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母乳の感染防御機能に関する研究　－咽頭粘液分泌型IgA濃度の栄養別比較－

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母乳の感染防御機能に関する研究

ー咽頭粘液IgA濃度の栄養別比較ー

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Secretary IgA levels in pharyngeal mucous among infants with different feeding methods at the age of 1-6 months

Key words: sIgA, pharyngeal mucous, breast feeding and respiratory infection

Abstract

Breast milk secretary immunoglobulin A (BMsIgA) has been thought to coat mucosal surface of gastrointestinal tract in recipient infants, protecting infants from invasion of pathogenic bacteria. It has been observed that the prevalence of respiratory tract infection in breastfed infant is also lower than that in formula-fed infant. We thought that surface of pharyngeal mucosa of recipient infants is also coated by BMsIgA as well as the surface of gastrointestinal tract in mucosa. In order to confirm this we estimated the level of sIgA in pharyngeal mucous among infants with different feeding methods. Throat swab collected from 22 one-month old infants (8 exclusively breastfed infants, 9 mixed-fed infants and 5 formula-fed infants), 19 two-months old infants (10 exclusively breastfed infants, 6 mixed-fed infants and 3 formula-fed infants) and 11 three to six- months old infants (6 exclusively breastfed infants, 4 mixed-fed infants and 1 formula-fed infant) were evaluated by enzyme immunoassay method for sIgA after emulsified in saline. The amount of sIgA for 1 mg of wet mucous specimen's weight was calculated from the collected mucous weight and the concentration of sIgA in emulsified saline. Concentration of protein in emulsified saline was also measured by a

dye-binding assay (Bio-Rad). The amount of protein for 1mg of wet mucous specimen's was also calculated from the collected mucous weight and the concentration of protein in saline.

The mean amount of sIgA weight(μ g) in saline / wet mucous weight(mg) and protein weight (μ g) in saline / wet mucous weight(mg), and ratio of sIgA for protein (sIgA/protein ratio $\times$ 100 is percentage of IgA to protein) were calculated in each infants. The results of the infants aged 1-2 months were compared by feeding methods of infants. The mean amount of sIgA (μ g) in saline/ wet mucous weight(mg) in breastfed, partially breast-fed and formula-fed infants were 0.15$\pm$0.03,0.19$\pm$0.04 and 0.12$\pm$0.03, respectively. The mean and standard error of protein (μ g) in saline / wet mucous weight(mg) in breastfed, partially breast-fed and formula-fed infants were 3.1 $\pm$0.3, 3.0$\pm$0.3 and 2.7$\pm$0.3, respectively. The mean and standard error of the ratio of sIgA/protein in breastfed, mixed-fed and formula-fed infants were 0.055 $\pm$0.010, 0.065$\pm$0.017 and 0.045$\pm$0.010 respectively. There was not significant difference in those values among infants with different feeding methods.

The results showed that the levels of sIgA among infants with different feeding methods were not different. The speculation of BMsIgA binding and

coating in the pharyngeal mucosa of recipient infants could not be confirmed quantitatively. Based on this preliminary study, we conclude that BMsIgA does not influence the level of sIgA of pharyngeal mucous in recipient infants.

Introduction

A secretary immunoglobulin A (sIgA) is the major protective immunoglobulin of external secretions such as gastrointestinal、respiratory and urogenital tracts, being produced by plasma cell in mucosa. Infants begin to product sIgA after birth. With regard to the development of sIgA secretion there are several reports, which are studied about intestinal and salivary sIgA. Perkkio & Savilahti (1) reported that only 24/mm square immunoglobulin containing cells were seen in rectal specimen from 12 day-old infant. After then, the mean number of IgA containing cells increased with age up to 6 months. The newborns may produce only amounts of sIgA and reaches adult levels gradually belong with the immunological development. According to the report of Gleeson et al (2), salivary IgA were not detected at birth but the median levels of salivary IgA increased rapidly to peak levels by 6 weeks of age. The concentration decreased by 3 months to levels that remained stable throughout

the study period (~ 800 days, that is 30 months). The salivary IgA was measured as total IgA of monomer and dimmer construction in their report. This pattern of appearance of salivery IgA is partly consisted with the population of the neonatal intestine with IgA-containing plasma cells and indicated a rapid maturation process of the human mucosal immune system. However when we discuss about humoral immunity, production of sIgA rather than total IgA (sIgA + monomer IgA) is better considered. Serum IgA of which levels are 10 μ g/dl in serum of young infants may exudates into mucous. Therefore the level of the serum IgA may influence the level of sIgA but they are not correlated with sIgA content of mucous. IgA committed cell included in membrane-associated lymphoid tissue develops into IgA introducing cell by antigen's stimulation. The process of developing into IgA producing cell from B cell need cytokine produced by helper T cell which is activated by antigen presenting macrophage. After IgA producing cell secrete IgA, secreted IgA become dimmer construction by conjugating secretary component that is sIgA. Now sIgA go through mucosal epithelial cell and secreted in mucous at mucosal surface. Therefore ability of sIgA secretion depends on immunological development of both T cell and B cell. Production of sIgA is not sufficient

enough during in young infants who are immature in immunity.

