Attenuated immune response to tetanus toxoid in young healthy men protected against tetanus

Elena Danilova a, Alexey Shiryayev b, Einar Klæboe Kristoffersen c, Haakon Sjursen d, *

a Institute of Medicine, Centre for International Health, University of Bergen, Norway
b North State Medical University, Arkhangelsk, Russia
c Department of Microbiology and Immunology, The Gade Institute, University of Bergen, Haukeland Hospital, Norway
d Institute of Medicine, University of Bergen, Haukeland University Hospital, N-5021 Bergen, Norway

Received 25 January 2005; received in revised form 25 May 2005; accepted 27 May 2005

Available online 15 June 2005

Abstract

Tetanus booster is a routine procedure of tetanus prevention in populations with high risk of injury, independent of the levels of protection. But the immune response in already protected individuals is not well studied. We describe the kinetics of booster response in individuals by measuring tetanus antitoxin levels by indirect ELISA. A 6-month follow up was performed on 60 boosted individuals tested before, 1 week, 1, 2, 3 and 6 months after the booster. High initial protection (mean titer 1.08 IU/ml) and less than 3-fold increase after 1 month were observed. After 1 month of stable antitoxin levels, the levels slowly decreased and reached a mean titer of 1.78 IU/ml after 6 months. Individuals with initial levels <1 IU/ml had booster response after the first month twice as high compared to those with initial level ≥1 IU/ml. However, in both groups, the decline from 1 to 6 months was about 2-fold. Individuals already protected against tetanus exhibited an attenuated, short-lasting booster response to tetanus toxoid. This was more pronounced in individuals with pre-booster levels ≥1 IU/ml, who did not improve immune protection after the booster.

Keywords: Booster response; Tetanus antitoxin kinetics; Adults immunisation

1. Introduction

In the industrialised world, tetanus is a serious but very rare disease due to high hygienic standards and extensive vaccination programmes. In the former USSR, childhood immunisation against tetanus was introduced in the 1950s. In 1961, mass childhood and adult’s booster immunisation were established. Since 1975, no cases of neonatal tetanus have been registered [1]. During the last decade, the incidence rate has been low, varying from 0.033 to 0.6 per 100 000. About 70 cases have been reported annually; half of them developed fatal outcome. In 2002–2003, the incidence rate was even lower, 0.02 per 100 000. Conventionally, tetanus toxoid is given in combination with diphtheria toxoid and pertussis vaccine for the primary vaccination. Boosters against tetanus are provided by Td vaccine (tetanus–diphtheria) or tetanus antitoxin alone. In recent years, there have been a number of alterations and additions in Russian National Vaccination Programme mainly connected with diphtheria antiepidemic measures. This has resulted in increasing frequencies of Td boosters. Different aspects of diphtheria booster response have been studied. In particular, the negative correlation between pre-booster diphtheria antitoxin levels and booster response to the toxoid has been shown [2,3]. However, little attention was paid to tetanus protection and booster response.

The main objective of this study was to investigate the kinetics of tetanus booster response in individuals with...
known diphtheria protection and course of diphtheria booster immune response.

2. Materials and methods

2.1. Subjects

Sixty healthy young Russian men (18.7 mean age) were followed for 6 months after a routine Td (5 Lf of diphtheria and tetanus toxoids, BIOMED, Russia) booster vaccination [4]. According to the national immunisation programme (prior to January 2002), three priming injection followed by three tetanus toxoid boosters were given by the age of 17. The recorded vaccination history was available only for 36.6% of the participants confirming three booster doses.

Blood samples were taken before the booster doses were given and after 1 week, and then 1, 2, 3 and 6 months after. Sera were separated from clotted blood and stored at −20°C until tested.

