Postbooster Antibodies from Humans as Source of Diphtheria Antitoxin

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Diphtheria antitoxin for therapeutic use is in limited supply. A potential source might be affinity-purified antibodies originally derived from plasma of adults who received a booster dose of a vaccine containing diphtheria toxoid. These antibodies might be useful for treating even severe cases of diphtheria.

Although diphtheria is an almost forgotten disease in industrialized countries, sporadic cases still occur. Possible reasons for these cases include partial failure of vaccine compliance, antivaccine campaigns, inadequate booster regimens, and immunosenescence. Health authority interest in this disease was rekindled after a nonvaccinated boy in Spain died of systemic diphtheria in June 2015 and 9 cases of cutaneous diphtheria among refugees were notified by Denmark, Sweden, and Germany in 2015 (1). According to the World Health Organization, 7,321 cases of diphtheria were reported worldwide in 2014. In the early 1890s, Emil von Behring used serum from a hyperimmune horse (challenged with sublethal dose of Corynebacterium diphtheriae) to develop equine diphtheria antitoxin (DAT), which seemed to confer passive immunity to patients with diphtheria (2). Subsequently, use of equine DAT to treat this disease became common. Uncontrolled but large studies of mortality rates from that time suggested effectiveness of equine DAT use; however, double-blinded randomized studies conducted by Adolf Bingel in 1918 concluded that equine DAT offered no benefit over serum from nonhyperimmune horses (not challenged with C. diphtheriae) (2). Although modern efficacy studies are lacking, equine DAT is still the recommended treatment for diphtheria, listed among the World Health Organization essential medicines (3). When administered early in the clinical course of disease, treatment with DAT can be lifesaving for patients with toxin-induced systemic symptoms.

A large proportion of European countries do not stockpile DAT, and many countries have experienced difficulties replacing expired stockpiles (3,4). As highlighted by the European Centre for Disease Prevention and Control (1), the current lack of DAT in the European Union is a concern. DAT is not produced or licensed in the United States or in most European countries; it is imported from Brazil under an Investigational New Drug protocol (5).

Equine DAT can induce anaphylactic reactions (a test for sensitivity to DAT should be conducted before each administration) (5). The European Centre for Disease Prevention and Control and the US Centers for Disease Control and Prevention encourage searching for new providers of equine DAT and promote the development of alternative antitoxins of human origin. The definitive solution will probably come from monoclonal antibodies (4) or synthetic molecules such as nucleic acid aptamers. These new molecules could constitute an unlimited source of DAT, with a low risk for hypersensitivity reactions. Unfortunately, these alternatives are not yet available and will need to undergo thorough regulatory processes before being approved for use in humans. We therefore describe the potential role of human plasma from vaccinated volunteers as a source of DAT.

Plasma from vaccinated persons is used to produce Anthrasil (Cangene Corporation, Winnipeg, Manitoba, Canada), a fully human polyclonal antiantitoxin intravenous immunoglobulin (IVIG) licensed in the United States. Antitetanus immunoglobulin is produced from plasma of young volunteers who received a booster dose of the tetanus–diphtheria vaccine.

The successful implementation of vaccination programs in industrialized and many developing countries indicates that most of these populations have antibodies against the diphtheria toxin. Nonetheless, the geometric mean concentration of IgG against diphtheria toxin in plasma of vaccinated adults who received the last dose of tetanus–diphtheria vaccine in their adolescence is not much over 0.3 IU/mL (6). For diphtheria treatment, 20,000–100,000 IU of DAT is needed; the dose depends on disease severity (5). In consequence, producing DAT from plasma obtained from the general population could not be cost-effective because large volumes would be needed to obtain a dose of DAT with enough potency for clinical use.

An alternative could be to obtain plasma from adult donors who recently received a booster dose of vaccine. Researchers have observed that during the diphtheria epidemic that emerged in the newly independent states of the...
that this approach was useful for concentrating DAT from
for research and development purposes (14). In consequence, this approach could be used to purify DAT
from plasma of revaccinated persons or from commercial
immunoglobulins (i.e., the antitetanus immunoglobulin
itself or nonspecific IVIG), which contains variable
concentrations of DAT (online Technical Appendix, http://
wwwnc.cdc.gov/EID/article/22/7/15-1670-Techapp1.pdf).
This concentrated DAT could be useful for treating diph-
theria of any severity in adults and children, with very low
risk of inducing hypersensitivity reactions.

