

Changes of Tetanus Specific IgG, IgM and IgG Subclasses after DPT Vaccination

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We evaluated tetanus specific IgG, IgM, IgG subclasses after DPT vaccination in infants and children. Tetanus toxoid specific IgG, IgM, IgG subclasses were measured to characterize the isotope profile of antibody against tetanus toxoid. The values of the tetanus specific IgG in the positive group were significantly increased compared to those of the control group, and were significantly increased after two inoculation. tetanus specific IgG was very low in adults and neonates. In our tetanus specific IgG subclasses study, forty-five of 56 cases (80%) showed predominantly IgG1 antibody responses to tetanus toxoid, while twenty-five of 56 cases (45%) showed IgG4 responses. Both IgG1 and IgG4 responses were demonstrated in 17 cases (30%). So we suggest that IgG was mainly involved in humoral immune response after DPT vaccination, and IgG1 may play an important role among IgG subclasses. IgG4, alone or together with IgG1, can also play a role in immune response to tetanus toxoid.

Key Words: Tetanus specific IgG, IgM, IgG subclasses.

Tetanus is caused by infection of a wound, or the umbilical stump in neonates, with spores of the strict anaerobe, *Clostridium tetani*.

The disease is the result of increased activity of both alpha and gamma-motor neurons due to the inhibition of neurotransmitters by a potent protein exotoxin, tetanospasmin.

In Korea, the occurrence of neonatal tetanus has been markedly decreased due to improvements in environmental sanitation and delivery care and also a national campaign for the elimination of neonatal tetanus under the auspices of the Korean Pediatric Association (KPA) which was initiated in 1971 (Choi

1972). Nevertheless, many cases of neonatal tetanus are still occurring in developing countries.

In an earlier study, we evaluated tetanus toxoid specific immunoglobulin (IgG, IgM) after DPT vaccination. It was demonstrated that IgG was mainly involved in humoral immune responses against tetanus toxoid after DPT vaccination. Tetanus toxoid specific IgG was significantly increased after two inoculations, which was maintained for about 2 years and decreased slowly thereafter (Shin 1987). The present study was also performed to evaluate tetanus specific antibody response after DPT vaccination in infants and children.

Tetanus toxoid specific IgG, IgM and IgG subclasses (IgG1, IgG2, IgG3, IgG4) were measured to characterize the isotope profile of antibody against tetanus toxoid.

MATERIALS AND METHOD

Subjects and Specimen

One hundred and ninety six healthy infants and children were enrolled in the study during a 7 month period in 1986. All of them were delivered at the hospital, and visited out well baby clinic for vac-

Received December 7, 1988

Accepted March 14, 1989

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Abbreviations

ELISA; enzyme linked immunosorbent assay

Ig ; immunoglobulin

PBST; phosphate buffer saline-Tween 20, PH 7.4

BSA ; bovine serum albumin

FHA ; filamentous hemagglutinin

DPT ; diphtheria pertussis tetanus

GSB ; group B streptococcus

Table 1. Values of Tetanus specific IgG and IgM after DPT vaccination in groups

No. of cases: 196

	Group I (n=28)	Group II (n=7)	Group III (n=12)	Group IV (n=71)	Group V (n=36)	Group VI (n=42)
IgG	0.044±0.002	0.169±0.099	0.513±0.091*	0.729±0.078*	0.539±0.074*	0.078±0.007
IgM	0.040±0.008	0.037±0.005	0.076±0.008	0.109±0.024	0.113±0.019	0.144±0.014

Group I : Neonates and no vaccination group

Group II : DPT vaccination: 1 dose

Group III: DPT vaccination: 2 doses

Group IV: DPT vaccination: more than 3 doses (less than 7 years old)

Group V : DPT vaccination: more than 3 doses (between 7-15 years old)

Group VI: Adult group (23-25 years old)

* P<0.01, The values are mean optical density±SE

cination.

Physical examination of body weight and height revealed these subjects to be within the normal range for their age.

We divided them into six groups according to the frequency of vaccination, and evaluated the values of tetanus specific IgG and IgM of these groups (Table 1).

