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Assays for Thyroid-stimulating Antibodies and Thyrotropin-binding Inhibitory Immunoglobulins in Children with Graves' Disease

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Abstract. Studies on thyrotropin receptor autoantibodies (TRAb) by measurement of both thyroid-stimulating antibodies (TSAb) and thyrotropin-binding inhibitory immunoglobulins (TBII) in serum from children with Graves' disease are limited in number of studies. The aim of this study was to investigate the levels of serum TSAb and TBII in children with Graves' disease, and to evaluate the clinical significance of these antibodies. We measured the serum TSAb and TBII at diagnosis and during management in 65 children with Graves' disease. Patients were divided into four groups according to their metabolic state: those with untreated active Graves' disease, those receiving treatment with antithyroid drugs, those in remission, and those in relapse. At diagnosis, both TSAb and TBII assays had high sensitivities and high specificities. In follow-up, the levels of both TSAb and TBII paralleled the course of the disease. There was a strong positive correlation between TSAb and TBII. TBII levels were significantly higher in the patients with ophthalmopathy than those without ophthalmopathy in untreated Graves' children. It was concluded that TSAb and TBII measurements are valuable in the diagnosis and management of children with Graves' disease.

Key words: Graves' disease, Child, Thyrotropin receptor autoantibodies (TRAb), Thyroid-stimulating antibodies (TSAb), Thyrotropin-binding inhibitory immunoglobulins (TBII)

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THE pathogenetic role of thyrotropin receptor autoantibodies (TRAb) in the serum of patients with Graves' disease has been widely accepted [1, 2]. Today, two kinds of assays are used to detect TRAb [3, 4]. One is based on the competition between the antibody and thyrotropin (TSH) for binding to the TSH receptor [thyrotropin-binding inhibitory immunoglobulins (TBII)]. The other is a functional assay that measures the production of cAMP in response to a TSH receptor interaction with stimulating antibodies [thyroid-stimulating antibodies (TSAb)] or blocking antibodies [thyroid stimulation blocking antibodies (TSBAb)]. The competitive assay does not distinguish between the TRAb that stimulates or blocks the TSH receptor. Only functional assays can identify whether the antibody is a stimulating antibody or a blocking antibody.

TRAb has a heterogeneous nature and multiple forms of TRAb are found [3, 5–7]. TSAb and TBII reflect different aspects of TRAb, hence the simultaneous measurement of both TSAb and TBII is clinically useful [3, 8, 9].

However, studies on TRAb by measurement of both TSAb and TBII in serum from children with Graves' disease are limited in number [10–13], and the use of TRAb testing in routine clinical practice in diagnosis and follow-up of Graves' disease is debated [14, 15]. The aim of the present study was to investigate the prevalence of serum TSAb and TBII in children with Graves' disease, and to evaluate the clinical significance of these antibodies. Furthermore, to investigate the influence of age on disease characteristics, we com-

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pared our experience with the clinical and biochemical findings in younger (age <13 yr) and older (age ≥13 yr) children with untreated Graves' disease.

Subjects and Methods

Patients

Sixty-five serum samples obtained from children with Graves' disease were studied. Their median age at diagnosis was 12.4 years (range 5.1 to 17.5 years). A diagnosis of Graves' disease was made on the basis of clinical and biochemical evidence of hyperthyroidism (suppressed TSH, elevated 3,5,3'-triiodothyronine (T_3), thyroxine (T_4), free T_3 or free T_4, goiter, increased thyroidal uptake of ^{123}I, and signs of Graves' ophthalmopathy when present). Patients who had eyelid retraction, eyelid lag, or proptosis were defined as subjects with ophthalmopathy. Patients were divided into four groups according to their metabolic state: those with untreated active Graves' disease (group I, n = 35), those receiving treatment with antithyroid drugs for longer than 4 weeks (group II, n = 19), those in remission (group III, n = 6), and those in relapse (group IV, n = 5). Sera for control measurements were obtained from 34 euthyroid individuals undergoing treatment of non-thyroidal illness (growth hormone deficiency, type 2 diabetes mellitus) at the Pediatric Endocrine Clinic of Kitasato University Hospital (group V, n = 34). The clinical data of the patients are summarized in Table 1.

Serum thyroid hormones and TSH were measured at the time the patients were seen. Before December 2001, T_3, T_4 and TSH were measured by immunoradiometric assay kits with T-3 Riabead, T-4 Riabead and TSH Riabead (Abbott Japan, Chiba, Japan), respectively, and free T_3 and free T_4 were measured with Amerlite-MAB FT_3 and Amerlite-MAB FT_4 (Ortho-Clinical Diagnostics, Buckinghamshire, UK), respectively. After December 2001, T_3, T_4, free T_3, free T_4 and TSH were measured by electrochemiluminescence immunoassay system with Elecsys T_3, Elecsys T_4, Elecsys free T_3, Elecsys free T_4 and Elecsys TSH, respectively (Roche Diagnostics GmbH, Mannheim, Germany). The reference ranges used for serum T_3, T_4, free T_3, free T_4 and TSH were 70 to 180 ng/dl, 5.2 to 12.4 μg/dl, 2.5 to 4.3 pg/ml, 1.0 to 1.8 ng/dl, and 0.30 to 4.00 mU/L before December 2001, and after then, 80 to 160 ng/dl, 6.10 to 12.4 μg/dl, 2.30 to 4.30 pg/ml, 0.90 to 1.70 ng/dl, and 0.500 to 5.00 mU/L, respectively.

