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Reaction of the Sera of the Korean Children Free from Hib Invasive Diseases against H. influenzae type B Capsular Polysaccharide Antigen

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The purpose of our experiment is to examine the level of anti-Haemophilus influenza polysaccharide antibody titer in the Korean population. Using ELISA, the level of Hib-PS antibodies in 384 infants and children who were all free from Hib invasive diseases, was tested. And the blood of 50 mothers within 24 hours of delivery and cord blood from their respective full-term neonates was also tested. The transport of Hib-PS IgG and IgG subclasses in paired sera from mothers and neonates was also measured. The titer of Hib-PS IgG varies with age. At birth the mean optical density of cord blood was 1.028 ; however, it declined to 0.609 up to 6 months and further decline was noted up to 2 years to 0.488. Then the mean O.D. remained around 0.5 from 3 to 14 years of age. The mean O.D. of Hib-PS IgG in the mothers blood was 0.856. The ratio of mean O.D. of anti-Hib PS IgG antibody in the cord blood to that in the maternal blood was 1.20. The mean optical densities of IgG subclasses were : 1.18 for anti-Hib PS IgG_1, 1.07 for anti-Hib PS IgG_2, 1.01 for anti-Hib PS IgG_3, and 1.09 for anti-Hib PS IgG_4. The sera from Korean children of almost all age groups reacted to Hib-PS antigen on ELISA. Also the active transport of anti-Hib PS IgG antibody through placenta was observed. Among four IgG subclasses, only IgG_1 transport had significant experimental meaning.

Key Words : *H. influenzae, anti-Hib-PS antibodies*

INTRODUCTION

Hemophilus influenzae type b (Hib) is an encapsulated gram-negative bacterium, and one of the most important causes of bacterial meningitis in infants and children in most industrialized and developing countries (Cochi et al., 1985 ; Rosenthal et al., 1988). It is also a common cause of epiglottitis, suppurative arthritis, cellulitis, bacteremia and pneumonia. Resistance to Hib infection depends upon the host defenses, such as mucosal factors, activation of complement-mediated responses, mucosal and humoral antibodies, phagocytosis and killing by macrophages and polymorphonuclear cells, and cell mediated immunity. Fothergill and Wright (Fothergill and Wright, 1933) in 1933 hypothesized that bactericidal activity was responsi-

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ble for immunity to Hib meningitis. Considerable evidence disclosed that antibody to the type b capsular polysaccharide has the most important protective specificity (Schneerson et al., 1971 ; Peltola et al., 1977 ; Peltola et al., 1984) : it activates complement, opsonophagocytosis, bactericidal effect, and protects animals from lethal Hib challenge (Weinberg et al., 1986 ; Noel et al., 1988).

The acquisition of the antibody is influenced by the duration and type of exposure, the rate of antigen clearance and, most importantly, the age of the individual. It has been understood that maternally acquired antibody is present in infants less than 6 months of age, and the antibody is "naturally" acquired by most children between 3 and 5 years of age(Kuklinska and kuklinska, 1984). This explains a peak incidence of Hib diseases in infants and young children.

The polysaccharide capsule of Hib(Hib-PS) is a carbohydrate antigen and similar carbohydrate antigens have been reported to elicit IgG antibodies relatively restricted to the IgG_2 subclass in man, and children with selective deficiency of IgG_2 are prone to develop recurrent infections caused by encapsulated bacteria such as Hib or *Streptococcus pneumoniae* (Shackelford et al., 1986). Several investigators have found that the transport of IgG_1 exceeds that of IgG_2 and the affinity of the trophoblast Fc receptors for IgG_1 was found to be higher than for IgG_2 (McNabb et al., 1976).

In Korea, the prevalence of Hib infection in children and the natural immunity to Hib in the general population have not been studied. In this situation, routine immunization of commercially available Hib-conjugate vaccine could not be recommended.

The aim of this study is to check the O.D. of sera against Hib-PS antigen in age groups varying from newborns to adults in the Korean population and to measure the ratio of trasport of Hib IgG and its subclasses in mother and neonate paired sera.

