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Dynamics of Transplacental Transmission of Pertussis Antibodies in Premature and Full Term Infants

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INTRODUCTION

Pertussis is an infection of respiratory tract caused by *Bordetella pertussis* [1-8]. The disease is highly contagious and is still one of the major infectious diseases of children in the world [1, 9-12]. Premature infants may be extremely vulnerable to pertussis infection since they are usually suffering from insufficient pulmonary function, which is one of the major complication of premature infants, and since they are in some degree of immunocompromized state [13, 14].

The purposes of this study are (1) to delineate the current status of epidemiology of pertussis and pertussis vaccination in the developing and developed countries in the world with emphasis on Japan, (2) to investigate the transplacental transmission of pertussis antibodies in premature infants of various gestational age and birth weight, as well in full-term newborn babies with normal birth weight.

MATERIALS AND METHODS

1. Samples

Cord blood samples of full term infants and serum samples of their mothers were collected from the 125 pairs of infants and mothers who were admitted at Hadano Red Cross Hospital between 1991-1993. Cord blood samples were collected from 36 premature infants with gestational age between 30-36 weeks and from 17 with gestational age between 24-29 weeks who were admitted to the neonatal intensive care unit (NICU) of Tokai University Hospital and serum samples from their mothers were obtained within 5 days after delivery. The samples were stored at −20 ℃ until assayed. Informed consent was obtained.

2. Antibody assay

Pertussis toxin (PT) and filamentous haemagglutinin (FHA) are common components of acellular pertussis vaccines manufactured and distributed from six manufacturers in Japan. Hence, we developed a simple and sensitive assay method for evaluation of serum anti-PT and anti-FHA antibodies by the use of ELISA technique. The method was published elsewhere [15]. In short, the polystyrene balls were soaked in highly purified PT or FHA and allowed to stand at 4 ℃ overnight and washed with 0.01 M PBS, pH 7.4, containing 0.1 % Tween 20 and then soaked in 10 % sheep serum in 0.01 M PBS, pH 7.4, containing 0.1 % Tween 20 at 45 ℃ for 2 hours. The ball was washed as described above. Test serum or standard solution and PT-coated PS ball or FHA-coated PS ball were paced in wells of microtiter plate specially made for the balls. The plate was incubated at 37 ℃ for 60 min and was washed with the wash solution. Antibody conjugate was added to the wells. The plate

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was incubated at room temperature for 30 min and was washed with the wash solution. The balls were then placed in the test tubes. Substrate reagent was added to the tubes and the tubes were allowed to stand at room temperature for 30 min. Then stopper reagent was added to the tubes, and absorbance at 492 nm of the substrate solution was measured. The whole procedure could be completed within 3 hours.

3. Statistical analysis

Both anti-PT antibody levels and anti-FHA antibody levels were expressed by logarithmic values. Correlation coefficient between anti-PT antibody levels of mothers' sera vs. those of cord blood and correlation coefficient between anti-FHA antibody levels of mothers' sera vs. those of cord blood were calculated.

Transplacental transmission rate of anti-PT and anti-FHA antibodies were expressed by logarithmic value of the cord blood level divided by logarithmic value of the mother' s serum level corresponding to each cord blood.

Paired t test was used to compare antibody levels of full term babies and their mothers. Unpaired t test was used to compare anti-PT antibody levels and anti-FHA antibody levels among other populations and to compare transmission rates.

RESULTS

As shown in Figure 1, anti-PT antibody levels of mothers' sera and those of cord blood of full term infants correlated well, and the correlation coefficient was 0.83. As shown in Figure 2, anti-FHA antibody levels of mothers' sera also correlated very well with those of cord blood of full term infants, and the correlation coefficient was 0.84.

Table 1 depicts anti-PT and anti-FHA antibody levels of cord blood of full term and premature infants and their mother's serum. In full term infants, geometrical mean titer (GMT) of cord blood anti-PT and anti-FHA antibody was 7.4 EU/ml (95 % CI: 5.9-9.2) and 11.3 EU/ml (95 % CI: 9.2-13.7), respectively and were significantly higher than that of their mothers' sera, which was 5.0 EU/ml (95 % CI: 9.2-13.7) ($p < 0.0001$) and 7.5 EU/ml (95 % CI: 4.4-12.5) ($p < 0.0001$), respectively. In 30-36 week premature infants, GMT of cord blood anti-PT antibody and anti-FHA antibody was 5.4 EU/ml (95 % CI: 3.8-8.4) and 17.1 EU/ml (95 % CI: 12.4-25.6), respectively. Those values were