On the other hand breast milk contains sIgA abundantly. The sIgA content in colostrums is more than 1000 mg/ dl, and in mature milk the sIgA content is 100 mg/dl. BMsIgA has been thought to coat the surface of gastrointestinal tract mucosa of recipient infants, because of following two reasons. Firstly, sIgA resist to digestive enzyme. Secondary, sIgA has high affinity to mucosal mucin. Thus it is speculated that BMsIgA can bind to mucin in mucosa of gastrointestinal tract and prevent pathogen's invasion at the mucosal surface. Because it is thought that BMsIgA has an effect such as inhibition of bacterial adherence, neutralization of toxins and prevention of absorption of antigens through mucosal surface. This speculation of IgA binding is one of the interpretations why prevalence of diarrhea in breastfed infant is lower than that in formula-fed infants. Empirically, we have observed that the prevalence of infectious diseases and allergic diseases of breastfed infant are lower than that of formula-fed infants.

With regard to the infectious diseases, it has been empirically observed that the prevalence of respiratory diseases and otitis media are lower in breastfed infant than those in formula-fed infant as well as infection of

gastrointestinal tract. The main pathogens that cause respiratory tract infection such as bronchopneumonia and otitis media in infants are *Streptococcus pneumoniae* and *Haemophilus influenzae.* BMsIgA may resist to them, for BMsIgA is not destructive into Fab and Fc fragment by IgA protease produced by pathogen such as *Streptococcus pneumoniae* and *Haemophilus influenzae*

We thought that BMsIgA bind to mucin in mucosa of oral pharynx and coat mucosal surface, which is continuous to nasal pharynx in recipient infants. Thus BMsIgA protects infants from invasion of pathogenic bacteria or viruses that cause respiratory tract infection on the mucosal surface of oral pharynx. It is speculated that the levels of mucous sIgA are higher in breastfed infants (mucous sIgA of oral pharynx is measured as sum of sIgA derived from breast milk binding in the pharynx and sIgA produced by infant self) than those in formula-fed infants. In order to confirm this the concentration of mucous sIgA collected by using throat swab were measured among infants with different feeding methods.

Subject and methods

1. Subject and samples collecting

Between July 1998 and June 2000, 22 healthy infants (comprising aged one month; 8 exclusively breastfed, 9 partially breast-fed and 5 exclusively-formula-fed infants, 19 infants aged two months; 10 exclusively breastfed, 6 partially breast-fed and 4 exclusively-formula-fed infants, and 11 infants aged three to six months; 6 exclusively breastfed, 4 partially breast-fed and 1 exclusively-formula-fed infant) who were: aged 1-6 months; full term; home care infants and not receiving antibiotics were recruited for the study. Informed consent for taking throat swab was obtained from infants' mothers. Low birth weight infant and those with symptoms of respiratory tract disease were excluded from the subjects.

(1) Category of subjects by feeding methods

The infants were divided into three groups according to their feeding methods as follows:

Breastfed= exclusively breastfed infants aged one to two months. In the infants of aged 3 to 6 months, semisolid food was usually introduced in addition to breast milk.

Partially breast-fed = Partially breast-fed infants aged one to two months. In the infants of aged 3 to 6 months, semisolid food were usually introduced adding

to breast and formula milk

Exclusively formula-fed = exclusively or mainly formula-fed infants aged one to two months. In the infants of aged 3 to 6 months, semisolid food was usually introduced in addition to formula milk.

Formula milk were Hohoemi or Sukoyaka that were produced Japan's popular milk company, which are Yukijirusi (snow brand) and Meijinyugyo. Contents and percentage of each nutrient of them are almost the same. They are made according to the regulation of Japan.

(2) Samples' (pharyngeal mucous) collecting and weighing

Throat swab samples were obtained before feeding with a sterile cotton-tipped applicator stick wiped around the oral part of Waldeyer's ring (around oral tonsil which are quite immature) without saliva contamination. Throat swab specimens are measured weight as whole cotton-tipped applicator stick before and after wiping pharyngeal mucosa to calculate the amount (weight) of mucous specimens. Equipment used for weighing is Electronic balance FA200 (A & Day Company, Japan) of which readability is 1mg.

Collected mucous weight was calculated as the difference of cotton swab weight between before and after wiping the mucosal surface. This collecting

method and weighing is according to the microbiological study to collect the mucous by using throat swab. The mean weight of samples was about 20 mg in such microbiological study.

2. Measurement of sIgA and protein in emulsified saline

(1) Measurement of sIgA

Throat swab including absorbed mucous was emulsified in 1ml sterile saline and sIgA concentration was measured by duplicated enzyme immunoassay method. EIAS-IgA Kit(M B L) was used. An enzyme-labeled antihuman IgA detects antibodies to human secretary component bound on polystyrene ball.

First reaction: Polystyrene ball is added into a test tube, which include mucous samples.

Second reaction: After washing the polystyrene ball, peroxidase labeled antihuman IgA (rabbit IgG/Fab) is reacted. As substrate O-phenylendiamine and H2O2 are added.

H2SO4 is added to stop reaction. Absorbance of 2,2'-diamino-azobenzol is read at 492nm by electro optical photometer(Nihonkouden). Measurement range is 0.07-71 μ g/ml. Measurement was duplicated. Measurement method is

summarized as follows.

a) Mucous emulsified saline 10 μ l+ Buffer 0.4ml+Anti human secretary component binding polystyrene ball

↓

Incubate for one hour at 37degree

↓

Aspirate buffer and add PBS 1ml to wash the polystyrene ball

↓

Washing is repeated 2 times

b) After aspirate the PBS, enzyme labeled (conjugated) antihuman IgA 0.5ml is added and set for one hour at room temperature.

↓

Washing is repeated 3 times

c) Add polystyrene ball into test tube including substrate (0-phenylendiamine 0.5ml + H_2O_2) for the enzyme:

↓

Incubate for 30 minutes at room temperature

d) A color reaction

After stop the reaction by H2SO4 2 ml, Absorbance is read at 492 nm. Then sIgA concentration was read from standard calibration curve.

