Serum Interleukin-8 Levels in Leprosy Patients

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Background **: Interleukin(IL)-8 is a potent chemotactic factor for neutrophils which was induced by tumor necrosis factor-α and IL-1. Serum IL-8 level was known to be associated with the poor prognosis of tuberculosis and IL-8 mRNA was increased in the tissue of erythema nodosum leprosum.**

Objective **: Our purpose was to investigate whether the serum IL-8 levels of leprosy patients are different from those of controls and whether the severity of the leprosy is associated with the serum IL-8 level.**

Methods **: Twenty eight patients with leprosy and fourteen healthy adults were used in this study. Serum IL-8 levels were detected by enzyme immunoassay.**

Results **: There were no statistically significant differences in the serum IL-8 levels between the twenty eight leprosy patients and the fourteen healthy control serums (29.99$\pm$53.14 vs 2.52$\pm$7.53) nor was the detection rate between the groups (9/28 vs 1/14) significant. There were also no statistically significant differences between the serum IL-8 concentrations of the patients with high bacterial indexes and the patients with low bacterial indexes (30.22$\pm$63.64 vs 29.73 $\pm$41.14).**

Conclusion **: Serum levels and the detection rate of IL-8 in the leprosy patients were not different from the control group and the severity of the disease was not associated with the IL-8 levels. (Ann Dermatol 11(1) 1~4, 1999).**

Key Words : IL-8, Leprosy

INTRODUCTION

Leprosy is a granulomatous skin disease in which the immunological response to leprosy is differentiated by the responsiveness to lepromin[1]. Cytokines have been known to play an important role in the formation of polar types of leprosy[1]. In lepromatous leprosy, less production of interleukin(IL)-2 and interferon(IFN)-γ, more abundant production of IL-4 and IL-10 compared to tuberculoid leprosy were reported[2]. During the treatment of lepromatous leprosy patients with IFN-γ, erythema nodosum leprosum(ENL) was observed[3] and IL-8 mRNA was increased in the ENL lesion[4]. A tumor necrosis factor(TNF)-α was responsible for the induction of ENL reaction[3,5] which was known to be suppressed by thalidomide, an inhibitor of TNF[6]. IL-8 is a potent chemotactic factor for neutrophils which was induced by TNF-α and IL-1[7]. Recently serum IL-8 levels became associated with a poor prognosis for tuberculosis[8]. Therefore we set out to investigate whether serum IL-8 levels in leprosy patients are associated with the severity of the disease.

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MATERIALS AND METHODS

Twenty eight leprosy patients (mean age; 50.6 years, M:F=25:3) and fourteen healthy controls (mean age; 42.8 years, M:F= 1:13) were studied. The leprosy patients were all LL types. The clinical data of the leprosy patients were summarized (Table 1). The other associated diseases in the patients were tuberculosis in two patients, chronic hepatitis accompanying treatment with thalidomide in one patient, chronic gastritis in one patient, bone exposure in one patient and osteomyelitis in one patient. Blood samples were taken from the leprosy patients and the control group, then centrifuged at 4°C and the serum samples were stored at -70°C until testing. A serum IL-8 concentration was detected by Quantikine human IL-8 immunoassay(R&D systems, Minneapolis, MN, U.S.A.) using two antibodies. In polystyrene microtiter plates which were coated with monoclonal antibody against IL-8, serum samples and IL-8 standard solutions were added at room temperature for two hours, then the wells were washed. The horseradish peroxidase conjugated polyclonal antibody against IL-8 was added and incubated at room temperature for two hours, then washed. The solution containing hydrogen peroxide and tetramethyl benzidine was added. After 20 minutes of reaction at room temperature, the color reaction was stopped by the addition of 2N H_2SO_4, and the absorbance was detected at 450 nm which was corrected at 620 nm within 30 minutes by the Titertek ELISA reader. A standard curve was drawn by using recombinant IL-8 and

