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BRIEF COMMUNICATION

Age-Related Changes of Serum Soluble Interleukin 9 Receptor (sIL-9Rα) in Healthy Subjects

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Abstract Most cytokine receptors including interleukin (IL)-9 have soluble counterparts in body fluids. We planned to investigate the pathophysiological significance of the serum soluble IL-9 receptor (sIL-9R) level. We determined the serum sIL-9Rα chain (sIL-9Rα) levels in 96 healthy Japanese individuals to establish a control value by means of specific human sIL-9Rα ELISA, followed by a preliminary application in a patient with diarrhea positive hemolytic uremic syndrome. Age was negatively correlated with the sIL-9Rα level (Spearman $r = -0.241$, $n = 96$, $p = 0.0180$). The serum sIL-9Rα level showed a progressive decline to the normal adult level by the age of 30. The serum sIL-9Rα level of the patient with HUS was markedly higher than those of the age-matched control from the onset of the disease. Because of the remarkable age-dependent variability of sIL-9Rα in healthy subjects, disease-related changes, as well as therapy-dependent alterations, should be considered with caution. Thus, it is recommended that when the serum sIL-9Rα levels of patients are evaluated, the values should be compared with those of age-matched controls. The established control value will be used to discriminate between normal and the pathological conditions in our future studies.

Keywords Soluble interleukin 9 receptor α chain (sIL-9Rα) · Hemolytic uremic syndrome (HUS) · ELISA

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Introduction

The interleukin (IL)-9 has largely been regarded as a Th2 cytokine that makes multifocal contributions to allergic and inflammatory disease [1]. Serum IL-9 levels are related to symptom severity in patients with allergic diseases [2, 3]. Recent data suggest that under certain conditions relevant to the development and maintenance of allergic inflammation, a distinct population of IL-9-producing 'Th9' helper T cells can exist [4].

IL-9 exerts its action by binding to the IL-9 receptor (IL-9R), a heterodimer consisting of the IL-9Rα and the common γ chain [5]. IL-9 and the IL-9Rα can affect differentiation of human T cells, eosinophils, and mast cells [6].

Most cytokine receptors including IL-9 have soluble counterparts in body fluids, that are formed either by proteolytic cleavage of the membrane-anchored receptor (IL-2R, etc.) or by alternative splicing of mRNA (IL-4R, etc.) [7]. The soluble receptors retain their ligand-binding capacity, allowing them to serve as antagonists to the membrane receptors and as carrier proteins [7–9]. Thus, soluble cytokine receptors might modulate immune reactions.

Although the pathological significance of most soluble cytokine receptors is still unclear, the balance between soluble Th1 type cytokine receptor (IL-2 receptor and IFN-γ receptor) and soluble Th2 type cytokine receptor (IL-4 receptor) in the serum showed considerable variation

according to disease states in our previous study [10–12]. We suggested that the simultaneous evaluation of multiple kinds of soluble cytokine receptors may provide useful information on the disease state. There is no information on the soluble IL-9 receptor in disease states.

We planned to investigate the pathophysiological significance of soluble Th9 type cytokine receptor (sIL-9Rα) in the serum. Diagnostic application of the measurement of the serum sIL-9Rα level depends critically on the samples used as control. The level of some soluble cytokine receptors in body fluids of healthy individuals changes with age [12–14]. At present, there is no information regarding the level of serum sIL-9Rα in healthy subjects.

In the present study, to investigate the correlation between the level of serum sIL-9Rα and the disease state, the serum sIL-9Rα level in healthy Japanese subjects was determined by enzyme-linked immunosorbent assay (ELISA), followed by a preliminary application in a patient with diarrhea positive (D+) hemolytic uremic syndrome (HUS).

Materials and Methods

Healthy Japanese Subjects

Serum samples were obtained from 96 healthy Japanese subjects that were at a mass screening examination. All subjects had been free of infectious diseases and were not undergoing any medical treatment. The age distribution of the subjects was 1–67 years old (median: 22.0 years old). Serum samples were immediately frozen and stored at −70 °C until use. The purpose, procedures, and benefits of our project were explained to the participants or their parents, and we obtained their consent for our study.

Patient

The patient, a boy who was 8 years of age in August 2002, presented with the typical clinical signs and symptoms of D+ HUS, such as diarrhea, vomiting, and hematochezia. At the time of admission, his blood urea nitrogen was 23.4 mg/dl and serum creatinine 1.01 mg/dl. His hematocrit level was 34.4 %, hemoglobin 12.1 g/dl, white blood count 14,500/μl, and platelet count 40,000/μl. Extensive microbiologic investigation revealed evidence of a Shiga toxin-producing *Escherichia coli* serotype O157 infection. Hemolytic uremic syndrome was diagnosed according to the diagnostic criteria proposed by the Japanese Society for Pediatric Nephrology [15]: the presence of hemolytic anemia (hemoglobin (Hb) < 10 g/dl), acute renal dysfunction (oliguria, anuria, or an increase in serum creatinine levels corrected for age), and thrombocytopenia (platelet count (PLT) $< 10 \times 10^4$/μl).

