EXPRESSION OF IL-6, sgp130, IL-8 AND THEIR RECEPTORS IN ACUTE PROMYELOCYTIC LEUKEMIA DURING ALL-TRANS RETINOIC ACID INDUCTION TREATMENT

ZENG Huilan 曾慧兰,[1] ZHANG Xueguang 张学光,[2] CHEN Zixing 陈子兴[2]
TAO Ruifang 陶瑞芳,[2] QIU Yuehua 邱月华,[2] ZHANG Yi 张毅[2]
SUN Aining 孙爱宁,[2] WANG Aiqing 王爱青,[2]
WANG Wei 王玮,[2] LIN Baojue 林宝爵[2]

[1] *First Affiliated Hospital, Jinan University, Medical College, Guangzhou 510630, China*
[2] *Suzhou Medical College, Suzhou Jiangsu Province 215007, China*

ABSTRACT

Objective: To evaluate the expression and its clinical significance of interleukin 6 (IL-6), soluble glycoprotein 130 (sgp130), interleukin 8 (IL-8) and type A interleukin 8 receptor (IL-8RA) in acute promyelocytic leukemia (APL) patients during all-trans retinoic acid (ATRA) induction treatment. Methods: Plasma and bone marrow mononuclear cell (MNC) culture supernatant IL-6, sgp130, IL-8 concentration of 18 cases with APL were kinetically measured *in vivo* and *in vitro* (ELISA). Bone marrow MNC IL-8RA was measured by flow cytometry after being cultured with ATRA (10^{-6}mmol/L). Results: Plasma IL-6, sgp130, IL-8 levels were higher than normal ($P<0.05$), IL-6, sgp130 levels correlated with white blood cell (WBC) counts ($P<0.05$) while IL-8 levels correlated with body temperature ($P<0.05$) at initial diagnosis. After 72-hour incubation with ATRA, concentration of IL-6 of bone marrow MNC culture supernatant did not change, that of sgp130 mildly decreased, and IL-8 significantly decreased while the positive rate of IL-8RA on bone marrow MNC increased. During ATRA treatment, plasma IL-6 changes were correlated with WBC counts. Peak levels of IL-6 and WBC were lower in patients who received intermittent therapy than those who received continuous therapy. Plasma IL-6 and IL-8 were increased when complicated with infection and IL-8 seemed more sensitive. Conclusion: Plasma IL-6, sgp130, IL-8 levels may reflect patients' responsiveness to ATRA treatment, and could be used to predict hyperleukocytosis and intercurrent infection. ATRA induces APL cell differentiation possibly via sgp130 signal transducer chain. Measurement of sgp130 had certain meaning to prognosis.

Key words: Leukemia, Premyelocytic, Retinoic acid, IL-6, Glycoprotein-130, IL-8, Receptor

Interleukin 6 (IL-6) and interleukin 8 (IL-8) are infective cytokines whose abnormal expressions are recently found to be correlated with some certain pathologic process as of acute promyelocytic leukemia (APL). We have previously found the relation of plasma IL-6 levels and hyperleukocytosis induced by all-trans retinoic acid (ATRA) treatment in APL patients.[1] Glycoprotein 130 (gp130) plays an important role in IL-6 signal transduction. In this study, serum and bone marrow monocuclear cell (MNC) culture supernatant IL-6, sgp130, IL-8 concentrations of 18 cases with APL were kinetically measured *in vivo* and *in vitro*. Bone marrow MNC IL-8RA was measured by flow cytometry after being cultured with ATRA (10^{-6} mmol/L). We demonstrated the correlation between the antiproliferative action of ATRA on APL cells and expression of IL-6 system signal transduction during ATRA treatment so that we could evaluate its clinical significance.

METHODS

Patients

Eighteen patients with APL were diagnosed according to the FAB cytological classification criteria, cytogenetic criteria and molecular criteria. There were 10 male patients, and 8 female patients. Their average age was 33 years old (14–55 years old).

