IMMEDIATE AND LONGER TERM IMMUNOGENICITY OF A SINGLE DOSE OF THE COMBINED *HAEMOPHILUS INFLUENZAE* TYPE B-*NEISSERIA MENINGITIDIS* SEROGROUP C-TETANUS TOXOID CONJUGATE VACCINE IN PRIMED TODDLERS 12 TO 18 MONTHS OF AGE

Robert Booy, MB BS, MSc, MD, Peter Richmond, MB BS,† Terry Nolan, MB BS, PhD,‡§ Jodie McVernon, MB BS, PhD,‡§ Helen Marshall, MB BS, MPH,¶ Michael Nissen, MB BS,‖ Graham Reynolds, MD,** John B. Ziegler, MD,†† Leon Heron, MB ChB, MPH,* Stephen Lambert, MB BS, MAppEpid,‖ Magalie Caubet, MA,‡‡ Narcisa Mesaros, MD,‡‡ and Dominique Boutriau, MD‡‡*

Abstract: Hib-primed but MenC-naive toddlers (N = 433) were randomized to receive 1 dose of Hib-MenC-TT or separate Hib-TT and MenC-CRM_{197} vaccines. One month later, noninferiority was demonstrated for serum bactericidal anti-MenC antibodies (rSBA) and Hib antipolyribosylribitol phosphate (PRP) antibodies; >99% in both groups had rSBA titer ≥8 or anti-PRP concentration ≥0.15 μg/mL. After 12 months, rSBA titer ≥8 persisted in 86.7% and 76.4%, and anti-PRP concentration ≥0.15 μg/mL persisted in 98.8% and 100% of children, respectively.

Key Words: *Haemophilus influenzae* type b, *Neisseria meningitidis* serogroup C, antibody persistence, toddlers

Accepted for publication September 9, 2010.

From the *National Centre for Immunisation Research and Surveillance (NCIRS), The Children's Hospital at Westmead, Sydney, Australia; †School of Paediatrics and Child Health, Princess Margaret Hospital for Children, University of Western Australia, Perth, Australia; ‡Murdoch Children's Research Institute, Royal Children's Hospital, Victoria, Australia; §School of Population Health, University of Melbourne, Carlton, Australia; ¶Paediatric Trials Unit, Women's and Children's Hospital and University of Adelaide, Adelaide, South Australia, Australia; ‖Queensland Pediatric Infectious Diseases Laboratory, Royal Children's Hospital, Herston, Queensland, Australia; **Paediatric and Child Health, Canberra Hospital, Canberra, Australia; ††Sydney Children's Hospital, Randwick, Australia; and ‡‡GlaxoSmithKline Biologicals, Wavre/Rixensart, Belgium.

Study number: 106445/106446; www.ClinicalTrials.gov: NCT00326118.

Supported by GlaxoSmithKline Biologicals (GSK), Rixensart, Belgium. R.B., T.N., J.B.Z., P.R., J.McV., H.M., M.N., G.R., L.H., and S.L. are investigators in studies funded by GSK. Occasionally funded by organizations such as Commonwealth Serum Laboratiories (CSL), Roche, Sanofi, GSK, and Pfizer (Wyeth) to attend and present at scientific meetings (to R.B.). Any funding received is directed to a research account at The Children's Hospital at Westmead and is not personally accepted by R.B. Received travel support from GSK, CSL, Novartis, Sanofi-Pasteur, and Wyeth for participation in investigator meetings or for the presentation of scientific data at research meetings, and honoraria for participation in a GSK independent data monitoring and safety board for an unrelated vaccine (to T.N.). Supported by Institutional funding from GSK for investigator-led epidemiological studies and received travel support from GSK and other vaccine companies to present scientific data and chair workshops (to P.R.). Sponsored by a range of vaccine manufacturers, and received travel grants from vaccine companies to attend and chair scientific meetings and honoraria for membership of vaccine advisory boards (to J.McV., investigator on vaccine studies). Supported by travel grants from GSK and other companies to attend scientific meetings to present independent scientific data (to H.M., investigator on vaccine studies and been a member of vaccine advisory boards for GSK and other companies); from GSK and Pfizer (Wyeth) for participation in investigator meetings or for the presentation of scientific data at research meetings (to M.N. and G.R.); and from Wyeth, Nutricia, and Octapharma for participation in investigator meetings or to attend, chair, and present at scientific meetings (to J.Z., investigator in industry sponsored vaccine and other clinical trials). Sponsored by GSK and other companies, and received support for conference attendance from GSK (to L.H., member of a vaccine advisory board for Novartis). The money is directed to a research account at the Children's Hospital at Westmead. Supported by travel grants from GSK and other companies for participation in investigator meetings or for the presentation of scientific data at research meetings, and honoraria for being a member of vaccine advisory boards for GSK and other companies (to S.L.).

Authors R.B., P.R., T.N., J.McV., H.M., M.N., G.R., J.B.Z., L.H., and S.L. have no shares, paid employment, or consultancies with GSK or other pharmaceutical companies.

D.B., M.C., and N.M. are employees of GSK. D.B. reports ownership of stock options and declares that she is designated inventor on a variety of patents owned by GSK.

Menitorix, Priorix, and Hiberix are trademarks of the GlaxoSmithKline group of companies. Meningitec is a trademark of Wyeth.

Address for correspondence: Robert Booy, MB BS, MSc, MD, National Centre for Immunisation Research and Surveillance (NCIRS), The Children's Hospital at Westmead, Sydney, NSW 2145, Australia. E-mail: robertb2@chw.edu.au.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pidj.com).

Copyright © 2011 by Lippincott Williams & Wilkins

DOI: 10.1097/INF.0b013e31820013d2

The combined *Haemophilus influenzae* type b-*Neisseria meningitidis* serogroup C-tetanus toxoid conjugate vaccine (Hib-MenC-TT; Menitorix; GlaxoSmithKline Biologicals, Rixensart, Belgium) has been shown to be immunogenic with an acceptable safety profile when administered as a 3-dose primary vaccination course in infants[1–3] and booster dose in the second year of life.[4–6] The present study evaluated Hib-MenC-TT when given as a single dose to toddlers aged 12 to 18 months, primed in infancy with a Hib vaccine but no MenC vaccine (routine in Australia), and with MMR coadministered. Immunogenicity was measured prevaccination, and 1 month and 1 year postvaccination. The safety and reactogenicity of the Hib-MenC-TT vaccine was also evaluated (Text and Figs., Supplemental Digital Content 1, http://links.lww.com/INF/A685).

METHODS

This was an open, randomized, controlled, multicenter study conducted between 2006 and 2008. Healthy Australian children aged 12 to 18 months, who had received routine childhood vaccinations in infancy at least 6 months before, were enrolled. All had been primed with either 2 doses of a Hib-outer membrane protein (Hib-OMP) containing vaccine or 3 doses of a diphtheria-tetanus-acellular pertussis/Hib-TT (DTPa/Hib-TT) containing vaccine. Children were assigned randomly (3:1 ratio) to receive MMR (Priorix) coadministered with either 1 dose of Hib-MenC-TT (Menitorix) (Hib-MenC group) or Hib-TT (Hiberix) plus MenC-CRM_{197} vaccine (Meningitec) (Hib+MCC group). Assessments of long-term protection are planned at yearly intervals up to 5 years after vaccination. This report presents antibody responses and persistence data up to 1 year after vaccination.

Serum samples were tested using a serum bactericidal assay with baby rabbit complement against *N. meningitidis* serogroup C (rSBA) and enzyme-linked immunosorbent assays for IgG antibodies against meningococcal polysaccharide C (data not shown) and Hib polysaccharide polyribosylribitol phosphate (PRP). For rSBA, a titer of 8 is considered seroprotective[7] and the more conservative cut-off of ≥128 was also evaluated. For anti-PRP antibodies, cut-offs of 0.15 and 1.0 μg/mL were considered as protective response correlates.[8,9]

A sample size of 432 children (324 in Hib-MenC group and 108 in Hib+MCC group) gave 90.2% power to demonstrate noninferiority of Hib-MenC-TT conjugate vaccine to separately administered Hib-TT and MenC-CRM_{197} vaccines in terms of the percentage of children with rSBA titer ≥8 and percentage of children with anti-PRP concentration ≥0.15 μg/mL 1 month postvaccination. The noninferiority coprimary objectives would be demonstrated if the lower limits of the standardized asymptotic 95% confidence interval (95% CI) for the differences between groups (Hib-MenC group − Hib+MCC group) were above −10% for both antigens.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

The differences in the percentage of children with antibody titers or concentrations greater than or equal to the relevant antibody cut-off value were also compared; 95% CIs of differences not including 0 were regarded as indicative that a significant difference might exist between groups. For differences in geometric mean titer (GMT) or geometric mean concentration (GMC) ratio (Hib-MenC group over Hib+MCC group), 95% CIs not including 1 indicated that a significant difference might exist. Exploratory subgroup analyses were also performed by type of primary Hib vaccination (DTPa/Hib-TT or Hib-OMP).

RESULTS

A total of 433 toddlers (mean age, 12.5 months; range, 12–17 months) were enrolled and 392 were included in the according-to-protocol cohort for immunogenicity (Fig., Supplemental Digital Content 2, http://links.lww.com/INF/A686). There were no major differences in demographic characteristics between the 2 groups (Table, Supplemental Digital Content 3, http://links.lww.com/INF/A687). The mean age at the year 1 assessment was 24.6 months; 38 children did not participate because of ineligibility (2 children), lost to follow-up (9), and other reasons (none associated with an adverse event; 27). Therefore, 395 children were included in the total enrolled cohort for year 1, of whom 357 were included in the according-to-protocol cohort for persistence at year 1 (Fig., Supplemental Digital Content 2, http://links.lww.com/INF/A686).

The noninferiority coprimary objectives were reached: 1 month after vaccination, 99.6% and 100% of children in the Hib-MenC group and Hib+MCC group, respectively had rSBA titer ≥8 (95% CI for difference, −1.99% to 3.43%). All children in both groups had anti-PRP concentration ≥0.15 μg/mL (95% CI for difference, −1.30% to 3.71%).

The rSBA GMT 1 month postvaccination tended to be lower in the Hib-MenC group than in the Hib+MCC group (Table, Supplemental Digital Content 4, http://links.lww.com/INF/A688) without reaching statistical significance (95% CI on GMT ratio, 0.57–1.02). After 12 months, the rSBA GMT tended to be higher in the Hib-MenC group (95% CI on GMT ratio, 0.97–2.14) and a significantly higher percentage of this group had rSBA titers ≥8 (86.7% vs. 76.4%; 95% CI for difference, 1.35%–20.89%). One month and 12 months after vaccination, there were no significant differences between groups in percentage of children with rSBA titer ≥128 (95% CI for difference, −9.14% to 5.13% and −6.68% to 17.03%, respectively).

Before vaccination, anti-PRP concentration ≥0.15 μg/mL persisted in more than 76% of children, with similar anti-PRP GMCs in both groups (Table, Supplemental Digital Content 5, http://links.lww.com/INF/A689). One month postvaccination, the Hib booster response was robust in both groups, inducing a 106-fold increase in GMC in the Hib-MenC group and 157-fold increase in the Hib+MCC group from prevaccination values. Anti-PRP concentration ≥1.0 μg/mL was recorded in 97.9% of the Hib-MenC group and all children in the Hib+MCC group. The anti-PRP GMC was statistically significantly lower in the Hib-MenC group than in the Hib+MCC group (95% CI on GMC ratio, 0.47–0.84). Twelve months after vaccination, anti-PRP concentration ≥0.15 μg/mL persisted in more than 98% of children in both groups (Table, Supplemental Digital Content 5, http://links.lww.com/INF/A689), with no significant difference between groups (95% CI for difference, −3.41% to 2.90%). There were no significant differences in percentage of children with anti-PRP concentration ≥1.0 μg/mL (95% CI for difference, −13.46% to 3.31%) or anti-PRP GMC (95% CI on GMC ratio, 0.53–1.02).

Exploratory analyses of children split according to their Hib priming schedule confirmed robust increases in anti-PRP GMCs in all subgroups 1 month postvaccination (Table, Supplemental Digital Content 5, http://links.lww.com/INF/A689). In comparison with DTPa/Hib-TT primed children, Hib-OMP primed children had higher anti-PRP antibody concentrations at enrollment and higher anti-PRP antibody concentrations 1 month and 1 year following vaccination with Hib-MenC-TT or Hib-TT plus MenC-CRM_{197}. Comparison of the Hib-MenC and Hib+MCC groups showed statistically significantly higher anti-PRP concentrations in the Hib+MCC group in DTPa/Hib-TT-primed children at 1 and 12 months (95% CI on GMC ratio, 0.47–0.89 after 1 month; 0.49–0.99 after 12 months). For rSBA titers, no difference was observed between children primed with Hib-OMP or with Hib-TT (data not shown).

DISCUSSION

The results of this study provide evidence that the combined Hib-MenC-TT vaccine is noninferior to separately administered Hib-TT and MenC-CRM_{197} vaccines in terms of proportions of children seroprotected against the MenC and Hib components. One month postvaccination, more than 99% of children in both groups had protective rSBA titers (≥8) and all had protective anti-PRP concentrations (≥0.15 μg/mL). The rSBA seroprotection rates in our study were similar to that seen previously in a UK study of MenC vaccine naive toddlers aged 12 to 18 months who received a single dose of MenC-TT vaccine at the same time as MMR but no Hib vaccine.[10] One year after vaccination, seroprotective rSBA titers persisted in 86.7% of the Hib-MenC group and 76.4% of the control group, and more than 98% in both groups had persistent seroprotective anti-PRP concentrations. The proportion with persistent seroprotective rSBA titers was higher than rates (33%–38%) reported after 1 year in a UK study of children primed with MenC-CRM_{197} and boosted with Hib-MenC-TT at 12 to 15 months of age.[11] We have no single explanation for this difference but the 2 studies were conducted in different countries, with different priming vaccination schedules, and used different laboratories. Notably, rSBA titer persistence in our study was consistent with that in the UK group primed with MenC-TT and boosted with Hib-MenC-TT (85%),[11] and the persistence rate (95%) reported in another study 18 months after MenC-TT priming and Hib-MenC-TT booster administration.[5]

It is interesting to note that the rSBA GMT 1 month postvaccination tended to be lower after Hib-MenC-TT immunization than with separate administration of Hib-TT and MenC-CRM_{197} conjugate vaccines. However, at 12 months postvaccination, the rSBA GMT tended to be higher, with a significantly higher percentage of children with seroprotective titers ≥8 in the Hib-MenC group. It has been previously observed that the Hib-MenC-TT vaccine induces bactericidal antibodies that persist longer than those induced by MenC-CRM_{197}.[2,4,5] Similarly, in another study in toddlers, the persistence of bactericidal antibodies was lower after immunization with 2 different MenC-CRM_{197} vaccines compared with MenC-TT vaccination.[10] However, as well as carrier type, there are other important differences between the Hib-MenC-TT vaccine and MenC conjugate vaccine used in our study in terms of antigen content (5 μg in Hib-MenC-TT, 10 μg in the MenC-CRM_{197} vaccine) and adjuvantation (MenC-CRM_{197} vaccine formulation includes aluminum). Moreover, a limitation of this study is that no treatment group received MenC-TT vaccine for comparison. A MenC-CRM_{197} vaccine was

© 2011 Lippincott Williams & Wilkins

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

chosen as control because this was commonly used in Australian routine vaccination programs at the time the study was conducted.