Measurement of Human sIL-9Rα by ELISA

The concentration of sIL-9Rα was measured by ELISA as follows. Microtiter plates (Nunc Immunoplate Maxisorp, Thermo Fisher Scientific, Inc., Roskilde, Denmark) were coated with 50 μl of 1 μg/ml human IL-9 Rα subunit affinity purified polyclonal antibody, goat IgG (R&D systems Inc., MN, USA) in carbonate buffer (0.02 mol/l, pH 9.5) overnight at 4 °C. Thereafter, the wells were blocked with 200 μl of phosphate buffered saline (PBS) containing 1 % BSA and 5 % sucrose for 90 min at room temperature. The plates were then washed three times with PBS containing 0.05 % Tween 20 (PBS-T). Recombinant (r) human IL-9 Rα subunit (R&D systems Inc. MN, USA), which was used to construct a standard curve (0–25 ng/ml), was serially diluted with 0.1 % BSA/PBS-T. 50 μl of each serum sample were incubated in the wells for 2 h at room temperature. After the incubation, the plates were washed and biotinylated human IL-9 Rα subunit affinity purified polyclonal antibody, goat IgG (50 μl, 500 ng/ml, R&D systems Inc., MN, USA) was added and incubated for 2 h at room temperature. The plates were washed, and 50 μl of horseradish peroxidase (HRP)-conjugated anti-biotin goat polyclonal antibody (Vector Laboratories, Inc., CA, USA) diluted 1/2,000 in 0.5 % BSA/PBS-T was added. Incubation was carried out for 1 h at room temperature. Finally, the substrate (SureBlue TMB Microwell Peroxidase Substrate, KPL, MD, USA) was added and allowed to incubate at room temperature for 5 min. The reaction was stopped with 2N H_2SO_4, and the absorbance at 450 nm was measured in a microplate reader Model 680 (BioRad Laboratories, CA, USA). Data reduction and the calculation of sample sIL-9Rα values were carried out with a data analysis software package (Microplate Manager, Bio-Rad). The detection limit of the assay was 0.020 ng/ml. The intra-assay and inter-assay coefficients of variation were 4.0 and 3.3 %, respectively. The mean recovery rate of the serum samples ranged from 85.4 to 111.0 % (Table 1). This ELISA shows no cross-reactivity with any of the cytokine receptors tested (sIL-2Rα, sIL-4Rα, sIL-6α, and sIL-13RI).

Table 1 Recovery test

Standard spiked value (ng)	*n*	Recovery (%)	Range (%)
12.5	7	96.4	89.9–103.6
32.5	7	98.8	85.4–111.0

A spike recovery sample determines the effect of the constituents of serum sample on the results

Results

Serum sIL-9Rα Levels of A Healthy Japanese Population

The sIL-9Rα levels of serum samples from 96 healthy Japanese subjects were determined. Scatter plots of the serum sIL-9Rα levels are shown in Fig. 1. The Spearman's rank correlation coefficient was used to discover the strength of a link between age and serum sIL-9Rα level. There was a statistical significant correlation between age and sIL-9Rα level (Spearman $r = -0.241$, $n = 96$, $p = 0.0180$).

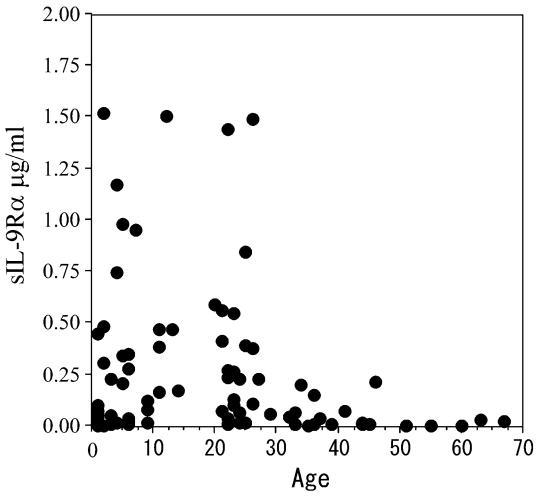

Fig. 1 Age-related profile of serum sIL-9Rα levels. The serum sIL-9Rα levels of 43 healthy children and 53 healthy adults are plotted. Spearman's correlation test was applied to evaluate any possible correlation between serum sIL-9Rα level and age. There was a statistical significant correlation between age and sIL-9Rα level (Spearman $r = -0.241$, $n = 96$, $p = 0.0180$)

Finally, there were no significant differences between the serum sIL-9Rα levels of males and females (median values, males: 0.041 μg/ml, $n = 61$, females: 0.167 μg/ml, $n = 35$, $p = 0.1943$).