Furthermore, group I was divided into younger (age <13 yr, n = 15) and older (age ≥13 yr, n = 20) patients.

Autoantibody measurements

Sera for TSAb and TBII measurements were collected and stored at –30°C until assay.

TSAb Assays. TSAb was measured as a percent increase in cAMP production by porcine thyroid cells with TSAb assay kit Yamasa (Yamasa Co., Choshi, Chiba, Japan) [16, 17]. Sera whose TSAb levels were

Table 1. Clinical data for the patients in the five groups

Group No.	n	Patients with Graves' disease and controls	Age (yrs)	Gender (female : male)	Number of patients with ophthalmopathy at diagnosis (%)	Basal TSH (mU/L)	T_3 (ng/dl)	T_4 (μg/dl)	free T_3 (pg/ml)	free T_4 (ng/dl)
I	35	Untreated Graves' disease	13.1 (6.6–17.5)	30 : 5	42.9	0.05>(n = 32) 0.005>(n = 3)	482 (213–1020)	19.7 (11.7–36.5)	17.5 (6.4–34.2)	7.6 (2.4–12.3)
II	19	Treated Graves' disease	14.9 (6.9–17.7)	16 : 3	52.6	1.91 (n = 12) (0.10–7.5) 0.05>(n = 7)	178 (145–736)	6.6 (4.4–17.7)	3.8 (2.9–9.1)	1.1 (0.5–6.4)
III	6	Graves' disease in remission	16.6 (15.1–18.5)	6 : 0	16.7	1.95 (0.70–5.0)	118 (65–147)	8.0 (6.3–8.9)	3.3 (1.9–4.0)	1.0 (0.9–3.9)
IV	5	Graves' disease in relapse	15.2 (11.5–19.9)	5 : 0	40.0	0.06 (n = 4) (0.02–0.53) 0.05>(n = 1)	460 (198–949)	22.6 (9.3–24.6)	12.9 (4.0–20)	5.6 (1.4–8.0)
V	34	Controls	11.3 (6.8–18.6)	15 : 19	—	1.37 (0.52–3.24)	185 (175–221)	8.8 (5.9–10.2)	3.6 (3.2–4.3)	1.3 (1.0–1.6)

Values given are medians, with the range in parentheses.

higher than 1000% were diluted and measured again. The intra- and interassay coefficients of variation of TSAb were 3.3% and 19.4%, respectively.

TBII assays. TBII was measured by a radioreceptor assay kit using porcine thyroid membrane with quantitative TRAb ^{125}I RRA kit (DiaSorin Inc., Stillwater, MN, USA) following the manufacturer's instructions, and the results were expressed in units (U) based on the WHO standard (LATS, MRC Research Standard B, Code 65/122) [18]. Briefly, 50 µl of standard solution or serum and 50 µl of solubilized porcine thyroid membrane were mixed and incubated for 15 min at room temperature, and then 100 µl of ^{125}I-labeled bovine TSH was added and incubated for 2 h at room temperature. Then, 1 ml of chilled precipitation solution was added. Tubes were centrifuged at 2200 g at 4°C for 30 min, and radioactivity in the pellets was measured. Sera whose TBII levels were higher than 100 U/L were diluted and measured again. The intra- and interassay coefficients of variation of TBII were 8.8% and 8.6%, respectively.

Statistical analyses

To define the clinical cut-off level for positive serum with TSH receptor antibodies, we performed receiver-operating characteristic (ROC) plot analysis of the data from the patients in group I (untreated Graves' disease) and group V (controls) [19, 20]. The sensitivity and specificity were plotted on an ROC curve. The sensitivity (true positive ratio) was calculated from the 35 untreated Graves' patients in group I. The specificity (true negative ratio) was calculated from 34 controls in group V. Statistical analysis was performed using Mann-Whitney rank sum analysis for comparison of the autoantibody levels in the different groups determined with one assay. Correlation analysis was performed with Pearson's correlation.

Results

Fig. 1 shows plots of the sensitivity and specificity of the TSAb assay and those of the TBII assay. From ROC curves, 180% was chosen as the cut-off value for TSAb and 8 U/L for TBII. Of the 35 untreated Graves' patients, 33 (94.3%) had positive TSAb, and 32 (91.4%) had positive TBII.