All participants in this study had been primed in infancy with a Hib-containing vaccine, either Hib-OMP or a DTPa/Hib-TT combination vaccine. One month after the booster dose, all subjects had anti-PRP concentrations ≥0.15 μg/mL. In both the Hib-TT group and the Hib-MenC-TT group, the Hib-OMP primed subjects of the groups demonstrated significantly higher postboost anti-PRP antibody levels (GMCs, 212 μg/mL and 150 μg/mL, respectively) compared with the DTPa/Hib-TT primed subjects of the groups (GMCs, 46 μg/mL and 29 μg/mL, respectively). The trend for a difference in Hib booster responses following Hib-TT (10 μg Hib-TT) versus Hib-MenC-TT (5 μg Hib-TT) might relate to the Hib dosage. A previous study found priming with Hib-OMP followed by Hib-CRM_{197} booster induced a stronger immune response than Hib-OMP boosting.[12] In relation to our study, Hib-OMP priming followed by Hib-TT boosting may create the environment of PRP-related B-cell memory and TT-related T-cell memory, enabling strong Hib booster responses. Previously, it was found that, in a 2-4-6 schedule, an early dose with Hib-OMP versus Hib-TT induced higher responses with subsequent Hib-TT immunization,[13] which is in line with our findings. However, whatever the type of Hib primary vaccination in this study, Hib booster vaccination elicited robust increases in anti-PRP GMCs and the majority of children had anti-PRP concentrations ≥1.0 μg/mL.

In conclusion, a single dose of the combined Hib-MenC-TT conjugate vaccine was as immunogenic as the licensed Hib-TT and MenC-CRM_{197} vaccines administered simultaneously in different sites to Hib-primed/MenC-unprimed toddlers. Moreover, the Hib-MenC-TT vaccine was well tolerated and incidences of solicited local symptoms, redness and swelling, and irritability were significantly lower in the Hib-MenC group compared with the Hib+MCC group (Text and Figs., Supplemental Digital Content 1, http://links.lww.com/INF/A685). Where consideration is given to implementing a single MenC dose in toddlers at the time of Hib boosting, the present study data suggest the Hib-MenC-TT vaccine can be used without compromising immunogenicity for the Hib or MenC antigens, while reducing the number of injections required. Data from the ongoing 5-year follow-up of these subjects will add to a growing body of information regarding long-term persistence of MenC antibody, relevant to considering potential requirements for an adolescent booster dose.

ACKNOWLEDGMENTS

The authors thank all the children and their parents as well as the staff members and nurses who were involved in this study. From Sydney: Annemarie Egan, Rosemary Joyce, Kylee Gibbins, Lyn Barnes, Laura Rost, Pamela Cheung, Jennifer Murphy, Camille Lang, Elizabeth Deegan, Elizabeth Clarke, Carol Shineberg, Edwina Jacobs, Stephen George, Katherine Hale, Mary Iskander, Nicholas Wood, Monica Lahra, Joseph Khouri, Gulam Khandaker, Tony Yee, Briget Evans, Susan Smith, Vanessa Bonett; Perth: Jennifer Kent and Larissa Rhind (site coordinators), Jan Adams, Dr. Gabriela Dixon, Lisa Montgomery, Dr. Karen Prosser, Dr. Tanya Stoney; Melbourne: Marita Kefford (site coordinator), Dr. Karyn Alexander, Michelle Boglis, Janet Briggs, Clare Brophy, Elizabeth Christie, Dr. Jenny Davey, Jane Gibson, Erin Hill, Alice Holloway, Dr. Lana Horng, Ruth Lawrence, Stephanie Lenko, Betty Lim, Sarah Macnee, AnnMarie McEvoy, Liz McGrath, Elanna Nolan, Jacinta O'Keefe, Mairead Phelan, Dr. Nicole Rose, Jane Ryrie, Charan Sandhu, Deb Saunders, Jacinta Sonego, Judith Spotswood, Dr. Loretta Thorn, Marie West; Brisbane: Aaron Buckner (site coordinator), Dr. Raymond Chuk, Lisa Mulhearn; Canberra Hospital: Dr. Sandra Gillet; Adelaide: Diana Weber, Louise DeGaris, Chris Heath, Michelle Clarke, Jane Tidswell, Dr. Jan Walker, Dr. Susan Evans. The authors also thank Koen Maleux for serology analysis, Sheila Woods for report writing, Drs Caterina Hatzifoti and Tatjana Mijatovic for publication management, and Dr. Joanne Knowles for drafting the manuscript on behalf of GlaxoSmithKline Biologicals. Finally, the authors are indebted to Dr Jacqueline Miller for critical reviewing of the manuscript and expert comments provided. GlaxoSmithKline Biologicals was involved in all stages of the study conduct and analysis. GlaxoSmithKline Biologicals also underwrote all costs associated with the development and publishing of the present manuscript. All authors contributed to the study and writing of the manuscript and the corresponding author had full access to the data and final responsibility for submission of the publication.

REFERENCES

1. Pace D, Snape M, Westcar S, et al. A new combination *Haemophilus influenzae* type b and *Neisseria meningitidis* serogroup C-tetanus toxoid conjugate vaccine for primary immunization of infants. *Pediatr Infect Dis J*. 2007;26:1057–1059.
2. Schmitt HJ, Maechler G, Habermehl P, et al. Immunogenicity, reactogenicity, and immune memory after primary vaccination with a novel *Haemophilus influenzae-Neisseria meningitidis* serogroup C conjugate vaccine. *Clin Vaccine Immunol*. 2007;14:426–434.
3. Tejedor JC, Moro M, Ruiz-Contreras J, et al. Immunogenicity and reactogenicity of primary immunization with a novel combined *Haemophilus influenzae* type b and *Neisseria meningitidis* serogroup C-tetanus toxoid conjugate vaccine coadministered with a diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated poliovirus vaccine at 2, 4 and 6 months. *Pediatr Infect Dis J*. 2007;26:1–7.
4. Pace D, Snape M, Westcar S, et al. A novel combined Hib-MenC-TT glycoconjugate vaccine as a booster dose for toddlers: a phase 3 open randomised controlled trial. *Arch Dis Child*. 2008;93:963–970.
5. Tejedor JC, Moro M, Merino JM, et al. Immunogenicity and reactogenicity of a booster dose of a novel combined *Haemophilus influenzae* type b-*Neisseria meningitidis* serogroup C-tetanus toxoid conjugate vaccine given to toddlers of 13–14 months of age with antibody persistence up to 31 months of age. *Pediatr Infect Dis J*. 2008;27:579–588.
6. Carmona A, Miranda M, Barrio F, et al. Reactogenicity and immunogenicity of combined *Haemophilus influenzae* type b-meningococcal serogroup C conjugate vaccine booster dose coadministered with measles, mumps, and rubella vaccine. *Pediatr Infect Dis J*. 2010;29:269–271.
7. Borrow R, Balmer P, Miller E. Meningococcal surrogates of protection—serum bactericidal antibody activity. *Vaccine*. 2005;23:2222–2227.
8. Käyhty H, Peltola H, Karanko V, et al. The protective level of serum antibodies to the capsular polysaccharide of *Haemophilus influenzae* type b. *J Infect Dis*. 1983;147:1100.
9. Anderson P. The protective level of serum antibodies to the capsular polysaccharide of *Haemophilus influenzae* type b. *J Infect Dis*. 1984;149:1034–1035.
10. Richmond P, Borrow R, Goldblatt D, et al. Ability of 3 different meningococcal C conjugate vaccines to induce immunologic memory after a single dose in UK toddlers. *J Infect Dis*. 2001;183:160–163.
11. Borrow R, Andrews N, Findlow H, et al. Kinetics of antibody persistence following administration of a combination meningococcal serogroup C and *Haemophilus influenzae* type b conjugate vaccine in healthy infants in the United Kingdom primed with a monovalent meningococcal serogroup C vaccine. *Clin Vaccine Immunol*. 2010;17:154–159.
12. Reid R, Santosham M, Croll J, et al. Antibody response of Navajo children primed with PRP-OMP vaccine to booster doses of PRP-OMP vs. HbOC vaccine. *Pediatr Infect Dis J*. 1993;12:812–815.
13. Greenberg DP, Lieberman JM, Marcy SM, et al. Enhanced antibody responses in infants given different sequences of heterogeneous *Haemophilus influenzae* type b conjugate vaccines. *J Pediatr*. 1995;126:206–211.

 © 2011 Lippincott Williams & Wilkins

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

LESSONS LEARNED FROM A MEASLES OUTBREAK IN ANTWERP, BELGIUM

2007–2008

Carine Asnong, MD, Koen Van Herck, MD, PhD,† Tinne Lernout, MD,‡ Heidi Theeten, MD,† and Pierre Van Damme, MD, PhD†*

Abstract: A 2007 to 2008 measles outbreak in Antwerp, Belgium, identified the orthodox Jewish communities as a new risk group. This study analyzes vaccination data of 949 school children of 4 belief systems to assess the completeness and timeliness of their measles-mumps-rubella vaccination. Orthodox Jewish children show a 4-fold lower chance of complete vaccination, a delayed start, and increased temporal spacing of childhood vaccinations. Not only belief issues but difficulties to access the regular vaccination program also seem to be the main reason.

Key Words: measles, outbreak, vaccination coverage, Belgium

Accepted for publication August 31, 2010.

From the *School Health Center of the Province of Antwerp, Antwerp, Belgium; †Centre for the Evaluation of Vaccination, Vaccine and Infectious Disease Institute, University of Antwerp, Antwerp, Belgium; and ‡Unit of Infectious Diseases, Scientific Institute of Public Health, Brussels, Belgium.

Address for correspondence: Pierre van Damme, MD, PhD, Centre for the Evaluation of Vaccination, Vaccine and Infectious Disease Institute, University of Antwerp, Antwerp, Belgium, Universiteitsplein 1–2610, Antwerp, Belgium. E-mail: pierre.vandamme@ua.ac.be.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pidj.com).

DOI: 10.1097/INF.0b013e3181fbf5b7

Routine national childhood vaccination programs aim at a 95% vaccination coverage for 2 doses of the measles-mumps-rubella (MMR) vaccine. Suboptimal coverage rates cause a slowly increasing pool of unprotected individuals in the absence of measles circulation and natural boosting, whereas frequent international travel facilitates the import of new measles cases.[1,2] Continuing reports of measles outbreaks, often originating in known risk groups for measles transmission, such as Roma populations and anthroposophic (AN) communities, threaten the WHO's goal to eliminate measles from the European region by 2010. Between August 2007 and May 2008, a measles outbreak affected the culturally closed orthodox Jewish (OJ) communities of Antwerp,[3] triggered by 2 import cases of measles from the United Kingdom. Overall vaccination coverage for the first dose of MMR in the province of Antwerp was 89% to 97% in different surveyed age cohorts,[4] but separate information for this particular group was not available. In Belgium, school health centers (SHC) play an important role in the prevention and control of infectious diseases for the school-aged population (age, 3–18 years). They monitor the vaccination status and administer more than 80% of vaccinations in school children,[5,6] free of charge. SHC also notify public health authorities in case of (suspected) infectious disease in their schools and offer vaccination to unprotected school contacts. This study uses vaccination data from an SHC involved in the Antwerp measles outbreak to assess the completeness and timeliness of MMR vaccination in Antwerp school children from 4 different belief systems: OJ, modern Jewish (MJ), AN, and mainstream (MS).

METHODS

For this study, we collected vaccination data from 949 children in nursery and primary schools supported by the SHC of the Province of Antwerp (SHCA) in the school year 2006 to 2007 (ie, before the outbreak). In each of the 4 different types of schools (OJ, MJ, AN, MS), we selected 3 age groups on the basis of regular contact schedule within the SHC. In the first year of nursery school (age 3), children are examined for the first time and infant vaccination data are checked. Subsequent contacts are scheduled in first and fifth grade. To obtain a better understanding of the general vaccination behavior in the OJ communities, not only data on the MMR vaccinations (recommended at 12 months and 10–13 years of age) were collected, but also on the primary diphtheria-tetanus-pertussis (DTP) vaccinations (recommended at 8, 12, 16 weeks, and 15 months of age).

Between December 2008 and February 2009, data collection was executed in 3 consecutive steps. Vaccinnet, a web-based vaccine ordering system linked to a vaccination database available to vaccinators in Flanders (northern Belgium),[7] was used as the primary data source. Incomplete records were supplemented by an internal database (used by SHC only) in a second step, and the individual paper SHCA records of children in a third step. There was no direct communication with other vaccinators to obtain any missing data. Vaccination data were validated for timeliness, according to the guidelines of the Belgian Superior Health Council.[8] All invalid doses were excluded from the study. Statistical analysis was performed in Excel and SPSS 15. Differences were considered as statistically significant when $P < 0.05$. The completeness and timeliness of MMR and primary DTP vaccinations were compared between the schools of different belief systems using cross-tabulation and Pearson χ^2 test. Completeness for MMR was defined as at least 1 dose of MMR vaccine. Completeness for primary DTP was defined as 4 valid doses of DTP vaccine when started before the age of 5 months (recommended), or 3 valid doses when started after the age of 5 months. Timeliness for MMR was defined as at least 1 dose of MMR vaccine before the age of 18 months. Timeliness for primary DTP was defined as 3 or 4 valid doses of DTP before the age of 18–24 months. We used 2 cut-off points to get a better view on vaccination delay. The potential effects of gender, age group, school, and belief system of the school on the completeness and timeliness of MMR and primary DTP vaccinations were assessed with multiple logistic regression analyses. Of the 137 measles cases notified to the public health authorities in this outbreak, 21 cases (15%) were detected by the SHCA, concerning 21 children attending 4 different OJ schools. According to public health guidelines, catch-up vaccination was offered to all unprotected school contacts. Therefore, the vaccination status of all children attending the schools involved in the outbreak had to be verified. Consent forms and questionnaires to identify possible contraindications had to be distributed and recollected. A telephone hotline was organized to answer the questions of parents and school personnel. Records of MMR catch-up vaccinations administered in the affected OJ schools were used to assess the uptake of the subsequent vaccination campaigns by the SHCA.

RESULTS

Analysis of Vaccination Data. We collected the vaccination data of 949 children, 482 girls and 467 boys, attending 8 different schools (Table, Supplemental Digital Content 1, http://links.lww.com/INF/A622, shows the distribution of children in the different schools and age groups).