MATERIALS AND METHODS

Serum

Venous blood was collected from 384 infants and children free from Hib invasive disease who were admitted to Yonsei University and Ewha Womans University Hospital for elective surgery. Venous blood was collected from fifty mothers within 24 hours of delivery, and cord blood from their respective full-term neonates in the same hospital. Sera were frozen at −20℃ until tested. All samples were run in duplicate. Mother-neonate pairs of sera were run in adjacent rows on the same microtiter plates.

Anti-Hib IgG antibody assay

Anti-Hib PS antibody was measured by ELISA. The antigen was polyribosylribitolphosphate-tyramine (PRP-tyramine) which was kindly provided by Dr. M. H. Nahm at Washington University School of Medicine, St Louis, MO, USA.

Each well on a 96 well polystyrene plate (Immunoplate, Nunc, Denmark) was coated with 100 ul of a 0.1 ug/ml solution of PRP-tyramine in 0.05 M carbonate buffer, pH 9.6, for 18 hours at 36℃ and washed with PTN(0.01 M phosphate buffer saline containing 0.05% Tween 20). Then it was blocked with PTNB (PTN containing 1% bovine serum albumin) at 36℃ for 1 h and washed three times with PTN. The plates were incubated at 36℃ for 1 h with 1 : 50 diluted sera in duplicate wells for 1 h and washed three times with PTN. To each well, one hundred ul of a 1 : 1,000 diluted alkaline phosphatase conjugated goat anti-human immunoglobulin G (Sigma, St. Louis, MO) was added. After 1 h incubation at 36℃, the plates were washed five times with PTN, then they were charged at 36℃ for 1 h with 0.1 ml of a *p*-nitrophenyl phosphate (1 mg/ml in 10% diethanolamine buffer, pH 9.8). Spectrophotometer was used to measure the absorbance at 410nm.

To eliminate the inter-plate variation, we have tested standard antigen and pooled sera along with the samples on every plate. In case that interplate OD variation was over 0.09, we discarded the results and repeated the tests.

Anti-Hib IgG subclass antibodies assay

ELISA was used to measure anti-Hib polysaccharide IgG_1, IgG_2, IgG_3, and IgG_4 antibodies. Each well on a 96 well polystyrene plate was coated with 100 ul of a 0.1 ug/ml PRP-tyramine solution in 0.05 M carbonate buffer, pH 9.6 and incubated at 36℃ for 16 h. The plates were then washed with PTN and blocked with PTNB for 1 h. One hundred ul of serum (1 : 10 dilution with PTNB for IgG_1 and IgG_2 and whole serum for IgG_3 and IgG_4) was added in duplicate wells and incubated at 36℃ for 1 h. The plates were washed three times with PTN and 0.1 ml of a 1 : 1,000 dilution of biotin

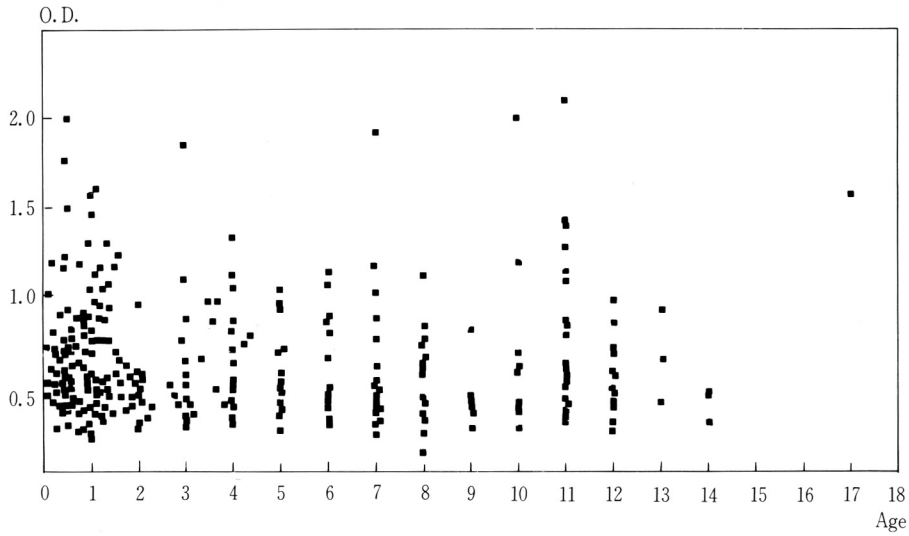

Fig. 1. Anti-Hemophilus capsular polysaccharide IgG antibody levels in healthy children by age groups.