Therefore in this method dilution rate of sIgA into saline is depending on the amount of collected pharyngeal mucous and amount of emulsified mucous samples. Amount of collected pharyngeal mucous which were slightly different from each samples. Although standard deviation was small, calculation of sIgA weight for mucous wet weight was used to compare the IgA levels of each group with different feeding methods as describing later.

(2) Protein measurement

Sample solutions are assayed in duplicate as well as measurement of sIgA. After incubation at room temperature for 5 minutes measured absorbance at 595nm. The value of concentration was read according to standard curve for the Protein Assay (Bio-Rad). Measurement method is summarized as follows:

a) Mucous emulsified saline 0.1ml

+

Bio-Rad reagent 5 ml

↓

Incubate for 5 minutes at room temperature

b) Absorption at OD595 is read. Then protein concentration was read from standard calibration curve.

Therefore dilution rate were also different from each samples that are depending on the amount of pharyngeal mucous as well as sIgA concentrations.

3. Calculation of sIgA and protein (μ g)/ mucous wet weight (mg), and sIgA/protein ratio

(1) Calculation of sIgA (μ g)/ mucous wet weight(mg)

The concentration of sIgA in saline depends on the amount of mucous collected from pharyngeal mucosa. It is assumed that one out of ten mucous of cotton swab is emulsified into saline. Therefore the weight of sIgA (μ g) / wet mucous weight (mg) will be derived from the mucous weight of collected pharyngeal mucosa (M) and the concentration of sIgA emulsified in saline(sIgA) as follows.

SIgA weight (μ g) / mucous wet weight (mg) = (IgA/ M) $\times$ 10

(2) Calculation of protein/ mucous wet weight (mg)

The concentration of protein in saline depends on the amount of mucous

collected from pharyngeal mucosa. As aforementioned it is assumed that one out of ten mucous of cotton swab is emulsified into saline. Therefore the weight of protein(μ g) / wet mucous weight (mg) will be derived from the mucous weight of collected pharyngeal mucosa (M) and the concentration of protein emulsified in saline(Pro) as follows.

protein weight (μ g) / mucous wet weight (mg) = (Pro/ M) $\times$ 10

(3) Calculation of sIgA /protein ratio

It is needed to correct two factors that influence the sIgA content of mucous; first is the amount of collected mucous and second is rate of emulsified sIgA into saline. In addition to these two factors, there may be natural factors that affect the content of sIgA in mucous. The concentration of sIgA is adjusted to a uniform protein level of pharyngeal mucous by expressing them as sIgA /protein ratio. The sIgA /protein ratio seems to be good indicator of sIgA levels of pharyngeal mucous. According to my speculation extragenous sIgA such as BMsIgA will increase the value of the ratio.

4. Descriptive and analytical statistics

Descriptive and analytical statistics were computed by using micro soft

Excel.

Mean values and standard deviation of weight of mucous, concentration of sIgA and protein, sIgA(μ g) /mucous(mg) and protein(μ g)/mucous(mg) and sIgA /protein ratio were observed in each infants group. Integrated infants groups of 1·2 months are compared by analysis of valiance.

Results

1. Description of data on each infant

All data on each infant are showed in Table 1~3 by age groups of 1, 2 and 3~6 months. The wet weight of collected pharyngeal mucous, the concentration of sIgA and protein, calculated value of sIgA(μ g) /wet mucous (mg) , Protein(μ g) /wet mucous (mg) and sIgA/ protein ratio are presented

2. Descriptive statistics of sIgA levels

Descriptive statistics of sIgA levels in each age group are presented by sex.

(1) One·month old infants (Table 4)

1) Wet weight of collected pharyngeal mucous

The mean and standard deviation of collected mucous weight in breastfed,

partially

breast-fed and formula-fed infants, irrespective of sex, were 16.5±4.2, 15.0±4.8 and 18.6±11.4, respectively.

2) Concentration of sIgA in saline

The mean and standard deviation of the sIgA concentration in breastfed, partially breast-fed and formula-fed infants, irrespective of sex were, 2.16±1.18, 2.77±2.19 and 1.53±0.89, respectively. The sIgA concentration does not usually distributed normal distribution. Although geometric mean and standard deviation is better considered, only arithmetic mean and standard deviation are presented.

3) Concentration of protein in saline

The mean and standard deviation of the protein concentration in breastfed,

partially breast-fed and formula-fed infants, irrespective, of sex were, 45.7± 13.1, 43.8±11.1 and 41.5±2.6, respectively. The variation of concentration of protein was relatively small except for the group of formula-fed infants.

4) SIgA(μg) /wet mucous (mg)

The mean and standard deviation of sIgA(μg) / mucous wet weight(mg)

in breastfed, partially breast-fed and formula-fed infants were, irrespective of sex, 0.13±0.10, 0.20±0.17 and 0.11±0.07, respectively. The variation of sIgA(μg) / wet mucous (mg) were relatively large.

5) Protein(μg)/ wet mucous(mg)

The mean and standard deviation of protein(μg)/ mucous(mg) in breastfed, partially breast-fed and formula-fed infants, irrespective of sex, were 3.1±1.4, 3.1±1.0 and 2.7±1.1, respectively.

6) SIgA / protein ratio

The mean and standard deviation of sIgA / protein ratio in breastfed, partially breast-fed and formula-fed infants, irrespective of sex, were 0.051±0.031, 0.073±0.073 and 0.037±0.022, respectively. SIgA constitute as much as 4-7 % of total protein of throat swab.