Table 1 shows statistically significant differences in both completeness and timeliness of MMR and primary DTP vaccinations between schools from the different belief systems. Whereas the MS school approached the Antwerp general vaccination coverage of 96.6% for the first dose of MMR and 97.9% for DTP,[4] the OJ and MJ schools scored 15% and 14% to 20% lower, respec-

© 2011 Lippincott Williams & Wilkins

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

TABLE 1. Completeness and Timeliness of MMR and Primary DTP Vaccinations for the Different Belief Systems

Dependent Variable	Belief System*	Yes† %	No† %	Odds Ratio (95% CI)‡
At least 1 MMR (n = 949)	OJ	77.9	22.1	0.28 (0.15–0.51)§
	MJ	78.1	21.9	0.27 (0.14–0.53)§
	AN	49.5	50.5	0.07 (0.04–0.14)§
	MS	93.0	7.0	1
At least 1 MMR <18 mo (n = 949)	OJ	45.5	54.5	0.20 (0.13–0.30)§
	MJ	65.8	34.2	0.47 (0.30–0.75)§
	AN	35.8	64.2	0.13 (0.07–0.22)§
	MS	78.5	21.5	1
Primary DTP complete (n = 949)	OJ	69.1	30.9	0.27 (0.16–0.44)§
	MJ	75.5	24.5	0.38 (0.22–0.66)§
	AN	50.5	49.5	0.12 (0.07–0.22)§
	MS	89.0	11.0	1
Primary DTP complete <18 mo (n = 688)	OJ	63.2	36.8	0.10 (0.05–0.20)§
	MJ	89.9	10.1	0.49 (0.21–1.16)
	AN	81.1	18.2	0.26 (0.10–0.69)§
	MS	94.9	5.1	1
Primary DTP complete <24 mo (n = 688)	OJ	86.3	13.7	0.21 (0.08–0.54)§
	MJ	95.3	4.7	0.60 (0.19–1.96)
	AN	92.7	7.3	0.39 (0.10–1.54)
	MS	97.2	2.8	1

*Orthodox Jewish (OJ), modern Jewish (MJ), anthroposophical (AN), mainstream (MS).
†Crosstabs, Pearson $\chi^2 < 0.001$.
‡Multivariate logistic regression analysis, using the MS belief system as a reference.
§$P < 0.05$.
MMR indicates measles-mumps-rubella; DDT, diphtheria-tetanus-pertussis; CI, confidence interval.

tively. About 1 in 2 children of the AN school was completely vaccinated for MMR and DTP. Logistic regression analysis confirmed these findings. Children from the MS school had a 4- to 10-fold higher chance of being completely vaccinated in comparison with children from OJ and AN schools, respectively. When looking at the timeliness of the administered vaccinations, percentages were generally lower, most remarkably for the OJ group. Our results show that, at the age of 18 months, more than half of the OJ children were not immunized against MMR and about one-third were not completely vaccinated against DTP. There was a remarkable catch-up by 24 months of age, but the coverage rate was still 10% lower than at the MS school.

SHC Actions: Vaccination Campaigns. A total of 578 children received catch-up MMR vaccination (Table, Supplemental Digital Content 2, http://links.lww.com/INF/A623, gives a detailed overview of the vaccination campaigns in the affected OJ schools). Two schools were affected early in the measles outbreak. They were approached twice with a vaccination campaign as the outbreak was still ongoing after the first campaign. The second wave yielded a higher response than the first one, bringing the total uptake in these schools to 55% to 57%.

DISCUSSION

The goal of this study was to assess the completeness and timeliness of MMR vaccination in Antwerp school children from 4 different belief systems. Our results clearly demonstrate a vaccination gap between MS and OJ school children in both completeness and timeliness of MMR vaccination, showing a delayed start and increased temporal spacing of childhood vaccinations. Similar results for DTP vaccination suggest that this gap cannot be explained by fear for the MMR vaccine and its alleged link to autism.[9] Although we did not investigate the vaccination data for other vaccines, a similarly low vaccination coverage can be expected for other childhood vaccines, such as hepatitis B and meningococcal C vaccines. As underscored by the results of the catch-up vaccination campaigns, there are no fundamental or religious objections against vaccination in this community. Our working experience within the OJ schools indicates that misinformation about vaccination, language barriers, family size, a preference for family doctors (general practitioner [GP]), practicing alternative medicine, frequent travel, and move, … play a major role. This study was limited to vaccination data from children of official OJ schools. A large number of OJ children, especially adolescents, attend private religious schools. They are not supported by a SHC and thus not entitled to free and systematic follow-up of vaccinations. Their parents have to rely on paid visits to a GP or pediatrician. The vaccination gap in this subgroup is probably even more dramatic. The rather low vaccination coverage in the MJ schools may reflect the limitation of our data collection (no direct communication with other vaccinators). The majority of the paper SHCA records of MJ school children explicitly stated that vaccination records were kept by private vaccinators. At that time, less than 50% of these vaccinators registered in Vaccinnet,[7] and transfer of these vaccination records to the SHC is often neglected. Our results for the AN school support the many reports of low vaccination coverage and outbreaks of infectious disease within this population.[10]

Future Challenges. To address the vaccination gap in the OJ communities information campaigns about vaccination need to be tailored to their social and cultural characteristics and organized at a local level, since modern media are rarely used. Regular and personal communication about the importance of vaccinations between these large Jewish families and any possible vaccinator is extremely important to counteract any alleged contraindications for vaccination and check and complete the vaccination status of all children. This requires concerted efforts from well-baby clinics (children 0–3 years of age), SHC (children 3–18 years of age), as well as GP's and pediatricians. In addition, a solution should be found for the OJ private religious schools. Without the support of a SHC or a comparable organization, vaccination coverage in this subgroup is less likely to improve. A round-table conference in

 © 2011 Lippincott Williams & Wilkins

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

Antwerp in 2009 about this issue did not result in any concrete actions so far.

Opportunities to Meet These Challenges. Since the measles outbreak in 2007 to 2008, the SHCA considers vaccination issues in the OJ communities as a top priority. A more proactive attitude has been adopted: instead of waiting for a spontaneous notification from the school or parents, teams actively inquire for new cases of measles or other infectious diseases and investigate any rumors circulating in these communities. In June 2009, a Flemish ministerial decree added measles to the list of infectious diseases with mandatory notification by all health professionals in Flanders (northern Belgium), thus facilitating epidemiologic and laboratory investigations of new measles cases. Vaccination registration in Vaccinnet[7] has become more easily accessible to all vaccinators in Flanders. Around 90% of recent vaccinations in infants and school children are registered within Vaccinnet. This improves the quality of vaccination monitoring.

CONCLUSIONS

This study is a first attempt to analyze the problematic vaccination coverage and vaccination behavior in the OJ communities, a so far unknown risk group identified by the Antwerp measles outbreak. It generated a wave of intensified vigilance and a number of actions at different levels of the national measles elimination plan. However, continuous efforts to reach this specific OJ risk group and to find any additional small risk groups for measles transmission remain necessary.

ACKNOWLEDGMENTS

The authors thank their SHC colleagues for their enthusiasm in tackling this measles outbreak and implementing the lessons learned in their daily practice.

REFERENCES

1. WHO. Eliminating measles and rubella and preventing congenital rubella infection. WHO European Region strategic plan 2005–2010. Available at: http://www.euro.who.int/_data/assets/pdf_file/0008/79028/E87772.pdf. Accessed August 26, 2010.
2. Muscat M, Bang H, Wohlfahrt J, et al. Measles in Europe: an epidemiological assessment. *Lancet*. 2009;373:383–389.
3. Lernout T, Kissling E, Hutse V, et al. An outbreak of measles in orthodox Jewish communities in Antwerp, Belgium, 2007–2008: different reasons for accumulation of susceptibles. *Euro Surveill*. 2009;14:pii=19087. Available at: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19087. Accessed August 26, 2010.
4. Hoppenbrouwers K, Vandermeulen C, Roelants M, et al. Vaccination coverage survey in infants and adolescents in Flanders in 2008. Feb 2009. Available at: http://www.zorg-en-gezondheid.be/v2_default.aspx?id=22769. Accessed August 26, 2010.
5. Theeten H, Vandermeulen C, Roelants M, et al. Coverage of recommended vaccines in children at 7–8 years of age in Flanders, Belgium. *Acta Paediatrica*. 2009;98:1307–1312.
6. Vandermeulen C, Roelants M, Theeten H, et al. Vaccination coverage in 14-year-old adolescents: documentation, timeliness and sociodemographic determinants. *Pediatrics*. 2008;121:e428–e434.
7. Top G, Paeps A, Wildemeersch D. Vaccinnet, a vaccine ordering system linked to a vaccination database in Flanders, Belgium. Poster Eurovaccine December 11, 2009. Available at: http://www.ecdc.europa.eu/en/activities/diseaseprogrammes/Eurovaccine/Pages/virtual_poster_session.aspx. Accessed August 26, 2010.
8. Belgian Superior Health Council. Vaccinatiegids, Brussel, 2009, nr. 8586. Available at: http://www.health.fgov.be/HGR_CSS/vaccinatie. Accessed August 26, 2010.
9. Wakefield A, Murch S, Anthony A, et al. Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children. *Lancet*. 1998;351:637–641.
10. Van Velsen E, de Coster E, van Binnendijk R, et al. Measles outbreak in an anthroposophical community in The Hague, the Netherlands, June-July 2008. *Euro Surveill*. 2008;13:ppi=18945. Available at: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18945. Accessed August 26, 2010.

THE CONTRIBUTION OF INFECTIONS TO NEONATAL DEATHS IN ENGLAND AND WALES

Sarita J. Depani, MRCPCH, Shamez Ladhani, MRCPCH,*† Paul T. Heath, FRCPCH,* Theresa L. Lamagni, PhD,† Alan P. Johnson, PhD,† Richard G. Pebody, FFPHM, PhD,† Mary E. Ramsay, FFPHM,† and Mike Sharland, FRCPCH**

Abstract: This study used anonymized death certificate data to determine the contribution of specific infections to neonatal deaths in England and Wales between 2003 and 2005. Infection was recorded in 11% of deaths, with two-thirds occurring in premature neonates. Group B *Streptococcus* was indicated in 32% of death certificates that specified a bacterial infection and in 11% of all infection-related deaths.

Key Words: neonatal mortality, infection, prevention, pathogens

Accepted for publication September 29, 2010.

From the *Paediatric Infectious Diseases Unit, St George's Hospital, London, United Kingdom; and †Centre for Infections, Health Protection Agency, London, United Kingdom.

Address for correspondence: Shamez Ladhani, MRCPCH, Immunisation Department, Centre for Infections, Health Protection Agency, 61 Colindale Avenue, London NW9 5EQ, United Kingdom. E-mail: shamez.ladhani@hpa.org.uk.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pidj.com).

DOI: 10.1097/INF.0b013e3182102249

The neonatal death rate (deaths among infants aged <28 days) in England and Wales has remained constant at 3.4 to 3.9 per 1000 live-births over the past decade.[1] This rate is substantially lower than the global neonatal mortality rate of 30 per 1000 live-births.[2] In England and Wales, as other industrialized countries, the most significant cause of neonatal mortality is prematurity and its complications, with infection playing a smaller role.[2] National surveillance through routine reporting of births and deaths,[1] or reporting of invasive isolates by hospital laboratories help define the contribution of specific pathogens to the burden of infection in different age groups, but provide little clinical or outcome data,[3] whereas reports focusing on specific hospitals cannot be extrapolated to the general population. This study used anonymized extracts of death registrations in England and Wales to determine the contribution of infections to neonatal deaths and to describe associations between specific pathogens and comorbidities, including prematurity.

METHODS

The completion of a death certificate by a clinician is a legal requirement in England and Wales. The methodology for this study has been described elsewhere.[4] Briefly, the Health Protection Agency routinely receives anonymized infectious disease-related death registration data from the Office for National Statistics (ONS) for public health surveillance purposes. For this study, an automated search of anonymized death registrations was undertaken for infants aged <28 days who died between January 2003 and December 2005, using predefined ICD-10 codes for infection (A00-B99, G00-G09, I00-I02, J00-J22, L00-L08, O75.3, O85, O86, O98, P35-P39). The ICD-10 codes and the corresponding

© 2011 Lippincott Williams & Wilkins

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

death certificate terms were then manually scrutinized and any record that did not indicate an infection was excluded. Neonates with no gestational age recorded on the death certificate were assumed to have been born at term, while neonates with no reported comorbidities were considered to have been previously healthy and were analyzed separately from those with comorbidities recorded on the death certificate. National neonatal death statistics for 2003–2005 were obtained from the ONS website.[1] Proportions were compared using χ^2 test or Fisher exact test, as appropriate. Ethical approval was not required because all data were anonymized at source.

RESULTS

There were 6700 neonatal deaths in England and Wales between 2003 and 2005 (neonatal mortality rate, 3.5/1000 live-births). Automated screening of death certificates identified 851 potential infection-related neonatal deaths, but manual scrutiny excluded 23 cases that were not infection-related. In a further 60 cases, the death certificate noted infection in the mother only with no further information on the neonate—these cases were not included in subsequent analyses despite a high likelihood that they were also infection-related. There were, therefore, 768 infection-related deaths recorded in neonates over the 3-year period, accounting for 11% of all neonatal deaths (0.4/1000 live births). Of these, 280 (36%) were recorded in term, 129 (17%) in preterm (28–36 weeks gestation), and 359 (47%) in extremely preterm (22–28 weeks) neonates. Overall, 57% of all neonates and 49% of term neonates with an infection-related death had at least one comorbidity recorded on the death certificate (Table, Supplemental Digital Content 1, http://links.lww.com/INF/A755).

Type of Infection. Septicemia was the most commonly recorded clinical presentation (594 cases, 77%), followed by pneumonia (100 cases, 13%) and meningitis (42 cases, 6%). The proportion of neonates with reported septicemia increased with the degree of prematurity: 162/280 (58%) of term versus 105/129 (81%) of preterm versus 327/359 (91%) of extremely preterm neonates (χ^2 for linear trend = 97.5; $P < 0.01$). In contrast, pneumonia was more commonly reported among term neonates (88/280 [31%] vs. 7/129 [5%] vs. 15/359 (4%] cases), as was meningitis (29/280 [10%] vs. 8/129 [6%] vs. 5/359 [1%] cases).

Pathogens. Data for preterm neonates with and without comorbidities were combined because the pattern of reported infections was similar (Table, Supplemental Digital Content 2, http://links.lww.com/INF/A756). A pathogen was recorded in 339 (44%) neonates—273 (81%) were bacterial, 37 (11%) fungal, and 29 (9%) viral. Among bacterial deaths, Gram-positive pathogens were reported in 58% of cases and the rate was higher among term neonates with no recorded comorbidities than in extremely preterm neonates (52/65 [80%] vs. 58/120 [48%]; $\chi^2 = 17.5$; $P < 0.001$). Group B *Streptococcus* was reported in almost one-third (87/273 cases, 32%) of neonatal deaths where a bacterial infection was specified and in 11% (87/768 cases) of all infection-related neonatal deaths. A higher proportion of deaths among term neonates with no recorded comorbidities were attributed to group B streptococci than among premature neonates (58/111 [52%] vs. 29/162 [18%] of bacterial infections; $\chi^2 = 35.8$; $P < 0.0001$). In contrast, staphylococcal infections, primarily coagulase-negative staphylococci, were more commonly reported among deaths in extremely premature neonates. Deaths due to Gram-negative pathogens were reported mainly among preterm and extremely preterm neonates (84/111 cases, 76%). *Escherichia coli* was reported in one-third (37/110 cases, 34%) of neonatal deaths caused by Gram-negative pathogens. In a further third, a "Gram-negative" infection was recorded on the death certificate, but the responsible pathogen was not mentioned. Viral infections were uncommonly reported, except herpes simplex virus which almost exclusively affected term neonates with no recorded comorbidities. Deaths because of respiratory syncytial virus were also rarely reported, irrespective of prematurity status, whereas fungal infections were reported mainly among extremely preterm neonates and were mostly due to *Candida* spp.

