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High IL-18 (interferon- γ inducing factor) concentration in a purine nucleoside phosphorylase deficient patient

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Abstract

The plasma concentration of IL-18 (interferon- γ inducing factor) was severely increased in a 3 year old boy with purine nucleoside phosphorylase (PNP) deficiency. The presence and activity of IL-18 were confirmed by immunoblotting and bioassay, respectively. These results suggest that IL-18 may be abundantly produced and secreted into plasma by PNP deficient macrophages in PNP deficiency.

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Keywords: purine nucleoside phosphorylase deficiency; interferon- γ inducing factor; cytokines

A deficiency of purine nucleoside phosphorylase (PNP) has sometimes been found in patients with severe susceptibility to infection. In patients with PNP deficiency, deoxyguanosine, one of the substrates of PNP, increases in plasma and urine, and dGTP is detected in erythrocytes, suggesting that an increase in the concentration of deoxyguanosine leads to accumulation of dGTP in cells, which may be toxic for T cells.¹ B and T cell functions have been investigated in patients with PNP deficiency,¹ while cytokines secreted into blood

have not. We measured plasma cytokines in a patient with PNP deficiency and found a severe increase of IL-18 in plasma, leading to further investigation.

Case report

Since the age of 1 year, the patient (a 3 year old boy) has had notable hypouricaemia (5.6 μ M), frequent episodes of antibiotic resistant urinary tract infection, and occasional respiratory infections. The product of a consanguineous partnership and uneventful term pregnancy, the patient had a neurogenic bladder with a left vesicourinary reflux and developmental delay. His laboratory data related to purine metabolism has been described previously² and is typical of those in PNP deficient patients. His PNP deficiency is attributable to a mutation of arginine codon (CGA) to terminal codon (TGA) (amino acid position 24) in exon 2 of the PNP gene. The patient is homozygous and his parents are both heterozygotes for this mutation.² Four blood samples were taken at the age of 3 years at the time of intercurrent urinary tract infections. The first sample was at 3 years and 17 weeks old, the second one week later, the third at 3 years and 22 weeks, and the fourth at 3 years and 28 weeks. The first and second samples were used for immunological tests (table 1) and the third and fourth to determine the serum concentration of cytokines. Leukocyte counts, lymphocyte subset, immunoglobulin levels, and mitogen response analyses were conducted using standard laboratory methods. IL-18 and other cytokine immunoassays were performed as previously described³ or according to manufacturer's instructions (Otsuka Assay Research Laboratory, Tokushima, Japan).

Plasma IL-18 concentration was also determined by induction of interferon- γ (IFN γ) production by T cells from the tonsil of a tonsillectomy patient cultured at 2×10^6 cells/ml for four days with IL-12 (100 or 200 ng/ml) and various concentrations of IL-18 or diluted plasma (1:4) from the patient or normal control either in the presence or absence of anti-IL-18 antibody. IFN γ in the culture supernatants was measured by specific enzyme linked immunosorbent assay (ELISA) (Bio Source International, Camarillo, California, USA).

Results

Table 1 shows immunological data. The plasma concentration of IL-18 was 216 ng/ml

Table 1 Laboratory data in PNP deficient patient

	Normal range (%)	%	Absolute ($\times 10^6/l$)
White cell count (S1)			16.8
Neutrophils (S1)		91.0	15.3
Lymphocytes (S1)		2.2	0.4
White cell count (S2)			14.2
Neutrophils (S2)		87.9	12.5
Lymphocytes (S2)		3.0	0.4
CD1a (S1)	< 1	< 1	0.004
CD2 (S1)	78-87	72.4	0.27
CD3 (S1)	62-79	15.9	0.06
CD4 (S1)	35-53	18.3	0.07
CD5 (S1)	58-76	24.5	0.09
CD7 (S1)	65-83	74.0	0.27
CD8 (S1)	19-32	20.1	0.07
CD10 (S2)	< 5	7.5	0.03
CD19 (S2)	8-14	1.5	0.007
CD20 (S2)	9-16	6.7	0.03
CD38 (S2)	2-10	80.1	0.34
HLA-DR (S1)	9-18	41.4	0.15
HLA-DR (S2)	9-18	37.9	0.16
IgG (mg/dl)	Normal range	Patient	
	800-1700	1607	
IgE (mg/dl)	0.01-0.14	76.6	
Mitogen stimulation by PHA on lymphocytes (sample 1)			
	PHA	Mean (SD) (cpm)	SI
Patient	-	353 (87)	1.00
	+	1398 (491)	3.96
Control	-	1468 (147)	1.0
	+	38271 (3989)	26.1

S, Sample, PHA, phytohaemagglutinin, cpm, counts per minute, SI, stimulation index.

Figure 1 IFN γ production by human T cells treated with IL-12 treated either with plasma of the PNP deficient patient or from a normal control. (Ab, antibody.)

(sample 3) and 66 ng/ml (sample 4). These concentrations are extraordinarily high compared with those in patients with various infectious diseases, rheumatoid arthritis, gout, and normal subjects (< 10, < 2, < 0.5, and < 0.2 ng/ml, respectively). The plasma concentrations of other cytokines were measured using sample 3. The plasma concentration of IFN γ was raised (84 pg/ml, normal range < 20). In contrast, the plasma concentrations of IL-4, IL-5, TNF α , IL-1 α , IL-1 β , IL-6, IL-2, IL-8, and granulocyte macrophage colony stimulating factor (GM-CSF) were all normal in the patient, although M-CSF (1843 pg/ml, normal < 1800) was slightly raised.

An immunoblot analysis of plasma with anti-IL-18 monoclonal antibody showed an 18 kDa band and other non-specific bands in the patient with PNP deficiency, while it showed only non-specific bands in a healthy subject (data not shown).

The IFN γ response (23.6 ng/ml) in the biological assay using the patient's plasma (sample 4) corresponds to a value of 72 ng/ml for plasma IL-18 derived from a standard curve (not shown), a value similar to that found by immunoassay (fig 1). The addition of anti-IL-18 antibody to the biological assay system completely blocked the secretion of IFN γ from IL-12 treated T cells in the patient's plasma (fig 1).

Discussion

We have demonstrated decreased CD3 positive and CD19 positive lymphocyte numbers, and

mitogen responses in our patient. However, plasma concentrations of IgG and IgE were normal, suggesting PNP deficiency preferentially affected T cell function in this case (table 1). The most intriguing finding in this study was the extraordinarily high plasma concentrations of IL-18. The immunoblot analysis of IL-18 in PNP deficient plasma showed that the detected cytokine was an 18 kDa polypeptide consistent with its mature form. The biological assay of IL-18 in PNP deficient plasma showed that the cytokine was biologically active. This increase may have been induced by the excessive action of natural killer (NK) cells and B cells in our patient who lacked T cell immune function.⁴

In patients with PNP deficiency, IL-18 may be excessively synthesised as precursor protein and actively processed to mature protein by caspase-1.⁴ Although IL-18 seems to play an important role in defence against infection with microbes that activate macrophages and NK cells to produce IL-12 and IL-18, we suggest that the very high plasma IL-18 in our patient was not a response to infection as it was much greater than levels in patients with inflammatory diseases, such as rheumatoid arthritis, and other infectious diseases. Furthermore, IL-1 β and IL-6, which are also major products of macrophages and similarly induced by infectious diseases, were not raised in the plasma of this patient. We propose that in PNP deficiency the production and secretion of IL-18 is increased perhaps because PNP deficient T cells fail to regulate the macrophages or NK cells that produce the cytokine.

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