(2) Two months- old infants (Table 5)

1) Wet weight of collected pharyngeal mucous

The mean and standard deviation of collected mucous weight in breastfed, partially

breast-fed and formula-fed infants, irrespective ,of sex were, 15.2±6.4, 19.5±8.6 and 20.0±7, respectively.

2) Concentration of sIgA in saline

The mean and standard deviation of concentration of sIgA in saline in breastfed,

partially breast-fed and formula-fed infants, irrespective of sex were, 2.12±1.87, 2.85±2.86 and 2.53±1.65, respectively.

3) Concentration of protein in saline

The mean and standard deviation of concentration of protein in saline in breastfed, partially breast-fed and formula-fed infants, irrespective of sex were,43.6±14.8,49.6±5.5 and 48.5±10.3, respectively.

4) SIgA(μg) / wet mucous (mg)

The mean and standard deviation of sIgA(μg) / wet mucous (mg) in breastfed, partially breast-fed and formula-fed infants, irrespective of sex were, 0.16±0.17, 0.18±0.017 and 0.17±0.10, respectively.

5) Protein(μg)/ wet mucous (mg)

The mean and standard deviation of protein(μg)/ mucous(mg) in breastfed, partially breast-fed and formula-fed infants irrespective of sex, were

3.2±1.4, 3.0±1.2 and 2.6±0.9, respectively. The variation was small relative to sIgA(μ g) / wet mucous (mg).

6) SIgA/protein ratio

The mean and standard deviation of sIgA / protein ratio (μ g) in breastfed, partially breast-fed and formula-fed infants, irrespective, of sex were, 0.058± 0.053, 0.054±0.052 and 0.058±0.040, respectively. SIgA constitute as much as 5-6 % of total protein of throat swab.

(3) Three to six months- old infants

1) Wet weight of collected pharyngeal mucous (Table 6)

The mean and standard deviation of wet weight of collected pharyngeal mucous in breast-fed, partially breastfed and formula-fed infant, irrespective of sex, were 23.7±12.7, 35.0±12.3 and 11.0 (n=1), respectively.

2) Concentration of sIgA in saline

The mean and standard deviation of concentration of sIgA in saline in breast-fed and formula-fed infant, irrespective of sex, were 1.42±0.70, 1.51± 1.10 and 3.00 (n=1), respectively

3) Concentration of protein in saline

The mean and standard deviation of concentration of protein in saline in breast-fed, partially breastfed and formula-fed infant, irrespective of sex, were 32.4±9.3, 51.1±4.2 and 41.9 (n=1), respectively

4) SIgA(μ g)/ wet mucous (mg)

The mean and standard deviation of sIgA(μ g)/ wet mucous (mg) in breast-fed, partially breastfed and formula-fed infant, irrespective of sex, were 0.05±0.02, 0.04±0.03 and 0.27 (n=1), respectively

5) Protein(μ g)/ wet mucous (mg)

The mean and standard deviation of protein(μ g)/ wet mucous (mg) in breast-fed, partially breastfed and formula-fed infant, irrespective of sex, were 2.0±1.5, 1.6±0.6 and 3.8 (n=1), respectively

6) SIgA/protein ratio

The mean and standard deviation of sIgA/protein ratio in breast-fed, partially breastfed and formula-fed infants, irrespective of sex, were 0.045± 0.019, 0.031±0.023 and 0.07 (n=1), respectively. SIgA constitute as much as 2-7 % of total protein of throat swab.

3. Analytical statistics of sIgA levels in one to two months- old infants (Table 7)

Two groups of one month and two months-old infants were integrated into one group because of following reasons. Firstly both groups showed the same tendency in terms of the level of sIgA. Secondary the numbers of group have to be large enough to compare by feeding methods statistically.

1) Wet weight of collected pharyngeal mucous

The mean and standard error of wet weight of collected pharyngeal mucous in breast-fed, partially breastfed and formula-fed infants, irrespective of sex, were 15.8±1.3, 16.8±1.7 and 19.1±3.4, respectively.

2) Concentration of sIgA in saline

The mean and standard error of wet weight of collected pharyngeal mucous in breast-fed, partially breastfed and formula-fed infants, irrespective of sex, were 2.14±0.37, 2.80±0.61 and 1.91±0.43, respectively. The concentration of SIgA did not differ among the different feeding methods with the highest value was found in partially breastfed infants.

3) Concentration of protein in saline

The mean and standard error of concentration of protein in saline in breast-fed, partially breastfed and formula-fed infants, irrespective of sex, were 44.5±3.2, 46.1±2.5, and 44.1±2.4, respectively. As shown in Table 7, the

mean concentration of total protein was not different among infants with different feeding methods.

4) SIgA (μ g)/ wet mucous (mg)

The mean and standard error of sIgA (μ g)/ wet mucous (mg) in breast-fed, partially breastfed and formula-fed infants, irrespective of sex, were 0.15±0.03, 0.19±0.04, and 0.12±0.03, respectively.

5) Protein(μ g)/ wet mucous (mg)

The mean and standard error of protein(μ g)/ wet mucous (mg) in breast-fed and formula-fed infants, irrespective of sex, were 3.0±0.3, 3.0±0.3, and 2.7±0.3, respectively.

6) SIgA/protein ratio

The mean and standard error of concentration of protein in saline in breast-fed, partilly breastfed and formula-fed infants, irrespective of sex, were 0.055±0.010, 0.065±0.017, and 0.045±0.010, respectively. SIgA for protein ratio was not significantly different among infants with the different feeding methods. SIgA constitute as much as 5-7 % of total protein of mucous.

