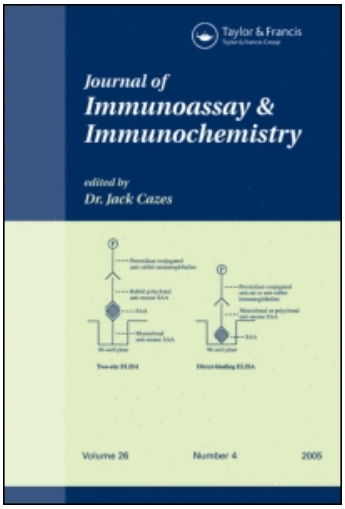

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[a] Laboratory of Immunology and Microbiology, Faculty of Pharmacy, Chiba Institute of Science, Choshi, Chiba, Japan [b] Department of Pediatrics, Nagoya Daini Red Cross Hospital, Nagoya, Aichi, Japan [c] Department of Pediatrics, Seirei Hamamatsu General Hospital, Hamamatsu, Shizuoka, Japan

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Age-Dependent Decrease in Serum Soluble Interferon-Gamma Receptor (sIFN-γR) in Healthy Japanese Individuals; Population Study of Serum sIFN-γR Level in Japanese

Yoshihiro Okamoto,[1] Kyoko Seta,[1] Izumi Nakajo,[1] Yoshimitsu Gotoh,[2] Takuhito Nagai,[2] Naoya Fujita,[3] Takashi Fukui,[1] and Toshiyuki Masuzawa[1]

[1]Laboratory of Immunology and Microbiology, Faculty of Pharmacy, Chiba Institute of Science, Choshi, Chiba, Japan
[2]Department of Pediatrics, Nagoya Daini Red Cross Hospital, Nagoya, Aichi, Japan
[3]Department of Pediatrics, Seirei Hamamatsu General Hospital, Hamamatsu, Shizuoka, Japan

Abstract: We planned to investigate the clinical significance of serum soluble interferon-gamma receptor (sIFN-γR) level in pediatric patients. The diagnostic application of the measurement of serum sIFN-γR level depends critically on the control value. However, there is no information of the control value of serum sIFN-γR for children. In the present study, we determined the serum sIFN-γR level of healthy Japanese children using an ELISA. The serum sIFN-γR level of children (0–14 years old) was significantly higher than that of adults (over 15 years old) ($p < 0.01$, $n = 104$). Thus, it is recommended that, when the serum sIFN-γR level of patients is evaluated, it should be compared against age-matched controls. We also preliminarily applied this assay as a diagnostic parameter for the patients with diarrhea positive (D+) hemolytic uremic syndrome (HUS).

Keywords: ELISA; Hemolytic uremic syndrome (HUS); Human; Soluble interferon-γ receptor (sIFN-γR)

Correspondence: Yoshihiro Okamoto, Faculty of Pharmacy, Chiba Institute of Science, 3 Shiomi-cho, Choshi, Chiba 288-0025, Japan. E-mail: yokamoto@cis.ac.jp

INTRODUCTION

Interferon-γ (IFN-γ) is a Th1 type cytokine produced by activated T-cells and natural killer (NK) cells, and is required for the development of cytotoxic T lymphocytes.[1–3] IFN-γ induces MHC class I and class II antigens on various cells,[4–6] thereby increasing the antigen-presenting capacity of cells and activating cellular immune responses.[4–6]

Most cytokine receptors, including IFN-γ, have soluble counterparts in body fluids that are formed either by proteolytic cleavage of the membrane-anchored receptor (IL-2R, IL-1R, TNF-R) or by alternative splicing of mRNA (IL-4R, IL-5R, IL-7R, GCSF-R, GM-CSF-R).[7]

The soluble receptors retain their ligand-binding capacity, allowing them to serve as antagonists to the membrane receptors and as carrier proteins.[7]

The pathological significance of most soluble cytokine receptors is still unclear. There is little information, especially on the soluble IFN-γ receptor in disease states. The IFN-γ receptor belongs to the type II cytokine receptor family.[8,9] The functional IFN-γ receptor consists of the heterodimers IFN-γR1 and -R2. The soluble version of the extracellular domain of IFN-γR1 (sIFN-γR) has been found in body fluids.[10] Toren et al. reported that serum sIFN-γR levels increased during engraftment and GVHD.[11] Elevated plasma levels of sIFN-γR have been detected in patients with rheumatoid arthritis (RA).[12]

We planned to further investigate the clinical significance of the serum sIFN-γR level in pediatric patients. Diagnostic application of the measurement of the serum sIFN-γR level depends critically on the samples used as control. The level of soluble cytokine receptors in body fluids of healthy individuals changes with age.[13–15] At present, there is no information regarding the level of serum sIFN-γR in healthy children.