DISCUSSION

Infection was recorded on the death certificates of 11% of neonatal deaths in England and Wales, and two-thirds of infection-related deaths were reported among preterm and extremely preterm neonates. Group B *Streptococcus* was the single most important pathogen associated with death, not least because it mainly affected neonates with no recorded comorbidities. In contrast, Gram-negative bacteria, fungal infections, and coagulase-negative staphylococci were more commonly reported among preterm neonatal deaths although, where a pathogen was specified on the death certificate, group B *Streptococcus* was equal to *Candida* as the most common pathogen, even in this group.

The limitations of data collection using death certificates are well-documented.[4–6] In particular, the contribution of specific pathogens to death and the role of any documented comorbidities must be interpreted with caution because the information contained within death certificates is known to be limited and may be incomplete. In some cases, an infection may not be stated on the death certificate when it clearly contributed to the death,[5] particularly in children with multiple comorbidities where an infectious cause may not have been identified at the time of death or the infection was not considered to directly contribute to death.[6] In addition, certifiers may only state the clinical manifestation of the infection (eg, pneumonia) or may preferentially state specific organisms (eg, methicillin-resistant *Staphylococcus aureus*).[6] In our study, the contribution of infection may also have been underestimated if the predefined ICD-10 codes used to extract infection-related deaths were not comprehensive enough. Finally, it was not possible to determine the age at onset of infection within the neonatal period, which may be important in identifying potential prevention strategies.

While acknowledging these limitations, our study provides minimum estimates for the contribution of infections to neonatal deaths and, because it is population-based, provides a broad overview of the types of infections associated with both term and preterm neonatal deaths. Of note, our estimates are almost 3-fold higher than the 3.6% reported by the ONS in 2005, perhaps because of the different methodology used to ascertain and/or categorize the cause of death in this age-group.[7] On the other hand, our estimates are similar to the 9.8% reported by the 2007 Confidential Enquiry into Child and Maternal Health.[8] The overall infection-related neonatal death rate in the 2007 Confidential Enquiry into Child and Maternal Health report was 0.3 per 1000 live-births, with 120/212 deaths (57%) occurring in the first week of life (ie, early neonatal deaths), although the pathogens responsible and any associations with prematurity or other comorbidities are not described.[8]

Group B *Streptococcus* was identified as the most common pathogen recorded on neonatal death certificates, accounting for 1 in 9 infection-related neonatal deaths and supporting the call for improved strategies for preventing this infection.[9] Interestingly, invasive methicillin-resistant *S. aureus* infections in neonates are extremely uncommon in the United Kingdom,[10] but it was recorded in 4 of the 9 *S. aureus*-related deaths, although in 19 other cases, the staphylococcal species was not recorded (Table, Supplemental Digital Content 2, http://links.lww.com/INF/A756).

© 2011 Lippincott Williams & Wilkins
Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

Similarly, neonatal *Listeria* infections are rare in England and Wales,[11] but this pathogen was reported in 1.1% of infection-related death certificates in this age group.

Gram-negative bacterial infections and fungal infections were more common among preterm and extremely preterm neonates and are likely to reflect their relatively immature immune system, as well as increased exposure to risk factors such as prolonged hospital stays, indwelling catheters, and prolonged use of multiple broad-spectrum antibiotics.[12–14]

In conclusion, we have for the first time established a minimum estimate of the contribution of specific infections to neonatal deaths in England and Wales. An integrated approach to linking microbiological, prescribing and clinical outcome data would provide a strong evidence base to prioritize research and identify key interventions to reduce infection-related deaths in this highly vulnerable age group.

REFERENCES

1. Office for National Statistics. Vital Statistics Summary, England and Wales. *Health Stat Q*. 2009;43:26.
2. Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: When? Where? Why? *Lancet*. 2005;365:891–900.
3. Henderson KL, Johnson AP, Muller-Pebody B, et al. The changing aetiology of paediatric bacteraemia in England and Wales, 1998–2007. *J Med Microbiol*. 2010;59(pt 2):213–219.
4. Ladhani S, Pebody RG, Ramsay ME, et al. Continuing impact of infectious diseases on childhood deaths in England and Wales, 2003–2005. *Pediatr Infect Dis J*. 2010;29:310–313.
5. Health Protection Agency. National confidential study of deaths following methicillin-resistant *Staphylococcus aureus* infection. 2007. London, Health Protection Agency. Available at: http://www.hpa.nhs.uk/web/HPAwebFile/HPAweb_C/1194947311070. Accessed September 1, 2010.
6. Wheller L, Rooney C, Griffiths C. Death certification following MRSA bacteraemia, England, 2004–05. *Health Stat Q*. 2009;41:13–20.
7. Office for National Statistics. Infant and perinatal mortality by social and biological factors, 2005. *Health Stat Q*. 2006;32:82–86. Available at: www.statistics.gov.uk/downloads/theme_health/HSQ32.pdf. Accessed September 1, 2010.
8. Confidential Enquiry into Maternal and Child Health. Confidential Enquiry into Maternal and Child Health (CEMACH) Perinatal Mortality 2007: United Kingdom. 2009. London. Available at: http://www.cmace.org.uk/Publications/CEMACH-Publications/Maternal-and-Perinatal-Health.aspx. Accessed September 1, 2010.
9. Vergnano S, Embleton ND, Collinson A, et al. Missed opportunities for preventing GBS infections. *Arch Dis Child Fetal Neonatal Ed*. 2010;95:F72–F73.
10. Johnson AP, Sharland M, Goodall CM, et al. Enhanced surveillance of methicillin-resistant *Staphylococcus aureus* (MRSA) bacteraemia in children in the UK and Ireland. *Arch Dis Child*. 2010;95:781–785.
11. Gillespie IA, McLauchlin J, Grant KA, et al. Changing pattern of human listeriosis, England and Wales, 2001–2004. *Emerg Infect Dis*. 2006;12:1361–1366.
12. Curtis C, Shetty N. Recent trends and prevention of infection in the neonatal intensive care unit. *Curr Opin Infect Dis*. 2008;21:350–356.
13. Stoll BJ, Hansen N. Infections in VLBW infants: studies from the NICHD Neonatal Research Network. *Semin Perinatol*. 2003;27:293–301.
14. Stoll BJ, Hansen N, Fanaroff AA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. *Pediatrics*. 2002;110:285–291.

ALTERED GENTAMICIN SERUM CONCENTRATIONS IN OBESE PEDIATRIC PATIENTS

Jamie J. Choi, PharmD, Brady S. Moffett, PharmD, MPH,* Erin J. McDade, PharmD, BCPS,* and Debra L. Palazzi, MD†*

Abstract: We performed a retrospective case-cohort study of 50 obese pediatric patients aged 2 to 18 years who received scheduled gentamicin therapy, with serum peak and trough concentrations measured. Obese pediatric patients had significantly higher gentamicin serum peak and trough concentrations despite receiving significantly lower mg/kg of actual body weight doses of gentamicin.

Key Words: gentamicin, aminoglycosides, obesity, pediatrics, pharmacokinetics

Accepted for publication September 28, 2010.

From the *Department of Pharmacy, Texas Children's Hospital, Houston, TX; and †Department of Pediatrics, Infectious Diseases Section, Baylor College of Medicine and Texas Children's Hospital, Houston, TX.

Address for correspondence: Jamie J. Choi, PharmD, Department of Pharmacy, Texas Children's Hospital, 6621 Fannin St, MC 2–2510, Houston, TX 77030. E-mail: jachoi@texaschildrens.org.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pidj.com).

DOI: 10.1097/INF.0b013e3181ff023e

The prevalence of obesity in the United States has increased to 10.4% in children aged 2 to 5 years and 18.7% in children aged 6 to 19 years.[1] Because of the pharmacokinetic alterations that obesity can impose on medications, pediatric clinicians often are challenged in finding appropriate treatment doses, especially when using antimicrobials.[2] When dosed based on actual body weight (ABW), obese pediatric patients may be at risk for over- (ie, gentamicin dosed by mg/kg) or underdose (ie, vancomycin if 1 g/dose in life-threatening infection) of antimicrobial agents, as the pharmacokinetic parameters for those medications may be altered in obesity.[2] Over- or underdosing of medications in this patient population may result in increased morbidity or mortality. Because gentamicin is a concentration-dependent drug, it is thought to be critical to obtain therapeutic serum concentrations early in the course of therapy and minimize supratherapeutic trough concentrations, to minimize toxicity.[3] The excess amounts of adipose tissue that are present in obese patients can significantly alter the volume of distribution (Vd) of gentamicin as gentamicin is primarily distributed in the extracellular fluid space. The differences in Vd of gentamicin have been characterized in obese adult patients; however, there is little information in obese pediatric patients.[4–6]

Obesity implies excess amounts of adipose tissue without a proportional increase in extracellular fluid. We hypothesized that obese pediatric patients would have smaller Vd per ABW for gentamicin and, consequently, would have higher serum peak (C_{peak}) and trough concentrations (C_{trough}) than healthy weight patients. The purpose of this study was to determine whether obese pediatric patients had altered gentamicin serum concentrations compared with healthy weight pediatric patients. Second, we aimed to determine whether the development of nephrotoxicity, length of hospital stay, and duration of gentamicin therapy differs between obese and healthy weight pediatric patients.

MATERIALS AND METHODS

A retrospective cohort study was performed to determine differences in gentamicin C_{peak} and C_{trough} between obese and healthy weight pediatric patients. Institutional review board approval was obtained through Baylor College of Medicine before the initiation of study. Between January 2006 and June 2009, patients were included if they were obese, 2 to 18 years of age, had been treated with gentamicin in a nonintensive care inpatient unit, and had gentamicin C_{peak} and C_{trough} appropriately drawn at steady state while being admitted to Texas Children's Hospital.

© 2011 Lippincott Williams & Wilkins

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

Patients were excluded if they had physician-documented renal insufficiency, an undetectable gentamicin C_{trough} (<0.6 μg/mL), or cystic fibrosis.

Patients with C_{peak} and C_{trough} obtained around the third gentamicin dose on an every 8-hour schedule were considered to be at steady-state. In our institution, C_{peak} of 6 to 10 μg/mL and C_{trough} <2 μg/mL are considered therapeutic. Creatinine clearance was calculated according to the Schwartz equation, although this equation has not been validated in obese children.[7] Obesity was defined as a body mass index (BMI) greater than or equal to the 95th percentile for age and gender, whereas healthy weight was defined as a BMI greater than or equal to the 5th percentile and less than the 85th percentile.[8] The following data were collected from each patient's medical record: demographic data (age, height, weight, and race), primary indication for gentamicin, serum creatinine at the beginning and end of therapy, gentamicin C_{peak} and C_{trough} values, times of blood sample collections, times of corresponding and previous gentamicin dose, number of gentamicin doses administered before measured gentamicin concentrations, dates of admission and discharge, and dates of gentamicin therapy. Individual therapeutic outcomes were not recorded. Sawchuk-Zaske Method was used to calculate pharmacokinetic parameters and to extrapolate gentamicin C_{peak} and C_{trough} values adjusted for appropriate times and doses.[9] Nephrotoxicity was defined as a decrease in the estimated creatinine clearance by >25% based on pRIFLE criteria (Table, Supplemental Digital Content 1, http://links.lww.com/INF/A637).[10]

Obese patients were matched with healthy weight patients 1:1 for age, gender, and primary indication for gentamicin. Patient charts were reviewed in reverse chronological order from 2009 to 2006. The first 25 obese patients were selected as cases, and the first 25 matching healthy weight patients were selected as controls.

Twenty-five case-control pairs (50 patients) were enrolled to detect a 2 ± 2 (mean ± SD) μg/mL difference in gentamicin concentration between obese and healthy weight groups, with a power of 80% and an alpha of 0.05. Baseline characteristics were summarized using descriptive statistics. Differences between groups were evaluated using Student *t* test for parametric data and Wilcoxon rank sum test for nonparametric data. χ^2 test was used for categorical data. Pharmacokinetic parameters were calculated from C_{peak} and C_{trough}. Predicted C_{peak} and C_{trough} were calculated on the basis of current recommended dose of 2.5 mg/kg/dose every 8 hours.

RESULTS

Fifty patients (25 cases and 25 controls) were enrolled. There were more white patients in the healthy weight group and a greater number of Hispanic or Latino patients in the obese group. The mean BMI of the healthy weight group was two-thirds that of the obese group ($P < 0.05$) (Table, Supplemental Digital Content 2, http://links.lww.com/INF/A638). The initial mg/kg (ABW) dose of gentamicin was significantly lower but the extrapolated C_{peak} was significantly higher in the obese group compared with the healthy weight group (Table 1). Obese patients had smaller Vd than healthy weight patients ($P < 0.01$). No differences were found in the incidence of nephrotoxicity (16.7% vs. 20%; $P = 0.85$), length of hospital stay (median, 8 days [range: 2–19] vs. 9 days [range: 2–26]; $P = 0.51$), or duration of gentamicin therapy (8.15 ± 4.60 days vs. 8.71 ± 4.87 days; $P = 0.68$) between obese and healthy weight patients. The predicted C_{peak} and C_{trough} calculated based on gentamicin 2.5 mg/kg/dose every 8 hours were both significantly higher in the obese group than in the healthy weight group (peak: 11.39 ± 2.72 μg/mL vs. 7.90 ± 1.91 μg/mL; $P < 0.01$; trough: 1.36 ± 0.82 μg/mL vs. 0.84 ± 0.32 μg/mL; $P < 0.01$). There were 4 (17.4%) obese patients and no healthy weight patients who had a predicted C_{trough} greater than 2 μg/mL ($P < 0.05$).

TABLE 1. Primary Outcomes

	Obese (n = 25) (Mean ± SD)	Healthy Weight (n = 25) (Mean ± SD)	*P*
Initial gentamicin dose (mg/kg) every 8 hours	1.86 ± 0.43	2.25 ± 0.41	<0.01
Ke (hr^{-1})	0.32 ± 0.06	0.33 ± 0.04	0.53
$t_{1/2}$ (h)	2.25 ± 0.44	2.16 ± 0.26	0.36
Extrapolated peak at 30 minutes post infusion (μg/mL)	8.17 ± 2.02	7.06 ± 1.52	<0.05
Extrapolated trough (μg/mL)	0.95 ± 0.58	0.74 ± 0.24	0.11
Vd (L/kg)	0.20 ± 0.05	0.28 ± 0.07	<0.01

SD indicates standard deviation; n, number of patients; Ke, elimination constant; $t_{1/2}$, half-life; Vd, volume of distribution.

DISCUSSION

In adults there is a difference in the Vd between healthy weight and obese patients, often requiring an adjustment in the initial aminoglycoside dose for obese patients, but there is little literature evaluating the differences in serum concentrations of gentamicin in obese pediatric patients. Despite receiving significantly lower initial mg/kg (ABW) doses of gentamicin, the obese pediatric patients in our study had higher C_{peak} and C_{trough} compared with those of the healthy weight patients. Moreover, when predicted C_{peak} and C_{trough} were calculated based on the current recommended gentamicin dose of 2.5 mg/kg every 8 hours, both concentrations were even higher. However, obese patients in our study did not have a higher incidence of nephrotoxicity, probably because they often did not receive the full 2.5 mg/kg (ABW)/dose of gentamicin for 2 possible reasons. At our institution, there is a recommended maximum gentamicin dose of 120 mg; therefore, many older obese patients would have received a lower mg/kg (ABW) dose of drug when given 120 mg. Alternatively, some patients may have empirically received a smaller dose according to the physician's clinical judgment, as the calculated dose may have seemed higher than normal for the patient's age. If both obese and healthy weight patients had received the equivalent mg/kg (ABW) dose of gentamicin, the incidence of nephrotoxicity may have been higher in the obese group. It was not possible to evaluate all patients' creatinine clearance due to unavailability of data; however, since all patients improved clinically and had similar durations of gentamicin therapy, it is unlikely any nephrotoxicity that occurred would have been different between the 2 groups.