Discussion

SIgA plays an essential role in the local defense mechanism and is part of the immunological system. In older literature sIgA is called an "antiseptic paint" or extraneous coat" of the intestinal mucosa, because sIgA is always found in the same locations as extracellular mucus on the epithelial and glandular surfaces of the gut. SIgA is painting the surface of respiratory and urinary tract mucosa as well as digestive mucosa. On the other hand infant-feeding methods is one of the first and most significant influences on the pattern of development of the mucosal immune system. IgA is foremost of protective proteins such as lactoferrin and lysozymes in human milk. With regard to the influence of breast milk, BMsIgA antibodies are directed against a wide variety of microbial antigens, protecting infants from the infections.

There are some reports about influence of breast milk on the level of sIgA in gastrointestinal tract so far.

1) The levels of sIgA in feces

Hanebrge (3) and Koutras & Vigorta(4) reported studies about immunoglobulin in feces from infants fed human or bovine milk. According to Hanebrge the concentration of sIgA in extracts of feces from infant receiving

breast milk were higher than in feces from infants fed formula milk. In his report rabbit antisera to human secretary component IgA was measured. According to Koutras & Vigorta , the levels of fecal sIgA in breast milk were also higher than that in formula-fed infant. With regard to the sIgA in the gastrointestinal tract, Koutras and Vijorita speculated that this is not simply due to the presence of IgA in breast milk but also the stimulatory effect of breast milk on the gastrointestinal humoral immunological development.

At birth no fecal sIgA was detected in all infants. The BMsIgA may pass through the whole in gastrointestinal tract of recipient infants after start of feeding. At 2, 4 and 8 weeks fecal sIgA in breast milk were significantly higher than in formula feeding. However those reported results do not indicate that BMsIgA bind in mucosa for a time and present at same location.

2) The levels of salivary IgA

Gleeson et al (2) investigated the effects of feeding on the developmental pattern of mucosal immunity. According to their report, the median levels of salivary IgA for the formula-fed group were consistently higher than median levels for the breast and partially breastfed-fed groups for all age classes from birth to 6 weeks of age. There was not significant difference in the concentration

of albumin among infants with different feeding groups. They excluded the specimens contaminated with breast milk that was identified by immunoelctrophoresis.

In the study of Kuitnen et al (5), there was no statistically significant difference of the salivary IgA between breast milk and cow's milk-fed infants. However, breast-fed infants had higher levels of feces IgA than formula-fed infants had in the study group. Greeson et al (6) also observed that salivary IgA was significantly greater during infection period compared with non-infection period. Although it is not clear if the salivery IgA was measured as total IgA in their report, the results indicate that the active secretary nature of IgA in response to environmental antigens such as formula milk or infectious microorganism. Because the albumin concentrations in saliva that reflect a passive leak of serum albumin, was not changed during infection.

When BMsIgA coat recipient infant's pharyngeal mucosa just like as a secreted sIgA of mucous, we can measure sIgA as a sum of BMsIgA and sIgA produced by infant self. We thought BMsIgA is able to bind and present in the same locations of oral pharynx as extragenous sIgA of mucous on the mucosal surface as well as gastrointestinal tract. I speculate the level of sIgA in mucous

collected by throat swab in breastfed infants is higher than that in formula-fed infants.

Discussions of the study is as follows.

1. Are the subjects appropriate for the study?

The most appropriate subjects are thought to be infants including neonates to confirm the influence of BMsIgA on the level of sIgA in recipient infant, for IgA production of neonates is not begun in neonate or amount of production is very small. Thus we can easily compare the influence of extragenous sIgA such as BMsIgA. As a study design prospective cohort study is ideal. However it was difficult to get informed consent from mothers of neonates. It is also impossible to carry experimental study for infants to assign the different feeding methods at random. In the present study, subjects were one to six-months old infants. Observational and cross sectional analysis was carried out in order to evaluate the level of BMsIgA among infants with different feeding methods. The study was a preliminary report to generate hypothesis about sIgA levels of pharyngeal mucous in breastfed infants.

With regard to the formula, the contents and percentage of each nutrient of formula are regulated by regulation in Japan. Although there are 4 major

company of milk production, the contents are almost the same. Therefore We did not make subgroup of the infants according to the formula.

The numbers of studied infants are not big enough to compare statistically when they divided into subgroups by age and sex. Therefore one and two-months old infants are integrated to compare statistically at the present time. Furthermore, in addition to those limitations of the present project report, it has following limits.

(1) The comparison by gender was not carried out because of small number of male.

(2) Although the prospective study design study is ideal. The repeated investigation on selected infants was not carried out.

(3) The throat swab immediately after feeding was not get, because mothers dislike vomiting of infants that often introduced by throat swab. Although naso-pharynx is better as sample collecting location in terms of respiratory tract infection, oral pharynx is selected for convenience.

2. Can throat swab collect enough mucous ?

It is difficult to collect appropriate specimens to detect antibody in mucous.

