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To determine the immunogenicity and safety of a single dose of diphtheria toxoid among adults, blood samples for detecting serum antitoxin levels were obtained from 18- to 59-year-old subjects (n = 248) before and 30 days after immunization with Td (tetanus-diphtheria toxoids; manufactured by Serum Institute of India). By day 30, the seroprevalence of antitoxin levels >0.1 IU/mL increased from 22.6% to 81.5%; median antitoxin levels increased from 0.01 to 4.0 IU/mL. These parameters were lowest among subjects who were 40–59 years old, especially among those 40–49 years old. Adverse reactions (local redness, swelling, induration, fever >39°C) were reported by 5.3% of participants. Our findings suggest that, in general, one dose of the Indian-produced Td vaccine is efficacious and safe in inducing an adequate immune response against diphtheria in adults; however, in Georgia, persons 40–59 years old, especially those 40–49 years old, will require additional doses of toxoid to achieve protective levels of antitoxin.

Since 1990, a large-scale resurgence of diphtheria began in the Russian Federation and subsequently spread to involve virtually all of the Newly Independent States (NIS) of the former Soviet Union. Routine diphtheria vaccination was introduced in the Republic of Georgia in the late 1950s. After almost 4 decades of successful control, epidemic diphtheria returned to the country in 1993; 28 cases of diphtheria were reported in 1993, 312 in 1994, 429 in 1995 [1], and 348 in 1996 [2].

The 1994 Strategy for Diphtheria Control in the NIS, which was outlined by the Regional Office for Europe, World Health Organization (WHO), and UNICEF, urged that population immunity be raised as rapidly as possible by mass vaccination of persons 3–59 years of age with at least one dose of diphtheria toxoid [3]. However, concerns remained about specific subpopulations known or suspected to be improperly vaccinated: the official vaccination policy had not been uniformly implemented throughout the Soviet republics, record keeping had been inconsistent and of variable quality, a long list of mostly false contraindications had been widely used to avoid vaccination, and an optimal vaccine and vaccination schedule for protection of adults had not been established. Additional questions arose concerning what might be the most efficacious vaccine potency and what number of vaccine doses would be required to protect adults.

We conducted a study to determine the immunogenicity and safety of a single dose of diphtheria toxoid among adults in the Republic of Georgia.

Methods

The present study was conducted in November and December 1995, while mass vaccination campaigns with tetanus-diphtheria toxoids (Td; vaccines with reduced content of diphtheria toxoid) were being implemented in the predominantly rural districts of Telavi and Gurjaani of Kakheti Region in Eastern Georgia. Study participants were recruited during a 5-day period on a first-come first-serve basis from apparently healthy volunteers (18–59 years old) presenting to receive vaccine at government clinics. Participants were excluded if they had a valid contraindication to vaccination or had received Td vaccine within the last 5 years.

The first blood specimen was obtained by venipuncture, and participants received Td vaccine (Serum Institute of India; Pune, India) formulated to contain <5 limit of flocculation units (Lf) of diphtheria toxoid. A second blood specimen was obtained 30 days later. Blood was separated at the study site, and the serum specimens were stored frozen in the Republic of Georgia until shipment.

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The study protocol was reviewed and approved by the Institutional Review Board of the CDC and by the Ministry of Health, Tbilisi. Informed consent was obtained from all participants.

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on dry ice to the virology laboratory at St. Christopher’s Hospital for Children, Philadelphia, for laboratory analysis. After the results of diphtheria antitoxin testing became available, study participants whose postvaccination level of diphtheria antitoxin was <0.1 IU/mL were contacted and offered an additional dose of Td vaccine.

A standard questionnaire was administered on day 30 to determine the frequency and nature of adverse events following administration of the study vaccine. Adverse events included redness ≥50 mm in diameter, swelling, or induration at the injection site within 7 days after immunization and body temperature >39°C or other severe systemic reactions within 48 h of vaccination.