Formulas, or "correction factors," for determining an appropriate weight for dosing gentamicin in obese adult patients have been published.[11] The formulas used for adults are based on the physiological finding that adipose tissue contains 50% to 60% less extracellular fluid volume than lean tissue, which may not be applicable to pediatric patients.[6,11] Pediatric patient physiology can change dramatically over time, with body water composition decreasing as age increases which suggests that a single "correction factor" may not be appropriate.[4] While both obese adults and obese children have a high BMI, the ABW-to-ideal body weight (IBW) ratio differs between children and adults. In obese adults the

© 2011 Lippincott Williams & Wilkins
Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

ABW:IBW is generally greater than 2, whereas ABW–IBW ratio in children may be less than 2 depending on their age.[11] The range of ABW–IBW ratio in the obese patients in our study was 1.16 to 2.22. This is further evidence that a "correction factor" for obese pediatric patients may be difficult to elucidate.

In conclusion, obese pediatric patients had significantly higher gentamicin C_{peak} and C_{trough} despite receiving significantly lower mg/kg of ABW doses of gentamicin. Although it is not possible to determine an appropriate "correction factor" for dosing gentamicin in obese children, it appears that the empirical reduction and individualization of the gentamicin dose may be appropriate and that therapeutic drug monitoring is necessary in this patient population. Further prospective research with a larger sample size is warranted to determine appropriate methods for empirical dose adjustment.

REFERENCES

1. Ogden CL, Carroll MD, Curten LR, et al. Prevalence of high body mass index in US children and adolescents, 2007–2008. *JAMA*. 2010;303:242–249.
2. Kendrick JG, Carr RR, Ensome MH. Pharmacokinetics and drug dosing in obese children. *J Pediatr Pharmacol Ther*. 2010;15:94–109.
3. Moore RD, Smith CR, Lietman PS. Association of aminoglycoside plasma levels with therapeutic outcome in gram-negative pneumonia. *Am J Med*. 1984;77:657–662.
4. Shevchuk YM, Taylor DM. Aminoglycoside volume of distribution in pediatric patients. *Ann Pharmacother*. 1990;24:273–276.
5. Schwartz SN, Pazin GJ, Lyon JA, et al. A controlled investigation of the pharmacokinetics of gentamicin and tobramycin in obese subjects. *J Infect Dis*. 1978;138:499–505.
6. Koshida R, Nakashima E, Taniguchi N, et al. Prediction of the distribution volumes of cefazolin and tobramycin in obese children based on physiological pharmacokinetic concepts. *Pharm Res*. 1989;6:486–491.
7. Schwartz GJ, Brion LP, Spitzer A. The use of plasma creatinine concentration for estimating glomerular filtration rate in infants, children and adolescents. *Pediatr Clin North Am*. 1987;34:571–590.
8. Centers for Disease Control and Prevention. About BMI for children and teens. Atlanta, GA: Centers for Disease Control and Prevention. Available at: http://www.cdc.gov/healthyweight/assessing/bmi/childrens_bmi/about_childrens_bmi.html. Accessed July 29, 2009.
9. Sawchuk RJ, Zaske DE, Cipolle RJ, et al. Kinetic model for gentamicin dosing with the use of individual patient parameters. *Clin Pharmacol Ther*. 1977;21:3:362–369.
10. Akcan-Arikan A, Zappitelli M, Loftis LL, et al. Modified RIFLE criteria in critically ill children with acute kidney injury. *Kidney Int*. 2007;71:1028–1031.
11. Traynor AM, Nafziger AN, Bertino JS Jr. Aminoglycoside dosing weight correction factors for patients of various body sizes. *Antimicrob Agents Chemother*. 1995;39:545–548.

THE NATURAL HISTORY OF CONTEMPORARY *STAPHYLOCOCCUS AUREUS* NASAL COLONIZATION IN COMMUNITY CHILDREN

Stephanie A. Fritz, MD, MSCI, Melissa J. Krauss, MPH,† Emma K. Epplin, BS,* Carey-Ann Burnham, PhD,*‡ Jane Garbutt, MB ChB,*§ W. Michael Dunne, PhD,‡ David A. Hunstad, MD,*¶ and Gregory A. Storch, MD*§*

Abstract: The natural history of contemporary *Staphylococcus aureus* nasal colonization was evaluated in community children during a 1-year period. Methicillin-susceptible *S. aureus* nasal carriage was more persistent than methicillin-resistant *S. aureus* nasal carriage, which was usually self-limited. Children with persistent staphylococcal colonization often carried identical strains. Identification of persistent methicillin-resistant *S. aureus* carriers might inform strategies for decolonization and reduction of staphylococcal transmission.

Key Words: *Staphylococcus aureus,* colonization, methicillin resistance

Accepted for publication September 22, 2010.

From the *Department of Pediatrics, Washington University School of Medicine, St. Louis, MO; †Division of Biostatistics, Washington University School of Medicine, St. Louis, MO; and Departments of ‡Pathology and Immunology, §Medicine, and ¶Molecular Microbiology, Washington University School of Medicine, St. Louis, MO.

Supported by National Institutes of Health (NIH) grant T32-HD007507; and by grants UL1-RR024992 and K30-RR022251 from the National Center for Research Resources (NCRR), a component of the NIH, and NIH Roadmap for Medical Research.

The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official view of NCRR or NIH.

Address for correspondence: Stephanie A. Fritz, MD, MSCI, Department of Pediatrics, Washington University School of Medicine, 660 South Euclid Ave, Campus Box 8116, St. Louis, MO 63110. E-mail: Fritz_S@kids.wustl.edu.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pidj.com).

DOI: 10.1097/INF.0b013e3181fe075e

The past decade has seen the emergence of community-associated (CA) strains of methicillin-resistant *Staphylococcus aureus* (MRSA) that affect individuals without traditional risk factors for MRSA acquisition.[1] The natural history and carriage states of nasal colonization with contemporary CA-*S. aureus* strains may differ from that of traditional *S. aureus* strains circulating in the past, dynamics that must be understood to develop strategies to prevent transmission of this pathogen. We investigated the natural history of contemporary *S. aureus* nasal colonization in community children, quantified colonization persistence using molecular typing, and identified current risk factors for prolonged *S. aureus* nasal colonization.

PATIENTS AND METHODS

Between October 2005 and June 2006, 1300 children ages birth to 18 years were recruited from community pediatric practices within a practice-based research network.[2] Both anterior nares were cultured to determine *S. aureus* colonization status, and persistence was tracked for 1 year by repeat culturing. A questionnaire was administered to identify epidemiologic risk factors associated with *S. aureus* nasal colonization. All procedures were approved by the Washington University Human Research Protection Office. Written, informed parental consent was obtained and written assent was provided by children of a developmentally appropriate age.

From the original 1300 study participants, 3 cohorts were developed according to baseline nasal colonization status (ie, MRSA, methicillin-susceptible *S. aureus* [MSSA], or no *S. aureus*), with an equal distribution of participants based on age group, pediatric practice from which the child was enrolled, and date of enrollment. Investigators and participants were blinded to participants' colonization status throughout the study. A collaborator outside the study team performed frequency matching and selected cohort subjects.

Participants were telephoned at 3, 6, and 12 months following study enrollment to ascertain the development of skin and soft tissue infection (SSTI) of any cause in participants or other household members. Information was also obtained regarding the use of interim antibiotics or decolonization measures (eg, intranasal mupirocin, chlorhexidine, or bleach baths). At each time point,

© 2011 Lippincott Williams & Wilkins

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

a collection packet was mailed to each participant, containing a culture swab (BD CultureSwab MaxV; Becton Dickinson, Sparks, MD), a biohazard compliant diagnostic mailing box (Infecon Diagnostic Mailer; Com-Pac International, Inc, Carbondale, IL), and instructions for collecting and returning nasal specimens.

Specimens were plated on trypticase soy agar with 5% sheep blood (Becton Dickinson), and incubated at 35°C in 5% CO_2. *S. aureus* identification and antibiotic susceptibility testing were performed as previously described.[2] All swabs yielded normal respiratory flora, indicating that specimens were in fact obtained from the anterior nares.

Methods for staphylococcal DNA extraction, repetitive sequence-based polymerase chain reaction (rep-PCR), and statistical analysis are provided as Appendix, Supplemental Digital Content 1, http://links.lww.com/INF/A629.

RESULTS

Of 105 participants in the longitudinal cohorts, 32 (30.5%) were colonized in the anterior nares with MRSA, 37 (35.2%) were colonized with MSSA, and 36 (34.3%) were not colonized with *S. aureus* at enrollment (Table, Supplemental Digital Content 2, http://links.lww.com/INF/A630, summarizes the characteristics of these 3 groups). Thirty-nine (37.1%) participants were lost to follow-up and were excluded from analysis. Of the remaining 66 participants, at baseline, 25 (38%) were colonized with MRSA, 23 (35%) were colonized with MSSA, and 18 (27%) were not colonized.

Of the 25 participants with baseline MRSA nasal colonization, 41% remained colonized with MRSA at 3 months, 30% at 6 months, and 18% at 12 months (Fig. 1). In contrast, of the 23 participants with baseline MSSA nasal colonization, subsequent rates of MSSA colonization were 74%, 63%, and 56% at 3, 6, and 12 months, respectively. Among participants with baseline MRSA colonization, 6 (24%) carried MSSA at least once during the follow-up period, whereas only 1 (4%) of those with baseline MSSA colonization carried MRSA at a subsequent sampling. Of the 18 participants not initially colonized with *S. aureus*, 6 (33%) carried MSSA at least once during follow-up and 1 (6%) carried MRSA at a subsequent sampling.

Of 66 participants with follow-up data, 38 provided ≥3 consecutive samples. Among these, *S. aureus* (MRSA or MSSA) was present at 3 consecutive samplings in 13 (34%). Of these 38 participants, 19 (50%) had intermittent colonization (colonized with *S. aureus* at ≥1 sampling and not colonized at ≥1 intervening sampling), whereas 6 (16%) did not carry *S. aureus* at any point.

Heterogeneity of strains (determined by rep-PCR) was observed among individual participants enrolled from within each pediatric practice, although several clones appeared to predominate (Fig., Supplemental Digital Content 3, http://links.lww.com/INF/A631, is a dendrogram of *S. aureus* isolates recovered from participants recruited from a single practice).

Of the 66 participants, 13 (20%) had a positive nares culture for MRSA at least once during follow-up (7 colonized once, 5 twice, and 1 three times). Univariate analyses of risk factors for MRSA and MSSA nasal colonization and for *S. aureus* colonization at ≥2 longitudinal samplings, are shown in the Table, Supplemental Digital Content 4, http://links.lww.com/INF/A632. Factors independently associated with longitudinal MRSA colonization in the multivariable model included prior MRSA colonization (adjusted odds ratio [aOR], 12.5; 95% confidence interval [CI], 2.1–75.7, P = 0.007), Medicaid or no health insurance (aOR, 10.2; 95% CI, 1.7–61.3, P = 0.01), healthcare worker in the household (aOR, 5.9; 95% CI, 1.3–27.6, P = 0.02), and interval SSTI in a household member (aOR, 6.5; 95% CI, 1.0–42.8, P = 0.05).

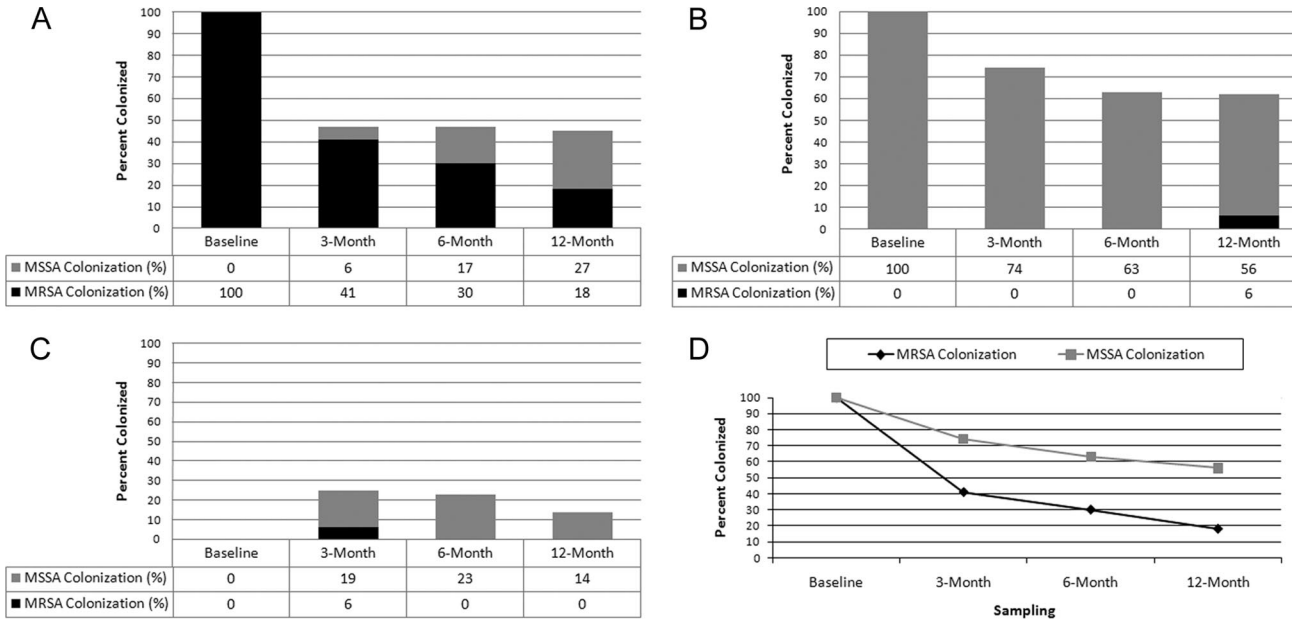

FIGURE 1. Rates of MRSA and MSSA nasal colonization over the 1-year longitudinal study. A, Rates of MRSA (black bars) and MSSA colonization (gray bars) in participants with baseline MRSA colonization. B, Rates of MRSA and MSSA colonization in participants with baseline MSSA colonization. C, Rates of MRSA and MSSA colonization in participants not colonized with *S. aureus* at baseline. D, Comparison of longitudinal MRSA colonization in participants with baseline MRSA colonization (black line) and longitudinal MSSA colonization in participants with baseline MSSA colonization (gray line).

 © 2011 Lippincott Williams & Wilkins

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

Twenty-nine (44%) participants had a positive nares culture for MSSA at least once during follow-up (13 once, 9 twice, 7 three times). In the multivariable model, prior MSSA colonization (aOR, 16.2; 95% CI, 5.9–44.4, $P < 0.001$), school attendance (aOR, 3.8; 95% CI, 1.4–10.8, $P = 0.01$), and fingernail biting (aOR, 3.1; 95% CI, 1.0–9.5, $P = 0.05$) remained significant risk factors for longitudinal MSSA colonization.

