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Concentration and avidity of anti-tetanus antibodies in mother-infant pairs: Relation to immunization time

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Abstract

The concentration and avidity of anti-tetanus antibodies in two groups of mother-infant pairs were compared. Mothers immunized during pregnancy and their newborns (group A) had significantly higher antibody concentrations than mothers immunized at least a year before their last pregnancy and their newborns (group B) as measured by an indirect enzyme immunoassay (EIA) procedure. Antibody avidity of samples was measured by an inhibition EIA technique and urea denaturation test. Although antibody avidity was higher in group B, the differences were not significant. These findings may represent a secondary antibody response to a protein antigen, when considering that all mothers in both groups had received a primary tetanus vaccination during childhood. In mothers with a history of primary tetanus immunization, a single booster dose of tetanus toxoid during pregnancy is enough to induce protective levels of antibodies with reasonably high avidity in both mother and newborn.

Keywords: Tetanus toxoid; Immunization; Antibody; Concentration; Avidity; Enzyme immunoassay

1. Introduction

The use of tetanus toxoid (TT) vaccine in pregnant women has been shown to reduce both maternal and neonatal mortality rates [1]. The neonates are passively protected by transplacentally transferred vaccine-specific maternal IgG antibodies. The transfer of IgG is an active process mediated by receptors on placental syncytiotrophoblasts specific to the Fc portion of the IgG molecule [2].

The affinity of antibodies for antigens is as important as their concentration for an effective immune response [3]. High affinity antibody is superior to low affinity antibody in many biological reactions such as immune elimination of antigen, complement fixation and toxin neutralization [4]. There have been a number of reports where maternal and neonatal anti-TT antibody levels were measured [5–7]. However, little is known about the affinity and affinity distribution of anti-TT antibodies in mother-infant pairs, and this is the subject of our report.

2. Materials and methods

2.1. Subjects and samples

Paired maternal and cord blood samples were obtained from 50 mothers and their newborn infants at birth between March and April 1993. Informed

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consent was obtained in all cases. The mean age of the mothers was 24.6 years (range 16–40 years). All participants were healthy and delivered normal babies at term. Twenty-nine mothers had received 1 ($n = 4$) or 2 ($n = 25$) injections of 7.5 Lf/ml alum absorbed TT in the last trimester of their pregnancy (group A); 12 mothers had been immunized 1 to 16 years (mean = 7.83 years) before their last pregnancy (group B), and 9 mothers had never been immunized (group C). All mothers in the first two groups, however, had received TT immunization in childhood. Sera were seperated within 2 h of collection, coded and stored in aliquots at −20°C until assayed.

2.2. Antigen and tetanus immunoglobulin

Concentrated alum-free tetanus toxoid (TT, 500 Lf/ml) was kindly provided by Dr. Mustafa Ulusoy (Refik Saydam Health Institute, Ankara Turkey). Purified tetanus human immunoglobulin (TIG, Tetaglobuline®, 250 IU/ml, Pasteur Merieux, Lyon, France) was used in enzyme immunoassay (EIA) reactions to establish standard dose-response curves.

2.3. Detection of anti-tetanus antibodies

The wells of microtiter plates were coated with 100 μl of 3 Lf/ml (≈ 10 μg/ml protein) TT in 0.05 M carbonate buffer (pH 9.6) overnight at 4°C. TBST-BSA (0.3% bovine serum albumin (BSA) in Tris buffered saline-Tween 20 (10 mM Tris.Cl pH 8.0, 140 mM NaCl, 0.05% Tween 20)) was used as blocking buffer, serum diluent and washing solution. After blocking for 2 h at room temperature, 100 μl from each serial three-fold dilutions of TIG starting at 30 IU/ml was pipetted into corresponding wells. Serum samples of mother-infant pairs were diluted 1/100 in TBST-BSA and 100 μl were used in the experiments. Following incubation at 37°C for 1 h and subsequent washing, wells were incubated with 100 μl/well of a 1/2000 dilution of goat anti-human IgG alkaline phosphatase conjugate at 37°C for 1 h. After washing, the plates were incubated with 100 μl/well of 1 mg/ml substrate (ortho phenylenediamine) in 0.05 M carbonate buffer (pH 9.6) containing 5 mM $MgCl_2$ at room temperature. Absorbances were read at 405/650 nm using a Bio-Tek El 312e (Bio-Tek, USA) kinetic reader. A standard dose-response curve was established by plotting absorbance read versus TIG concentration. Antibody concentrations in serum samples were determined using this standard curve.