The distributions of the autoantibody levels of the

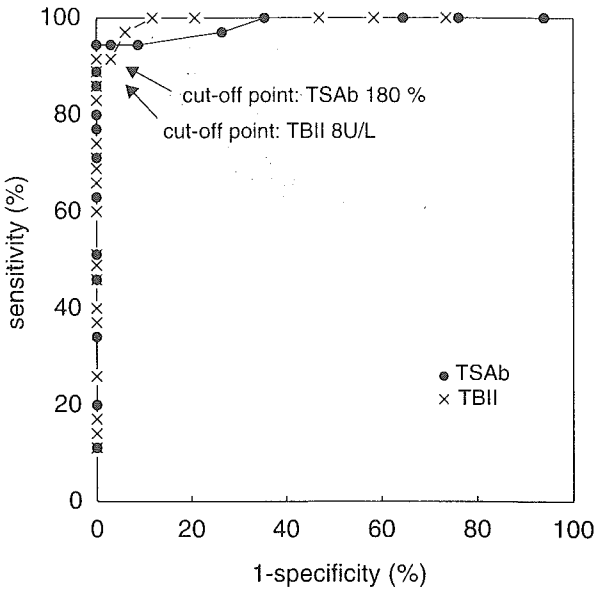

Fig. 1. ROC curves of TSAb and TBII. Closed dots, TSAb; cross, TBII. Each cut-off is indicated by an *arrow*.

TSAb assay and that of the TBII assay in groups I–V are shown in Figs. 2A and 2B, respectively. There were significantly higher autoantibody levels in groups I, II and IV than in the control group in both assays (by Mann-Whitney rank sum analysis: groups I and II, $p<0.01$; group IV, $p<0.05$). There were significantly lower autoantibody levels in group II than in group I in both assays ($p<0.01$). All of the subjects in group III had both negative TSAb and TBII, and all but one in group IV had both positive TSAb and TBII.

Two patients in group I displayed neither TSAb nor TBII. They had mild hyperthyroidism. They did not exhibit ophthalmopathy.

There was a strong positive correlation between the data obtained in the TSAb assay and those obtained in the TBII assay in group I as shown in Fig. 3 ($r = 0.80$, $p<0.001$). There were also strong positive correlations between the data obtained in these two assays in groups II, IV, and all Graves' children ($r = 0.74$; $n = 19$; $p<0.001$, $r = 0.92$; $n = 5$; $p<0.05$ and $r = 0.80$; $n = 65$; $p<0.001$, respectively; data not shown).

In addition, in Fig. 3, the closed dots indicate the data of patients with ophthalmopathy, and the open dots indicate those of patients without ophthalmopathy in group I. TSAb levels were not significantly higher in the patients with ophthalmopathy (median, 525.9%; range, 201.1%–4568.7%) than those without ophthalmopathy (median, 428.3%; range, 137.2%–918.5%).

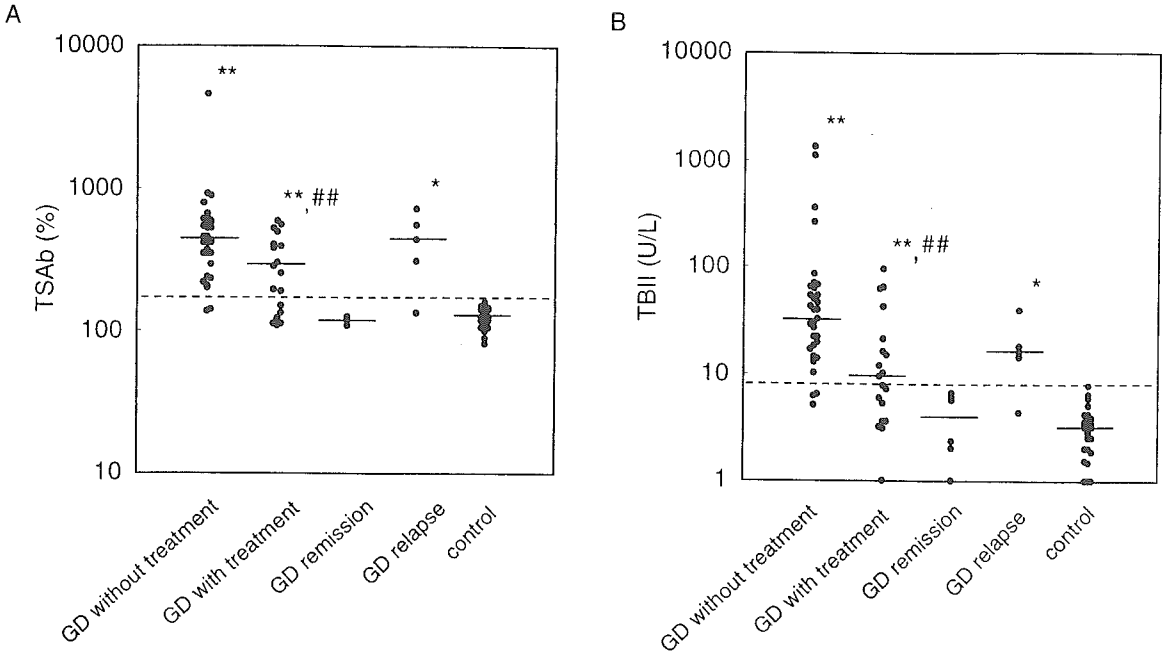

Fig. 2. A: Distribution of TSAb in different groups of patients. GD, Graves' disease. *Horizontal line* indicates median value for each group. The cut-off is shown by the *dotted line*. **, $p<0.01$ *vs.* control group; *, $p<0.05$ *vs.* control group; ##, $p<0.01$ *vs.* group I. B: Distribution of TBII in different groups of patients. GD, Graves' disease. *Horizontal line* indicates median value for each group. The cut-off is shown by the *dotted line*. **, $p<0.01$ *vs.* control group; *, $p<0.05$ *vs.* control group; ##, $p<0.01$ *vs.* group I.