Therapy

Patients were divided into two groups at random. In the continuous group ATRA was administered at a dose of 45 mg/m^2/day in three divided doses till

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Correspondence to: Zeng Huilan, Department of Hematology, The First Affiliated Hospital, Jinan University, Medical College, Guangzhou 510630, China; Phone: (0086-20)-85516832 ext. 6344/3135; Fax: (0086-20)-85516819; E-mail: zenghL666@163.net

complete remission (CR) in bone marrow. While in the intermittent group ATRA was taken for one week, discontinued one week, then given another one week and so on till CR, the daily dosage used in this group per day was the same as in the continuous group. Those non-remission (NR) patients were added with chemotherapy. When suffered DIC, heparin was given (75mg/day) continuously intravenously.

In vitro Induction

Mononuclear cells (MNCs) of fresh bone marrow were purified by Ficoll-Hypaque density centrifugation. MNCs (initial cell concentration was 1×10^6 cells/ml) were cultured with or without ATRA (10^{-6} mmol/L) at 37°C for 72 hours in RPMI 1640 medium with, 10% fetal calf serum (FCS) at humidified 5% CO_2 atmosphere. IL-8RA on MNC membrane was examined every day. Supernatants were collected and stored at −20°C which were to be examined for IL-6, sgp130, IL-8.

Plasma Samples

Peripheral blood samples were collected and heparinized before treatment and after treatment every 3–5 days. Plasma samples were obtained by centrifugation and stored at −20℃. Control samples were obtained from healthy blood donors.

IL-6, sgp130, IL-8 Analysis

IL-6, sgp130, IL-8 concentrations in plasma and culture supernatants were estimated by ELISA kit from Immunotech Co., France. The results of IL-6 were expressed in ng/ml and IL-8 in ng/ml. Sgp130 levels were detected by sgp130 ELISA kit produced by Institute of Biological Technique, Suzhou Medical College. The results were expressed in μg/ml.

Statistical Analysis

t test, Spearmann rank analysis was used.

RESULTS

Plasma Levels of IL-6, sgp130, IL-8 before and after Treatment

Plasma levels of IL-6, sgp130, IL-8 of the patients were significantly higher than the control group. IL-6, sgp130 levels were positively correlated with WBC counts at diagnosis. The levels of IL-8 were positively correlated to body temperature ($P<0.05$). The sgp130 levels of those who reached CR were significantly decreased, while NR not significantly changed. IL 6 levels were not significantly correlated to effectiveness ($P>0.05$). (Table 1).

Table 1. Plasma levels of IL-6 (ng/L), sgp130 (μg/ml), IL-8 (ng/L) ($\bar{x} \pm s$)

	Normal	Before treatment	CR	NR	WBC high	WBC not high	T>38.5°C	T<38.5°C
Cases	40	18	7	3	4	14	4	14
IL 6	8.31±	47.26±	21.11±	242.90±	72.38±	36.92+	42.15+	37.28±
	6.32	25.21	112.43	23 04	34.01	23.47	15.13	14.12
..Sgp130	410.28±	487.27 ±	392.44 ±	463.27 +	488.64+	431.87−	438.42±	421.56±
	54.52	87.25	9.58	71.31	212.31	161.72	143.56	151.28
IL-8	32.82±	70.15±			47.58±	81.65±	102.12±	64.01±
	15.13	34.92			22.13	48.43	51.22	24.57

Compared with control, $P<0.05$

Effect of ATRA on APL Cells *in vitro*

IL-6, sgp130, IL-8 secretion was tested in culture supernatant after incubation with or without ATRA (10^{-6}mmol/L). In the absence of ATRA incubation, APL cell production of IL 6 did not change significantly compared to levels obtained in RPMI1640 alone, sgp130 levels decreased mildly, IL 8 decreased significantly, while IL-8RA positive rate on cell membrane significantly increased when incubated with ATRA.