There are a few study of sIgA other than salivery IgA and there is not a report of the concentration of mucus sIgA. Most studies have been carried out about sIgA of saliva from the buccal cavity mainly so far. The salivary glands have been recognized as a part of the common mucosal immune system. SIgA of saliva is undetectable at birth, rising during the first year then leveled off through out childhood. Measurement of salivary secretary IgA may be useful for assessing the development in mucosal immunity in infants. However interpretation of concentration needs some careful correction. Because the concentration of sIgA may be modified by many physiologic factors such as alterations in flow rate or dehydration. Gleeson et al (7) described that the level of albumin or total protein in saliva have been used as a correction factor to adjust for the influence of physiological variations such as flow rate on the concentration of individual protein in saliva. Furthermore, in infants the saliva collection by gentle suction from the buccal cavity of the mouth with the aid of a soft plastic pipette is difficult.

In the present study we used throat swab as a method to collect a sample of pharyngeal mucus to measure mucosal sIgA. In the study of mucous, mucous collection is very difficult. As Professor Bo Darer wrote to me,

absorption of mucous onto cotton lead to assay problems. Quantification is difficult. We tried quantificate the collected mucous by measuring the weight of applicator. Although 20 mg of mucous was collected by throat cotton swabs, we had to assumpt that 1/5 - 1/10 are emulsified into saline.

There has been no report of sIgA concentration of throat swab so far. The method used in the study seems to be useful for collecting mucous specimens from the throat of infants. Variability of weight of sample specimens of throat swab was relatively small. SIgA concentration can be adjusted by calculating the content of sIgA per mg of mucous. Furthermore the concentration of total protein was measured for assessing and correcting for possible influencing factors. Variability of the total protein was also small relative to sIgA concentration.

3. Is measurement method of sIgA appropriate?

The sum of sIgA derived from breast milk and sIgA produced by infant self have to be measured. Antigen for secretary component seems to be appropriate to detect sIgA. We measured sIgA after emulsified into saline. This results in assay problems. It is assumed that one out of ten mucous specimens are

emulsified into saline.

4. Which is appropriate indicator of mucous sIgA?

The amount of pharyngeal mucous collected by throat swab are different from samples. Therefore the weighing of collected mucous was necessary. The difference of weight between before and after collecting pharyngeal mucosal samples was used for calculation. The IgA concentration was measured and weight of sIgA was estimated by assuming that 1/10 of mucous was emulsified into saline. SIgA /mucous was estimated to correct the influence on the difference of the amount of collected mucous. There is another factor that influence of sIgA levels. That is the rate of emulsion from absorbed mucous of cotton into saline. Therefore, as an indicator of pharyngeal mucous sIgA, sIgA/protein ratio seems to be appropriate.

5. How was the results of sIgA /protein ratio among infants with different feeding methods?

The levels of sIgA in pharyngeal mucous IgA expressed by sIgA/protein ratio were 3·7 % for protein in pharyngeal mucous. The ratio did not differ

among infants with different feeding methods. Although we did not measure serum IgA concentration, measurement of sIgA of throat swab provides sum of the IgA provided local mucous secretion and received from material BMsIgA. These results may therefore suggest that sIgA via mother's breast milk does not stay in the same locations of mucosa of recipient infants in spite of sIgA's high affinity to mucosal mucin. BMsIgA may present in pharyngeal epithelial mucous of recipient infants, but it seems to be transitional and do not influence the level of sIgA of mucous quantitatively.

On the bases of above discussions, we conclude that the ratio of sIgA/protein in pharyngeal mucous of the present study was similar level of that in saliva, being not different among infants with different feeding methods. Because present study has limitation of the small number of subjects, further detailed studies are necessary to confirm this in a larger trial in the future.

In terms of the role of breast-feeding on the development of immune response, breast milk may mainly stimulate the production of specific sIgA. The ontogeny patterns for specific sIgA antibodies differ from the pattern for salivery total amount of sIgA. For example salivery IgA antibodies to *Escherichia coli* O

antigens remain low in children from developed countries, and do not parallel the ontogeny patterns for total salivery IgA. It is reasonable to observe qualitative difference of sIgA as well as total sIgA for assessing the immunological development among infants with different feeding methods. Breast milk may stimulate and produce specific sIgA in recipient infants but that does not influence the level of total sIgA concentration.

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Table 1. SIgA levels in pharyngeal mucous of one-month old infants

Breastfed infants

NO	Sex	Wt	IgA	Protein	IgA/M	Pro/M	IgA/Pro
1	F	13	4.30	60.5	0.33	4.65	0.071
2	M	14	3.00	55.0	0.21	3.93	0.055
3	F	10	0.70	52.5	0.07	5.25	0.013
4	F	19	2.20	51.0	0.12	2.68	0.043
5	M	18	1.85	53.0	0.10	2.94	0.035
6	F	15	0.70	35.0	0.02	2.33	0.020
7	F	20	2.13	37.0	0.12	1.85	0.058
8	F	23	2.43	21.5	0.11	1.37	0.113

Partially breastfed (mixed-fed) infant

NO	Sex	Wt	IgA	Protein	IgA/M	Pro/M	IgA/Pro
1	F	12	3.95	47.5	0.33	3.96	0.083
2	M	10	0.55	52.7	0.06	5.27	0.010
3	F	14	1.50	45.0	0.11	3.21	0.033
4	F	16	0.15	41.5	0.01	2.59	0.004
5	F	18	5.80	60.0	0.32	3.33	0.097
6	F	23	0.70	46.5	0.03	2.02	0.015
7	F	21	4.15	47.3	0.20	2.25	0.088
8	F	10	2.55	30.0	0.26	3.00	0.085
9	M	11	5.60	23.3	0.51	2.12	0.240