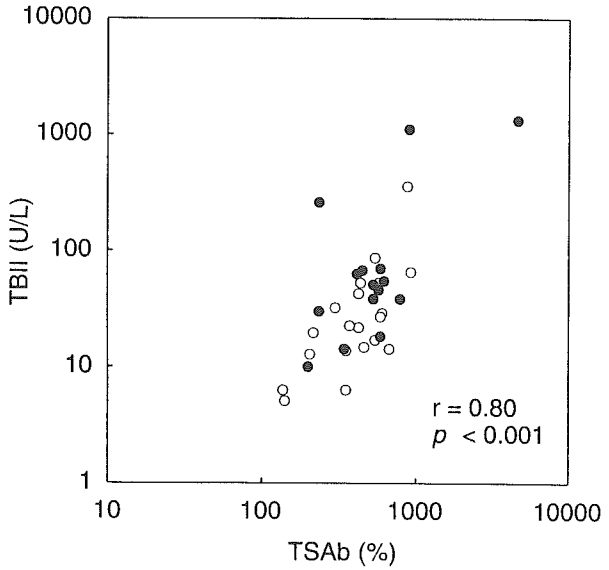

Fig. 3. Correlation of TSAb and TBII in patients with untreated Graves' disease (group I). Closed dots, patients with ophthalmopathy; open dots, patients without ophthalmopathy.

TBII levels were significantly higher in the patients with ophthalmopathy (median, 50.5 U/L; range, 10.1 U/L–1342.5 U/L) than those without ophthalmopathy (median, 22.2 U/L; range, 5.1 U/L–351.9 U/L, $p<0.02$). There were no significant differences of the levels of thyroid hormones between those with ophthalmopathy and those without ophthalmopathy in group I (data not shown).

The serum thyroid hormones, serum TSH, the incidence of ophthalmopathy, and the titers of both TSAb and TBII were not statistically different between younger (age <13 yr) and older (age ≥13 yr) patients in group I.

Discussion

From the ROC curves, it was indicated that both TSAb and TBII assays had high sensitivity and high specificity at diagnosis. In follow-up, the levels of both TSAb and TBII in groups I–IV paralleled the course of the disease, hence, TSAb and TBII measurements are valuable in the diagnosis and follow-up of children with Graves' disease. Both TSAb and TBII assays are commercially available and do not require culture facilities, and so their use is not limited to specialized laboratories. Although the use of TSAb and TBII testing in routine clinical practice in diagnosis

and follow-up of Graves' disease remains controversial [14, 15], these methods may be the most sensitive and specific procedures for the diagnosis and management of Graves' disease.

Two patients with negative TSAb and negative TBII in group I had mild disease, which is similar to that reported by others [21, 22].

We observed a strong positive correlation between TSAb and TBII in groups I, II, IV, and all Graves' children. It is reported that children with atrophic autoimmune thyroiditis (AAT) did not possess TSBAb, which are often found in adult patients with AAT [23]. Similarly, it is possible that children with Graves' disease seldom possess TSBAb, and so the correlations between TSAb and TBII might be strongly positive. Another possible reason for our strong correlation is the characteristics of our methods. The TSAb assay and TBII assay which we used were semi-quantitative assay and quantitative assay, respectively, so they can detect high levels without bluntness.

The finding of 42.9% incidence of ophthalmopathy in group I is similar to that found in other reports of juvenile Graves' disease [24–26]. In group I, the children with ophthalmopathy had significantly higher TBII titers than those without ophthalmopathy, which is similar to that of children with Graves' disease reported by others [27]. In group I, TSAb levels were not significantly higher in the Graves' children with ophthalmopathy than those without ophthalmopathy. However, it is reported that TSAb was correlated with the severity of ophthalmopathy in adult Graves' disease [28, 29]. The clinical and biological findings of Graves' disease, including ophthalmopathy, are different with age [4, 24, 25, 27, 30–33], and so it is possible that this difference might be due to age. However, we examined only the presence of ophthalmopathy, and not its severity, hence further evaluation is needed.

In the present study, no difference was seen between younger and older patients in group I. This is probably due to the small number of patients in each group.

In conclusion, both TSAb and TBII measurements are valuable in the diagnosis and follow-up of children with Graves' disease.