A potential drawback of affinity purification is that the
obtained DAT could be denatured by acid elution. This risk
could be minimized by immediately neutralizing pH by
adding 1 mol/L Tris, followed by dialysis with phosphate-
buffered saline. The obtained product should undergo the
same biological agent removal processes as those used
for standard IVIG (i.e., chemical inactivation, heat inac-
tivation, nanofiltration, and precipitations). Neutralization
potency of DAT obtained from human plasma should be
assigned according to the Vero cell cytotoxicity assay and
the guinea pig lethality model; the 1st International Stan-
dard for Diphtheria Antitoxin Human should be used as the
reference antitoxin (National Institute for Biological Stan-
dards and Control code 10/262).

A limitation of using DAT obtained from human plasma
is the potential cost. Some developing countries, where
most cases of diphtheria occur, could not afford it. Produc-
tion costs and the price of each dose of human DAT could
be reduced by using as source the same plasma obtained
from the donors recruited to produce the antitetanus im-
munoglobulin. Industrialized countries could also donate
doses of this human DAT to developing countries.

### Table. Seroepidemiologic studies assessing levels of antitetanus antibodies in adults who received a booster dose of vaccine*

| Study population | Immunogenicity, GMC IU/mL (95% CI) |
|------------------|-----------------------------------|
| Mean age, y (SD or range) | Vaccine | Before booster | After booster |
| Ref. | | | |
| (8) | 40.1 (13.63) | 1.44 | 0.5 mL Tdap (Boostrix; GlaxoSmitKline Biologicals, Rixensart, Belgium) | 0.4 (0.4–0.4) | 4.7 (4.4–5.1) |
| 40.4 (13.48) | 728 | 0.5 mL Tdap (Adacel; Sanofi Pasteur, Swiftwater, PA, USA) | 0.5 (0.4–0.5) | 5.0 (4.6–5.4) |
| (9) | 31.7 (15–69) | 64 | 0.5 mL of Tdap (Sanofi Pasteur Limited, Toronto, ON, Canada) after previous vaccination with MCV4D (Menactra; Sanofi Pasteur, Swiftwater, PA, USA) | 4.45 (2.77–7.15) | 8.70 (6.59–11.5) |
| 379 | 0.5 mL Tdap (Sanofi Pasteur Limited, Toronto) | | | 0.13 (0.11–0.16) | 2.17 (1.84–2.56) |
| (10) | 19.4 (1.2) | 55 | 0.2 mL DTap (Kaketsuke, Kumamoto, Japan) | 0.22 (0.16–0.30) | 4.29 (3.53–5.21) |
| 19.4 (0.8) | 56 | 0.5 mL DTap (Kaketsuke) | 0.21 (0.15–0.30) | 6.28 (4.86–8.11) |
| (11) | 66.0 (59–91) | 252 | 0.5 mL Tdap (Repevax; Sanofi Pasteur MSD | 0.04 (0.03–0.06) | 1.09 (0.81–1.46) |
| 24.0 (20–33) | 21 | GmbH, Leimen, Germany | 0.14 (0.05–0.33) | 4.16 (2.36–7.34) |
| (12) | 21.1 (0.31) | 74 | 0.5 mL Tdap (Boostrix) | 0.3 (0.2–0.4) | 6.0 (4.7–7.7) |
| (13) | 26.5 (18–52) | 401 | 0.5 mL Tdap (Statens Serum Institut, Copenhagen, Denmark) | 0.11 (0.9–1.14) | 4.60 (4.03–5.26) |
| 26.1 (18–55) | 399 | 0.5 mL dTeBooster (Statens Serum Institut) | 0.11 (0.09–0.14) | 5.54 (4.00–5.15) |

*DTap, diphtheria, tetanus, and pertussis vaccine (for children >6 years of age); GMC, geometric mean concentration; Ref., reference; Tdap, tetanus, diphtheria, and pertussis vaccine (for children >11 years of age and adults).
Plasma from young adults receiving a booster dose of vaccine could represent a potential source of human DAT. Antigen-affinity antibody purification could help to produce a highly concentrated DAT from this plasma, useful for treating even the most severe forms of diphtheria. This approach could help mitigate the limited access to this essential medicine.