Table 1. Clinical data of leprosy patients

Patient no.	Age/Sex	B.I.	Duration of disease	Therapy
1.	60/F	3+	19 years	Dapsone, rifampin, clofazimine
2.	70/M	2+	N.A.	Dapsone, rifampin, clofazimine
3.	55/M	3+	35 years	Dapsone, rifampin, clofazimine
4.	49/M	2+	29 years	Dapsone, rifampin, clofazimine
5.	63/M	1+	8 years	Dapsone, rifampin, prothionamide
6.	44/F	0	36 years	Dapsone, rifampin, clofazimine
7.	54/M	2+	28 years	Dapsone, rifampin, clofazimine
8.	45/M	3+	4 years	Dapsone, rifampin, clofazimine
9.	32/M	2+	6 years	Dapsone, rifampin, clofazimine
10.	48/M	3+	15 years	Dapsone, rifampin, clofazimine
11.	38/M	1+	9 years	Dapsone, rifampin, clofazimine
12.	32/M	4+	9 years	Dapsone, rifampin, clofazimine
13.	59/M	2+	42 years	Dapsone, rifampin, clofazimine
14.	80/F	1+	9 years	Dapsone, rifampin, clofazimine
15.	43/M	1+	29 years	Dapsone, rifampin, clofazimine
16.	69/M	1+	8 years	Dapsone, rifampin, clofazimine
17.	70/M	1+	37 years	Dapsone, clofazimine
18.	46/M	3+	32 years	Dapsone, rifampin, clofazimine
19.	63/M	1+	13 years	Dapsone, rifampin, clofazimine
20.	36/M	4+	22 years	Dapsone, rifampin, clofazimine
21.	63/M	3+	3 years	Dapsone, rifampin, clofazimine
22.	47/M	3+	2 years	Dapsone, rifampin, clofazimine
23.	41/M	2+	7 years	Dapsone, rifampin, clofazimine
24.	33/M	1+	8 years	Dapsone, rifampin, clofazimine
25.	30/M	1+	9 years	Dapsone, rifampin
26.	56/M	1+	N.A.	Dapsone, rifampin, clofazimine
27.	53/M	1+	23 years	Dapsone, rifampin, clofazimine
28.	37/M	1+	37 years	Dapsone, rifampin, clofazimine

B.I., bacterial index; N.A., not available

Table 2. Serum IL-8 detection in leprosy patients and controls

	IL-8 detection (number)		
	Yes	No	Totals
Control	1	13	14
Leprosy	9	19	28
Totals	10	32	42

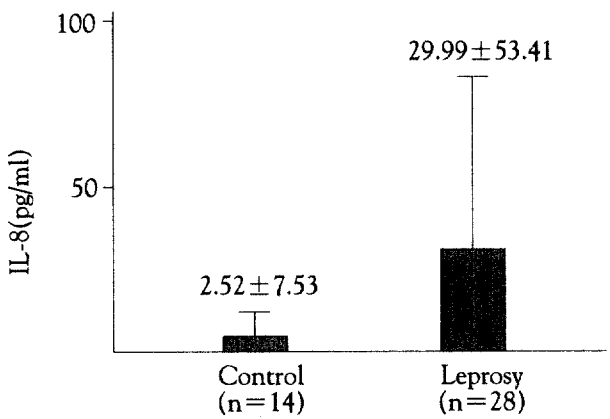

Fig. 1. Serum IL-8 concentrations in the leprosy patients compared with the levels in the healthy control group (mean±SD).

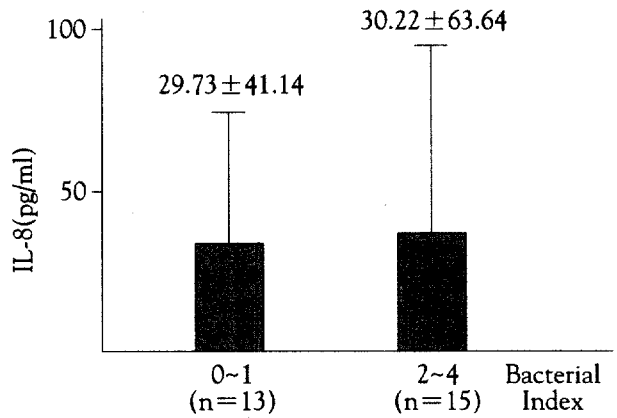

Fig. 2. Serum IL-8 concentrations in the leprosy patients with high bacterial indexes compared with the levels in the patients with low bacterial indexes (mean ±SD).

the detection unit was pg/ml. The sample concentrations were calculated from O.D. values by a Novapath microplate manager(Bio-Rad, Richmond, CA, U.S.A.). The detection limit of this method was 18.1 pg/ml. Therefore, the sample values lower than 18.1 pg/ml were counted as non-detectable. For statistical analysis, the Mann-Whitney rank-sum test was used to compare the differences in IL-8 concentration between the groups and the Fisher exact test was used to compare the differences in the detection rate.

RESULTS

The number of serum samples above the detection limit among 28 leprosy patients was 9, but the number in the 14 control serums was 1. However there were no statistically significant differences between the detection rates(Table 2, $p>0.05$). Serum IL-8 concentrations of the twenty eight patients of leprosy (29.99± 53.14) were compared with the levels of fourteen healthy control serums(2.52± 7.53). There were no statistically significant differences between the groups (Fig. 1, $p>0.1$). Serum IL-8 concentrations of the patients with high bacterial indexes (2-4) were also compared with the levels of the patients with low bacterial indexes (0-1). There were no statistically significant differences between the groups (Fig. 2, $p>0.1$).