Formula-fed infants

NO	Sex	Wt	IgA	Protein	IgA/M	Pro/M	IgA/Pro
1	F	38	0.35	43.0	0.01	1.13	0.008
2	F	15	2.80	42.8	0.19	2.85	0.065
3	M	10	1.85	36.8	0.19	3.68	0.050
4	F	11	1.30	42.7	0.12	3.88	0.030
5	M	19	1.35	42.0	0.07	2.21	0.032

Wt: weight of collected pharyngeal mucous (mg)

IgA: concentration of sIgA in saline (μ g/ml)

Protein: concentration of protein in saline (μ g/ml)

IgA/M: content of sIgA (μ g) in saline / weight of collected pharyngeal mucous (mg)

Pro/M: content of protein (μ g) in saline/ weight of collected pharyngeal mucous (mg)

IgA/Pro: ratio of sIgA / protein in saline

Table 2. SIgA levels in pharyngeal mucous of two-months old infants

Breastfed infants

NO	Sex	Wt	IgA	Protein	IgA/M	Pro/M	IgA/Pro
1	F	11	0.30	44.3	0.03	4.03	0.007
2	M	10	0.11	51.2	0.01	5.12	0.002
3	M	10	5.80	47.3	0.58	4.73	0.123
4	F	11	1.30	49.0	0.12	4.45	0.027
5	F	11	1.60	40.0	0.15	3.64	0.040
6	F	27	1.35	70.0	0.05	2.59	0.019
7	M	24	2.05	54.0	0.09	2.25	0.038
8	M	11	1.60	15.0	0.15	1.36	0.107
9	F	17	5.10	32.5	0.30	1.91	0.157
10	F	20	1.95	32.5	0.01	1.63	0.060

Partially breastfed (mixed-fed) infants

NO	Sex	Wt	IgA	Protein	IgA/M	Pro/M	IgA/Pro
1	F	23	7.20	52.7	0.31	2.29	0.137
2	M	13	5.60	57.5	0.43	4.42	0.097
3	F	24	0.70	44.0	0.03	1.83	0.016
4	F	12	0.70	42.7	0.06	3.56	0.016
5	F	33	0.70	50.8	0.02	1.54	0.014
6	M	12	2.20	49.7	0.18	4.14	0.044

Formula-fed infants

NO	Sex	Wt	IgA	Protein	IgA/M	Pro/M	IgA/Pro
1	F	28	3.90	45.5	0.14	1.63	0.086
2	F	13	3.00	40.0	0.23	3.08	0.075
3	F	19	0.70	60.0	0.04	3.16	0.012

Wt: weight of collected pharyngeal mucous (mg)

IgA: concentration of sIgA in saline (μ g/ml)

Protein: concentration of protein in saline (μ g/ml)

IgA/M: content of sIgA (μ g) in saline / weight of collected pharyngeal mucous (mg)

Pro/M: content of protein (μ g) in saline/ weight of collected pharyngeal mucous (mg)

IgA/Pro: ratio of sIgA / protein in saline

Table 3. SIgA levels in pharyngeal mucous of three to six-months old infants

Breastfed infants

NO	Sex	Wt	IgA	Protein	IgA/M	Pro/M	IgA/Pro
1	M	10	1.95	42.5	0.02	4.25	0.046
2	M	39	2.45	41.9	0.06	1.07	0.058
3	F	17	0.70	30.0	0.04	1.76	0.023
4	F	36	1.15	18.5	0.03	0.51	0.062
5	M	11	0.70	35.0	0.06	3.18	0.020
6	F	29	1.57	26.5	0.05	0.91	0.059

Partially breastfed (mixed-fed) infants

NO	Sex	Wt	IgA	Protein	IgA/M	Pro/M	IgA/Pro
1	M	37	2.70	48.1	0.07	1.30	0.056
2	F	22	0.12	52.1	0.01	2.37	0.002
3	F	51	1.95	47.5	0.04	0.93	0.041
4	M	30	1.30	56.5	0.04	1.88	0.023

Formula-fed infants

NO	Sex	Wt	IgA	Protein	IgA/M	Pro/M	IgA/Pro
1	M	11	3.00	41.9	0.27	3.81	0.070

Wt: weight of collected pharyngeal mucous (mg)

IgA: concentration of sIgA in saline (μ g/ml)

Protein: concentration of protein in saline (μ g/ml)

IgA/M: content of sIgA (μ g) in saline / weight of collected pharyngeal mucous (mg)

Pro/M: content of protein (μ g) in saline/ weight of collected pharyngeal mucous (mg)

IgA/Pro: ratio of sIgA / protein in saline

Table 4. SIgA levels in pharyngeal mucous of infants aged one month by feeding methods and sex

Mean±Standard deviation

Feeding	Sex	No	Wt	IgA	Protein	IgA/M	Pro/M	IgA/Pro
Breastfed	Male	2	16.0±2.8	2.43±0.81	54.0±1.4	0.16±0.08	3.4±0.7	0.045±0.014
	Female	6	16.7±4.8	2.08±1.33	42.9±14.3	0.12±0.10	3.0±1.6	0.053±0.036
	Total	8	16.5±4.2	2.16±1.18	45.7±13.1	0.13±0.10	3.1±1.4	0.051±0.031
Mixed-fed	Male	2	10.5±0.7	3.08±3.57	38.0±20.8	0.28±0.32	3.7±2.2	0.125±0.163
	Female	7	16.3±4.7	2.69±2.05	45.4±8.9	0.18±0.13	2.9±0.7	0.058±0.039
	Total	9	15.0±4.8	2.77±2.19	43.8±11.1	0.20±0.17	3.1±1.0	0.073±0.073
Formula-fed	Male	2	14.5±6.4	1.60±0.35	39.4±3.7	0.13±0.08	3.0±1.0	0.041±0.012
	Female	3	21.3±14.6	1.48±1.24	42.8±0.2	0.11±0.09	2.6±1.4	0.034±0.029
	Total	5	18.6±11.4	1.53±0.89	41.5±2.6	0.11±0.07	2.7±1.1	0.037±0.022