To evaluate the distribution of tetanus specific IgG subclasses, we selected 66 cases; 56 cases (positive group) showed a high titer of tetanus specific IgG (O.D: above 0.4) and 10 cases (negative control group) showed a low titer of tetanus specific IgG (O.D: below 0.1). After taking sera samples, 2% sodium azide (10ul/ml serum) was added, and all sera were preserved at -70°C until antibody assays were performed.

Human tetanus immunoglobulin (250 IU/ml, Green, Cross Lab., Korea) and reference serum obtained from an adult with the highest relative tetanus antibody found from a screening test were used as positive controls. Buffer, as well as serum with no detectable antibodies, was included in each assay as a negative control. All assays were performed in duplicate.

Antibody Assays

Tetanus specific IgG, IgM and IgG subclass (IgG1, IgG2, IgG3, IgG4) levels were measured by ELISA as postulated by Voller et al (1976).

Tetanus specific IgG & IgM: Micro-ELISA plates (Immunlon II, Dynatech Lab., USA) were coated with 100ul of tetanus toxoid (0.2 Lf/ml, Green-Cross Lab., Korea).

The plates were then washed with PBST three times, and then 100ul of serum (1:100 dilution with PBST containing 0.5% BSA) was added to duplicate

wells. The plates were incubated for 2 hours at room temperature, and then washed with PBST three times. one hundred ul of goat anti-human IgG or IgM conjugated with alkaline phosphatase (1:1000 dilution with PBST, Sigma Chemical Co., USA) was added to the wells. The plates were incubated in the dark for 1 hours at room temperature, and again washed with PBST three times. One hundred microliter of p-nitrophenyl phosphate disodium (1mg/ml, Sigma Chemical Co., USA) in 10% diethanolamine buffer (pH 9.8) was added as substrate. The ELISA plates were incubated in the dark for 1 hour at room temperature, and optical densities were measured with an ELISA reader (Dynatech Co., USA) at 410 nm.

Tetanus specific IgG subclasses: Micro-ELISA plates (Dynatech Lab., USA) were incubated overnight at 4°C with 100ul of tetanus toxoid (0.4 Lf/ml), Green-Cross Lab., Korea).

The plates were then washed with PBST three times. One hundred ul of serum (1:20 dilution with PBST containing 0.5% BSA) was added in duplicate wells and the plates were incubated for 2 hours at room temperature, and then washed PBST three times. one hundred ul of monoclonal mouse anti-human IgG subclass antibodies (Unipath, England) were added to the wells (IgG1, IgG3; 200ng/100ul/well in PBST, IgG2, IgG4; 100ng/100ul/well in PBST) and incubated for 2 hours at room temperature. After three more washings with PBST, One hundred ul of rabbit anti-mouse antibody conjugated with alkaline phosphatase (1:1000 dilution with PBST, Sigma Chemical Co., USA) was added and the plates were incubated in the dark for 1 hour at room temperature.

Finally, the enzyme substrate application and reading were performed by the same method as in the tetanus specific IgG assay.

Statistical Method

The unpaired student test (two-tailed) was used to determine the significance of difference of IgG and IgG subclass level in the positive group and the negative group, and the relationship between IgG and IgG subclasses was analyzed by simple correlation test and regression test.

RESULTS

Tetanus Specific IgG & IgM

The values of tetanus specific IgG and IgM after DPT vaccination are shown in Table 1 and Fig. 1.

IgG was mainly involved in the humoral immune response against tetanus toxoid after DPT vaccination. The values of the tetanus specific IgG in the positive group were significantly increased compared to those of the control group, and were significantly increased after two inoculations. Tetanus specific IgG was very low in adults and neonates (Table 1, Fig. 1).

The values of the tetanus specific IgG in the positive group were significantly increased compared to those of the control group. (0.949 ± 0.039 VS 0.062 ± 0.008 , $p < 0.001$, Tab 2, Fig 2).

