Increased levels of p60 soluble tumor necrosis factor receptor in the sera of patients with Kawasaki disease

(分担研究：川崎病のサーベイランスに関する研究)

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To further understand the role of tumor necrosis factor (TNFα), we investigated whether p60 soluble TNF receptor (sTNF-R) shedding into the circulation increases during acute Kawasaki disease (KD). In addition, we studied the effect of pentoxifylline for KD patients on the levels of p60 sTNF-R in serum. Patients with KD had increased levels of p60 sTNF-R in serum samples obtained during acute stages. Moreover, KD patients with coronary artery lesion (CAL) had still higher levels of p60 sTNF-R than did those without CAL. We found a positive correlation between serum levels of p60 sTNF-R and levels of TNFα during acute KD prior to treatment. During the acute stage, KD patients treated with pentoxifylline underwent a greater decrease in their p60 sTNF-R levels than those without pentoxifylline.

Our findings suggest that p60 sTNF-R levels in serum form an important parameter for determining the severity of vascular damage during acute KD, and that pentoxifylline therapy for KD modulates the regulatory system of TNFα.

見出し語：Kawasaki disease　Coronary artery lesions　Soluble tumor necrosis factor receptor　Tumor necrosis factor α　Pentoxifylline treatment

Tumor necrosis factor α (TNFα) is an inducible cytokine produced primarily by monocytes/macrophages. Many biologic activities of TNFα appear to be involved in the pathogenesis of inflammation and vasculitis. TNFα could be easily measured in sera in pathological conditions but its true biological activity has been debated. It has been reported that human urine and serum, particularly in pathological conditions, contain proteins which can interfere with the functions of TNFα when tested in

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assays of cytotoxicity, using TNFα susceptible cell lines. Characterization of these inhibitors revealed two immunologically distinct TNFα binding proteins [1]. The same structures were found to be part of the extra-membranous fragments of two TNFα receptors with apparent molecular masses of 55,000-60,000 MW and 75,000-80,000 MW, respectively [2].

It has been reported that the levels of serum TNFα increased during the acute stage of Kawasaki disease (KD) and peripheral blood mononuclear cells from acute KD patients spontaneously secreted high levels of TNFα [3,4]. Recently, we have reported that KD patients had increased TNFα inhibitory activities in urine during the acute stage [5]. In addition, serum TNFα levels and TNFα inhibitory activities in urine are more evident in KD patients with coronary artery lesions (CAL) than in patients without CAL [3,5]. These reports suggest that TNFα plays an important role in the exacerbation of vascular damage in KD. In the present study, we investigated whether p60 soluble TNF receptor (sTNF-R) shedding into the circulation increases during acute KD. In addition, we compared p60 sTNF-R shedding in patients with acute KD with that in patients with measles, a representative acute febrile illness. Furthermore, we studied the effect of pentoxifylline for KD patients on the levels of p60 sTNF-R in serum since pentoxifylline has been used for treating some diseases, in which there is the increased TNFα production [6].

The patients met specific diagnostic criteria for KD. We included 24 boys and 22 girls 2 months to 4.6 years (mean, 1.8 years). After admission, the patients were treated with aspirin at a dosage of 30mg/kg/day, in 3 divided doses, until the thirtieth day after the onset of fever, when the dosage was reduced to 3-5mg/kg/day. All KD patients also received intravenous infusions of gamma globulin (IVGG; Venilon, Teijin Limited, Tokyo) in a 5% solution at a dosage of 200mg/kg/day for 5 consecutive days. Thirteen of the 46 KD patients were treated with pentoxifylline (Trental, Hoechst Japan, Tokyo) at a dosage of 20mg/kg/day, in 3 divided doses, until the thirtieth day after the onset of fever. The day of onset of fever was considered the first day of illness. Blood samples for examining levels of shed p60 sTNF-R in serum were taken on days 2-9 (mean±SD, 5.0±1.8) of illness prior to treatment (acute stage) and on days 20-80 (mean, 33.8±13.6), of illness (convalescent stage). Serum TNFα levels were also measured in 18 of the 46 patients during the acute stage prior to treatment. In 13 patients treated with aspirin and IVGG (group G), and in all patients treated with aspirin, IVGG and pentoxifylline (group P), serum p60 sTNF-R levels were measured on days 7-13 (mean, 9.80±1.8 days), after