Kinetic Studies of IL-6, sgp130, IL-8 *in vivo*

Spearmann rank analysis to 83 samples from the continuous group demonstrated that plasma IL-6 levels during ATRA treatment were strongly correlated to the increase of WBC counts, especially to promyelocytes. IL-8 levels were correlated to myelocytes and body temperature ($P<0.05$) (Table 2). Among 13 cases in continuous group, in 7 cases the WBC was significantly increased while in 4 cases it did not increase. IL-6 levels of those 7 cases began to increase on the 3–10th day, reached peak levels on the 10–12th day and the high levels lasted about 3 24 days. IL-6 levels of those 4 cases were significantly lower than of those 7 cases. Among the intermittent

group (5 cases), the WBC in 3 cases increased, IL-6 levels were also correlated to WBC changes. IL-6 peak levels occurred on the 15–18th day (122.5 ng/L), which was lower than that of the continuous group (214.2 ng/L). Time to WBC peak levels and WBC peak levels (9.3×10^9/L) were also lower than those in the continuous group (37.3×10^9/L) (Figure 1).

Relation between IL-6, IL-8 and Infection

Fevers (T>38°C) were observed in 18 cases, in of 6/18 of those cases pathogenic bacteria were found, 13/18 cases only demonstrated clinical infective syndromes and signs, 6/18 cases no evidence of infection was found. 25 samples from those during a fever period were compared to those from no fever period (including 1–3 days before fever). IL-6, IL-8 levels increased in different extents during fever, especially significant when pathogenic evidences were found. IL-8 changes seemed more sensitive before distinct infective syndromes occurred (Table 3).

Table 2. Correlation between plasma IL-6(ng/L), IL-8(ng/L) levels and myelocytes

		WBC	Promyelocyte	Myelocyte	Lymphocyte	Platelet	Body Temperature
IL-6	r	0.276	1.228	1.198	1.173	1.181	1.206
	P value	<0.05	<0.05	>0.05	>0.05	>0.05	>0.05
IL-8	r	0.198	0.209	0.253	0.166	0.178	0.317
	P value	>0.05	<0.05	<0.05	>0.05	>0.05	<0.01

Spearmann rank analysis: $r_s0.05(81)=0.217$, $r_s0.01(81)=0.283$

Table 3. Correlation of plasma IL-6(ng/L), IL-8(ng/L) ($\bar{x}\pm s$) levels and infection

	Pathogen(+−)	Syndrome/sign(+)	Cause unknown	Temperature normal	Before fever
Samples	6	13	6	20	18
IL-6	112.34±57.31	56.18±29.73	43.15±23.92	39.43±17.12	38.33±15.62
IL-8	234.16±72.18	198.47±68.32	92.33±36.14	73.28±29.31	98.24±37.12