2.4. Titration of serum samples

Serum samples were titrated across the TT coated (0.3 Lf/ml) plates in 1/3 log dilutions and incubated for 1 h at 37°C. The EIA procedure was identical to that used for detection of anti-TT antibodies. The dilution of antibody resulting in an absorbance of approximately 50% of the maximum absorbance value was used for the determination of avidity index and average affinity of antibodies [8].

2.5. Avidity index method

A modification of the urea denaturation test [9] was used to determine avidity index (AI) of the 36 paired serum samples, 27 of which were from group A and 9 from group B. Serum samples at the appropriate dilution were incubated for 1 h at 37°C, followed by rinsing off once with wash buffer. Half of the wells were then filled with wash buffer and half with 8 M urea dissolved in wash buffer. The wells were soaked for 5 min, and the procedure repeated twice prior to washing twice with wash buffer. The same EIA procedure was performed as described above. The AI was calculated from the absorbance values of the wash buffer-soaked wells (A) and the absorbance values of the urea-soaked wells (B) using the formula:

$$AI = (B/A) \times 100$$

2.6. Determination of antibody affinity

Serum samples that had anti-TT concentrations over 0.1 IU/ml were randomly chosen from group A ($n = 4$), and group B ($n = 6$) for affinity determination in an inhibition EIA format as described by Rath et al. [10]. Briefly, constant amounts of serum at the appropriate dilution were incubated with different concentrations of TT ranging from 346 nM to 0.63 nM (assuming the MW of TT is 150 000), in the wells coated with 0.3 Lf/ml of TT, in a final volume

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of 200 μl for 1 h at 37°C. The EIA format as described above was then followed to determine the free antigen concentration required to saturate half of the antigen combining sites ($I_{0.5}$) as a measure of average affinity of antibodies in solution.

2.7. Estimation of affinity heterogeneity

The percentile inhibition contributed at each concentration of free antigen was calculated and plotted in the form of histograms [11]. The values were also used to determine Shannon's coefficient (SC) as a mathematical expression of affinity heterogeneity [8] using the formula:

$$I = -kG \sum_{j=1}^{j=n} pj \ln pj$$

where I = Shannon's coefficient, $k = 1/\ln 2$, G = number of intervals used, pj = percentile inhibition contributed by a given inhibitor antigen concentration expressed as a fraction.

2.8. Statistics

Mann-Whitney U-test was used for the comparison of the means of groups with different immunization times. The relationship between antibody concentration and avidity of mother-infant pairs was determined by Spearman rank correlation analysis.

3. Results

3.1. EIA experiments

The anti-TT EIA experiments were performed in a kinetic manner and repeated three times independently. The standard dose-response curves were fit by 4-parameter equations on a linear/log scale, and gave linear responses between 0.003 IU/ml and 10 IU/ml (data not shown). The assay was highly specific, as judged by the negativity of all 9 paired samples from the non-immunized group (i.e. group C). The intra-assay and inter-assay coefficients of variation were less than 5% (data not shown).

3.2. The effect of immunization time on antibody concentrations

The anti-TT concentrations of mothers in group A were significantly higher than those in group B ($P = 0.035$) (Table 1). In both groups, there was a significant correlation between the antibody levels of mothers and their children (rho = 0.935, $P = 0.0001$). Accordingly, infants in group A had higher antibody levels than infants in group B, and the difference was significant ($P = 0.028$). All serum samples in group A had anti-TT concentrations higher than 0.1 IU/ml, which is a magnitude higher than the minimal protective antibody concentration pro-

Table 1
The concentration and avidity of IgG antibody responses in mothers vaccinated with tetanus toxoid during pregnancy and infants (group A), and mothers vaccinated at least a year before their last pregnancy and infants (group B)

	Mothers		Infants	
	Group A	Group B	Group A	Group B
Anti-TT (IU/ml)	0.88 ± 0.84 [b]	0.56 ± 0.97	0.79 ± 0.56 [b]	0.43 ± 0.49
(*n*) [a]	(29)	(12)	(29)	(12)
Avidity index (AI)	78.4 ± 10.1	76.8 ± 9.6	78.7 ± 8.0	77.9 ± 10.2
(*n*)	(27)	(9)	(27)	(9)
$I_{0.5}$ (nM^{-1}) [c]	13.9 ± 7.8	7.2 ± 2.4	15.0 ± 11.1	9.0 ± 3.3
(*n*)	(4)	(6)	(4)	(6)
Shannon's coefficient	27.3 ± 2.2	27.0 ± 2.9	28.4 ± 1.8	26.9 ± 1.4
(*n*)	(4)	(6)	(4)	(6)

[a] Corresponds to number of serum samples tested.
[b] Significant difference between groups A and B ($P < 0.05$).
[c] The concentration of inhibitor antigen required to saturate half of the antigen combining sites.