Tetanus Specific IgG Subclasses

The values of tetanus specific IgG1 were significantly higher in the positive group than those of the control group (0.247 ± 0.022 VS 0.027 ± 0.004 , $p < 0.001$, Table 2, Fig. 2). The relationship between IgG and IgG1 was tested by simple correlation test and regression test. there was a close correlation between the level of IgG and IgG1 (Fig. 3). The values of tetanus specific IgG4 were also increased in the positive group compared to those of the control group, but there was no statistical significance (0.189 ± 0.038 VS 0.009 ± 0.001 , $p > 0.01$, Table 2, Fig. 2, 6).

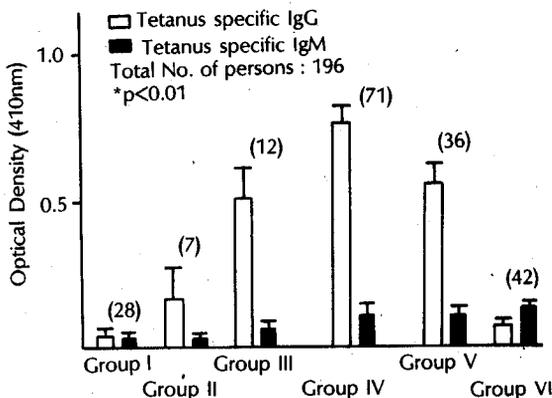


Fig. 1. Changes of tetanus specific IgG and IgM responses after DPT vaccination.

- Group I : Neonates and no vaccination group
 - Group II: DPT vaccination: 1 dose
 - Group III: DPT vaccination: 2 doses
 - Group IV: DPT vaccination: more than 3 doses (less than 7 years old)
 - Group V: DPT vaccination: more than 3 doses (between 7-15 years old)
 - Group VI: Adult group (23-25 years old)
- Figures in parenthesis indicate number of person. Each column & bar represents mean \pm SE respectively.

There was no significant difference in tetanus specific IgG2 and IgG3 levels between those groups (0.015 ± 0.001 VS 0.008 ± 0.001 in IgG2, 0.018 ± 0.002 VS 0.009 ± 0.001 in IgG3, $p > 0.001$, Tab2, Fig 4, 5).

Of all 56 positive cases, an optical density above 0.1 of tetanus specific IgG1 was observed in 45 cases (80.4%), and of tetanus specific IgG4 in 25 cases (44.6%). In 17 cases (30.4%), both tetanus specific IgG1 and IgG4 were increased simultaneously. These results show that IgG1 and IgG4 may play an important role in immune response against tetanus toxoid, either

Table 2. Values of Tetanus specific IgG and IgG subclasses after DPT vaccination

	IgG	IgG1	IgG2	IgG3	IgG4
Positive group (n=56)	$0.949 \pm 0.039^*$	$0.247 \pm 0.022^*$	0.015 ± 0.001	0.018 ± 0.002	0.189 ± 0.038
Control group (n=10)	0.062 ± 0.008	0.027 ± 0.004	0.008 ± 0.001	0.009 ± 0.001	0.009 ± 0.001

No. of cases: 66

Positive group: The optical density of tetanus specific IgG > 0.4
 Control group: The optical density of tetanus specific IgG < 0.1
 * $P < 0.01$, The values are mean optical density \pm SE

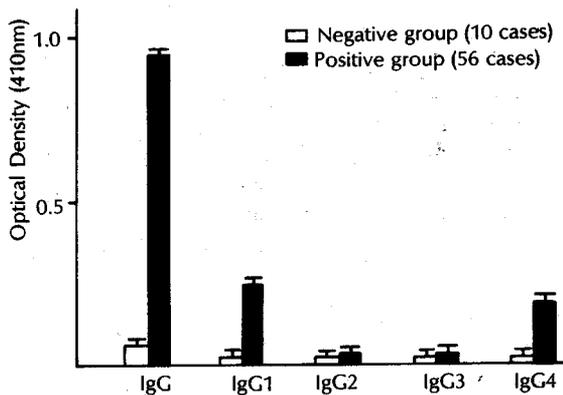


Fig. 2. Values of tetanus specific IgG and IgG subclasses after DPT vaccination.