labelled monoclonal mouse anti-human IgG subclass antibodies (Sigma, St. Louis, MO) was added. After 1 h incubation at 36℃, the plates were washed five times with PTN, and 0.1 ml of a 1 : 4,000 dilution of streptavidin peroxidase conjugated rabbit anti-mouse immunoglobulins (Sigma, St. Louis, MO) was added. The plates were washed five times with PTN, and incubated with 0.1 ml of a 0.4 mg/ml O-phenylene diamine hydrochloride in phosphate-citrate buffer, pH 5.0 for 20 minutes at room temperature. Lastly to stop the reaction, 50 ul of 2 N H_2SO_4 was added, and the absorbance at 490 nm was measured.

Statistical analysis

Student t-test for paired samples was used to compare the means of OD of the respective IgG and IgG subclasses in cord blood and maternal sera.

RESULTS

Anti-Hib-PS IgG antibody

As shown in Figure 1, the O.D. of sera against Hib-PS antigen varies with age. At birth, the mean optical density in cord blood was 1.028, but the titer declined to 0.609 up to 6 months of age and further decline was noted up to 2 years of age to 0.488.

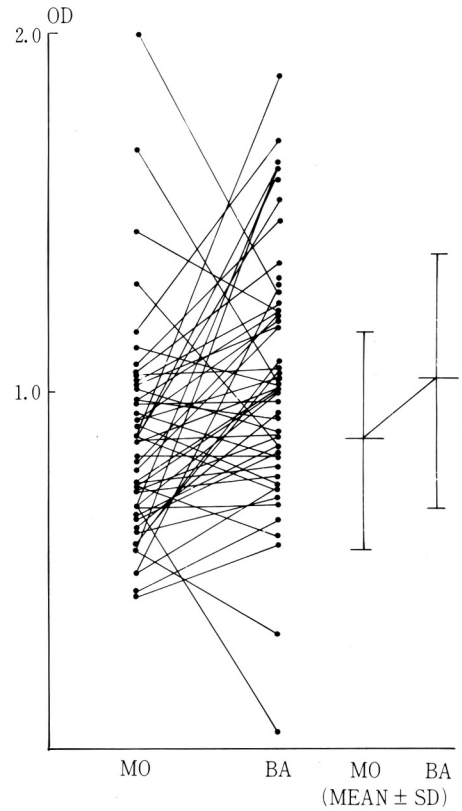

Fig. 2. Transplacental transfer of maternal IgG antibody (MO) against Hemophilus capsular polysaccharide to neonate (BA) ($P<0.05$)

Then the O.D. titier started to increase over 0.5 and then maintained a plateau until 14 years of age. There were no significant differences of O.D. between males and females in each age group (data not shown).

The mean O.D. of Hib-PS IgG of the mothers was 0.856, which was little higher than that of the teen age group. The mean cord/maternal O.D. ratio of anti-Hib PS IgG was 1.20 ; that is, the mean anti-Hib PS IgG was significantly higher in cord blood than in maternal blood ($P<0.05$), although there were some cases in which the ratio was reversed (Fig. 2).

Anti-Hib-PS IgG subclass antibodies in paired maternal and infant sera

To evaluate IgG subclass transportation of antibodies through placenta, anti-Hib PS IgG_1, IgG_2, IgG_3, and IgG_4 antibodies were measured by ELISA.