Preliminary Application to Clinical Cases of HUS

To demonstrate the usefulness of the control value, we measured the sIL-9Rα value of one pediatric patient with D+ HUS, as a preliminary study. The sIL-9Rα serum level and laboratory data of the 8 year-boy with D+ HUS are summarized in Table 1. The hemoglobin (Hb), platelet count, and serum creatinine (sCre) levels are markers for the diagnosis of HUS [15], and a higher white blood cell count and lower serum sodium concentration have been reported as risk factors for progression to severe HUS [15, 16]. The patient satisfied the diagnostic criteria for HUS. The serum sIL-9Rα level of the patient with HUS was markedly higher than those of healthy children from the onset of D+ HUS (Table 2).

Discussion

The diagnostic application of the measurement of serum sIL-9Rα level depends critically on the control value used. However, no information concerning the serum sIL-9Rα level of healthy population is available. The scatter plots of sIL-9Rα in the serum are shown in Fig. 1. Age was negatively correlated with the sIL-9Rα level (Spearman $r = -0.241$, $n = 96$, $p = 0.0180$). The serum sIL-9Rα level showed a progressive decline to the normal adult level by the age of 30. Because of the age-dependent variability in healthy subjects, disease-related changes, as well as therapy-dependent alterations, should be considered with

Table 2 Serum sIL-9Rα levels and laboratory data for a patient with D + HUS

Days after onset	WBC/μl	Platelet ($x10^4$/μl)	Hb (g/dl)	Na (mEq/l)	sCre (mg/dl)	sIL-9Rα (μg/ml)
0	14,500	4.0	12.1	135	1.01	2.214
5	10,900	1.6	5.3	137	2.46	1.204
12	4,100	23.4	6.5	138	0.71	2.057
Healthy control*						
Median						0.105
Lower quartile (Q_1)						0.020
Upper quartile (Q_3)						0.357
Clinical lab reference values**	3,500 ~ 8,000	12 ~ 40	#	135 ~ 147	0.3 ~ 1.0	

The normal level of hemoglobin is 13.7 ~ 16.9 for males. *WBC* white blood cell count, *Hb* hemoglobin, *Na* serum sodium concentration, *sCre* serum creatinine

* Control consists of 14 age-matched healthy subjects (median age; 8 years old, range 6–11)

** Reference values of healthy children from our institution. Each reference value is age-dependent

caution. Thus, it is recommended that when the serum sIL-9Rα levels of patients are evaluated, the values should be compared with those of age-matched controls.

Many researchers, including our group, have reported that the serum levels of soluble receptors of some cytokines decreased with age [12–14]. The reduction in soluble cytokine receptor levels with aging suggests enhanced proliferative activity of the immune system during early childhood, especially in connection with the thymus maturation of T cells [13].

Some kinds of circulating cytokines and the soluble receptors drastically increased with the onset of HUS, including TNF-α, FGF, IL-1β, IL-6, IL-8, IL-10, sTNFR1, sIL-2R, and sIL-4R [12, 17–23]. Thus, sIL-9R level of the patients with HUS should be shifted. To demonstrate the usefulness of the control value, we determined the serum sIL-9Rα level in an 8-year boy with D+ HUS as a preliminary application to demonstrate the propriety of our control value.

D+ HUS occurs after a prodrome of hemorrhagic colitis caused by a Shiga toxin-producing *E. coli* infection [24, 25]. The disease causes the destruction of red blood cells, damage to blood vessel walls, and in severe cases, kidney failure. HUS is a rare condition that mostly affects children under the age of 10; however, severe cases of HUS are life-threatening. If the child survives the initial stages of the disease, the long term prognosis is good. A useful test for the diagnosis of severe HUS is needed for pediatric patients.

The serum sIL-9Rα level as well as platelet count, Hb, and sCre of the patient with HUS was markedly higher than those of healthy control subjects from the onset of D+ HUS (Table 2). The shift of serum sIL-9R level with some diseases may be detectable by the assay with our reliable control values described in the present study. As far as we know, this is the first report on serum sIL-9Rα change with diseases.

In summary, this study determined the serum sIL-9Rα levels in healthy Japanese individuals by means of ELISA specific for human sIL-9Rα to establish a control value. Age was negatively correlated with the sIL-9Rα level. Thus, it is recommended that when the serum sIL-9Rα levels of patients are evaluated, the values should be compared with those of age-matched controls. The control value will be used to discriminate between normal and the pathological conditions in our future studies.

Conflict of interest The authors declared no financial or commercial conflict of interest.

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