In the present study, to investigate the correlation between the level of serum sIFN-γR and a disease state in children with diarrhea positive (D+) hemolytic uremic syndrome (HUS), the serum sIFN-γR levels of Japanese healthy children were determined by enzyme-linked immunosorbent assay (ELISA) as a reference value, followed by preliminary application of serum sIFN-γR in the patients with HUS.

EXPERIMENTAL

Subjects

Ten Japanese children with D + HUS (7 boys and 2 girls, median age; 5 years old), 54 healthy Japanese children matched for age and sex (34 boys and 20 girls, median age; 5.0 years old), and 50 healthy Japanese

adults (35 males and 15 females, median age; 34.5 years old) were included in the study.

Hemolytic uremic syndrome was defined according to the diagnostic criteria proposed by the Japanese Society for Pediatric Nephrology:[16] 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 $< 10 \times 10^4/\mu L$).

Serum samples were obtained from nonatopic healthy children undergoing a medical check for corrective surgery for hernia or circumcision repairs that were considered to be unrelated to diseases with altered sIFN-γR. Some samples were collected at a group medical examination in an elementary school. All children had been free of infectious diseases and were not undergoing any medical treatments. Specimens from healthy Japanese adults were collected during a group medical examinations.

Serum samples from the subjects were immediately frozen and stored at $-70^{\circ}C$.

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

Measurement of Human sIFN-γR1 with ELISA

The concentration of sIFN-γR1 was measured by ELISA as follows. Microtiter plates (Immunoplate Maxisorp, Nunc, Roskilde, Denmark) were coated with 50 μL of 2 μg/mL of monoclonal mouse anti-human sIFN-γR1 antibody (Clone GIR208, 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 300 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 sIFN-γR1 (R&D Systems, Inc. MN, USA), which was used to construct a standard curve (0–60 ng/mL), was serially diluted with 0.5% BSA/PBS-T. Fifty μL of each serum sample was incubated in the wells for 2 hours at room temperature. After the incubation, the plates were washed and biotinylated goat anti-human sIFN-γR1 (50 μL, 100 ng/mL, R&D Systems, Inc., MN, USA) was added and incubated for 2 hours 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 hour at room temperature. The plates were washed and 100 μL of 0.05 mol/L citrate phosphate buffer, pH 5.8

containing 0.4 μg/mL *o*-phenylendiamine (DAKO Japan, Kyoto, Japan) and 0.0013% hydrogen peroxide, was added to each well. After 15 min, the enzyme-substrate reaction was terminated by the addition of 100 μL 0.5 mol/L sulfuric acid. The absorbance at 490 nm was measured in a Microplate reader Model 680 (BioRad Laboratories, CA, USA). Data reduction and calculation of sample sIFN-γR values were carried out with an analysis software package (Microplate Manager, BioRad). The detection limit of the assay was 0.08 ng/mL. The intra-assay and inter-assay coefficients of variation were 4.0% and 3.3%, respectively. The mean recovery rate from serum samples ranged from 89 to 97%.

RESULTS

Serum Levels of sIFN-γR in a Healthy Japanese Population

The levels of sIFN-γR in the serum samples from healthy children ($n = 54$) and those of healthy adults ($n = 50$) are summarized in Table 1. sIFN-γR levels in the sera of children were significantly higher than those of adults ($p < 0.01$).

The scatter plots of sIFN-γR in the serum are shown in Figure 1. Spearman correlation rank coefficients (two-tailed) were used to evaluate the relationship between age and the sIFN-γR level. Age was negatively correlated with the sIFN-γR level (Spearman $r = -0.586$, $n = 104$, $p < 0.001$). The serum sIFN-γR level showed a progressive decline to the normal adult level by the age of 15. Finally, there was no significant difference between females and males (data not shown).