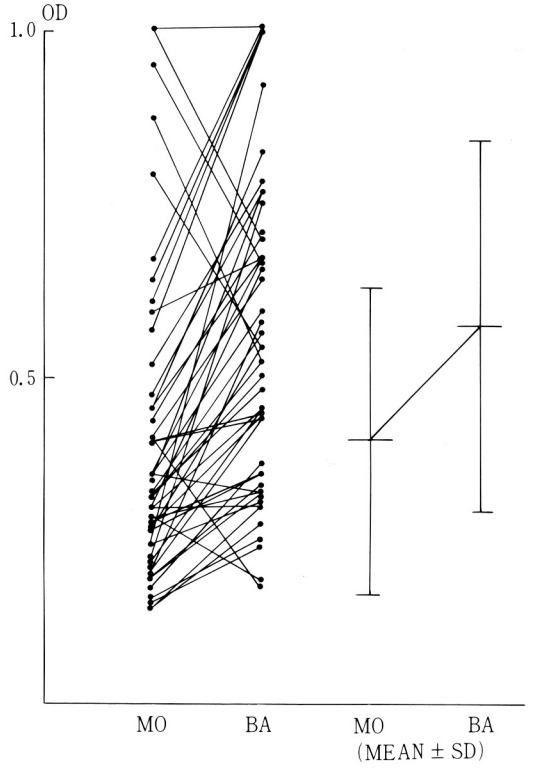

Fig. 3. Transplacental transfer of maternal IgG_1 antibody (MO) against Hemophilus capsular polysaccharide to neonate (BA) ($P<0.001$)

The transplacental transfer of maternal IgG_1 antibody against Hib-PS to neonates is shown in Figure 3, IgG_2 in Figure 4, IgG_3 in Figure 5 and IgG_4 in Figure 6. The mean cord/maternal O.D. ratio of anti-Hib PS IgG_1 was 1.18 and the mean O.D. of anti-Hib PS IgG_1 was significantly higher in cord blood than in maternal blood ($P<0.001$) (Fig. 3). The ratio of anti-Hib PS IgG_2 was 1.07, the ratio of IgG_3 was 1.01, and the ratio of IgG_4 was 1.09, and the values of anti-Hib PS IgG_2, IgG_3, and IgG_4 in cord blood was higher than those in maternal blood, but there were no statistical significances ($P>0.1$) (Fig 4,5, and 6).

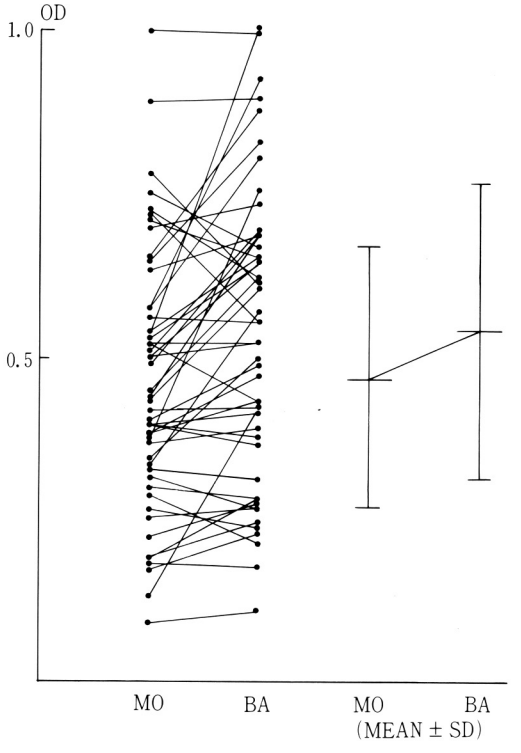

Fig. 4. Transplacental transfer of maternal IgG_2 antibody (MO) against Hemophilus capsular polysaccharide to neonate (BA) ($P>0.1$)

DISCUSSION

Fothergill and Wright (Fothergill and Wright, 1933) hypothesized that bactericidal activity was responsi-

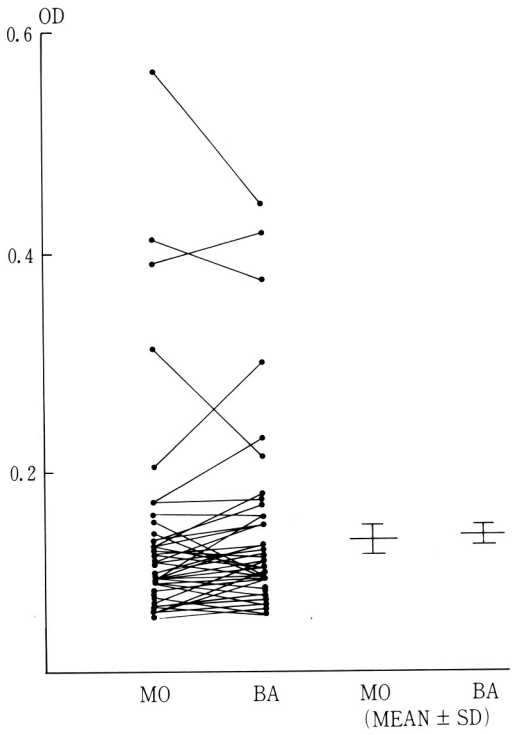

Fig. 5. Transplacental transfer of maternal IgG_3 antibody (MO) against Hemophilus capsular polysaccharide to neonate (BA) ($P>0.1$)

