Age-Dependent Decreases in Serum Soluble Interleukin-1 Receptor Type I (sIL-1RI) in Healthy Individuals: A Population Study of Serum sIL-1RI Levels in Japanese Subjects

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The levels of several soluble cytokine receptors in body fluids of healthy individuals change with age. Clinical application of the measurement of the serum soluble interleukin-1 receptor type I (sIL-1RI) level depends critically on the samples used as the controls. At present, there is no information regarding the levels of serum sIL-1RI in healthy subjects. The purpose of this study is to reveal the age-related changes that occur in the serum sIL-1RI

levels of healthy individuals. We determined the serum sIL-1RI levels of healthy Japanese children using ELISA. The serum sIL-1RI level of children (0–14 years) was significantly higher than that of adults (more than 15 years) ($P = 0.0138$, $n = 90$). Thus, it is recommended that when the serum sIL-1RI level of patients is evaluated, it should be compared against age-matched controls. *J. Clin. Lab. Anal.* 23:175–178, 2009. © 2009 Wiley-Liss, Inc.

Key words: soluble type I IL-1 receptor (sIL-1RI); human; ELISA

INTRODUCTION

Interleukin (IL)-1 and its receptors control immunological and inflammatory processes (1). Three members of the IL-1 family are known: IL-1 α , IL-1 β , and an IL-1 receptor antagonist (IL-1Ra). These ligands bind to two distinct types of receptors: the type I and type II IL-1 receptors (IL-1RI/IL-1RII). IL-1RI mediates the biological effects of IL-1, whereas IL-1RII does not transduce signals and apparently serves as a decoy receptor for IL-1 (2). Soluble forms of both types of IL-1R are generated by proteolytic cleavage of the membrane-anchored receptors (3). Naturally occurring soluble forms of IL-1 receptor type I (sIL-1RI) and type II (sIL-1RII) exist physiologically in body fluids (4,5). They are produced by mononuclear cells (6) and retain their ligand-binding capacity, allowing them to serve as natural buffers that bind to IL-1 α , IL-1 β , and IL-1Ra (7). Therefore, the presence of IL-1 soluble receptors modulates IL-1 activity (8–10).

Reportedly, the plasma sIL-1RII level increases during sepsis (11) and acute meningococcal infection (12).

Furthermore, higher levels of sIL-1RII have been detected in the synovial fluid of patients with joint inflammation (5). On the other hand, little information is available on the pathological changes that occur in sIL-1RI levels in body fluids. Therefore, the clinical relevance of sIL-1RI remains unclear. We planned to clarify this.

Clinical application of the measurement of the serum sIL-1RI level depends critically on the samples used as the controls. The levels of several soluble cytokine receptors in the body fluids of healthy individuals change with age (13–15). At present, there is no information regarding the levels of serum sIL-1RI in healthy subjects. The purpose of this study is to reveal the age-related changes that occur in serum sIL-1RI

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levels. We determined the serum sIL-1RI level of healthy Japanese subjects using an enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Subjects

Serum samples were obtained from nonatopic healthy children who were undergoing a medical checkup for corrective surgery for hernia or circumcision repairs, which were considered to be unrelated to diseases with altered immunological parameters. Some samples were collected at a group physical examination in an elementary school. Specimens from healthy Japanese adults were collected during a group physical examination. The subjects presenting inflammatory signs of a physical examination were excluded. Furthermore, health assessments also involve gathering information about a person's medical history and lifestyle and doing some laboratory tests.

The exclusion criteria were the use of any medication within the previous 3 months. By the time of the blood sampling, none of the study subjects reported or showed inflammatory or other concurrent diseases.

Serum samples from the subjects were frozen immediately and stored at -70°C .

The purpose, procedure, and benefits of this project were explained to the participants or their parents, and their informed consent was obtained.

Measurement of Human sIL-1RI by ELISA

The concentration of sIL-1RI was measured by ELISA as follows. Microtiter plates (Nunc Immoplate Maxisorp, Thermo Fisher Scientific, Inc., Roskilde, Denmark) were coated with $50\mu\text{l}$ of $2\mu\text{g/ml}$ monoclonal mouse anti-human sIL-1RI antibody (Clone 35730, R&D Systems Inc., Minneapolis, MN) in carbonate buffer (0.02mol/l , pH 9.5) overnight at 4°C . Thereafter, the wells were blocked with $300\mu\text{l}$ 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 sIL-1RI (R&D systems Inc.), which was used to construct a standard curve ($0\text{--}2,500\text{pg/ml}$), was serially diluted with 0.5% BSA/PBS-T. Fifty microliter of each serum sample was incubated in the wells for 2 hr at room temperature. After the incubation, the plates were washed, and biotinylated goat anti-human sIL-1RI ($50\mu\text{l}$, 100ng/ml , R&D systems Inc.) was added and incubated for 2 hr at room temperature. The plates were washed, and $50\mu\text{l}$ of horseradish peroxidase-conjugated antibiotin goat polyclonal antibody