DISCUSSION

Elevated serum IL-8 has been reported in tuberculosis[8], psoriasis[9], liver metastasis of colorectal cancer[10], sepsis[11] and systemic sclerosis[12]. Although TNF is a potent inducer of IL-8[7], M. *tuberculosis* induced IL-8 is due to interaction with monocytes and the bacilli instead of TNF[13]. Considering the weak inducing capability of several cytokines by M. *leprae*[14], direct induction of IL-8 by leprosy bacilli may be difficult. Although the serum samples of elevated IL-8 were detected more frequently in the patients' serum compared to the control serum (9/28 vs 1/14), there were no statistically significant differences in detection rates(Table 2,

P>0.05). There were no differences in the levels of IL-8 between the patients and the control group, as well as the IL-8 levels between the patients with high and low bacterial indexes, suggesting that serum IL-8 levels and the severity of the disease were not associated. IL-8 was detected in one patient (137.87 pg/ml) out of two ENL patients, suggesting serum IL-8 levels were not always detected although IL-8 mRNA was reported to increase in the tissue of ENL[4]. IL-8 was detected only in the patient with osteomyelitis (48 pg/ml). In the control group, four people had chronic gastritis, but IL-8 was not detected in the serum. Considering that the tissue infiltrations in L-type leprosy were mostly composed of macrophages, it is more relevant to test the monocyte/macrophage specific chemokines. Our results suggest that serum IL-8 levels are not elevated in leprosy and the severity of the disease is not associated with serum IL-8 levels which may be associated with other relevant factors.

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REFERENCES

1. Rea TH: Immune responses in leprosy, cytokines and new archetypes for dermatology. Clin Exp Dermatol 20:89-97, 1995.
2. Yamamura M, Uyemura K, Deans RJ, et al: Defining protective responses to pathogens: cytokine profiles in leprosy lesions. Science 254:277-279, 1991.
3. Sampaio EP, Moreira AL, Sarno EN, Malta AM, Kaplan G: Prolonged treatment with recombinant interferon induces erythema nodosum leprosum in lepromatous leprosy patients. J Exp Med 175:1729-1737, 1992.
4. Yamamura M, Wang XH, Ohmen JD, et al: Cytokine patterns of immunologically mediated tissue damage. J Immunol 149:1470-1475, 1992.
5. Barnes PF, Chatterjee D, Brennan PJ, Rea TH, Modlin RL: Tumor necrosis factor production in patients with leprosy. Infect Immun 60:1441-1446, 1992.
6. Sampaio EP, Sarno EN, Gailly R, Cohn ZA, Kaplan G: Thalidomide selectively inhibits tumor necrosis factor α production by stimulated human monocytes. J Exp Med 173:699-703,1991.
7. Matsushima K, Morishita K, Yoshimura T, et al: Molecular cloning of a human monocyte-derived neutrophil chemotactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin 1 and tumor necrosis factor. J Exp Med 167: 1883-1893, 1988.
8. Friedland JS, Hartley JC, Hartley C, Shattock R, Griffin GE: Proinflammatory cytokines and prognosis in tuberculosis and HIV infection. Lymphokine Cytokine Res 12:389, 1993, (English abstract).
9. Teranishi Y, Mizutani H, Murata M, Shimizu M, Matsushima K: Increased spontaneous production of IL-8 in peripheral blood monocytes from the psoriatic patient: relation to focal infection and response to treatments. J Dermatol Sci 10:8-15,1995.
10. Ueda T, Shimada E, Urakawa T: Serum levels of cytokines in patients with colorectal cancer: possible involvement of interleukin-6 and interleukin-8 in hematogenous metastasis. J Gastroenterol 29: 423-429, 1994.
11. Engel A, Kern WV, Murdter G, Kern P: Kinetics and correlation with body temperature of circulating interleukin-6, interleukin-8, tumor necrosis factor alpha and interleukin-1 beta in patients with fever and neutropenia. Infection 22: 160-164, 1994.
12. Reitamo S, Remitz A, Varga J, et al: Demonstration of interleukin 8 and autoantibodies to interleukin 8 in the serum of patients with systemic sclerosis and related disorders. Arch Dermatol 129:189-193, 1993.
13. Friedland JS, Remick DG, Shattock R, Griffin GE: Secretion of interleukin-8 following phagocytosis of Mycobacterium tuberculosis by human monocyte cell lines. Eur J Immunol 22:1373-1378, 1992.
14. Suzuki K, Fukutomi Y, Matsuoka M, et al: Differential production of interleukin 1 (IL-1), IL-6, tumor necrosis factor, and IL-1 receptor antagonist by human monocytes stimulated with Mycobacterium leprae and M. bovis BCG. Int J lepr 61:609-618, 1993.