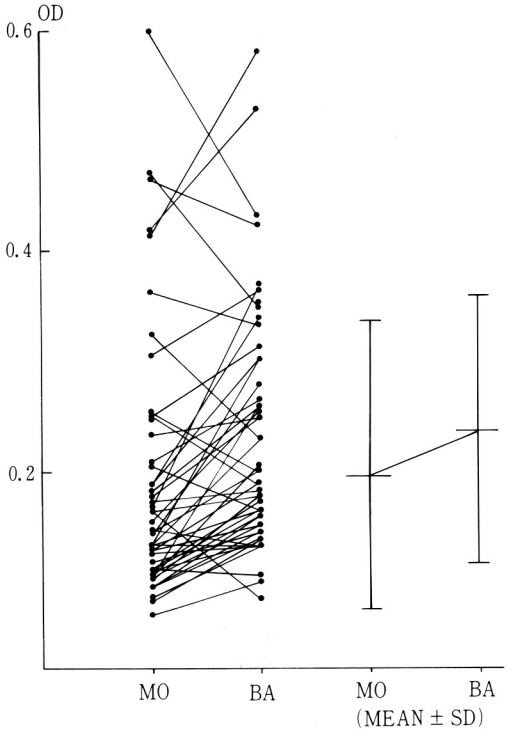

Fig. 6. Transplacental transfer of maternal IgG_4 antibody (MO) against Hemophilus capsular polysaccharide to neonate (BA) ($P>0.1$)

ble for immunity to Hib meningitis. And considerable evidence disclosed that serum antibody to the type b capsular polysaccharide, a linear polymer of ribose-(1→1)-ribitol-5-phosphate, is protective against invasive disease in general(Schneerson et al., 1971 ; Peltola et al., 1977 ; Peltola et al., 1984), and it can also opsonize (Noel et al., 1988) or directly lyse (Weinberg et al., 1986) the Hib organism in vitro.

The acquisition of this antibody is influenced by the duration and type of exposure, the rate of antigen clearance, and most importantly, the age of individuals. Although healthy young children less than 2 years of age respond well to various protein antigen, they show poor antibody response to polysaccharide (Robbins JB, 1978). It has been understood that maternally acquired antibody is present in infants less than 6 months of age, and the antibody is acquired naturally in most older children between the ages of 3 and 5 and in most adults. This explains a peak incidence of Hib diseases in infants and young children.

What is responsible for the natural production of these antibodies in late childhood or adulthood is not known. Antibodies to PRP are T-independent, and age-related immune responsiveness of T-independent antigens has been attributed to sequential maturation of different populations or lines of B lymphocytes. Contacts of an index case in a day care center had higher antibody titers compared to the control group(Redmond and Pichichero, 1984). Because these children were relatively well, it was suggested that nasopharyngeal carriage of *H. influenzae* type b without symptomatic infection can be immunogenic. Natural antibody may also result from colonization with enteric and/or nasopharyngeal organisms with antigens cross-reactive with capsular polysaccharide (Robbins JB et al., 1973).

There are groups at high risk for Hib disease including contacts of a case in a household (Ward et al., 1979) or day care center (Redmond and Pichichero, 1984), day care center attendees (Istre et al., 1985), twins (Kaplan and Mason, 1983), pa-

tients with sickle cell disease (Ward and Smith, 1976), certain racial groups (Ward et al., 1981 ; Losonsky et al., 1985 ; Ward et al., 1986), and certain immunocompromised patients. The reasons for this unusual disease incidence are not yet understood but may involve several factors, such as early exposure, increased bacterial pathogenicity, altered immunocompetence, or genetic susceptibility (Petersen et al., 1987).