IVGG administration. Two-dimensional echocardiography was used to detect coronary artery lesions (CAL); this was done twice a week after hospital admission. Coronary arteries with diameters of 4mm or greater were regarded as exhibiting CAL, according to the Research Committee on Kawasaki Disease (Ministry of Health and Welfare, Japan) diagnostic criteria for cardiovascular lesions in KD. Measles patients included 7 boys and 5 girls 9 months to 4.6 years (mean, 2.5 years). The day of onset of fever was considered the first day of illness. Blood samples were taken on days 3-6 (mean, 5.3±1.3) of illness (acute stage) and on days 12-113 (mean, 37.2±30.6) of illness (convalescent stage). Controls included 19 boys and 11 girls 5 months to 5.9 years (mean, 2.3 years). Control samples were tested in parallel with patient samples. The levels of p60 sTNF-R in serum were determined using a sTNF-R (60 kDa) ELISA kit (Bender MedSystems, Vienna, Austria). The principle of the method was an enzyme immunoassay. We previously developed a specific and sensitive sandwich enzyme immunoassay for human TNFα in serum [7]. Statistical analyses were performed using Student's t-test and the paired t-test.

During the 30 days after the onset of KD, 4 patients had CAL. Figure 1 shows p60 sTNF-R levels in serum samples from patients with acute-stage and convalescent-stage KD and measles, and in control subjects. These KD patients do not include pentoxiffylline-treated patients. The levels of p60 sTNF-R in serum were increased during the acute stage of KD preceding treatment (6.6±4.6 ng/ml), as compared with levels found in control subjects (1.5±0.5 ng/ml, $P<0.01$). These high levels diminished to normal range during the convalescent stage of KD (1.6±0.9 ng/ml). The levels of p60 sTNF-R in the 4 patients with CAL were higher than those in KD patients without CAL during the acute stage before treatment (13.8±5.2 versus 5.6±3.6 ng/ml; $P<0.01$). In addition, we found a positive correlation between serum levels of p60 sTNF-R and TNFα during the acute stage prior to treatment in 18 patients with KD ($r=0.51$, $P<0.05$), as shown in figure 2. There were no significant differences in p60 sTNF-R levels between the patients with acute-stage measles (1.8±0.5 ng/ml) and those with convalescent-stage measles (1.2±0.5 ng/ml) or control subjects.

We analyzed p60 sTNF-R levels in paired samples before and after treatment with IVGG during the acute stage in two therapy groups. Group G received aspirin and IVGG. Group P received the same course of aspirin and IVGG as group G, and in addition, pentoxifylline. These patients in two therapy groups had no CAL. Table 1 shows the rates of decrease for serum p60 sTNF-R during the acute stage of two therapy groups. The rates of decrease for serum p60 sTNF-R levels were calculated as follows:

$$\text{rate of decrease for sTNF-R (\%)} = \frac{\text{sTNF-R(B)-sTNF-R(A)}}{\text{sTNF-R(B)}} \times 100,$$

where sTNF-R is p60 sTNF-R level before (B) and after (A) IVGG treatment during the acute stage. Patients divided into the two treatment groups did not differ significantly with regard to age distribution, sex, duration of illness prior to entry into study, and p60 sTNF-R levels in serum prior to treatment. Group P underwent a greater decrease in their p60 sTNF-R levels than group G ($P<0.05$).