Serum antibody titers against diphtheria toxoid were determined by toxin neutralization in Vero cells, using a modification of the procedure described by Miyamura and colleagues [4, 5] and Deforest et al. [6]. Assays were done in 96-well microtiter plates, using doubling serum dilutions. Anti-diphtheria antibody titers were converted to international units per microliter after standardization with reference serum provided by the Center for Biologics Evaluation and Research, Food and Drug Administration (Rockville, MD) [7]. The lowest detection limit for the assay is 0.0038 IU/mL.

The data were analyzed by χ² analysis, Fisher’s exact test, and comparison of median antitoxin levels, using Epi Info [8]. For the purposes of this study, we defined an antitoxin level of ≥0.01 IU/mL as at least minimally protective, a level of ≥0.1 IU/mL as consistent with at least short-term protection, and a level of ≥1.0 IU/mL as providing long-term protection against diphtheria [9]. Seroconversion was defined as an increase of antitoxin level from <0.1 IU/mL on day 0 to ≥0.1 IU/mL on day 30.

## Results

A total of 314 participants (92 men and 222 women) were enrolled, of whom 248 (79.0%) returned 30 days later; thus, 248 paired serum samples were available for analysis. The differences in the drop-out rates between the age groups were not statistically significant.

The overall prevalence of protective antitoxin levels significantly increased between days 0 and 30 following Td vaccination (P < .001; table 1). Overall, 22.6% of participants had diphtheria antitoxin levels of ≥0.1 IU/mL before vaccination, compared with 81.5% on postvaccination day 30. By day 30, the overall median level of diphtheria antitoxin increased from 0.01 to 4.0 IU/mL (table 2). Fifty-six participants (23%) had antitoxin levels ≥0.1 IU/mL prior to Td administration. Seroconversion was documented in 146 (76%) of the remaining 192 participants.

Significant differences by age group in the distribution of protective antitoxin levels were noted. Prior to vaccination, the seroprevalence of antitoxin levels ≥0.01 and ≥0.1 IU/mL by age group was distributed unevenly (P < 10⁻⁶ for both cutoff levels) and was lowest for the 40- to 49-year-old age group. Seroprevalence of antitoxin levels ≥1.0 IU/mL was significantly higher among participants 18–29 years old, compared with older age groups (P < .0022, Fisher’s exact test) (table 1). Distribution of prevaccination median antitoxin levels followed the same pattern: The 40- to 49-year-old group had a median antitoxin level below the lower limit of detection of the neutralization assay (table 2).

With one exception, the increase in seroprevalence and median antitoxin levels by day 30 among study participants was significant (P < .001) for all age groups for all three cutoff levels. The increase in the seroprevalence of antitoxin levels ≥0.01 IU/mL in the 18-to 29-year-old age group was not significant due to a high seroprevalence on day 0 (tables 1–2). However, postvaccination antitoxin levels of a large proportion (34.5%) of 40- to 59-year-olds adults remained below the protective threshold of 0.1 IU/mL; 22.4% remained below the level of minimal protection (0.01 IU/mL), and the median postvaccination antitoxin level in the 40- to 49-year-old age group was much lower (0.35 IU/mL) compared with that for the other age groups. Seroprevalence of diphtheria antitoxin levels <0.1 IU/mL by age group before and 30 days after Td administration is given in figure 1.

The proportion of 146 participants who seroconverted was also unevenly distributed by age group (P < .001): All 22 subjects who were 18–29 years old seroconverted, 58 (90.6%) of 64 subjects who were 30–39 years old seroconverted, 39 (60%) of 65 subjects who were 40–49 years old seroconverted, and 27 (65.9%) of 41 subjects who were 50–59 years old seroconverted. Among the 46 persons who did not seroconvert after Td administration, 17 in the 40- to 49-year-old age group (65.4%) and 9 in the 50- to 59-year-old age group (64.3%) had antitoxin levels <0.01 IU/mL on day 30.

Analysis of seroprevalence among study participants by sex revealed a significantly higher proportion of prevaccina-

## Table 1. Distribution of diphtheria antitoxin levels in subjects of various age groups before and 30 days after receipt of tetanus-diphtheria toxoids vaccine, Republic of Georgia, 1995.