Of the 66 children, 37 with follow-up information (56%) were colonized with *S. aureus* (MRSA or MSSA) at ≥2 samplings. Independent risk factors for *S. aureus* colonization at ≥2 samplings by multivariable analysis were MSSA nasal colonization at baseline (aOR, 5.9; 95% CI, 1.5–23.4, $P = 0.01$), asthma (aOR, 6.0; 95% CI, 1.4–25.1, $P = 0.02$), and school attendance (aOR, 4.0; 95% CI, 1.2–13.7, $P = 0.03$). Of the 37 participants with colonization at ≥2 samplings, 22 (60%) carried an identical strain (by rep-PCR) throughout the study: 47% of those with baseline MRSA and 67% of those with baseline MSSA.

DISCUSSION

In the current epidemic of *S. aureus* infections in community children, an understanding of the natural history, dynamics, and determinants of CA-*S. aureus* carriage is essential to devise targeted approaches to interrupt CA-*S. aureus* transmission. The interplay of host and bacterial factors likely influences the staphylococcal carrier state, and optimal interaction of these factors may result in persistent carriage.

In the present study, 34% of participants carried *S. aureus* in the anterior nares at 3 consecutive samplings over 1 year (ie, were persistent carriers), whereas 50% were intermittently colonized and 16% were persistent noncarriers. In concordance with a study in which Nouwen et al inoculated staphylococcal carriers and noncarriers with multiple strains of *S. aureus*,[3] the majority (60%) of carriers in our study subsequently harbored an identical strain, arguing that an optimized host–microbe pair results in persistent carriage. We found that initial MRSA and MSSA colonizations were associated with subsequent colonization with these organisms, respectively, and individuals colonized at ≥2 time points were more likely to be prolonged carriers. However, our observation that nasal carriage of MRSA was less persistent than MSSA, coupled with the lower prevalence of MRSA colonization in the community compared with MSSA,[2] may reflect that a minority of individuals in the community offer a host environment that favors persistent carriage of contemporary MRSA. In support of this concept, host genetic polymorphisms associated with nasal *S. aureus* carriage were recently identified in a modest Amazonian cohort.[4]

Alternatively, factors intrinsic to each *S. aureus* clone may underlie differences in carriage patterns. For example, although novel gene content in contemporary MRSA strains might enhance cutaneous virulence,[5] traditional MSSA strains might be better equipped to compete for the colonization niche. In support of this hypothesis, we observed a trend toward more frequent strain substitution in children initially colonized with MRSA. One-fourth of these patients carried MSSA subsequently, whereas acquisition of MRSA by those initially colonized with MSSA was infrequent; baseline noncarriers also acquired MSSA more frequently than MRSA.

Although our study is an important gateway to understanding the natural history of contemporary *S. aureus* colonization in community children, it is limited by cohort size and sampling of only the nares; we and others have recently demonstrated that the groin and axilla are also important sites of CA-MRSA colonization.[6,7] Although rep-PCR does not assign specific sequence types, the conclusions of this study are applicable to the contemporary clinician managing patients with CA-*S. aureus* colonization and infection.

Because individuals with CA-MRSA colonization are at risk for subsequent SSTI,[8,9] and CA-MRSA SSTI are often recurrent, many practitioners prescribe decolonization measures in an effort to prevent recurrent CA-MRSA SSTI.[10] We found that contemporary MRSA colonization frequently resolves spontaneously, and that multiple positive cultures identify those individuals likely to remain colonized. Such information may help to target decolonization measures to those patients at highest risk for developing recurrent SSTI.

ACKNOWLEDGMENTS

The authors acknowledge the following Washington University Pediatric and Adolescent Ambulatory Research Consortium practices: Blue Fish Pediatrics, Children's Clinic, Crystal City Pediatrics, Esse Health—Creve Coeur, Esse Health—Webster Groves, Grace Hill—Soulard, Healthcare for Kids, Northwest Pediatrics, St. Louis Pediatric Practitioners, Southwest Pediatrics, and Tots thru Teens Pediatrics. The authors also thank Rachel Orscheln, MD, for assembling the cohorts to maintain blinding to colonization status for the investigators and participants; and Jennifer Loughman, PhD, for technical assistance.

REFERENCES

1. Kaplan SL, Hulten KG, Gonzalez BE, et al. Three-year surveillance of community-acquired *Staphylococcus aureus* infections in children. *Clin Infect Dis*. 2005;40:1785–1791.
2. Fritz SA, Garbutt J, Elward A, et al. Prevalence of and risk factors for community-acquired methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* colonization in children seen in a practice-based research network. *Pediatrics*. 2008;121:1090–1098.
3. Nouwen J, Boelens H, van Belkum A, et al. Human factor in *Staphylococcus aureus* nasal carriage. *Infect Immun*. 2004;72:6685–6688.
4. Ruimy R, Angebault C, Djossou F, et al. Are host genetics the predominant determinant of persistent nasal *Staphylococcus aureus* carriage in humans? *J Infect Dis*. 2010;202:924–934.
5. Diep BA, Gill SR, Chang RF, et al. Complete genome sequence of USA300, an epidemic clone of community-acquired methicillin-resistant *Staphylococcus aureus*. *Lancet*. 2006;367:731–739.
6. Fritz SA, Fritz JM, Mitchell K, et al. Sites of community-acquired *Staphylococcus aureus* colonization in patients presenting with skin and soft tissue infections. In: *Abstracts of the 46th Annual Meeting of the Infectious Diseases Society of America*; October 25–28, 2008; Washington, DC. Arlington, VA: Infectious Diseases Society of America; 2008. Abstract K-4075.
7. Yang ES, Tan J, Rieg G, et al. Body site colonization prevalence in patients with community-associated methicillin-resistant *Staphylococcus aureus* infections. In: *Final Program and Abstracts of the 45th Annual Meeting of the Infectious Diseases Society of America*; October 4–7, 2007; San Diego, CA. Arlington, VA: Infectious Diseases Society of America; 2007. Abstract 285.
8. Ellis MW, Hospenthal DR, Dooley DP, et al. Natural history of community-acquired methicillin-resistant *Staphylococcus aureus* colonization and infection in soldiers. *Clin Infect Dis*. 2004;39:971–979.
9. Fritz SA, Epplin EK, Garbutt J, et al. Skin infection in children colonized with community-associated methicillin-resistant *Staphylococcus aureus*. *J Infect*. 2009;59:394–401.
10. Creech CB, Beekmann SE, Chen Y, et al. Variability among pediatric infectious diseases specialists in the treatment and prevention of methicillin-resistant *Staphylococcus aureus* skin and soft tissue infections. *Pediatr Infect Dis J*. 2008;27:270–272.

© 2011 Lippincott Williams & Wilkins

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

THE CLINICAL SPECTRUM OF PATIENTS WITH DEFICIENCY OF *SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION-1*

Diana Averbuch, MD, Ariane Chapgier, PhD,†‡*
Stéphanie Boisson-Dupuis, PhD,†‡§
Jean-Laurent Casanova, MD, PhD,†‡§
*and Dan Engelhard, MD**

Abstract: *STAT-1* (*Signal Transducer and Activator of Transcription-1*) is a key signaling component of interferon gamma responses. We present long-term manifestations in siblings with a mutation in the *STAT1* gene, which include invasive salmonellosis, recurrent severe respiratory syncytial virus pneumonitis, and hepatosplenic mycobacterial disease, and we summarize all other reported cases with *STAT-1* deficiency.

Key Words: mycobacterial diseases, *Mycobacterium*, *Salmonella*, RSV, interleukin 12/23-interferon gamma (IFNγ) pathway

Accepted for publication September 22, 2010.

From the *Department of Pediatrics and the Pediatric AIDS Center and Pediatric Infectious Diseases, Hadassah–Hebrew University Medical Center, Ein-Kerem, Jerusalem, Israel; †Laboratory of Human Genetics of Infectious Diseases, Necker Branch, U980, Institut National de la Santé et de la Recherche Médicale (INSERM), Paris, France; ‡Necker Medical School, University Paris Descartes, Paris, France; and §Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, The Rockefeller University, New York, NY.

Address for correspondence: Dan Engelhard, MD, Pediatric Department and Pediatric AIDS Center and Pediatric Infectious Diseases, Hadassah–Hebrew University Medical Center, Ein-Kerem, Jerusalem, Israel. E-mail: engelhard@hadassah.org.il.

DOI: 10.1097/INF.0b013e3181fdff4a

Mendelian susceptibility to mycobacterial disease syndrome is characterized by a selective predisposition to infection by low-virulence mycobacteria, such as BCG vaccines and environmental mycobacteria, as a result of impairment of the interleukin 12/interleukin 23 (IL-12/IL-23)–interferon gamma (IFNγ) pathway.[1,2] Mutations in several genes have been found in affected patients.[1,2] Very few patients have been described with *STAT-1* deficiency, which is a key signaling component of IFNγ responses, and participates in IFNα/β cellular responses that mediate antiviral immunity. We present the long-term clinical manifestations of 2 siblings who have a recently found mutation in the *STAT1* gene,[3] and summarize the clinical manifestations of 7 other patients with *STAT-1* deficiency previously described.[4–7]

REPORTS OF PATIENTS WITH *STAT-1* DEFICIENCY

The 2 siblings were born to consanguineous parents of Muslim origin. Two other children of this family are healthy. The children received all routine vaccinations, including diphtheria, tetanus, pertussis, hepatitis A and B, measles, mumps, rubella, and annual parenteral influenza vaccine. They were not vaccinated with BCG.

The boy, currently 15 years old, has had mild asthma since he was 1 year old. At 2 years of age, he presented with prolonged fever, general deterioration, and hepatosplenomegaly. Laboratory results revealed white blood cell, 52,000 cells/mm^3; platelets, 18,000 cells/mm^3; hemoglobin, 6.7 g %; and impaired cholestatic liver function (gamma-glutamyl transpeptidase [GGT]), 1335 units (normal, 10–80); total bilirubin, 81 μmol/L (normal, 0–17); and direct bilirubin, 47 μmol/L (normal, 0). Blood cultures yielded *Salmonella* group D, which continued to grow for 10 days despite appropriate treatment with intravenous (i.v.) ampicillin and chloramphenicol. Abdominal ultrasound revealed gallbladder empyema. He recovered after 3 weeks' treatment. One month later, he was readmitted because of recurrent fever. His blood cultures were negative, but bone marrow culture yielded *Salmonella* D. He was treated with ceftriaxone for 2 weeks. At 4 years of age, he was hospitalized due to prolonged fever, rash, and hepatosplenomegaly. Serologic work-up showed positive cytomegalovirus (CMV) IgG and IgM, positive Epstein-Barr virus VCA IgM, and negative IgG and Epstein-Barr virus nuclear antigen (EBNA). He recovered without complications. During the following years, he had other infectious episodes, including: at 3 to 10 years, recurrent otitis media with 1 to 3 episodes a year; at 3 years of age, herpes simplex virus (HSV) gingivostomatitis and herpetic whitlow with 4 recurrences until 12 years; at 4 years of age, lobar pneumonia with a benign clinical course; at 5 to 7 years, molluscum contagiosum. At the age of 9 years, he developed fever and abdominal pain. Blood cultures yielded *Salmonella* B. He was treated with ceftriaxone for 3 weeks and oral cefixime for an additional 3 weeks, without relapse. His development is normal for his age, but he has short stature; at the age of 15 years, his weight is 44 kg (percentile 7%) and his height is 154.8 cm (percentile <3%).

His younger sister, currently 13 years old, has had mild asthma from an early age. She had the following infections: at 2 months, severe respiratory syncytial virus (RSV) pneumonitis; at 1 year, pneumonia with leukocytosis (60,000/mm^3), including eosinophilia (15,000/mm^3), with very slow recovery of 3 weeks. At 16 months, she was hospitalized for 5 days with severe gingivostomatitis and interstitial pneumonitis of unknown etiology (HSV negative). At 18 months, she developed bilobar pneumonia and at 2 years she developed varicella complicated by severe *Staphylococcus aureus* facial cellulitis, treated with parenteral clindamycin and acyclovir. At 4 years of age, she developed interstitial pneumonitis with hypoxia and generalized, including mediastinal and hilar lymphadenopathy. Laboratory tests revealed leukocytosis with 26,000 cells/mm^3, absolute neutrophil count of 18,200 cells/mm^3, and eosinophilia of 2100 cells/mm^3. PPD was negative. Lymph node biopsy showed nonspecific inflammation but no granulomas. Acid-fast stain and cultures for *Mycobacterium* were negative from gastric aspiration, bronchoalveolar lavage, and mediastinal lymph node biopsy. Bronchoalveolar lavage polymerase chain reaction for *Mycobacterium* was negative. Serology for CMV and Epstein-Barr virus showed evidence of past infection. *Mycoplasma* serology was negative. She was treated empirically with i.v. cefuroxime for 17 days with gradual improvement, and her blood counts, including eosinophil count, normalized. Two months later, she was hospitalized for suspected appendicitis. Appendectomy was performed, but the appendix was not inflamed.

At the age of 5 years, she was hospitalized because of 3 weeks' fever and headache. Urine culture yielded *Escherichia coli* and *Klebsiella pneumoniae*. Blood and bone marrow cultures were negative for *Mycobacterium* and any other bacteria. A computed tomography revealed sinusitis and nephronia. She was treated with i.v. cefuroxime for 3 weeks, as recovery was very slow. A few days after termination of the antibiotics, she developed meningitis and *Salmonella* D was cultured from her cerebrospinal fluid (CSF). She was treated with ceftriaxone for 2 weeks and oral cefixime for an additional 2 weeks. After 3 months, she was hospitalized due to bilateral pneumonia which was treated with parenteral ceftriaxone. During the following 6 months, she was hospitalized for aseptic meningitis and later due to severe *Shigella sonei* dysentery, treated with i.v. fluids and ceftriaxone. At 8 years of age, she developed severe interstitial RSV pneumonitis, requiring hospitalization with supplemental oxygen for 10 days. At 9 years, she developed

 © 2011 Lippincott Williams & Wilkins

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

TABLE 1. Summary of Patients With *STAT-1* Deficiency

Patient		Genetic Characteristics			Clinical Characteristics*				
					Infections				Outcome
Age at Presentation	Origin	Mutation/ Inheritance	Defect in STAT-1 Function	IFNγ-GAF/ IFNα-ISGF-3 Defect	Mycobacteria	*Salmonella*	Viruses	Other	
Pt. 1, 2 yr	Israel (this report)	P696S/AR	P/mRNA splicing	R/R	No	2 episodes of bacteremia	HSV gingivostomatitis; molluscum contagiosum	Recurrent otitis media; lobar pneumonia	Alive
Pt. 2, 1 yr	Israel (this report)	P696S/AR	P/mRNA splicing	R/R	Probable hepatosplenic	Meningitis	Recurrent RSV pneumonitis; aseptic meningitis	Recurrent pneumonia; cellulitis; urinary tract infection; chronic sinusitis; osteomyelitis	Alive
Pt. 3, during the first year of life	Germany[4]	E320Q/AD	P/DNA binding	D/R	Disseminated *Mycobacterium bovis*	No	High antibodies titers against mumps, varicella zoster, EBV, measles, rubella, influenza A, adeno, RSV	Not mentioned	Alive
Pt. 4, 2 yr	Germany[4]	Q463H/AD	P/DNA binding	D/R	Pulmonary *Mycobacterium avium*	No	High antibodies titers against EBV, RSV, parainfluenza I and III, adeno, influenza A/B	Not mentioned	Alive
Pt. 5, childhood	France[5]	L706S/AD	P/phosphorylation of tyrosine 701	D/R	Disseminated *M. bovis*	No	Suffered from many common benign viral infections	Not mentioned	Alive
Pt. 6, 6 yr	USA[5]	L706S/AD	P/phosphorylation of tyrosine 701	D/R	Pulmonary *M. avium*	No	No	Not mentioned	Alive
Pt. 7, 3 mo	Pakistan[6]	1928 insA/ AR	C/frame shift mutation leading to stop codon	D/D	Disseminated *M. bovis*	No	Laboratory isolation of Polio III; parainfluenza II with spontaneous clearance; no life threatening infections	Not mentioned	SCT at the age of 8 mo; died at the age of 11 mo due to severe hepatitis
Pt. 8, 3 mo	Saudi Arabia[7]	1757-1758-delAG/AR	C/premature stop codon at position 603	D/D	Disseminated *M. bovis*	No	Recurrent disseminated HSV-1 (CNS, skin) infection	Not mentioned	Died at the age of 16 mo of disseminated HSV-1
Pt. 9, 2 mo	Saudi Arabia[7]	L600P/AR	C/missense mutation in exon 20	D/D	Disseminated *M. bovis*	No	Suspected severe viral infection	Not mentioned	Died at the age of 12 mo of suspected viral infection

*Family history: Patient 3—His mother developed local BCG disease following vaccination at the age of 14 years. Maternal grandfather developed disseminated tuberculosis at the age of 16 years. Mother's paternal grandfather died at the age of 38 years of tuberculosis; Patient 4—His paternal grandfather developed tuberculosis at the age of 20 years and recovered only after 5 years of antibiotic treatment in the 1950s.