(Vector Laboratories, Inc., Burlingame, CA) diluted 1/2,000 in 0.5% BSA/PBS-T was added. Incubation was carried out for 1 hr at room temperature. Finally, the substrate (SureBlue TMB Microwell Peroxidase Substrate, KPL, MD) was added and allowed to incubate at room temperature for $3\text{min} \pm 30\text{sec}$. The reaction was stopped with $2\text{N H}_2\text{SO}_4$, and the absorbance was read at 450nm in a Microplate reader Model 680 (BioRad Laboratories, Hercules, CA). Data reduction and calculation of sample sIL-1RI values were carried out with an analysis software package (Microplate Manager, BioRad). The detection limit of the assay was 20pg/ml . The intra-assay and inter-assay coefficients of variation were 4.0 and 3.3%, respectively. The mean recovery rate from the serum samples ranged from 89 to 97%. In this assay, no interference was observed with recombinant human IL-1 α or IL-1 β .

Statistical Analysis

The results are presented as medians together with the lower (Q_1) and upper (Q_3) quartile values. Comparisons between two groups were performed using the Mann-Whitney U rank sum test. The Spearman test was used to evaluate the correlation between two quantitative variables. A value of $P < 0.05$ was considered statistically significant. The statistical analysis was performed with the aid of a computerized statistical package, Stat View-5.0 (SAS Institute Inc., Cary, NC).

RESULTS

Serum Levels of sIL-1RI in a Healthy Japanese Population

The upper age limit of pediatrics ranges from age 14 to 18, depending on the country. In Japan, the upper limit is usually 14 years. Therefore, we divided the subjects into two groups (under 14 years and more than 15 years). The levels of sIL-1RI in the serum samples from healthy children ($n = 42$) and those of healthy adults ($n = 48$) are summarized in Table 1. The sIL-1RI levels in the sera of the children were significantly higher than those of the adults ($P = 0.0138$).

Scatter plots of the sIL-1RI levels in the serum are shown in Figure 1. The serum sIL-1RI levels of 42 healthy children and 48 healthy adults are plotted. Spearman's correlation test was applied to evaluate the possible correlation between serum sIL-1RI level and age. Earlier age was correlated with a higher serum sIL-1RI level (Spearman $r = -0.551$, $n = 90$, $P < 0.001$).

Finally, there was no significant difference between females and males (data not shown).

TABLE 1. A Summary of the Serum sIL-1RI Levels in Healthy Subjects

	Children	Adults
Number of subjects	42	48
Male/Female	25/17	33/15
Age (years)		
Median	4.0	29.0
Lower quartile (Q_1)	2.0	23.0
Upper quartile (Q_3)	6.8	42.5
sIL-1RI (pg/ml)		
Median	68.46*	42.34
Lower quartile (Q_1)	30.52	9.74
Upper quartile (Q_3)	497.24	107.66

Age and sIL-1RI level are shown as median values and the lower (Q_1) and upper (Q_3) quartiles. The serum sIL-1RI levels of children (age; 1–14 years) were significantly higher than those of adults (age; 22–67 years). The Mann-Whitney U rank sum test was used to investigate significant differences. * $P = 0.0138$.

Fig. 1. The age-dependent decrease in the levels of serum sIL-1RI. The serum sIL-1RI levels of 42 healthy children and 48 healthy adults are plotted. Spearman's correlation test was applied to evaluate the possible correlation between serum sIL-1RI level and age. Earlier age was correlated with a higher serum sIL-1RI level (Spearman $r = -0.551$, $n = 90$, $P < 0.001$).

DISCUSSION

The physiological significance of sIL-1RI levels in body fluids remains poorly understood. This study is the first step toward clarifying the clinical relevance of serum sIL-1RI levels. Many researchers have reported that serum soluble cytokine receptor levels change with age (13–17). However, there is no information as to the serum sIL-1RI levels of healthy subjects. The purpose of this study is to reveal the age-related changes that occur in serum sIL-1RI levels in healthy Japanese subjects. The serum sIL-1RI levels of children were significantly higher than those of adults (Table 1). Furthermore, serum sIL-1RI levels decreased with age (Fig. 1). Thus, the serum sIL-1RI level of a pediatric patient cannot be compared with that of a normal adult control. Although it has been reported that the soluble form of IL-1RI

exists in body fluids (5,18), no trend in age-related serum sIL-1RI variation has yet been reported. These control data will be helpful as reference values for the evaluation of various clinical samples. In this study, we could not obtain the serum samples for ages between 15 and 21. The lack of data for the ages could not affect the age-related profile of serum sIL-1RI significantly. However, further investigation of subjects for ages between 15 and 21 are needed to determine the age-related changes in more detail.

In summary, this study determined the age-related profile of serum sIL-1RI levels in healthy Japanese individuals by means of an ELISA specific for human sIL-1RI. It was found that the serum sIL-1RI levels of children (0–14 years) are significantly higher than those of adults (more than 15 years). Thus, it is recommended that when the serum sIL-1RI levels of patients are evaluated, their values should be compared with those of age-matched controls. These data on healthy subjects are important for future clinical studies on the members of the IL-1 family.

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