<table>
<thead>
<tr>
<th>Age group, years (n)</th>
<th>% prevaccination seroprevalence</th>
<th>% postvaccination seroprevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥0.01 IU/mL</td>
<td>≥0.1 IU/mL</td>
</tr>
<tr>
<td>18–29 (62)</td>
<td>96.8</td>
<td>64.5</td>
</tr>
<tr>
<td>30–39 (70)</td>
<td>54.3</td>
<td>8.6</td>
</tr>
<tr>
<td>40–49 (69)</td>
<td>24.6</td>
<td>5.3</td>
</tr>
<tr>
<td>50–59 (47)</td>
<td>44.7</td>
<td>12.8</td>
</tr>
<tr>
<td>All age groups (248)</td>
<td>54.8</td>
<td>22.6</td>
</tr>
</tbody>
</table>

NOTE. Vaccine was formulated by Serum Institute of India (Pune, India) to contain ≤5 limit of flocculation units of diphtheria toxoid.
Table 2. Median diphtheria antitoxin levels in subjects of various age groups before and 30 days after receipt of tetanus-diphtheria toxoids vaccine, Republic of Georgia, 1995.

<table>
<thead>
<tr>
<th>Age group, years (n)</th>
<th>Prevaccination level</th>
<th>Postvaccination level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median antitoxin level (IU/mL)</td>
<td>95% CI</td>
</tr>
<tr>
<td>18-29 (62)</td>
<td>0.18</td>
<td>0.125-0.25</td>
</tr>
<tr>
<td>30-39 (70)</td>
<td>0.01</td>
<td>0.005-0.02</td>
</tr>
<tr>
<td>40-49 (69)</td>
<td>&lt;0.0038</td>
<td>&lt;0.0038</td>
</tr>
<tr>
<td>50-59 (47)</td>
<td>0.005</td>
<td>&lt;0.0038-0.01</td>
</tr>
<tr>
<td>All age groups (248)</td>
<td>0.01</td>
<td>0.075-0.02</td>
</tr>
</tbody>
</table>

NOTE. Vaccine was formulated by Serum Institute of India (Pune, India) to contain <5 limit of phospholipid units of diphtheria toxoid. CI, confidence interval.

Discussion

Following widespread vaccination against diphtheria in the Republic of Georgia in the late 1950s and early 1960s, diphtheria was controlled effectively and disappeared as a disease of major public health importance [10]. Vaccines were not only effective in controlling clinical respiratory diphtheria but probably interrupted the circulation of toxigenic Corynebacterium diphtheriae strains in the Republic of Georgia and other areas that had effective immunization programs. In the absence of natural exposure to and periodic boosting with toxigenic C. diphtheriae strains, vaccine-induced immunity against diphtheria toxin determines population immunity patterns. Because both vaccine coverage and vaccine effectiveness are <100% and immunity wanes in the absence of routine administration of...
booster vaccinations among adolescents and adults, cohorts of persons with increasing susceptibility to diphtheria develop. Eventually, such cohorts may again support the epidemic transmission of toxigenic *C. diphtheriae* strains, as was observed during the diphtheria epidemic in the Republic of Georgia.

In the Kakheti Region of the Republic of Georgia, prevaccination protective levels (≥0.1 IU/mL) of diphtheria antitoxin were found in 22.6% of adults tested; the seroprevalance was higher in recent serosurveys conducted in Ukraine (Kiev [11] and Odessa [12]) and Poland [13]. The low seroprevalence found in Georgia may be related to suboptimal immunization coverage levels in the past as well as to the lack of an effective cold chain and differences in exposure to natural diphtheria infection. Because seroprevalence levels were suboptimal among all age groups studied, these data support the recommendation of WHO/UNICEF that all persons 3–59 years old need to receive at least one dose of diphtheria toxoid to control the diphtheria epidemic in the NIS.

Although many adults tested were susceptible to diphtheria, cases among persons >40 years of age constituted only 9% of cases (29/324) during 1993–1995 in the two sites most affected by the disease (unpublished data, National Center for Disease Control of the Ministry of Health of Georgia). Other factors, such as exposure, likely contribute to the relatively low risk of clinical disease in this susceptible population [14].