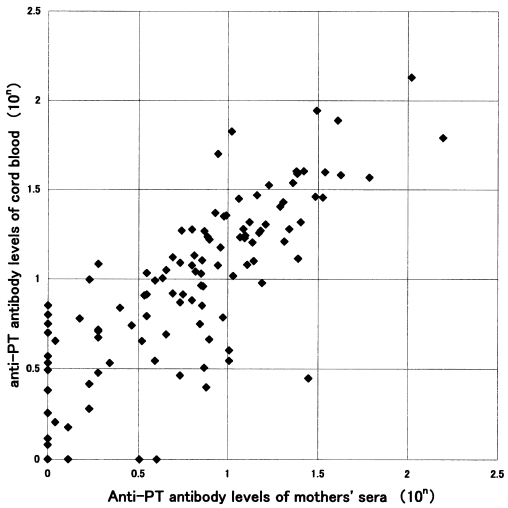

Fig. 1 Anti-PT antibody levels of mothers' sera vs. those of cord blood of full term infants.
Logarithmic values of anti-PT antibody levels of mothers' sera and those of cord blood were calculated, and the latter was plotted against the former.

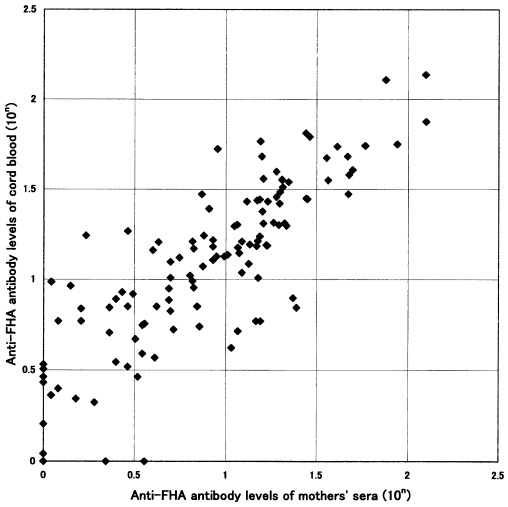

Fig. 2 Anti-FHA antibody levels of mothers' sera vs. those of cord blood of full term infants.
Logarithmic values of anti-FHA antibody levels of mothers' sera and those of cord blood were calculated, and the latter was plotted against the former.

Table 1 Anti-PT and anti-FHA antibody levels of cord blood and mothers' serum

Gestational age		anti-PT antibody levels*		anti-FHA antibody levels	
		Cord blood	Mother's serum	Cord blood	Mother's serum
Full term	sample number	125	125	125	125
	GMT	7.4**	5.0	11.3	7.5
	95 % CI	5.9-9.2	4.0-6.2	9.2-13.7	4.4-12.5
30-36 week	sample number	36	15	36	15
	GMT	5.4	7.2	17.1	15.9
	95 % CI	3.8-8.4	3.9-17.6	12.4-25.6	8.4-33.1
24-29 week	sample number	17	9	17	9
	GMT	3.4	3.8	14.2	13.7
	95 % CI	2.0-6.7	1.3-13.4	8.3-24.4	4.1-60.8

*antibody levels of equal or less than 1 EU/ml were calculated as 1 EU/ml.
**ELISA unit (EU)/ml

Table 2 Transplacental transmission rate of anti-PT and anti-FHA antibodies

Gestational age		Transplacental transmission rate* [Cord bood (10^n) / Mother's serum (10^n)]	
		anti-PT antibody levels	anti-FHA antibody levels
Full term	number of paris**	98	113
	mean	1.4	1.7
	95 % CI	1.1-1.8	1.2-2.1
30-36 week	number of paris	13	13
	mean	1.0	1.3
	95 % CI	0.8-1.2	1.0-1.5
24-29 week	number of paris	6	9
	mean	0.6	1.4
	95 % CI	0.4-0.8	0-3.0

*the transplacental transmission rates were expressed by results of logarithmic value (10^n) of cord blood divided by logarithmic value (10^n) of mothers' sera.
**pairs in which mothers' antibody levels were over 1 EU/ml were analysed.

almost equal to those of mothers' sera, which was 7.2 EU/ml (95 % CI: 3.9-17.6) and 15.9 EU/ml (95 % CI: 8.4-33.1), respectively. In 24-29 week premature infants, GMT of cord blood anti-PT antibody levels and anti-FHA antibody levels was 3.4 EU/ml (95 % CI: 2.0-6.7) and 14.2 EU/ml (95 % CI: 8.3-24.4), respectively. Those values were almost equal to those of mothers' sera, which were 3.8 EU/ml (95 % CI: 1.3-13.4) and 13.7 EU/ml (95 % CI: 4.1-60.8), respectively. Among the three groups of infants, GMT of cord blood anti-PT antibody of full term infants were the highest, averaging 7.4 EU/ml, those of 30-36 weeks premature infants intermediate, i.e., 5.4 EU/ml, and those of 24-29 weeks premature infants the lowest, i.e., 3.4 EU/ml. A significant difference was observed between anti-PT levels of premature infants 24-29 weeks and those of full term infants ($p < 0.05$). GMT of cord blood anti-FHA antibody of 30-36 week premature infants were the highest, averaging 17.1 EU/ml, those of 24-29 weeks premature infants intermediate, i,e., 14.2 EU/ml, and those of full term infants the lowest, i.e., 11.3 EU/ml. A significant difference was observed between anti-FHA levels of premature infants 30-36 weeks and those of full term infants ($p < 0.05$).