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posed by WHO [12]. However, antibody levels in group B were widely spread; some were around 0.01 IU/ml. Receiving single or double injections of TT during pregnancy did not seem to significantly affect concentration of anti-TT antibodies in mothers ($P = 0.343$ and $P = 1.000$, respectively), and in their children ($P = 0.704$ and $P = 0.733$, respectively).

3.3. The effect of immunization time on avidity indices

There were no significant differences in the avidity indices (AI) of serum samples from mother-infant pairs with different immunization histories (Table 1). The AI of paired samples were significantly correlated (rho = 0.863, $P = 0.001$). Receiving one or two doses of TT during pregnancy had no significant effect on the AI of the antibodies of mother-infant pairs ($P = 1.000$, and $P = 0.733$, respectively).

3.4. Average affinity and affinity distribution of anti-TT antibodies

The optimization of an inhibition EIA assay for the determination of average affinity and affinity distribution of polyclonal anti-TT antibodies has been published elsewhere [13]. The average affinities of both mother and infant samples in group A were slightly lower than those of group B (Table 1), but this difference was not found to be statistically significant at 95% confidence level ($P = 0.054$ and $P = 0.389$, respectively). There were no significant differences between the affinity distributions of antibodies in group A and B mothers ($P = 0.669$) and their children ($P = 0.201$). Shannon's coefficient values for mothers and infants had a strong correlation (rho = 0.806, $P = 0.016$), whereas no correlation was found for Ko values (rho = 0.133, $P = 0.719$). Shannon's coefficient values are shown in Table 1.

4. Discussion

In this study, we showed that immunization during pregnancy resulted in higher anti-TT antibody levels both in mothers and infants, but did not significantly affect the avidity and affinity heterogeneity of these antibodies. These findings may represent an already matured antibody response to a protein antigen, considering that all mothers in groups A and B had received primary vaccination. The main features of a successful secondary antibody response to T-dependent antigens include a shorter lag phase, production of antibodies at higher concentrations, isotype switching, affinity maturation and the development of memory [4]. Hedman et al. [14] have shown that the avidity of specific IgG increased dramatically in the first 6 months and less markedly between 6 and 12 months following rubella vaccination in previously non-immune subjects, whereas the initially high IgG avidity of previously immune vaccinees remained at an elevated level post-vaccination.

The avidity indices and concentrations of anti-TT antibodies in infants correlated well with those of mothers in both groups, suggesting the passive transfer of immunity to infants.

There was no correlation found between Ko values of mothers and infants, while Shannon's coefficient values were significantly correlated. This may be due to the fact that increases in the complexity of the antibody mixture bring about only small increases in the Shannon's coefficient, and therefore it may not be very sensitive to small changes in the affinity heterogeneity [8]. It should also be kept in mind that affinity measurements were made in only a small fraction of serum samples ($n = 10$), and therefore, statistical comparison might be misleading.

In Turkey, single dose booster immunization with TT has been recommended by the Ministry of Health for pregnant women with primary vaccination history. Our findings that some mothers in group B had antibody levels equal to or slightly higher than 0.01 IU/ml, and receiving single or double booster immunizations of TT during pregnancy (group A) did not significantly affect the concentration and avidity of anti-TT antibodies supports the current official recommendations. It seems that a single booster dose of TT provides antibodies with reasonably high affinity at protective concentrations. Accordingly, it has been reported that up to 30 years after primary vaccination, one reinforcing dose of alum absorbed TT was sufficient to induce protective titres of antitoxin [15].

However, there are many reasons to avoid multiple TT injections during pregnancy. Firstly, although

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it may enhance serum antibody titers, it could lead to an ineffective immune response [16]. Secondly, alum absorbed TT vaccines have been shown to induce specific IgE response [17] which may trigger hypersensitivity reactions. Thirdly, vaccines that require fewer doses are critical for success in immunization programs, especially in underdeveloped countries.

The predominant antibody response to TT is IgG_1, and to a lesser extent IgG_4. The latter has been shown to be associated with a low affinity antibody response in man which probably reflects differences in the regulation of isotype switching and affinity maturation at the T-cell level [18]. We did not attempt to characterize the distribution and relative affinites of IgG subtypes in mother-infant pairs, but it would be interesting to study how these parameters are affected with respect to immunization time and frequency. We are currently undertaking studies to address these questions.

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