STAT-1 indicates *signal transducer and activator of transcription 1*; IFNγ/GAF, interferon gamma/gamma-activating factor pathway; IFNα/ISGF3, interferon alpha/interferon stimulated gene factor 3 pathway; Pt, patient; P, partial; C, complete; AR, autosomal recessive; AD, autosomal dominant; R, recessive; D, dominant; RSV, respiratory syncytial virus; EBV, Epstein-Barr virus; HSV, herpes simplex virus; CNS, central nervous system; SCT, stem cell transplantation.

© 2011 Lippincott Williams & Wilkins

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

right anterior iliac crest osteomyelitis which was treated with 6 weeks of ceftriaxone. Blood cultures yielded no bacterial pathogens. At 10 years of age, she developed abdominal pain without fever. Abdominal ultrasonography revealed several hypodense lesions in liver and spleen. Spleen biopsy revealed granulomatous lesions compatible with mycobacterial disease. Acid-fast stain was negative and culture did not yield any organism. Gradual but complete resolution of the findings occurred after 12 months of therapy with azithromycin, rifampin, and ethambutol. At 11 years of age, she suffered another episode of severe interstitial RSV pneumonitis, requiring hospitalization with supplemental oxygen for 1 week.

While we were completing this manuscript, she developed abdominal pain. Ultrasound showed multiple lesions in her liver and spleen. Spleen biopsy revealed granulomas, whereas acid-fast smear was negative. We suspect another episode of mycobacterial infection.

During these years, the girl underwent investigation for recurrent infections and lymphadenopathy. Respiratory mucosal biopsy showed no evidence of immotile cilia. Serologic tests for C-ANCA and P-ANCA and antinuclear factor were negative, and C3 and C4 values were normal. Immunologic investigation, including IgE values and functions of T-cells, B-cells, and neutrophils were normal; serum immunoglobulins, including IgG subclass values were normal; and serology for HIV and HTLV-1, 2 was negative.

Investigation of the IL-12/IFNγ pathway in both children revealed a new mutation, P696S, in the *STAT1* gene, which causes abnormal splicing of *STAT1* mRNA and diminishes *STAT-1* expression, leading to impaired but not abolished responses after stimulation with IFNα and IFNγ.[3] This describes the first autosomal recessive mutation in the *STAT1* gene resulting in a partial deficiency. Our 2 sick children were homozygous for P696S/P696S, whereas both parents and another sibling, all healthy, were found heterozygous for this mutation (P696S/WT) and another sibling, healthy as well, was WT/WT.

Data on 7 other patients with *STAT-1* deficiency previously described in the literature are summarized in Table 1.

DISCUSSION

On the basis of the findings in our 2 patients, invasive salmonellosis joins mycobacterial and viral pathogens as significant pathogens in patients with *STAT-1* deficiency. Invasive salmonellosis occurs in less than half of the patients with Mendelian susceptibility to mycobacterial disease; it is significantly more frequent in patients with an IL-12/IL-23 pathway defect (44%) than those with an IFNγR1/IFNγR2/STAT-1 pathway defect (7%) ($P < 0.0001$).[2,8] The IL-12/IL-23 pathway activates different complexes than the IFNγ signaling pathways that participate in human host against *Salmonella*, and therefore invasive salmonellosis should occur less frequently in patients with *STAT-1* deficiency.[8] *Salmonella* has been shown to induce IL-27 which induces *STAT-1*-containing GAS-binding proteins and *STAT-1*-dependent IRF1 transcription; this response was impaired but not abolished in our patients, which may have contributed to the development of salmonellosis.[3]

Nontuberculous mycobacterial infection is a classic manifestation in patients who have IL-12/IL-23–IFNγ pathway deficiency. One of our patients had probable *Mycobacterium* infection and all 7 previously reported patients with *STAT-1* deficiency had disseminated *Mycobacterium bovis* (n = 5) or pulmonary *Mycobacterium avium* infections (n = 2). Despite the underlying immune deficiency, all the patients with this infection were controlled with antimicrobial therapy.

Viral infections may be lethal in complete *STAT-1* deficiency. The 3 patients (P7–9) with autosomal recessive mutations causing complete absence of *STAT-1* with impairment of both IFNγ and IFNα-induced immunity died of proven or suspected viral infection.[6,7] The other 4 children (P3–6) survived their viral infection; they had autosomal dominant mutations, causing partial *STAT-1* deficiency that are recessive for IFN-α sequence response elements, transcriptional activity, and antiviral immunity.[4,5] The course of viral infections in our children is consistent with the laboratory data published earlier,[3] which shows that the defect in the IFN-stimulated gene factor 3 antiviral immunity pathway (requiring only one STAT-1 molecule) is milder than that of the gamma-activating factor antimycobacterial pathway (requiring 2 STAT-1 molecules).

An interesting observation is the recurrent severe RSV pneumonitis in our female patient, which recurred at an older age than usual, and required prolonged hospitalization with supplemental oxygen. RSV is ubiquitous; it infects virtually 100% of young children in the first few years of life.[9] RSV infection was not reported in the other *STAT-1* deficient patients, including those with complete *STAT-1* deficiency, maybe because the latter died at about 1 year of age, before being exposed to RSV. Infection with RSV activates the IFNα pathway and is able to inhibit IFNα-dependent antiviral effects, resulting in establishment of productive infections in human airway epithelia.[10] *STAT-1* activation by both IFNα/β and IFNγ plays an important role in establishing a T_H1 immune response to RSV infection, and *STAT-1* deficient mice infected with RSV showed poor lung resistance and airway responsiveness.[11] Moreover, in vitro studies showed that the antiviral agent, ribavirin stimulates IFN-stimulated gene factor 3-induced IFNα antiviral response by stabilizing the binding of *STAT-1* to ISRE elements.[12] Nevertheless, the predisposition to severe and recurrent RSV pneumonitis in *STAT-1* deficient patients has still to be established.

The opportunity to follow these children for years enabled us to shed light on the clinical course of this unique immune deficiency. Worthy of note is our finding that the clinical course of both children has become milder over the years.

In conclusion, *STAT-1* deficiency should be suspected in children with invasive *Salmonella* infections or recurrent severe RSV pneumonitis, as well as unusually low-virulent mycobacterial infections, HSV, and other severe viral infections. Indeed, another family with a partial form of recessive *STAT-1* deficiency (caused by a distinct mutation) was recently diagnosed (Kong XF, Casanova JL, and Boisson-Dupuis S, unpublished data).

REFERENCES

1. Bustamante J, Boisson-Dupuis S, Jouanguy E, et al. Novel primary immunodeficiencies revealed by the investigation of paediatric infectious diseases. *Curr Opin Immunol*. 2008;20:39–48.
2. Filipe-Santos O, Bustamante J, Chapgier A, et al. Inborn errors of IL-12/23- and IFN-gamma-mediated immunity: molecular, cellular, and clinical features. *Semin Immunol*. 2006;18:347–361.
3. Chapgier A, Kong XF, Boisson-Dupuis S, et al. A partial form of recessive STAT1 deficiency in humans. *J Clin Invest*. 2009;119:1502–1514.
4. Chapgier A, Boisson-Dupuis S, Jouanguy E, et al. Novel STAT1 alleles in otherwise healthy patients with mycobacterial disease. *PLoS Genet*. 2006; 2:e131.
5. Dupuis S, Dargemont C, Fieschi C, et al. Impairment of mycobacterial but not viral immunity by a germline human STAT1 mutation. *Science*. 2001; 293:300–303.

 © 2011 Lippincott Williams & Wilkins

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

6. Chapgier A, Wynn RF, Jouanguy E, et al. Human complete Stat-1 deficiency is associated with defective type I and II IFN responses in vitro but immunity to some low virulence viruses in vivo. *J Immunol*. 2006;176: 5078–5083.
7. Dupuis S, Jouanguy E, Al-Hajjar S, et al. Impaired response to interferon-alpha/beta and lethal viral disease in human STAT1 deficiency. *Nat Genet*. 2003;33:388–391.
8. MacLennan C, Fieschi C, Lammas DA, et al. Interleukin (IL)-12 and IL-23 are key cytokines for immunity against *Salmonella* in humans. *J Infect Dis*. 2004;190:1755–1757.
9. Sigurs N. Epidemiologic and clinical evidence of a respiratory syncytial virus-reactive airway disease link. *Am J Respir Crit Care Med*. 2001;163:S2–S6.
10. Ramaswamy M, Shi L, Varga SM, et al. Respiratory syncytial virus nonstructural protein 2 specifically inhibits type I interferon signal transduction. *Virology*. 2006;344:328–339.
11. Durbin JE, Johnson TR, Durbin RK, et al. The role of IFN in respiratory syncytial virus pathogenesis. *J Immunol*. 2002;168:2944–2952.
12. Zhang Y, Jamaluddin M, Wang S, et al. Ribavirin treatment up-regulates antiviral gene expression via the interferon-stimulated response element in respiratory syncytial virus-infected epithelial cells. *J Virol*. 2003;77:5933–5947.

TICK-BORNE ENCEPHALITIS IN CHILDHOOD

RARE OR MISSED?

Magnus E. A. Hansson, MD,† Claes Örvell, MD, PhD,‡§ Mona-Lisa Engman, MD, PhD,*† Katarina Wide, MD, PhD,*† Lars Lindquist, MD, PhD,¶‖ Karl-Johan Lidefelt, MD, PhD,*† and Mikael Sundin, MD, PhD*†*

Abstract: Retrospective evaluation of medical history and 3635 anti-TBE (tick-borne encephalitis) serologies during the years 2003–2008 indicates that childhood TBE is characterized by vague symptoms. Clinical findings suggest a nonspecific inflammatory disease with restricted encephalitic profile compared with adult TBE. Childhood TBE might elude diagnosis, which is unsatisfactory because of potential long-term consequences.

Key Words: TBE, symptoms, adolescents, children

Accepted for publication September 23, 2010.

From the *Division of Pediatrics, Department of Clinical Science, Intervention and Technology (CLINTEC), Karolinska Institutet, Stockholm, Sweden; †Astrid Lindgren Children's Hospital, Karolinska University Hospital, Huddinge, Stockholm, Sweden; ‡Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden; §Virology Laboratory, Department of Microbiology, Karolinska University Laboratory, Karolinska University Hospital, Huddinge, Stockholm, Sweden; ¶Division of Infectious Diseases, Department of Medicine, Karolinska Institutet, Stockholm, Sweden; and ‖Department of Infectious Diseases, Karolinska University Hospital, Huddinge, Stockholm, Sweden.

Supported by Karolinska Institutet, the Stockholm County Council and the Swedish Association of Persons with Neurological Disabilities.

Address for correspondence: Mikael Sundin, MD, PhD, Division of Pediatrics, B57, Astrid Lindgren Children's Hospital, Karolinska University Hospital, SE-141 86 Stockholm, Sweden. E-mail: mikael.sundin@ki.se.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pidj.com).

DOI: 10.1097/INF.0b013e3181fe3b5a

In Europe, tick-borne encephalitis (TBE) is a common infection of the central nervous system with a 400% increase in reported cases during recent decades. TBE is caused by an RNA virus of the genus *Flavivirus*, also including the dengue, yellow fever, Japanese encephalitis, and West Nile viruses. The TBE virus (TBEV) is usually transmitted by bites of an infected tick. In most of Europe, the TBEV is endemic with seasonally distributed TBE cases due to temperature-dependant tick activity.[1,2] Generally, TBE is characterized by a biphasic course after an incubation period of ~1 to 2 weeks. Nonspecific flu-like symptoms 1 to 8 days are followed by an asymptomatic interval and thereafter symptoms from meningitis to meningoencephalomyelitis of varying severity occur.[1,3,4] Childhood TBE is considered to be a relatively mild condition with favorable outcome compared with adult TBE.[3,5,6] However, the general assumption of childhood TBE being insignificant is challenged by pediatric case-reports of severe acute TBE[7] and deficits in attention and psychomotor activity combined with electroencephalogram abnormalities as sequelae.[8] No specific treatment exists, but TBE is preventable by immunization.[1,4,9,10]

This study aimed to reevaluate symptomatology and clinical findings at diagnosis in childhood TBE. The algorithms for diagnostics are based on adult findings or studies in which children and adults are analyzed collectively. A better description of childhood TBE is essential for diagnostic accuracy and can lead to better understanding of the natural course of the disease in children.

PATIENTS AND METHODS

During 2003–2008, 3635 anti-TBE serologies were obtained at our hospital. These tests were requested by our own hospital, county hospitals, and general practitioners. Overall 998 patients (27%) were positive for anti-TBE immunoglobulin M (anti-TBE IgM) and/or G (anti-TBE IgG). These patients were asked retrospectively (May 2009) to fill in a questionnaire regarding symptoms prior to serology and to give consent to participate. Responses were obtained from 703 patients (70%), 32 (3.2%) were deceased and 13 (1.3%) could not be reached. Among respondents, 30 (4.3%) declined participation. Thus, 673 patients (67%), males/females 1/1.1, were available for further analysis. The study was approved by the Regional Ethics Review Board.

Serodiagnostic Testing for TBE. Serum anti-TBE IgM and G were determined (accredited test procedures) by enzyme immunoassays (Siemens Healthcare Diagnostics, Deerfield, IL) at the Virology Laboratory, Department of Microbiology in Huddinge, Karolinska University Laboratory. The serodiagnostic criterion was a positive anti-TBE IgM or increased titer (≥4-fold) of anti-TBE IgG between acute and convalescent samples.