Higher incidence rates for diphtheria have been reported among women than men in Georgia [10] and other NIS countries [15]. The lower prevaccination seroprevalence we found among women is generally consistent with the higher disease incidence among females in Georgia [10] and other NIS countries [15]. This difference cannot be explained by immunization of males during military service, as the analysis of antitoxin levels among men by previous military service did not reveal higher prevaccination antitoxin levels among those who served in the military in the past.

The Indian-produced Td vaccine, which contained ≤5 Lf of diphtheria toxoid, used in this study demonstrated an adequate safety profile. Adverse reactions reported by the study participants were generally less common than those reported in other immunogenicity studies in the NIS using comparable protocols [11, 12]. For example, 7% of the participants of the study conducted in Odessa reported swelling, 2% reported redness, and 5% reported fever >38°C [12]. Adverse reactions in the present study were also less common than in the small reactogenicity and immunogenicity trial of a Polish Td vaccine, which contained 2 Lf of diphtheria toxoid [13]; however, differences in data collection between the two studies do not allow direct comparison. In addition, because inquiry regarding adverse reactions was delayed until 30 days after vaccination and because safety data were not collected from participants who did not return after 30 days, our findings may represent an underestimate of the rate of adverse reactions.

Despite certain limitations (the study was not population based, women were over-represented in the sample, the study did not cover an urban population), the present survey demonstrated that one dose of Td vaccine was effective in inducing an effective immune response in most individuals, with the exception of 40- to 59-year-old adults. This age group had a considerably lower degree of protection following a single dose of Td vaccine. Similar age differences in susceptibility have been reported from serosurveys in Ukraine [11, 12]. These serologic findings are consistent with the high case-fatality rate among 40- to 49-year-old diphtheria patients in Georgia [10] and in the Russian Federation [16]. It is a likely explanation that these cohorts born after World War II were less exposed to toxigenic *C. diphtheriae* strains, decreasing the likelihood of developing natural immunity against diphtheria, and also were not vaccinated when the immunization programs were first established [16].

Persons 40–59 years old, especially those 40–49 years old, will require additional doses of diphtheria toxoid to achieve protective levels of antitoxin against diphtheria. The present study supports the recommendation to implement in the Republic of Georgia and other NIS a mass campaign of vaccination with at least one dose of diphtheria toxoid–containing vaccine to control the on-going epidemic. In addition, implementation of routine administration of booster doses of Td to adults every 10 years will be essential to ensure that diphtheria cannot reemerge in the future.

<table>
<thead>
<tr>
<th>Sex</th>
<th>% prevaccination seroprevalence</th>
<th>% postvaccination seroprevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n ≥0.01 IU/mL ≥0.1 IU/mL ≥1.0 IU/mL</td>
<td>n ≥0.01 IU/mL ≥0.1 IU/mL ≥1.0 IU/mL</td>
</tr>
<tr>
<td>Male</td>
<td>65 67.7% 38.6% 9.2% 92.3% 83.1% 72.3%</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>183 50.3% 16.9% 4.4% 88.5% 80.3% 61.2%</td>
<td></td>
</tr>
<tr>
<td>Previous military service</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>33 57.6% 27.3% 12.1% 97 78.8% 66.7%</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>32 78.1% 50% 6.2% 87.5% 87.5% 78.1%</td>
<td></td>
</tr>
</tbody>
</table>

a *P<.05.
b *P<.001.
Acknowledgments

This study is dedicated to the memory of the late Iain Hardy, who conceived and designed it, inspiring all who participated in carrying it out. We thank Tanja Popovic, Centers for Disease Control and Prevention (CDC), for her organizational support; the health authorities of the Kakheti Region for substantial local support; the trainees of CDC/Tbilisi (N. Mebonia, N. Mamuchishvili, L. Sturua, M. Tsereteli, R. Tskikauri, R. Gvetadze, and I. Khulordava) for participating in the field work and data entry; and Alicia Tortu, Virology Laboratory, St. Christopher’s Hospital for Children, for performing the serologic assays. We express special thanks to Melinda Wharton, Child Vaccine Preventable Disease Branch, National Immunization Program, CDC, for careful review of the manuscript and valuable comments.

References