Table 2 depicts the transplacental transmission rate of anti-PT and anti-FHA antibodies. Only the results of samples from paired infants and mothers were analyzed. The transplacental transmission rate of

anti-PT antibody was the highest in full term infants, averaging 1.4 (95 % CI: 1.1-1.8), intermediate in 30-36 weeks premature infants, 1.0 (95 % CI: 0.8-1.2), and the lowest in 24-29 weeks premature infants, 0.6 (95 % CI: 0.4-0.8). Significant differences were observed between the transplacental transmission rate of anti-PT antibody in premature infants 24-29 weeks vs. that of premature infants 30-36 weeks ($p < 0.01$), in premature infants 24-29 weeks vs. that of full term infants ($p < 0.0001$). A significant difference was also observed between the transmission rate of anti-PT antibody in premature infants 30-36 weeks vs. that of full term infants ($p < 0.0001$). The transplacental transmission rate of anti-FHA antibody was higher in full term infants, averaging 1.7 (95 % CI: 1.2-2.1), than that of 30-36 weeks premature infants, averaging 1.3 (95 % CI: 1.0-1.5) or that of 24-29 weeks premature infants, 1.1 (95 % CI: 0-3.0). However, the differences observed in the transmission rates of anti-FHA antibody were not statistically significant.

DISCUSSION

PT is the most important protective antigen of *B. pertussis* and the major component of acellular pertussis vaccines. *B. pertussis* is the only bacteria which produces and excretes PT as exotoxin. FHA, another component of acellular pertussis vaccines, is produced not only by *B. pertussis* but also by other bacteria. *B. parapertussis* does not produce PT but it produces FHA. Hence, anti-PT antibody levels reflect more precisely the protective ability against infection with *B. pertussis* than anti-FHA antibody levels. When acellular pertussis vaccines were developed, there was no method of assaying serum anti-PT or anti-FHA antibody levels. Then, Sato Y, NIH developed an ELISA method which uses a plate with 96 wells [16]. Although the method could measure the levels of anti-PT and anti-FHA antibodies after vaccination or after pertussis infection, it was not sensitive enough to be used for population survey or for studying transplacental tranmission of antibodies. This was because the levels of antibodies of general population are usually at much lower levels than those immediately after vaccination or natural infection. We developed the ELISA methods using polystyrene ball. Since the surface area of a ball was uniform, the reproducibility and the sensitivity of the assay were markedly higher than those of ELISA using a plate. The method used in this study was therefore more reproducible, simple and sensitive. It has been used for population surveys conducted every year in collaboration between NIH and Prefectural Institutes of Public Health in Japan. Kits for anti-PT and anti-FHA antibody assays using polystyrene balls are now commercially available from Wako Chemical Co., Tokyo, Osaka. As shown in Table 1, GMT values of both cord blood anti-PT antibody and anti-FHA antibody of 125 full term infants were higher than those of their mothers, suggesting the presence of active transplacental transport of antibodies and accumulation of antibodies in the body of fetus that occur during gestation. Anti-PT antibody levels of cord blood were the highest in full term infants and the lowest in 24-29 weeks premature infants. GMT of anti-FHA antibody of cord blood was the highest in 30-36 weeks premature infants and the lowest in full term infants.

Shown in Table 2 is a clear tendency in the transplacental transmission rate. Premature infants have lower transplacental transmission rate than full term infants with regards to both anti-PT antibody and anti-FHA antibody.

In addition to the method described in this study, we have tried a particle agglutionation (PA) method for the evaluation of pertussis antibodies [17]. However, this method was found not sensitive nor reproducible, and we concluded that PA may be used only in developing countries, where spectrophtometers are not easily available, for a rough evaluation of the acceptance rate of pertussis vaccines.

CONCLUSION

It was concluded that premature infants may be extremely vulnerable to infection with *B. pertussis* because they have insufficient transplacentally transmitted maternal antibody.

It is strongly recommended that any person who has severe cough do not enter NICU and that all personnels who work at NICU be administered with DPT.

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