2.2. Serological method

The tetanus antitoxin level was assessed in all samples by an indirect ELISA technique [5,6]. Briefly, 96-well microtiterplates (Nunc-Immuno plates MaxiSorp cat. no. 439454, NUNC, Denmark) were coated with tetanus toxoid (606 Lf/ml, purity 2020 Lf, 7 mg protein nitrogen) diluted to a concentration of 0.75 Lf/ml incubated at 37°C for 2 h, then at 4°C overnight. Following blocking by 3% bovine serum albumin (Roche Diagnostics GmbH Mannheim, Germany) in 0.067 M phosphate buffer saline, 0.05% tween 20 (Sigma Aldrich Chemie GmbH, Germany) (3% BSA-PBST) at 37°C, wells were incubated for 1 h at 37°C with duplicate 3-fold serial dilutions of the reference 3.3 IU/ml (WHO International Standard of Tetanus Immunoglobulin) or 2-fold dilutions of tested sera. After 1-h incubation at 37°C with goat anti-human, IgG peroxidase conjugated (Southern Biotech. Assoc. Inc, Birmingham, Alabama, USA) diluted 1:1000 in PBST. Between each step, the plates were thrice washed with 0.3% BSA-PBST. The optical density values were read by BIO-RAD microplate reader at 490 nm. Four-parameter logistic regression was used to construct the reference curve. The results were read by using the linear part of the reference curve and expressed as International Units per millilitre (IU/ml). The level of quantitation was 0.002 IU/ml. Antibody levels ≥0.2 IU/ml were considered protective [6].

2.3. Statistical analysis

After log 10 transformation of the data, the geometric mean concentrations (GMC), standard deviation (S.D.) and 95% confidence interval (95% CI) were computed. For comparison of the GMC, paired and independent t-tests were used. To investigate linear relation between paired levels, correlation coefficients were used. A booster effect was defined as a ratio between antibody levels after the booster and the initial levels. The correlation coefficient and the linear regression model were used to find a relationship between initial antibody levels and the booster effect after 1 and 6 months. “Back transformation” and original data scale were used where possible.

3. Results

Prior to the booster, nearly all participants were well protected: only one individual (1.6%) had antitoxin level <0.1 IU/ml [7], whereas four (6.6%) had levels lower than 0.2 IU/ml. 25–75 percentiles were 0.49–2.14 IU/ml. During the first week after the booster, antitoxin levels increased and the maximum was reached within 1 month (Table 1). During the next month, the antibody levels were stable and a decline started only 2 months after the booster vaccination and was not steep: 1.28-fold from the second to the third month. Six months after the booster, the antitoxin level was half that of the maximum at 1 month; however, it exceeded the initial level by 1.65-fold. A weak positive but statistically significant linear influence of the initial concentration on the antibody levels 1 months after the booster ($R^2 = 0.18, p = 0.003$) was revealed, but not after 6 months. However, the magnitude of booster effect correlated negatively with the initial antibody levels (Figs. 1 and 2). The ratio between 1- and 6-month levels did not correlate with the initial levels and very little with the maximum levels ($R^2 = 0.27$).

Fig. 1. The influence of initial antibody levels on the magnitude of booster response after 1 month. Linear regression model: booster response = 0.504 – 0.622 × initial antibody level, $p < 0.001$ (for logarithmic data).
Table 1  
The tetanus antitoxin levels before and after tetanus toxoid booster in initially protected young men.

<table>
<thead>
<tr>
<th>Day of examination</th>
<th>No. of subjects</th>
<th>GMC (95% CI)</th>
<th>Ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>60</td>
<td>1.08 (0.12–10.15)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1 week</td>
<td>55</td>
<td>1.99 (0.13–30.99)</td>
<td>1.84 (1.20–2.83)</td>
<td>0.006</td>
</tr>
<tr>
<td>1 month</td>
<td>46</td>
<td>3.52 (0.54–22.80)</td>
<td>2.72 (1.98–3.73)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2 months</td>
<td>44</td>
<td>3.26 (0.48–21.91)</td>
<td>2.63 (1.88–3.66)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3 months</td>
<td>59</td>
<td>2.58 (0.31–18.29)</td>
<td>2.13 (1.48–3.07)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6 months</td>
<td>60</td>
<td>1.78 (0.24–12.05)</td>
<td>1.65 (1.18–2.30)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

*a Geometric mean concentration.
*b 95% Confidence interval.
*c Ratio GMC of the month to GMC of the day 0.