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Effects of dioxins on the quantitative levels of immune components in infants

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Dioxins (polychlorinated dibenzo-*p*-dioxin (PCDD)+polychlorinated dibenzofuran (PCDF)) and polychlorinated biphenyls (PCBs) are potentially hazardous compounds and have structural similarity with thyroid hormones. Animal studies have demonstrated that PCDDs, PCDFs and PCBs can alter immune functions. However, in humans it is not yet elucidated whether dioxins contained in breast milk have any effects on the immune functions in infants. To investigate the effects of dioxins on the immune system, we compared the quantitative levels of immune components between a breast-fed group and bottle-fed group, in which dioxin concentration is almost zero. Ratios of immune cells, such as CD4+ and CD8+ T-lymphocytes, as well as B-lymphocytes (CD19+ and/or CD20+) and NK cells (CD16+, CD56+) in peripheral blood lymphocytes, serum immunoglobulin level, and level of specific IgE antibody to allergens in the venous blood at 12 months of age were assessed in a subgroup of 281 infants. The relationship of post-natal dioxin exposure via breast feeding with the ratio of immunological markers and the level of humoral antibodies up to 12 month of age was not demonstrated. In conclusion, it would appear that the content of dioxins in breast milk in the Japanese general population is not enough to induce any change in theses-examined immunological parameters during the first year of life, although long-term effects remain to be evaluated. *Toxicology and Industrial Health* 2006; **22:** 1–5.

Key words: *breast feeding; bottle feeding; dioxins; IgE; lymphocytes subsets*

Introduction

Polychlorinated-dibenzo-*p*-dioxin (PCDD), polychlorinated-dibenzofuran (PCDF), and coplanar-polychlorinated biphenyl (Co-PCB) compounds, hereafter referred to as dioxins, are tricycle aromatic compounds. They are mainly formed as byproducts of the synthesis of organochlorine chemicals and from the combustion of municipal and hazardous waste. In the late 1970s, the production and use of these compounds were banned because their adverse health effects had become evident.

Immune suppression is a common and extensively characterized sequela associated with acute 2,3,7,8,-tetrachloro-dibenzo-*p*-dioxin (TCDD) ex-

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posure in laboratory animals. Comprehensive cell-type fractionation-reconstitution studies have previously demonstrated the profound inhibition of B-cell function by TCDDs. According to direct addition studies utilizing primary cultured murine B cells, there is evidence suggesting that the suppression of antibody production by TCDDs may be closely associated with altered B-cell differentiation (Suh *et al.*, 1983). This finding is further supported by the observation that TCDDs only modestly inhibit B-cell proliferation.

There is a paucity of *in vivo* studies on the effects of dioxins on the immune system of humans (Tryphona *et al.*, 1998). Initial studies, showing that PCB and dioxins may be toxic to human immune function, were carried out on individuals accidentally exposed to these compounds (Patterson *et al.*, 1988). Weisglas-Kuperus *et al.* (1995) demonstrated that prenatal PCB/dioxin exposure was associated with changes in T cell subpopulations in the blood in Dutch infants.

It is not yet clearly determined, however, whether pre- and post-natal exposures to high background levels of PCDD, PCDF and PCB can alter the immune system in human infants, and whether the health of infants is adversely affected by these pollutants. In this study, we investigated the effects of dioxins contained in breast milk on the quantitative levels of various immune components in Japanese infants from birth to 12 months of age.

Subjects and methods

We collected breast milk from 415 mothers in 20 prefectures and cities in Japan at 30 days post-partum and quantified 14 isomers for PCDDs, 15 for PCDFs and 12 for coplanar PCBs (Co-PCBs). To express the toxic potency of the mixture of dioxins in breast milk samples, the toxic equivalency (TEQ) calculation, based on the new TEF re-evaluated by WHO in 1997, was used. The ages of the mothers were limited to 25 ± 34 years, and all mothers were primiparous and resided in the same area for more than five years.

At one year of age, blood samples were obtained from 281 breast-fed infants (breast-fed group) for the evaluation of immune functions. The breast-fed group was infants who had not received bottle-milk until one year of age. Blood samples were also obtained from 20 infants who were bottle-fed at one year of age, as a control group (bottle-fed group).

The fat content in human milk was determined by weighing, as described by Patterson *et al.* (1988). In brief, breast milk (50 mL) was mixed with saturated potassium citrate (10 mL), ethanol (100 mL), diethylether (50 mL) and hexane (120 mL) in a 500-mL separatory funnel and shaken vigorously for 10 min. The hexane phase was then removed and washed first with 2 mol/L NaOH followed by sulfuric acid. The hexane phase was then dried and weighed. The fat content of breast milk at five days post-partum was $3.0 \pm 1.4\%$, and at 30 days post-partum was $3.8 \pm 1.2\%$, and did not change thereafter (Matsuura, 2001a,b).

PCDDs, PCDFs and Co-PCBs in human milk were identified by GC/MS conducted at the Japan Food Research Laboratory (Matsuura, 2001b). Surface markers of peripheral blood monocytes (PBMCs) were quantified by flow cytometry (SRL, Tokyo, Japan) (Ip *et al.*, 1982).