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References
1. European Centre for Disease Prevention and Control. Rapid risk assessment: a case of diphtheria in Spain [cited 2015 Jul 7]. http://ecdc.europa.eu/en/publications/Publications/diphtheria-spain-rapid-risk-assessment-june-2015.pdf
2. Opinel A, Tröhler U, Gluud C, Gachelin G, Smith GD, Podolsky SH, et al. Commentary: the evolution of methods to assess the effects of treatments, illustrated by the development of treatments for diphtheria, 1825–1918. Int J Epidemiol. 2013;42:662–76. http://dx.doi.org/10.1093/ije/dyr162
3. Wagner KS, Stickings P, White JM, Neal S, Crowcroft NS, Sesardic D, et al. A review of the international issues surrounding the availability and demand for diphtheria antitoxin for therapeutic use. Vaccine. 2009;28:14–20. http://dx.doi.org/10.1016/j.vaccine.2009.09.094
4. Both L, White J, Mandal S, Efratiou A. Access to diphtheria antitoxin for therapy and diagnostics. Euro Surveill 2014;19 pii:20830
5. Centers for Disease Control and Prevention. Use of diphtheria antitoxin (DAT) for suspected diphtheria cases [cited 2015 Aug 19]. http://www.cdc.gov/diphtheria/downloads/protocol.pdf
6. Wagner KS, White JM, Andrews NJ, Borrow R, Stanford E, Newton E, et al. Immunity to tetanus and diphtheria in the UK in 2009. Vaccine. 2012;30:7111–7. http://dx.doi.org/10.1016/j.vaccine.2012.09.029
7. Bissumbhar B, Rakhmanova AG, Berbers GA, Iakolev A, Nosikova E, Melnick O, et al. Evaluation of diphtheria convalescent patients to serve as donors for the production of anti-diphtheria immunoglobulin preparations. Vaccine. 2004;22:1886–91. http://dx.doi.org/10.1016/j.vaccine.2003.11.006
8. Blatter M, Friedland LR, Weston WM, Li P, Howe B. Immunogenicity and safety of a tetanus toxoid, reduced diphtheria toxoid and three-component acellular pertussis vaccine in adults 19–64 years of age. Vaccine. 2009;27:765–72. http://dx.doi.org/10.1016/j.vaccine.2008.11.028
9. Halperin SA, McNeil S, Langley J, Blatter M, Dionne M, Embree J, et al. Tolerability and antibody response in adolescents and adults revaccinated with tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccine adsorbed (Tdap) 4–5 years after a previous dose. Vaccine. 2011;29:8459–65. http://dx.doi.org/10.1016/j.vaccine.2011.07.068
10. Hara M, Okada K, Yamaguchi Y, Uno S, Otsuka Y, Shimano C, et al. Immunogenicity and safety after booster vaccination of diphtheria, tetanus, and acellular pertussis in young adults: an open randomized controlled trial in Japan. Clin Vaccine Immunol. 2013;20:1799–804. http://dx.doi.org/10.1128/CVI.00490-13
11. Kaml M, Weiskirchner I, Keller M, Luft T, Hoster E, Hasford J, et al. Booster vaccination in the elderly: their success depends on the vaccine type applied earlier in life as well as on pre-vaccination antibody titers. Vaccine. 2006;24:6808–11. http://dx.doi.org/10.1016/j.vaccine.2006.06.037
12. Mertsola J, Van Der Meerens O, He Q, Linko-Parvinen A, Ramakrishnan G, Mannermaa L, et al. Decennial administration of a reduced antigen content diphtheria and tetanus toxoids and acellular pertussis vaccine in young adults. Clin Infect Dis. 2010;51:656–62. http://dx.doi.org/10.1086/655825
13. Thierry-Carstensen B, Jordan K, Uhlying HH, Dalby T, Sorensen C, Jensen AM, et al. A randomised, double-blind, non-inferiority clinical trial on the safety and immunogenicity of a tetanus, diphtheria and monocomponent acellular pertussis (Tdap) vaccine in comparison to a tetanus and diphtheria (Td) vaccine when given as booster vaccinations to healthy adults. Vaccine. 2012;30:5464–71. http://dx.doi.org/10.1016/j.vaccine.2012.06.073
14. Sutjita M, Hohmann A, Comacchio R, Bradley J. Polyspecific human and murine antibodies to diphtheria and tetanus toxoids and phospholipids. Clin Exp Immunol. 1988;73:191–7.
15. Estabrook MM, Jarvis GA, McLeod Griffiss J. Affinity-purified human immunoglobulin G that binds a lacto-N-neotetraose-dependent lipoooligosaccharide structure is bactericidal for serogroup B Neisseria meningitidis. Infect Immun. 2007;75:1025–33. http://dx.doi.org/10.1128/IAI.00882-06