Hib vaccine has been introduced since Hib infection causes many complications in the pediatric age group especially Hib Meningitis. Although there has been many studies of several *Haemophilus influenzae* type b conjugate vaccines in different populations, some researchers have suggested that Hib conjugate vaccines have differences in immunogenicity in different populations, and that vaccine might provide protection in one population but not in anothers (Granoff and Munson, 1986). After preventive effect of the newly developed vaccine has been proved, routine vaccination of Hib is now recommended in the United States and some European countries(Peter, 1992).

It was reported that Hib infection in neonates and children was relatively rare in Korea (Lee et al, 1983, Park et al, 1984). But these reports were made out only in local areas. General studies on Hib infection such as the prevalence or immunization rate have not been done in Korea yet. We have questioned the applicability to the general Korean population of assessments of vaccine efficacy in other populations. We tested the level of "natural" Hib-PS specific antibodies in age groups varying from newborns to adults in the Korean population. The values of anti-Hib-PS IgG varies with age. At birth, the O.D. in cord blood was 1.028, but the titer declined to 0.609 up to 6 months of age and further decline was noted up to 2 years of age as 0.488. Then the O.D. titer showed over 0.5 as a plateau until 14 years of age. But the mean O.D. value against Hib-PS antigen in the mothers' sera was 0.856. In general, antibody transferred from the mother maintained until 6 months after birth, but can be rarely detected after 3 years. And the half life of transferred IgG from the mother was not long enough to maintain its level up to 2 years. Whether theses antibody response to Hib-PS were from maternal transfer or from individual immune responses should be studied.

We also examined the cord/maternal O.D. ratio of anti-Hib PS antibodies in relation to their subclass composition. We used an anti-Hib PS subclass specific ELISA to measure antibodies in sera from unimmunized mothers and their infants. The mean cord/maternal concentration ratio of anti-Hib PS IgG_1 was 1.18 and it was significantly higher in cord blood than in maternal blood. The values of anti-Hib PS IgG_2, IgG_3, and IgG_4 in cord blood were higher than those in maternal blood. All four IgG subclasses appear to cross the placenta effectively, but there were no statistical significances. This finding is well corresponded to that of Shakelford (Shakelford PG et al., 1988).

The polysaccharide capsule of Hib is a carbohydrate antigen and similar carbohydrate antigens have been reported to elicit IgG antibodies relatively restricted to the IgG_2 subclass in man (Shackelford PG et al., 1988), in contrast to the predominance of IgG_1 among antibodies to proteins (Sarnesto et al., 1985) . The subclass compositions of anti-Hib PS have been reported as predominantly IgG_2. In this study, we found that the cord/maternal concentration ratio of IgG_1 exceeded that of IgG_2. This finding is compatible with that of Einhorn et al. (Einhorn et al., 1987). This study also showed that maternal IgG_2 level is higher than its IgG_1 level and neonatal IgG_1 is higher than its IgG_2 level. The greater cord/maternal O.D. ratio of anti-Hib PS IgG_1 compared to that of anti-Hib PS IgG_2 again supports the hypothesis that placental transport of antibodies is related to their subclass composition rather than their antigenic specificity. The findings of other investgators showed that antibodies to pneumococcal capsular polysaccharide, which are restricted to the IgG_2 subclass in adults (Barrett and Ayoub, 1986), appeared to have lower concentrations in cord blood than in maternal blood (Chudwin et al., 1985).

These data and those in most previous studies indicate that although all four kinds of IgG subclasses are effectively transported across the placenta, the transport of IgG_1 is more effective than that of IgG_2 (Morell et al., 1970 ; Morell et al., 1971 ; Schur et al., 1973 ; Oxelius, 1979). The basis for variation in placental transport of the IgG subclasses is unknown. I have been observed that Fc receptors are present in human placental tissue (Matre et al., 1975 ; Jenkinson et al., 1976), and that they bind to IgG_1 with higher affinity than to IgG_2 (McNabb et al., 1976).

It is known that O.D. level in ELISA correlates with antibody concentration in some extent. But the ideal way of evaluating protective immunity against Hib is

determination of serum concentration of specific anti-Hib antibody. In a strict sense, the results that we have drawn may not be used as a definite value of protective immunity against Hib. Therefore it is necessary to continue on the study using serum antibody concentration rather than O.D. in the future.

In conclusion, the sera from most pediatric age groups in Korea reacted to Hib-PS antigen on ELISA and further epidemiologic study must be done in this area.

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