Serum IgE concentrations were determined by chemiluminescent enzyme immunoassay (Matsui *et al.*, 2000). Specific IgE antibodies for house dust, milk and egg white were quantified by fluoroenzyme assay (CAP RAST FEIA, Pharmacia & Upjohn, Sweden). All parents who participated in this study gave their written informed consent.

The ratio of CD3, CD4, CD8, CD4/8, CD19, CD20 and CD86 between the breast-fed and bottle-fed groups was analysed by Student's t-test. The serum immunoglobulin levels of IgG, IgA, IgM and IgE between the breast-fed and bottle-fed groups were analysed by Student's t-test. Distribution of CAP-RAST scores between 0 and 1–6 was analysed by Fisher's exact test. Probability (P) values <0.05 were considered to be statistically significant.

Results

Effect of dioxins in breast milk on T cell ratio in PBMCs

The ratios of CD3+, CD4+, CD8+ or CD4+/CD8+ cells in PBMCs were compared between the breast-fed and bottle-fed groups (Table 1). No significant differences were demonstrated. The correlation between the concentration of dioxins in human milk and T cell ratio was also investigated

Table 1. The ratio of lymphocyte subsets in the breast-fed and bottle-fed groups.

	Breast-fed group ($N=281$)	Bottle-fed group ($N=20$)	P
CD3 (%)	73.1±7.2	69.9±6.1	0.053
CD4 (%)	50.1±8.4	48.6±6.7	0.436
CD8 (%)	24.1±5.8	24.1±7.4	0.861
CD4/CD8	2.2±0.9	2.3±1.2	0.718
CD19 (%)	14.7±5.6	15.9±6.7	0.362
CD20 (%)	14.3±5.6	15.9±5.8	0.242
CD86 (%)	0.8±0.5	1.2±0.9	0.078
IgG (mg/dL)	645.1±182.1	694.0±186.2	0.248
IgA (mg/dL)	34.2±22.6	36.6±19.1	0.644
IgM (mg/dL)	105.9±33.6	106.9±41.4	0.899
IgE (U/mL)	54.4±89.9	58.2±105.9	0.857

and no significant correlation was found between them (Figure 1A).

Effect of dioxins in breast milk on B cell ratio in PBMCs

The ratios of CD19+, CD20+ or CD86+ cells, which are the surface markers of activated B cells, were compared between the breast-fed and bottle-fed groups (Table 1). There was no significant correlation. The correlation between the concentration of dioxin in human milk and B cell ratio was also investigated and no significant correlation was found between them (Figure 1A).

Effect of dioxins in breast milk on NK cell ratio in PBMCs

The correlation of the ratio of NK cells (CD16+/CD56+) with the concentration of dioxins in human milk was examined and no significant correlation was found (Figure 1A).

Effect of dioxins in breast milk on the serum immunoglobulin levels

The serum immunoglobulin levels of IgG, IgA, IgM and IgE were compared between the breast-fed and bottle-fed groups (Table 1). No significant differences were demonstrated between them. The correlation between the concentration of dioxins in human milk and the serum immunoglobulin levels was also investigated and no significant correlation was found between them (Figure 1B).

The specific IgE antibody to house dust, milk and egg white was quantified (Table 2). It was not demonstrated that there was no significant correlation between the breast-fed and bottle-fed groups.

Discussion

In this study, we investigated the relationship of the concentration of dioxins contained in breast milk with the ratios of immune cells and immunoglobulin levels. It has been reported that *in vitro* dioxins suppress B cell differentiation. Furthermore, there are some *in vivo* studies suggesting an effect of dioxins on immune functions (Forawi *et al.*, 2004). Smoger *et al.* (1993) reported that for children born to mothers living in a TCDDs-contaminated environment in Time Beach (MO) during and after pregnancy, a decrease in CD4+ T cells and an increase in CD8+ T cells was detected in children from nine to 14 years of age. In one preliminary study conducted in Northern Quebec, the CD4+ :CD8+ T cell ratio of Inuit infants, whose mothers have increased levels of PCB and dioxins in their breast milk, decreased at six and 12 months of age (Dewailly *et al.*, 1993).

Svensson *et al.* (1994) reported that the consumption of fatty fish species, such as salmon and herring, from the Baltic Sea is an important source of human exposure to persistent organochlorine compounds, eg, PCDDs, PCDFs and co-PCBs. The high fatty-fish consumers had lower ratios and numbers of NK cells, identified by the CD56 marker, in peripheral blood than the non-consumers. The weekly intake of fatty fish correlated negatively with the ratio of NK cells. This indicates that accumulation of persistent organochlorine compounds in high fatty-fish consumers may adversely affect NK cell ratios. Weisglas-Kuperus *et al.* (1995) demonstrated that a high post-natal PCB/dioxin exposure is associated with an increase in the number of TcR..+ T cells at birth and with an increase in the number of CD8+, TcR..+ or TcR..+ T cells at 18 months of age. Nagayama *et al.* (1998) reported that the ratios of CD4+ to CD8+ T cells had a significant increasing tendency with the estimated total TEQ intakes.