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Technical Appendix

**Technical Appendix Table.** Content in diphtheria antitoxin in commercially available intravenous immunoglobulins

| Intravenous immunoglobulin          | Antidiphtheria IgG, IU/mL, mean, (SD or range) | No. lots evaluated | Method for evaluation of diphtheria antitoxin                                      | Reference |
|------------------------------------|------------------------------------------------|--------------------|-------------------------------------------------------------------------------------|-----------|
| Endobulin (Baxter)                 | 7.82 (5.2)                                     | 3                  | In-house ELISA with standard calibrated against “Diphtheria antitoxin human serum 91/534” NIBSC reagent | (1)       |
| Flebogamma 5% (Grifols)            | 19.9 (19.3)                                    | 6                  | NA                                                                                  | NA        |
| Octagam (Octapharma)               | 10.1 (6.5)                                     | 16                 | NA                                                                                  | NA        |
| Tegeline (LFB-Biomedicaments)      | 7.8 (4.1)                                      | 9                  | NA                                                                                  | NA        |
| Vigam (Bio Products Laboratory)    | 8.9 (5.4)                                      | 2                  | NA                                                                                  | NA        |
| Immunoglobulin (NA)                | 12.9 (6.9)                                     | 2                  | NA                                                                                  | NA        |
| Bivlgam (Biotest)                  | 18.2 (3.5)                                     | NA                 | NA                                                                                  | (2)       |
| TBSF (Taiwan Blood Services Foundation) | 4.46 (0.86)                                   | 8                  | ELISA from Virotech® (Genzyme/Sekisui®)                                             | (3)       |
| Carimune (CSL Behring)             | 3.6 (NA)                                       | NA                 | Diphtheria toxin neutralization assay                                                | (4)       |
| Flebogamma 10% DIF (Grifols)       | 13.7 (1.4)                                     | NA                 | NA                                                                                  | NA        |
| Gammagard S/D 5% (Baxter)          | 5 (NA)                                         | NA                 | Diphtheria toxin neutralization assay                                                | (4)       |
| Gammmaplex 5% (Bio Products Laboratory) | 2.2 (NA)                                     | NA                 | NA                                                                                  | NA        |
| Hizentra 20% (CSL Behring)         | 2.5 (NA)                                       | NA                 | NA                                                                                  | NA        |
| Octagam 5% (Octapharma)            | 5-30                                           | NA                 | NA                                                                                  | NA        |
| Privigen 10% (CSL Behring)         | 4.9 (3.8-7.3)                                  | NA                 | NA                                                                                  | NA        |
| Flebogamma 5% DIF (Grifols)        | 6.0 (1)                                        | 29                 | ELISA (manufacturer not provided)                                                   | (5)       |
| NA (CSL Behring)                   | 3.6 (1.1)                                      | 44                 | ELISA from Scimedx®                                                                | (6)       |

*NA, not available.

References

1. Nobre FA, Gonzalez IG, Simão RM, de Moraes Pinto MI, Costa-Carvalho BT. Antibody levels to tetanus, diphtheria, measles and varicella in patients with primary immunodeficiency undergoing intravenous immunoglobulin therapy: a prospective study. BMC Immunol. 2014;15:26. PubMed [http://dx.doi.org/10.1186/1471-2172-15-26](http://dx.doi.org/10.1186/1471-2172-15-26)
2. Wasserman RL. A new intravenous immunoglobulin (BIVIGAM®) for primary humoral immunodeficiency. Expert Rev Clin Immunol. 2014;10:325–37. PubMed
http://dx.doi.org/10.1586/1744666X.2014.891438

3. Wu C-Y, Wang H-C, Wang K-T, Yang-Chih Shih D, Lo C-F, Wang D-Y. Analyzing titers of antibodies against bacterial and viral antigens, and bacterial toxoids in the intravenous immunoglobulins utilized in Taiwan. Biologicals. 2013;41:88–92. PubMed

4. Siegel J. Immune globulins: therapeutic, pharmaceutical, cost, and administration considerations. Pharm Pract News [cited 2016 Apr 27]. http://www.pharmacypracticenews.com/download/PF131_ImmuneGlobulins_WM.pdf

5. Jorquera JI. Flebogamma 5% DIF development: rationale for a new option in intravenous immunoglobulin therapy. Clin Exp Immunol. 2009;157(Suppl 1):17–21. PubMed
http://dx.doi.org/10.1111/j.1365-2249.2009.03953.x

6. Lejtenyi D, Mazer B. Consistency of protective antibody levels across lots of intravenous immunoglobulin preparations. J Allergy Clin Immunol. 2008;121:254–5. PubMed
http://dx.doi.org/10.1016/j.jaci.2007.11.001