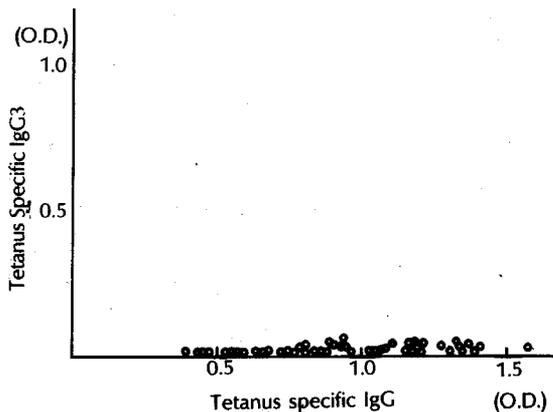


Fig. 5. Distribution of tetanus specific IgG & IgG3 in the positive group.

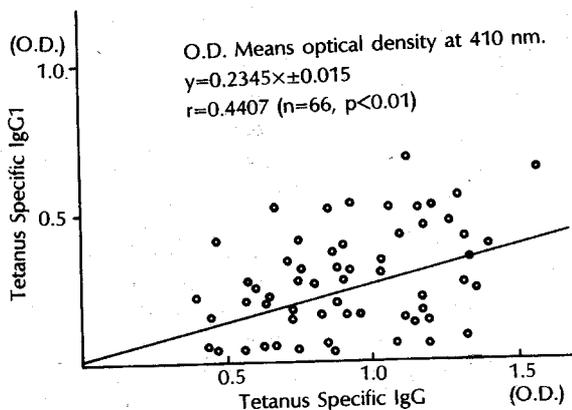


Fig. 3. Distribution of tetanus specific IgG & IgG1 in the positive group.

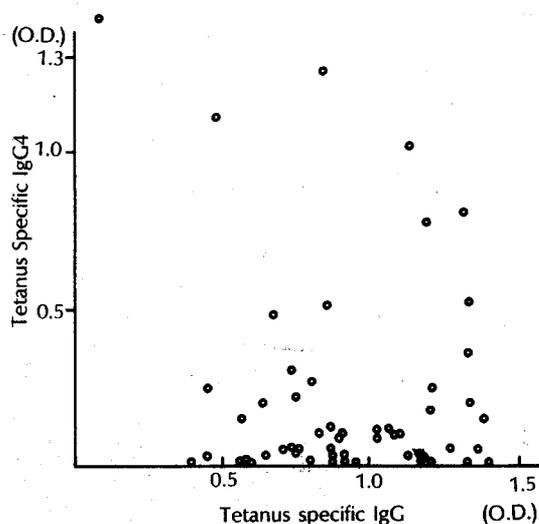


Fig. 6. Distribution of tetanus specific IgG & IgG4 in the positive group.

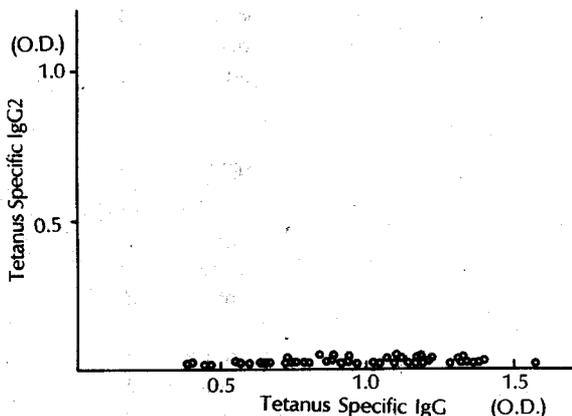


Fig. 4. Distribution of tetanus specific IgG & IgG2 in the positive group.

together or alone.

DISCUSSION

Although immune responses to tetanus toxoid can be elicited before birth (Thomas *et al.* 1983), these responses improve with age, especially after the first 2 months of age, and at least 2 inoculations of DPT vaccination are required for a significant increase of the tetanus specific IgG in serum (Shin *et al.* 1987).