In our study, it was not demonstrated that the ratios of T cell subpopulation and CD16+ CD56+ cells (NK cells) in PBMCs correlated with the concentration of dioxins in human milk, although the number of CD4+ and CD8+ T cells were not

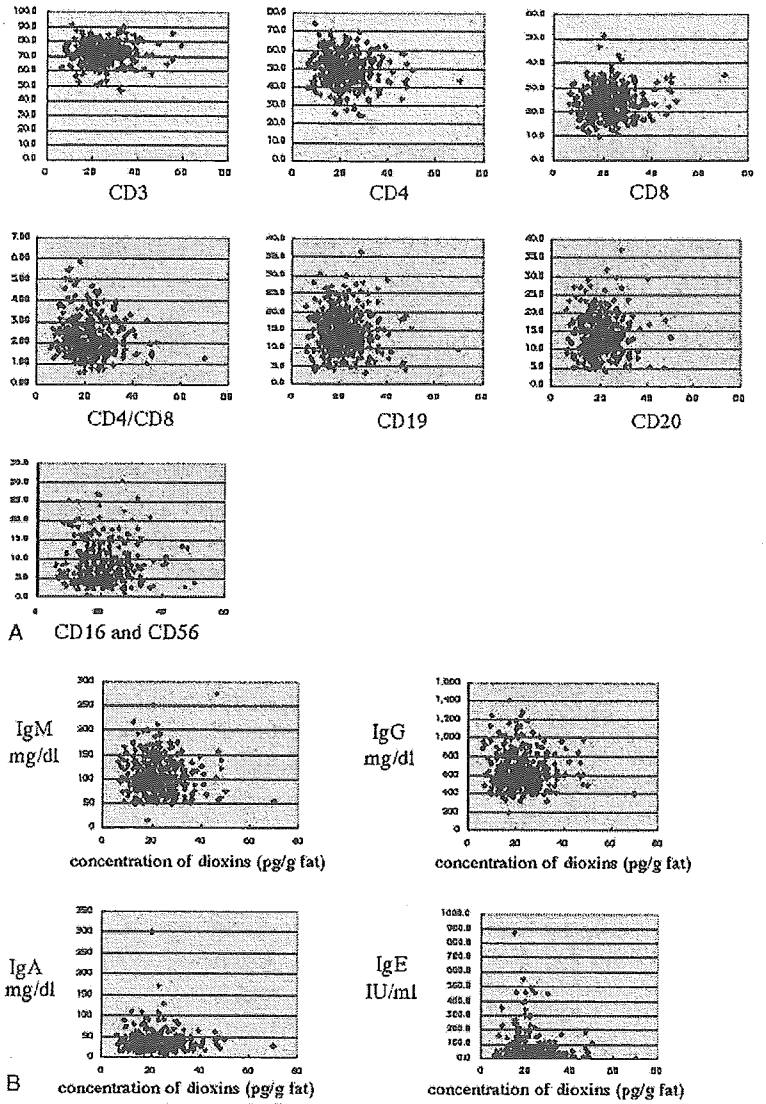

Figure 1. (A) Relationships between ratios of CD3+, CD4+, CD8+, CD4+/CD8+, CD19+, CD20+ and CD16+ CD56+ cells in PBMCs and the concentration of dioxins in human milk at 30 post-partum days. X-axis means the concentration of dioxins (pg/g fat). Y-axis means the percent of surface marker positive cells except CD4/CD8. In CD4/CD8, Y-axis means the ratio of CD4 per CD8. (B) Relationships between ratios of serum IgM, IgG, IgA and IgE and the concentration of dioxins in human milk at 30 post-partum days. X-axis means the estimated intake of dioxins (pg/g fat). Y-axis means the serum concentration of IgM (mg/dL), IgG (mg/dL), IgA (mg/dL) and IgE (IU/mL).

Table 2. Specific IgE antibody of breast-fed and bottle-fed groups.

	Breast-fed group							Bottle-fed group						*P*
	0[a]	1	2	3	4	5	6	0	1	2	3	4	5	6	
House dust	251[b]	8	10	5	1	1	0	19	0	1	0	0	0	0	0.568
Milk	238	12	20	5	1	0	0	17	0	2	1	0	0	0	0.558
Egg white	180	24	44	22	2	3	0	15	3	2	0	0	0	0	0.264

[a]Number (0–6) indicates CAP-RAST scores to each allergens.
[b]Number indicates the number of persons who have the CAP-RAST score.
Distribution of CAP-RAST scores between 0 and 1–6 was analysed by Fisher's exact test.

analysed. We could not find that the levels of serum immunoglobulins and specific IgE to allergen were significantly different between the breast-fed and bottle-fed groups. The difference between our data and those of Weisglas-Kuperus may be due to the following: the time at which immunological analysis was performed, and the amount of dioxins to which the subjects were exposed, that is, a higher concentration of dioxins in the early days after birth; the content of dioxins in breast milk was almost 2-fold higher in the Netherlands (30.75 pg TFQ/g fat) than in our study (14.8 ± 6.1 pg TFQ/g fat) (Matsuura *et al.*, 2001b).