Table 1. Summary of serum sIFN-γ receptor levels in healthy subjects

	Children	Adults
Number of subjects	54	50
Male/Female	34/20	35/15
Age [years old]		
median	5.0	34.5
IQR	6.0	20.0
sIFNγR [ng/ml]		
median	4.39*	1.36
IQR	5.53	1.70

Age and sIFN-γR level are shown as median value and inter-quartile range (IQR). Serum sIFN-γR levels of children (age; 1–14 years old) were significantly higher than those of adults (age; 22–67 years old). Mann-Whitney U rank sum test was used to investigate significant differences. *$p < 0.01$.

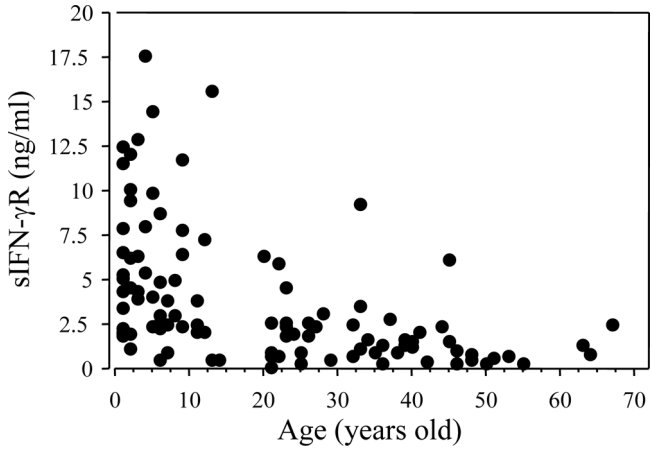

Figure 1. Age-dependent decrease in the level of serum sIFN-γR. Serum sIFN-γR levels of 54 healthy children and 50 healthy adults were plotted. Spearman's correlation test was applied to evaluate the possible correlation between serum sIFN-γR level and age. Age was negatively correlated with the sIFN-γR level (Spearman $r = -0.586$, $n = 104$, $p < 0.001$).

Preliminary Application to Clinical Cases of HUS

The serum levels of sIFN-γR and laboratory data for patients with HUS and healthy children are summarized in Table 2. The hemoglobin (Hb), platelet count, and serum creatinine (sCre) level are markers for the diagnosis of HUS,[16] and a higher white blood cell count and lower serum sodium concentration have been reported as risk factors for progression to severe HUS.[16,17] All patients satisfied the diagnostic criteria for HUS.

On the day of diagnosis, the serum sIFN-γR levels of the HUS group were significantly lower than those of the healthy children ($p < 0.01$). To facilitate a review of the results, a direct comparison between the HUS and healthy children is presented in Figure 2.

DISCUSSION

Many researchers, including our group, have reported that serum soluble cytokine receptor levels change with age.[13–15] However, there is no information as to the serum sIFN-γR levels of healthy subjects. First, to determine reference values for serum sIFN-γR, we carried out a population study on healthy Japanese. Serum sIFN-γR decreased with age (Figure 1). Thus, the serum sIFN-γR level of pediatric patients cannot

Table 2. Summary of serum soluble IFN-γ receptor levels and laboratory data for individual patients

Patients	Age years old	the day of diagnosis						
		Sex f/m	WBC/μL	platelet × 10^4/ μL	Hb g/dL	Na mEq/l	sCre mg/dL	sIFNγR ng/mL
HUS								
1	5	m	9400	2.2	7.5	131	1.50	1.91
2	3	f	11300	3.9	7.3	131	0.92	0.83
3	3	m	12500	1.8	9.2	139	0.43	3.98
4	6	m	11000	0.9	7.4	137	0.78	0.59
5	9	m	17800	6.5	4.2	136	0.65	1.00
6	2	m	13900	3.3	13.2	126	1.50	1.87
7	10	m	13700	2.2	4	137	10.19	2.08
8	8	f	15700	3.4	7.6	129	8.70	2.02
9	2	m	18800	9.4	12.5	131	2.13	3.68
median	5		13700	3.3	7.5	131	1.50	1.91**
IQR	5.5		5000	2.5	3.5	6.5	3.03	1.53**
Healthy children (n = 54)								
median	5							4.39**
IQR	6							5.53
Reference Value			3500 ~ 8000*	12 ~ 40*	#	135 ~ 147*	0.3 ~ 1.0*	

*Normal levels of healthy children from our institution. Each normal level depends on age. #Normal level of hemoglobin is 13.7 ~ 16.9 for males and 11.4 ~ 14.7 for females, respectively.