Mixed-fed: partially breastfed infant

Wt: weight of collected pharyngeal mucous (mg)

IgA: concentration of sIgA in saline (μg/ml)

Protein: concentration of protein in saline (μg/ml)

IgA/M: content of sIgA (μg) in saline / weight of collected pharyngeal mucous (mg)

Pro/M: content of protein (μg) in saline/ weight of collected pharyngeal mucous (mg)

IgA/Pro: ratio of sIgA / protein in saline

Table 5. SIgA levels in pharyngeal mucous of infants aged two month by feeding methods and sex

Mean±Standard deviation

Feeding	Sex	No	Wt	IgA	Protein	IgA/M	Pro/M	IgA/Pro
Breastfed	Male	4	13.8±6.8	2.39±2.42	41.9±18.1	0.21±0.26	3.4±1.8	0.068±0.057
	Female	6	16.2±6.5	1.93±1.65	44.7±14.0	0.12±0.10	3.0±1.2	0.052±0.054
	Total	10	15.2±6.4	2.12±1.87	43.6±14.8	0.16±0.17	3.2±1.4	0.058±0.053
Mixed-fed	Male	2	12.5±0.7	3.90±2.40	53.6±5.5	0.31±0.18	4.3±0.2	0.071±0.037
	Female	4	23.0±8.6	2.32±3.25	47.6±4.9	0.11±0.14	2.3±0.9	0.046±0.061
	Total	6	19.5±8.6	2.85±2.86	49.6±5.5	0.18±0.17	3.0±1.2	0.054±0.052
Formula-fed	Male	0	-	-	-	-	-	-
	Female	3	20.0±7.5	2.53±1.65	48.5±10.3	0.14±0.10	2.6±0.9	0.058±0.040
	Total	3	20.0±7.5	2.53±1.65	48.5±10.3	0.14±0.10	2.6±0.9	0.058±0.040

Mixed-fed: partially breastfed infant

Wt: weight of collected pharyngeal mucous (mg)

IgA: concentration of sIgA in saline (μ g/ml)

Protein: concentration of protein in saline (μ g/ml)

IgA/M: content of sIgA (μ g) in saline / weight of collected pharyngeal mucous (mg)

Pro/M: content of protein (μ g) in saline/ weight of collected pharyngeal mucous (mg)

IgA/Pro: ratio of sIgA / protein in saline

Table 6. SIgA levels in pharyngeal mucous of infants aged two month by feeding methods and sex method and sex

Three-month

Mean±Standard deviation

Feeding	Sex	No	Wt	IgA	Protein	IgA/M	Pro/M	IgA/Pro
Breastfed	Male	3	20.0±16.5	1.70±0.90	39.8±4.2	0.05±0.03	2.8±1.6	0.041±0.019
	Female	3	27.3±9.6	1.14±0.44	25.0±5.9	0.04±0.01	1.1±0.6	0.048±0.022
	Total	6	23.7±12.7	1.42±0.70	32.4±9.3	0.05±0.02	2.0±1.5	0.045±0.019
Mixed-fed	Male	2	33.5±4.9	2.00±1.00	52.3±5.9	0.06±0.02	1.6±0.4	0.040±0.023
	Female	2	36.5±20.5	1.04±1.29	49.8±3.3	0.02±0.02	1.7±1.0	0.022±0.028
	Total	4	35.0±12.3	1.51±1.10	51.1±4.2	0.04±0.03	1.6±0.6	0.031±0.023
Formula-fed	Male	1	11	3.00	41.9	0.27	3.8	0.072
	Female	0	-	-	-	-	-	-
	Total	1	11	3.00	41.9	0.27	3.8	0.072

Mixed-fed: partially breastfed infant

Wt: weight of collected pharyngeal mucous (mg)

IgA: concentration of sIgA in saline (μg/ml)

Protein: concentration of protein in saline (μg/ml)

IgA/M: content of sIgA (μg) in saline / weight of collected pharyngeal mucous (mg)

Pro/M: content of protein (μg) in saline/ weight of collected pharyngeal mucous (mg)

IgA/Pro: ratio of sIgA / protein in saline

Table7 Comparison of sIgA levels in infants with different feeding methods at the age of 1-2 months

Mean±Standard error

Feeding	No	Wt	IgA	Protein	IgA/M	Pro/M	IgA/Pro
Breastfed	18	15.8±1.3	2.14±0.37	44.5±3.2	0.15±0.03	3.1±0.3	0.055±0.010
Mixed fed	15	16.8±1.7	2.80±0.61	46.1±2.5	0.19±0.04	3.0±0.3	0.065±0.017
Formula fed	8	19.1±3.4	1.91±0.43	44.1±2.4	0.12±0.03	2.7±0.3	0.045±0.010

Mixed-fed: partially breastfed infant

Wt: weight of collected pharyngeal mucous (mg)

IgA: concentration of sIgA in saline (μ g/ml)

Protein: concentration of protein in saline (μ g/ml)

IgA/M: content of sIgA (μ g) in saline / weight of collected pharyngeal mucous (mg)

Pro/M: content of protein (μ g) in saline/ weight of collected pharyngeal mucous (mg)

IgA/Pro: ratio of sIgA / protein in saline