The sample size in this study was 281 in the breast-fed group and 20 in the bottle-fed group, which is a maximum size considering the budget for this study and the co-operation of the mothers. When we consider the value between the breast-fed and bottle-fed groups (shown in Table 1 as true difference), the power of CD3 and CD86 was higher than 50%, however, CD8, CD4/8, IgA, IgM and IgE was lower than 10%.

On the basis of the results of this study, we conclude that, although the infants were exposed to some amounts of dioxins in the breast milk in Japan, we could not find that the quantitative levels of immune components at one year of age was seriously impaired. However, long-term effects remain to be evaluated.

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栄養

少子化時代の食の重要性

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食生活の乱れやそれによる生活習慣病などが子どもたちにおよぼす影響は、はかりしれません。子どもたちの健全な育成のために、少子化対策の一環としての食育の提唱です。

メタボリックシンドロームと若い女性のやせ

疾病予防のために、普段の生活、なかでも食生活の重要性が認識されるようになってきました。この背景には最近のわが国の飽食状態を反映して肥満が増加し、インスリン抵抗性による糖尿病や動脈硬化、心血管異常の増加が問題となり、健康な生活を送るためには健全な栄養摂取が重要であることが認識されるようになったためと思われます。

最近、日本内科学会では、生活習慣病とよばれる諸疾患の原因となるメタボリックシンドロームの定義と、診断基準を発表しています。ここでは内臓脂肪の蓄積を重要視し、ウエスト周囲径が男性85cm以上、女性90cm以上で、かつ高トリグリセライド血症and/or低HDLコレステロール血症、高血圧、空腹時高血糖のうち2項目以上を示すものをメタボリックシンドロームとしています。

小児でも最近肥満の頻度の増加が問題になっています。しかし、女子大で教鞭を執るようになり若い女性に接する機会が増えてみると、肥満ばかりでなくやせが目立ってならなりません。成人ではBMI(body mass index:体重kg/身長m^2)は18.5～25が正常とされ、標準体重はBMIが22とされていますが、若い女性ではやせとはされないまでも20未満のやせ傾向が目立ちます。

若い女性のやせの影響

最近の出生児の平均体重の変化と低出生体重児の割合を図1、2に示しましたが、近年の出生体重の平均値の低下傾向は早産に対する医療や不妊症治療の進歩の結果だけでは説明がつかず、妊娠する女性のからだに変化が起こっている徴候ではないかと心配されます。

胎児期の低栄養状態が成人期や高齢期の糖尿病や高血圧などの発症に関与していることが明らかになったことからも、若い女性のやせ傾向は、少子化で一人ひとりがますます貴重となる次世代の社会を構成する人々の健康に、影響をおよぼすのではないかと懸念されます。

朝食を食べない若者が増え、インスタントラーメンやファーストフードなど栄養的に問題がある食事が多くなっていますが、このような食生活が将来の骨粗鬆症や生活習慣病などの発症に影響するだけでなく、このような若者が親となり育児を行うことになるのです。

食生活の乱れの影響

睡眠時間の乱れ、運動だけでなくからだを動かす機会も少なくなった生活、自然とのふれあいの欠如などの日常生活の変化は、コンビニや自動販売機で手軽に手に入れることができる飲料水やスナック、家庭での欠食や孤食など食生活の乱れも加わって、すでに子どもの生活に深く影響をおよぼしています。

少子化時代に子どもたちの健全な生育をどう支援するかが、少子化対策としてもっとも重要であるにもかかわらず、社会がこの重要性に気づかず対策が講じられないまま経過していることが、これまでに経験したことのない悲惨な少年犯罪や児童虐待が毎日のようにテレビや新聞で報道されている原因になっているのではないかと疑われます。

少子化対策としての食育

子どもたちを中心において、子どもには何が必要かを考えた上で、出生直後からの親と子への支援を考えることが少子化対策としてもっとも重要であると思われます。親と楽しく食事をすることは、子どもの生活の中でもっとも単純でしかも実現が可能な事柄であるはずです。

毎日は困難であっても、少なくとも週に何回かは、両親が早く帰ってきて子どもと一緒に料理を作り、会話を楽しみながら一緒に食べる機会を作ることは、会社の理解により働き方を少し変えるだけで実現が可能です。また、こうした機会が持てれば、子どもは生活を楽しむことができるようになり、食や料理に関心を持つようになります。近年話題になっている食育もこうした少子化対策の一環として考えていきたいものです。

図1　平均出生体重値の変化

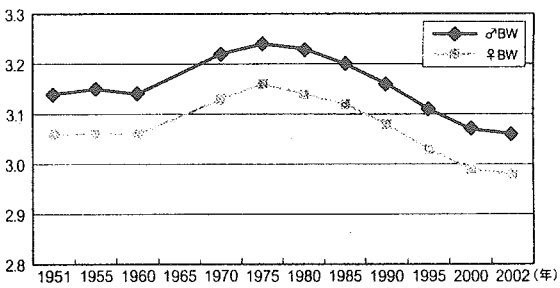

図2　低出生体重児・極低出生体重児の出生率の変化