Previous studies show that sTNF-R are the natural homeostatic regulators of TNFα action, and that the degree of TNFα-driven inflammatory activity depends on the balance of TNFα and TNF inhibitor (sTNF-R) [8,9]. It is therefore likely that high amounts of p60 sTNF-R are produced in a condition where circulating concentrations of TNFα are increased during acute KD. We have reported that the increases in peripheral blood CD14+ monocyte/macrophage counts, serum TNFα levels and levels of soluble intercellular adhesion molecule 1 in serum are more evident in KD patients with CAL than in patients without CAL [3,7]. Our findings suggest that p60 sTNF-R levels in serum, together with these other immunologic markers, form an important parameter for determining the severity of vascular damage during acute KD.

IVGG therapy has been reported as being effective in reducing the incidence of CAL in KD [10]. Although the incidence of CAL in KD has reduced after the routine application of this treatment, still CAL developed in approximately 10% of patients with KD in Japan. We studied the effect of pentoxifylline for KD patients on the levels of p60 sTNF-R in serum since pentoxifylline blocks TNFα production [6]. During the acute stage, KD patients treated with pentoxifylline had lower levels of p60 sTNF-R in serum than did those without pentoxifylline. It is likely that the decreased levels of p60 sTNF-R in KD patients treated with pentoxifylline reflect the decreased TNFα production during the acute stage. It is our speculation that pentoxifylline therapy may be effective in KD by modulating the regulatory system of TNFα. In 1992, 22 KD patients received aspirin, IVGG and pentoxifylline in our hospital and branch hospitals, and these patients had no CAL. However, these observations do not warrant the use of pentoxifylline in acute KD; that would be premature. A well-designed study is underway.

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REFERENCES

1.Engelmann H, Novick D, Wallach D: Two tumor necrosis factor-binding proteins purified from human urine: Evidence for immunological cross-reactivity with cell surface tumor necrosis factor receptors. J. Biol. Chem. 265; 1531-1536, 1990.

2.Seckinger P, Zhang JH, Hauptmann B, Dayer JM: Characterization of a tumor necrosis factor α (TNF-α) inhibitor: Evidence of immunological cross-reactivity with the TNF receptor. Proc. Natl. Acad. Sci. USA 87; 5188-5192, 1990.

3.Furukawa S, Matsubara T, Jujoh K, Yone K, Sugawara T,Sasai K, Kato H, Yabuta K: Peripheral blood monocyte/macrophages and serum tumor necrosis factor in Kawasaki disease. Clin. Immunol. Immunopathol. 48; 247-251, 1988.

4.Lang GA, Silverman ED, Laxer RM, Lau AS: Spontaneous tumor necrosis factor production in Kawasaki disease. J. Pediatr. 115; 939-943, 1989.

5.Matsubara T, Furukawa S, Suzuki J, Yone K, Yabuta K: Tumor necrosis factor-α inhibitory activity in urine of Kawasaki disease. Clin. Immunol. Immunopathol. 63; 285-288, 1992.

6.Hakin J, Mandell GL, Novick Jr. WJ: Pentoxifylline and analogues: Effects on leukocyte function. Basel, Karger, 1990.

7.Furukawa S, Imai K, Matsubara T, Yone K, Yachi A, Okumura K, Yabuta K: Increased levels of circulating intercellular adhesion molecule 1 in Kawasaki disease. Arthritis Rheum. 35; 672-677, 1992.

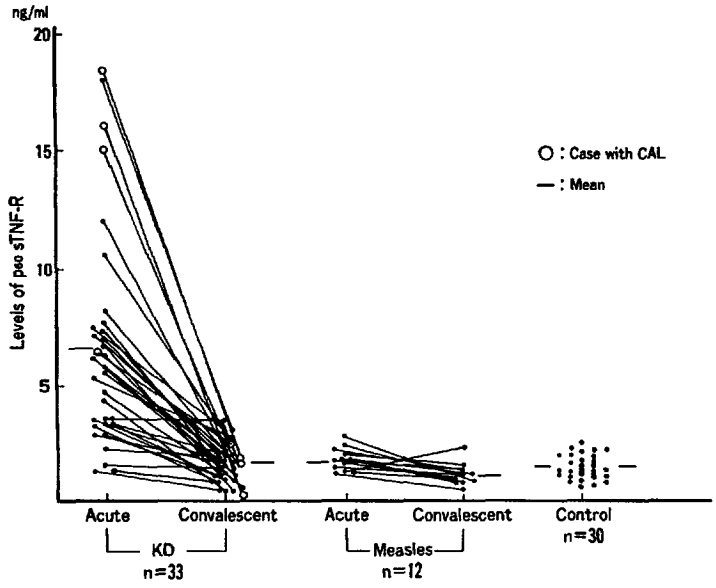
8.Girardin E, Roux-Lombard P, Grau GE, Suter P, Gallati H, The J5 study group, Dayer JM: Imbalance between tumour necrosis factor-alpha and soluble TNF receptor concentrations in severe meningococcaemia. Immunology 76; 20-23, 1992.