4. Discussion

In order to maintain protection against tetanus, tetanus toxoid is given routinely in childhood and adolescence, and thereafter as boosters in every 10 years, as well as a preventive measure after injuries. However, to our knowledge, the dynamics of the booster response in protected individuals is inadequately studied. With four exceptions, all the young men participating in this study had adequate levels of tetanus protection before the booster (>0.2 IU/ml). During the first week of the booster response, the GMC doubled and the maximum concentration was reached within 1 month. The increase within 1 month was less than 3-fold, which is lower than that demonstrated by others [8–11]. The participants had responded appropriately to diphtheria toxoid, so we had no reason to suspect any immune inadequacies among the participants [4]. We have no evidence of low efficacy of the tetanus vaccine. On the contrary, all subjects tested had significant pre-booster antitoxin levels. A specific tolerance towards tetanus toxoid has not been revealed. However, it has been shown that after five injections of toxoid, the decrease is steeper than after four, and that after six injections, the maximum level is reached and additional doses showed no further increase [12].

We did not reveal significant relationship between pre- and post-vaccination levels. However, an inverse correlation between initial antitoxin levels and booster immune response was demonstrated. Correspondingly, the booster effect in the low group was larger than in the high. Similar tendencies have been observed in other studies [9,12]. Aggerbeck et al. revealed a trend towards lower increase in post-vaccination concentrations with higher pre-vaccination concentration and with even a few non-responders with imi-
tial antitoxin levels above 10 IU/ml [9]. Olander et al. studied the booster response to tetanus toxoid carrier of pneumo-
coccal conjugate vaccine and the increase range in Finnish adults and children. Toddlers had seven times lower ini-
tial GMC compared to adults. Nevertheless, they developed a much stronger booster response after 1 month and even
reached levels significantly higher than the adults.

Compared to diphtheria booster where waning of anti-
body levels started immediately after the peak concentration,
tetanus maximum levels persisted longer than a month before
decreasing. This finding correlates with a previous study that
observed the maximum concentration waning little even after
3 months [8].

Overall, the decline from 1- to 6-month concentration was
about 2-fold, and after 6 months, only individuals with initial
antitoxin levels below 1 IU/ml had a booster effect ratio over
1. A similar rate of decline was observed by Aggerbeck et
al. [10]. From 1 to 12 months, a more considerable 4-fold
decline has been revealed [11]. Therefore, a further decline
among our participants can be speculated on.

The booster response in protected individuals generally
followed a typical curve except for a moderately prolonged
period of maximum levels. The response was more pro-
nounced in individuals with lower initial protection and was
longer maintained. Recently, a case of tetanus infection was
reported in an individual fully immunised and twice boosted
during the 5 years preceding his infection [14]. Unfortunately,
the anti-tetanus antibody level was not tested at the onset
during the 5 years preceding his infection [14]. Unfortunately,
the anti-tetanus antibody level was not tested at the onset
of disease. However, good response to booster given after
the cleaning of the infection was shown confirming normal
immune status of the patient. Other cases of tetanus infection
with histories of completed vaccination and boosters have
been reported [15–17]. The question arises whether these
breakthroughs happened because of insufficient vaccine effi-
cacy or due to some tolerance caused by frequent boosters?
For instance, frequent vaccinations may stimulate the for-
mation of specific regulatory T cells that suppress protective
immunity parallel to that found after allergy desensitisation
[18].

We found that half of the young men eligible for routine
vaccination had antitoxin level exceeding 1 IU/ml. It is not
an unusual situation that people with sufficient protection are
eligible for reinforcing doses. Thus, it has been found that
many more individuals admitted to an emergency department
with wounds where eligible for the booster according to their
vaccination histories than in fact were unprotected according
to serum antitoxin levels [19].

In conclusion, we have demonstrated a modest response
to tetanus toxoid in already protected individuals, whereas
an adequate anamnestic response in primed individuals with
undetectable antitoxin levels is well known. Taking into con-
sideration that the number of the adverse reactions to tetanus
toxoid increases with the number of injections [20] we rec-
ommend postponing the routine booster vaccination and pro-
viding it with a longer interval when the majority of the target
population has antitoxin level lower than 1 IU/ml.