disease. In contrast, IL-1ra levels were increased in the serum and the CSF. The search for the deficiency in immunomodulation in HLH has to continue.

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