For detection of the tetanus antibody, the passive hemagglutination and neutralization technique were

used. In the hemagglutination technique, the serum antitoxin was titrated in microtiter trays against sheep erythrocytes coated with tetanus toxoid. For the neutralization technique, the survival of mice was determined after injection of different serum dilutions of added tetanus toxin. A serum antitoxin concentration of 0.01 IU/ml corresponds to an antibody content of 1/100 of 1ml WHO standard serum, and this is deemed to be the lowest titer affording protection (Edsall 1949). Hemagglutination results corresponded to the *in vivo* results for antitoxin concentrations between 0.1 and 10 IU/ml, but outside these values the hemagglutination results were significantly higher (Simonsen *et al.* 1984).

ELISA was developed by Engvall and Perlmann in 1971 and has since been used to investigate humoral immune responses against various antigens. We studied the changes of tetanus specific IgG, IgM and IgG subclasses (IgG1, IgG2, IgG3, IgG4) by ELISA. It is commonly known that the same switch from the formation of IgM antibodies to that of IgG antibodies occur in humans (Peacock *et al.* 1973). Baroff *et al.* (1984) showed that IgM antibodies to FHA antigen were somewhat higher in the early immunization group, while IgG antibodies were lower.

Our study shows that IgG mainly played a role in humoral immune response to tetanus toxoid and also that a booster injection of Td in adults will be required for elevation and transfer of tetanus specific antibody. It has been recommended that immunization with the DPT vaccine is adequate for infants (Myers *et al.* 1982) and preterm infants (Bernbaum *et al.* 1985), but there is no supporting immunologic data in children.

Pichichero *et al.* (1986) suggested that all of the children in his study had a protective titer to diphtheria and tetanus toxin after the 18 month booster does of DPT vaccine as determined by the neutralizing method.

Barkin *et al.* (1984), demonstrated that immunization with a reduced dose provided equivalent antibody response, significantly reducing the incidence and severity of adverse reactions. Although a significant level of tetanus specific IgG was obtained after two doses of DPT vaccine, three doses are recommended to assure 100% protection (Barkin *et al.* 1985). IgG subclasses are isotypes of IgG. The history of IgG subclasses goes back to the publication of Dray in 1960 and the confirmation of his study by Terry and Fahey (1964) and Grey and Kunkel in 1964. Four subclasses of IgG (IgG1, IgG2, IgG3, IgG4) have been identified in humans which differ in antigenicity, number and position of disulfide bridges between the two r-chains and the angle of Fab arms (Daniel *et al.*

1987).

Oxelius (1979) showed that the IgG1 levels of newborns were consistently higher than those of their mothers, while the IgG2, IgG3, IgG4 levels were about the same. Siber *et al.* (1980) demonstrated that IgG2 was mainly increased against bacterial polysaccharide antigens. IgG3 response against hepatitis B surface antigen (Morell *et al.* 1983) and rubella (Skvaril *et al.* 1984), IgG4 response in allergic diseases (Heiner 1984), and the role of IgG3 in GBS infection (Kim 1985) have been reported.

Some investigators, relying on polyclonal antisera, reported predominantly IgG1 response to tetanus toxoid *in vivo* and *in vitro* with little activity in the other subclasses (Steven *et al.* 1983); however others found IgG4 anti-tetanus toxoid antibodies in 72% of normal sera (Seppala 1983).

Devey *et al.* (1985) used a set of monoclonal antibodies and found that most individuals (65%) produced predominantly IgG1 antibody response to tetanus toxoid, with the remainder producing either a predominantly IgG4 antibody response (29%) or an equal amount of IgG1 and IgG4 antibodies (6%). This is in agreement with Rubin *et al.* (1986).

Older studies relying on polyclonal antisera may have to be reevaluated using monoclonal subclass specific reagents, but Dengrove *et al.* (1986) demonstrated that anti-tetanus responses were evident in all four IgG subclasses.

In our study, forty-five of 56 cases (80%) showed predominantly IgG1 antibody response to tetanus toxoid, while twenty-five of 56 cases (45%) showed IgG4 response. Both IgG1 and IgG4 were mainly involved in humoral immune response after DPT vaccination, and IgG1 may play an important role among IgG subclasses. IgG4, alone or together with IgG1, can also play a role in immune response to tetanus toxoid.

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