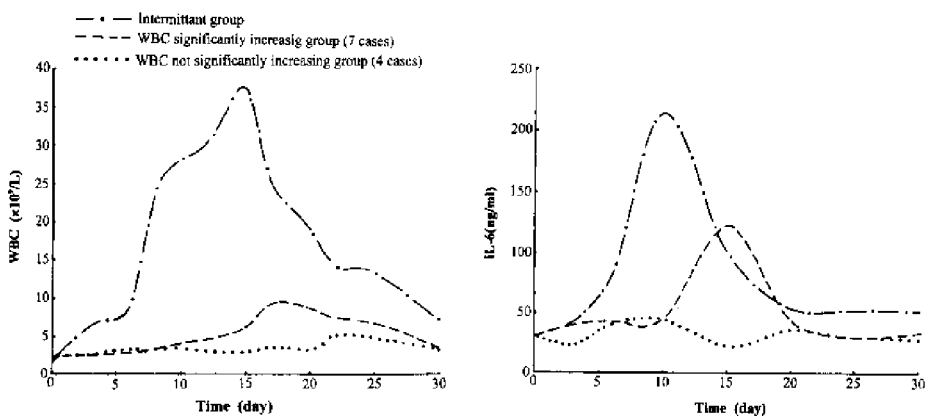

Fig. 1. Relation of plasma IL-6 levels and WBC counts in different groups

DISCUSSIION

Il-6 can stimulate granulocyte, monocyte and their colonys growth. As a megakaryocyte maturing factor, it also plays an important role in platelet-forming.[1] Gp130 is a trans-membrane protein; it also is the common signal transducer of leukemia-inhibitory factor (LIF), oncostatin M (OM), ciliary neurotrophic factor (CNTF) and IL-11. Interaction of IL-6 and IL-6R induces homodimerization of gp130 which activates intrinsic tyrosine kinases and then the IL-6 signal can be transduced and IL-6 biologic effectiveness can be produced.[2] In our study, IL-6 and sgp130 levels of 18 cases were significantly higher than normal and correlated to WBC. As reported by others before,[3] G-CSF was increased

earlier, and its peak levels had a shorter duration during ATRA treatment. These results suggested that G-CSF may have the same transduction chain as gp130, which can also induce the activation of nuclear factor IL-6 (NF-IL-6) and transduction of IL-6.[4] Changes of IL-6 may be the result of secondary stimulation of G-CSF through sgp130 transduction chain.

We all know that once APL relapses, ATRA re-induction treatments often failure. Pharmacokinetics research demonstrated that if ATRA were taken continuously for over one month, plasma drug concentration (Cpmax) and area under concentration-time curve (AUC) would decrease, t1/2β would increase, and apparent volume of distribution (V/F) illustrated that large amount drug was stored in deep tissues. Decrease of Cpmax and AUC can cause low effective concentration and poor therapeutic efficacy.[5] Castaigne, et al.[6] reduced dose of ATRA from 45 mg/m^2 to 25 mg/m^2. We also designed taking ATRA intermittently, not continuously.[7] The WBC peak counts and plasma IL-6 peak levels were significantly lower, and peak levels occurred later and lasted a shorter time. This result proved that there is a relationship between IL-6 and granulocytes. Intermittent treatment should be evaluated further.

ATRA had no influence in the production of IL-6 secreated by APL cells, as did our results. But we found sgp130 was significantly decreased. Sgp130 had been reported to partly suppress the proliferative effectiveness induced by IL-6.[2,9] Did the plasma sgp130 levels decrease when reaching CR just because of the proliferative signal blocking the tumor cells? Was sgp130 not significantly changed when NR just because of the proliferative signal not being blocked? The function and clinical implication of Il-6 and its sgp130 signal transduction in APL should be further researched.

IL-8 belongs to the CXC cytokine superfamily, whose major function is to interpose chemotaxic activation. IL-8RA is the high special affinity receptor of IL-8. APL cells can constitutionally express high levels of IL-8, while ATRA can specially suppress APL cells secreting IL-8 but not other cell types. On the other hand, we found that ATRA could strengthen the expression of IL-8RA, which suggested that with maturing of APL cells induced by ATRA, increased expression of IL-8RA and signal transduction were strengthened and so the sensitivity of granulocytes to IL-8RA. This suggested that with maturing of APL cells induced by ATRA, increased expression of IL-8RA, signal transduction were strengthened and so was the sensitivity of granulocytes to IL-8 chemotaxis. We also found that IL-8 levels were correlated to occurrence of hyperleukocytosis[10] but not to WBC changes. The influence of IL-8 to WBC counts might be through changing the characteristics such as chemiotaxis, migration and adhesion but not through proliferation or differentiation, so WBC counts did not significantly change. Plasma IL-6, IL-8 levels of those patients with granulocytosis after chemotherapy and complicated by infection were commonly increased and rapidly decreased once infection was controlled. But, TNF α, IL-1 β did not change as IL-6 and IL-8 did.[8] IL-8 levels had been reported to be the most sensitive sign for predicting infection among all cytokines. Our results of infection were similar to this; clearer the evidences were, higher IL-6 and IL-8 levels were, especially the IL-8 levels. IL-8 could be used as a predictor for infection.

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