9.Cope AP, Aderka D, Doherty M, Engelmann H, Gibbons,D, Jones AC, Brennan FM, Maini RN, Wallach D, Feldmann M: Increased levels of soluble tumor necrosis factor receptors in the sera and synovial fluid of patients with rheumatic diseases. Arthritis Rheum. 35; 1160-1169, 1992.

10.Furusho K, Kamiya T, Nakano H, Kiyosawa N, Shinomiya K, Hayashida T, Tamura T, Hirose O, Manabe Y, Yokoyama T, Kawarano M, Baba K, Baba K, Mori C: High-dose intravenous gammaglobulin for Kawasaki disease. Lancet II; 1055-1058, 1984.

Fig.1 Levels of p60 sTNF-R in serum samples from patients with acute-stage and convalescent-stage KD and measles, and in control subjects.

Fig.2 Correlation between serum levels of p60 sTNF-R and TNFα during the acute stage prior to treatment in patients with KD.



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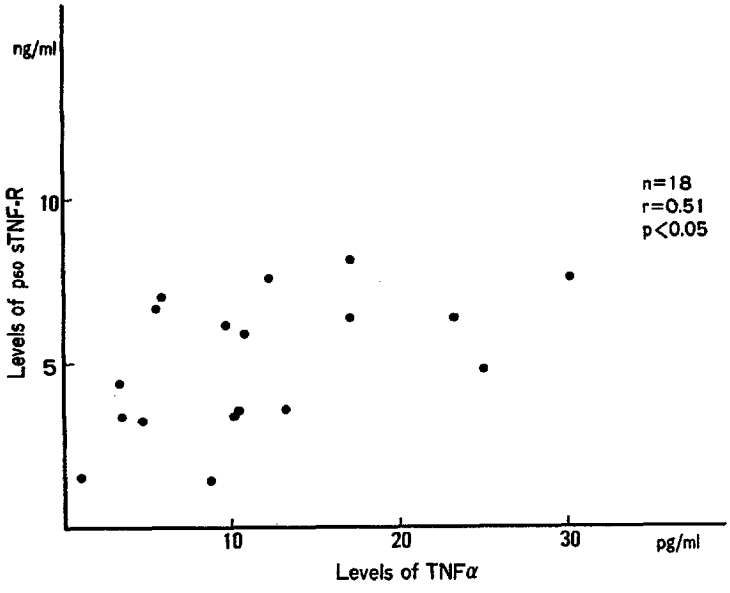

Fig.2 Correlation between serum levels of p60 sTNF-R and TNFα during the acute stage prior to treatment in patients with KD.

Table 1. Rate of decrease for serum p60 sTNF-R levels treated with and without pentoxifylline during acute KD

	Prior to treatment		After treatment		Rate of decrease for p60 sTNF-R, %
	Days from onset	p60 sTNF-R levels, ng/ml	Days from onset	p60 sTNF-R levels, ng/ml	
Group P n=13	4.3±1.2	5.3±2.1	9.6±2.0	2.3±1.4	57.1±20.9*
Group G n=13	5±0.8	4.8±2.3	10±1.7	3.1±1.9	31.7±35.4

Group P received aspirin, IVGG and pentoxifylline.
Group G received aspirin and IVGG.
Values are expressed as mean±SD.
* Significant at $p<0.05$ vs. group G.

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