**Significant differences between the HUS and healthy groups were determined by Mann-Whitney U rank sum test. **$p < 0.01$.

Hb, hemoglobin; sCre, serum creatinine; IQR, inter-quartile range.

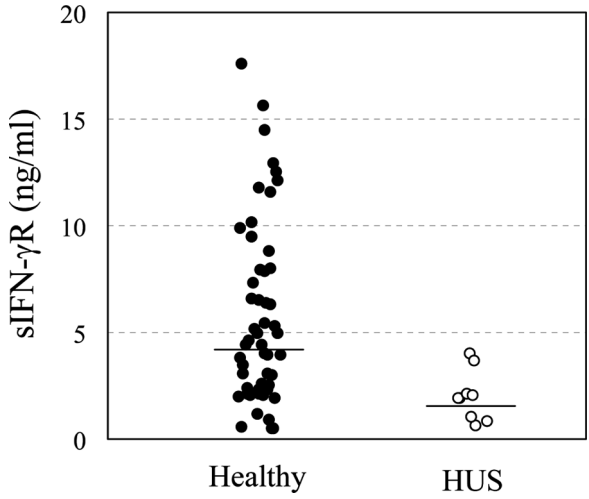

Figure 2. Serum sIFN-γR level of patients with D + HUS on the day of diagnosis. Dots indicate the serum sIFN-γR level of individual subjects (healthy children; n = 54 *vs* HUS; n = 9), and each horizontal bar represents the median values of each group. Significant differences were determined by the Mann-Whitney U rank sum test. $^{*}p < 0.01$.

be compared with that of normal adult controls. Although it has been reported that the soluble form of IFN-γR exists in body fluids,[10,12] a trend of age-related serum sIFN-γR variation has not yet been reported. Our data should be valuable as a reference value for the evaluation of various clinical samples. 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]

We determined the serum sIFN-γR levels in patients with diarrhea positive (D+) hemolytic uremic syndrome (HUS) as a preliminary application. D + HUS occurs after a prodrome of hemorrhagic colitis caused by verotoxin-producing *Escherichia coli* infection.[18,19] The disease causes the destruction of red blood cells, the damage of blood vessel walls, and, in severe cases, kidney failure. HUS is a rare condition mostly affecting 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 in pediatrics. Although some circulating cytokines, such as TNF-α, IL-1β, sIL-2R, IL-6, IL-8, and IL-10[20–26] are upregulated during thrombotic microangiopathy with HUS, changes in the sIFN-γR level have not been reported.

The serum sIFN-γR level of the HUS group was significantly lower than that of the healthy group ($p < 0.01$) (Table 2). Previously, we have reported that patients with HUS showed elevated serum sIL-2R and decreased serum sIL-4R levels.[15] Elevated plasma levels of sIFN-γR have been detected in patients with rheumatoid arthritis (RA)[12] and the patients with episodes of rejection after organ transplantation.[11]. Elevated serum levels of sIL-2R have also been reported in patents with RA and organ transplant rejection. However, patients with HUS showed opposite trends for sIL-2R and sIFN-γR levels. Although both IL-2 and IFN-γ belong to the Th1-type cytokines, the expression of their receptors might be differentially regulated. Further studies, including animal experiments, are needed to clarify the physiological and clinical significance of the changes in serum soluble cytokine receptors.

CONCLUSION

We determined the serum sIFN-γR level of healthy Japanese as a control value by means of ELISA specific for human sIFN-γR. It was found that the serum sIFN-γR level of children (0–14 years old) was significantly higher than that of adults (over 15 years old). Thus, it is recommended that when the serum sIFN-γR levels of patients are evaluated, the values should be compared with those of age-matched controls. The down-regulation of serum sIFN-γR in patients with HUS was found in a preliminary study. Although the clinical significance of sIFN-γR remains to be clarified, we demonstrated that the sIFN-γR level may be used as a diagnostic tool to determine the disease state.

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