Clinical Chemistry. Laboratory results (ie, blood count, inflammatory indices, and cerebrospinal fluid [CSF] analysis) other than anti-TBE IgM and G were collected from patient charts. Tests were performed at accredited laboratories, mainly the Karolinska University Laboratory. The reference intervals are congruent with <5% variation.

Statistics. Proportions were compared using Fisher exact test or, when sample size allowed, the χ^2-test. Individual samples were analyzed using the Mann-Whitney U test or the Kruskal-Wallis test followed by Dunn multiple comparison test when appropriate. Statistical significance was set at $P < 0.05$.

RESULTS

Of 3635 anti-TBE serologies, 699 (19.2%) were obtained from patients <16 years (Tables 1, 2). Sampling occurred from spring to late autumn with a peak in August. In total, 296 (8.1%) anti-TBE serologies suggested TBE. Interpretation of serology in relation to clinical picture by responsible clinician resulted in 287 (7.9%) diagnoses. Of 3635 serologies, 692 (19.1%) displayed negative anti-TBE IgM but positive IgG. Data from 444 available questionnaires showed that 116 (26.3%) patients had no previous flavivirus infection and were nonimmunized against flaviviruses, suggesting subclinical infection (Tables 1, 2; and Table, Supplemental Digital Content 1, http://links.lww.com/INF/A634).

Epidemiologic and Clinical Findings in Childhood TBE. Most children either resided in or visited TBE endemic areas and tick-bites were recalled by 75%. Nonspecific findings and symptoms (eg, elevated body temperature, headache, and malaise/fatigue) were more common in children, whereas those suggesting

© 2011 Lippincott Williams & Wilkins

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

TABLE 1. Serologic Findings per Age Group in All Patients Tested for TBE During 2003–2008

	≤6 yr n = 257	7–15 yr n = 442	≥16 yr n = 2916	Unknown n = 20	All n = 3635
Anti-TBE Ig G−/M−	220 (85.6%)	309 (69.9%)	2101 (72.1%)	17 (85.0%)	2647 (72.8%)
Anti-TBE Ig G−/M+	2 (0.8%)	1 (0.2%)	27 (0.9%)	0	30 (0.8%)
Anti-TBE Ig G+/M+	7 (2.7%)	29 (6.6%)	229 (7.9%)	1 (5.0%)	266 (7.3%)
Anti TBE Ig G+/M−	28 (10.9%)	103 (23.3%)	559 (19.1%)	2 (10.0%)	692 (19.1%)

TBE indicates tick-born encephalitis; −, negative; +, positive.

TABLE 2. Serologic Findings in Pediatric and Adult Patients Tested for TBE During 2003–2008

	≤15 yr n = 699	≥16 yr n = 2916	*P*
Anti-TBE Ig G−/M−	527 (75.7%)	2101 (72.1%)	n.s.
Anti-TBE Ig G−/M+	3 (0.4%)	27 (0.9%)	n.s.
Anti-TBE Ig G+/M+	36 (5.1%)	229 (7.9%)	0.017
Anti TBE Ig G+/M−	131 (8.7%)	559 (19.1%)	n.s.

TBE indicates tick-born encephalitis; −, negative; +, positive; n.s., no statistical significance.

encephalitic origin (eg, cognitive dysfunction, impaired general appearance, and motor abnormalities) occurred more frequently in adults. Meningeal signs (eg, sensory disturbances and neck stiffness) were found in comparable frequencies in children and adults. Biphasic course was less frequent in preschool children than in schoolchildren and adults (Table, Supplemental Digital Content 1, http://links.lww.com/INF/A634). Sensory disturbances, neck stiffness, vertigo/balance problems, and biphasic course were significantly more frequent in children with TBE than in non-TBE cases with completed questionnaires (Table, Supplemental Digital Content 2, http://links.lww.com/INF/A635). Duration of hospitalization was shorter in children than in adults. No deaths occurred in children (Table, Supplemental Digital Content 1, http://links.lww.com/INF/A634).

Laboratory Findings in Childhood TBE. Patients with TBE in different age groups displayed slightly elevated mean values in blood inflammatory indices without statistical difference—except for white blood cell count, which was significantly higher in children than in adults. Erythrocyte sedimentation rate and white blood cells were significantly elevated in children with TBE compared with non-TBE cases (Table, Supplemental Digital Content 2, http://links.lww.com/INF/A635). CSF analyses showed pleocytosis with mononuclear preponderance and a normal mean CSF glucose concentration in most patients, without statistically significant deviations between the age groups. Mean CSF albumin concentration was significantly higher in adults than in children. CSF glucose concentrations were significantly elevated in children with TBE compared with non-TBE cases (Table, Supplemental Digital Content 3, http://links.lww.com/INF/A636).

DISCUSSION

In the present study, anti-TBE serologies were consistent with the diagnosis in 5.5% of patients <16 years. Since the symptomatology is nonspecific, there is reason to believe that children and adults may elude diagnosis. Hence, 26.6% of patients with exclusively positive anti-TBE IgG had no medical history of flavivirus infection (N.B., TBEV is the only local Flavivirus) and were nonimmunized against flaviviruses. False positive anti-TBE serologies as an explanation in all these patients seem unlikely. Because the pitfalls and risk of bias when evaluating medical history retrospectively up to 6 years later, it can be argued that a significant number of patients might elude diagnosis due to subclinical infections.

The number of preschool children in this study is small. Since, according to the literature, childhood TBE is a rare disease particularly in preschool children,[1,3,6,11] there is reason to suspect too infrequent consideration of the diagnosis. This is corroborated by the fact that during 2008, serologies (at our hospital) indicating *Borrelia burgdorferi* infection were 5 times more common (25.9% vs. 5.7%) in preschool children than schoolchildren and adults (C. Örvell, MD, PhD, unpublished data, 2010). Therefore, young children are evidently more exposed to ticks than can be concluded from the anti-TBE serologies. Whether this is a result of a better clinical awareness of *B. burgdorferi* infections than TBE infections in childhood needs to be elucidated.

Our data indicate that the clinical presentation and course of childhood TBE contrast in several aspects with the disease seen in adults. Symptoms are vague and nonspecific, and difficulties in verbalization of symptoms further emphasize the diagnostic challenge in children. The most prominent symptom was headache, reported in 60% of the preschool children compared with 74.3% to 85.2% in older individuals. This contrasts with previous studies where headache was reported in >90% children and adults.[3–5,8,11] Furthermore, the biphasic course—a hallmark of TBE—was seen less frequently in preschool children than in older individuals.[3–5,11] Generally, adults tended to be more affected and display motor abnormalities to a greater extent than children, as described by others.[1,3,5] Again this indicates that childhood TBE is either characterized by vague symptoms easily eluding recognition or that symptoms usually described for adolescents/adults may be absent.

It has been hypothesized that TBEV induces a more pronounced encephalitic picture in adults than in children.[5,11,12] This is corroborated by our findings of more frequent cognitive dysfunction, motor abnormalities, and elevated CSF albumin concentrations in adults. The medical history, clinical findings, and laboratory data indicate that the childhood TBE is a nonspecific inflammatory disease with a restricted encephalitic profile. Such an age-dependant symptomatology, also seen in other viral diseases, eg, Epstein-Barr virus and varicella-zoster virus infections, shows that immune responses can be different in children and adults.[13,14]

The diagnostic problems could be partly overcome by careful medical history and clinical examination. A comparison of children with and without TBE showed that biphasic course, vertigo/balance problems, and neck stiffness should prompt the clinician to consider TBE. In view of severe clinical courses of TBE in adults and the poorly described risk of sequelae in childhood TBE,[1,3,4,7,8] there is reason to believe that children, in whom the diagnosis is probably missed to a large extent, would benefit from increased vigilance among medical care providers. Anti-TBE serologies should be performed more frequently because of the vague symptomatology and sparse clinical findings. Prospective studies are warranted to determine the incidence as well as morbidity and long-term consequences of childhood TBE.

© 2011 Lippincott Williams & Wilkins
Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

REFERENCES

1. Lindquist L, Vapalahti O. Tick-borne encephalitis. *Lancet*. 2008;371:1861–1871.
2. Mansfield KL, Johnson N, Phipps LP, et al. Tick-borne encephalitis virus—a review of an emerging zoonosis. *J Gen Virol*. 2009;90:1781–1794.
3. Kaiser R. The clinical and epidemiological profile of tick-borne encephalitis in southern Germany 1994–98: a prospective study of 656 patients. *Brain*. 1999;122(pt 11):2067–2078.
4. Gunther G, Haglund M, Lindquist L, et al. Tick-bone encephalitis in Sweden in relation to aseptic meningo-encephalitis of other etiology: a prospective study of clinical course and outcome. *J Neurol*. 1997;244:230–238.
5. Logar M, Arnez M, Kolbl J, et al. Comparison of the epidemiological and clinical features of tick-borne encephalitis in children and adults. *Infection*. 2000;28:74–77.
6. Zenz W, Pansi H, Zoehrer B, et al. Tick-borne encephalitis in children in Styria and Slovenia between 1980 and 2003. *Pediatr Infect Dis J*. 2005; 24:892–896.
7. Fritsch P, Gruber-Sedlmayr U, Pansi H, et al. Tick-borne encephalitis in Styrian children from 1981 to 2005: a retrospective study and a review of the literature. *Acta Paediatr*. 2008;97:535–538.
8. Schmolck H, Maritz E, Kletzin I, et al. Neurologic, neuropsychologic, and electroencephalographic findings after European tick-borne encephalitis in children. *J Child Neurol*. 2005;20:500–508.
9. Kunze U. Tick-borne encephalitis: from childhood to golden age does increased mobility mean increased risk? Conference report of the 11th meeting of the International Scientific Working Group on Tick-Borne Encephalitis (ISW-TBE). *Vaccine*. 2010;28:875–876.
10. Vene S, Haglund M, Lundkvist A, et al. Study of the serological response after vaccination against tick-borne encephalitis in Sweden. *Vaccine*. 2007; 25:366–372.
11. Lesnicar G, Poljak M, Seme K, et al. Pediatric tick-borne encephalitis in 371 cases from an endemic region in Slovenia, 1959 to 2000. *Pediatr Infect Dis J*. 2003;22:612–617.
12. Kaiser R, Holzmann H. Laboratory findings in tick-borne encephalitis—correlation with clinical outcome. *Infection*. 2000;28:78–84.
13. Tunbridge AJ, Breuer J, Jeffery KJ. Chickenpox in adults—clinical management. *J Infect*. 2008;57:95–102.
14. Vetsika EK, Callan M. Infectious mononucleosis and Epstein-Barr virus. *Expert Rev Mol Med*. 2004;6:1–16.

IMPAIRED AVIDITY MATURATION AFTER TETANUS TOXOID BOOSTER IN CHILDREN WITH DOWN SYNDROME

Maaike A. Kusters, MD, C. M. Jol-van der Zijde, BASC,† Maarten J. van Tol, PhD,† W. E. Bolz, MD,‡ L. A. Bok, MD,§ Marjan Visser, MD,¶ and Esther de Vries, MD, PhD**

Abstract: Down syndrome children show a decreased avidity of the antibody response after tetanus toxoid booster vaccination at 9 years of age suggesting impaired memory B cell selection in the germinal center. Clinicians need to be aware of this ongoing subtle immunologic deficit in Down syndrome.

Key Words: Down syndrome, tetanus toxoid, antibody formation, avidity, children

Accepted for publication September 30, 2010.

From the *Department of Pediatrics, Jeroen Bosch Hospital, ′s-Hertogenbosch, The Netherlands; †Department of Pediatrics, Leiden University Medical Center, Leiden, The Netherlands; ‡Department of Pediatrics, Elkerliek Hospital, Helmond, The Netherlands; §Department of Pediatrics, Máxima Medical Center, Veldhoven, The Netherlands; and ¶Department of Pediatrics, Beatrix Hospital, Gorinchem, The Netherlands.

Supported by a grant from the Jeroen Bosch Hospital Research Fund.

The authors have declared that they have no conflict of interest.

Address for correspondence: Esther de Vries, MD, PhD, Department of Pediatrics, Jeroen Bosch Hospital, PO Box 90153, 5200 ME ′s-Hertogenbosch, The Netherlands. E-mail: esid@estherdevries.nl or e.d.vries@jbz.nl.

DOI: 10.1097/INF.0b013e3181ff85a8

Children with Down syndrome (DS) show abnormal thymocyte maturation and T-lymphocyte development.[1] The increased frequency of hematological malignancies and auto-immune diseases but absence of opportunistic infections in DS suggests this is associated with a clinically relevant but mild T-lymphocyte deficiency.[2] The increased, mainly respiratory, infections in especially younger DS children suggest a possible B-lymphocyte problem.[3] Recently, we found a striking B-lymphocytopenia in DS,[4] and decreased immunoglobulin (Ig)G_2, IgG_4, and IgM but increased IgG_1, IgG_3, and total IgG serum levels after 5 years of age,[3] as had been described before.[5] It is not clear whether the low absolute B-lymphocyte numbers and their altered Ig-production are mainly caused by an intrinsic B-lymphocyte defect, which would fit the increased frequency of respiratory infections in DS,[5] or whether these changes are mainly accounted for by a T-lymphocyte defect leading to altered T-lymphocyte help and impaired T-B-lymphocyte collaboration.

Assaying specific antibody production against well-defined antigens can be used as an in vivo model to assess the capacity of the immune system. The response to T-cell-dependent antigens represents the combined outcome parameter of T-lymphocyte function, T-B interaction and B-lymphocyte function, whereas the response to T-cell independent antigens is largely if not only determined by B-lymphocyte function alone. Repeated vaccination with a T-cell dependent antigen activates selected clones of memory B-cells that produce high avidity immunoglobulins after somatic hypermutation.

A strong T-cell-dependent B-lymphocyte response is normally seen after exposure to tetanus toxoid (TT), a highly immunogenic protein antigen, with increased avidity after repeated boosting.[6] Disturbed responses may be indicative of a clinically relevant antibody deficiency. Earlier reports on tetanus vaccination in DS showed the ability to achieve the threshold of protective antibody levels at a random time point after vaccination, which is set at 0.2 IU/mL (= 1 μg/mL) specific anti-TT-IgG according to World Health Organization criteria.[7,8] However, peak anti-TT-responses at 3 to 4 weeks after booster vaccination and avidity maturation data, which will be disturbed in case of impaired T-B interaction processes in the germinal center, have never been reported in DS.

PATIENTS AND METHODS

TT is part of the Dutch immunization program at 2, 3, 4, and 11 months of age, with boosters at 4 and 9 years. In a cross-sectional study, paired sera of 22 DS children (4 years of age, n = 15; 9 years of age, n = 7) were obtained before and 3 to 4 weeks after regular TT-booster at 4 or 9 years of age (1 prevaccination serum missing at 4 years of age). Specific anti-TT-IgG and IgG-subclasses were measured by an antibody-capture enzyme-linked immunosorbent assay.[9] Since the IgG-response after TT-booster in healthy controls predominantly (>75%) consists of IgG_1 antibodies, the anti-TT avidity-enzyme-linked immunosorbent assay was performed for IgG_1 only.[9] Avidity is expressed as the relative index representing the molarity of NaSCN required to elute 50% of TT-bound IgG_1.[9] Results were compared with reference values obtained in 20 healthy age-matched controls (AMC) (for the DS children at 4 years of age) and 20 healthy adults (Ad-REF) (for the DS children at 9 years of age), respectively.[6] Statistical analysis was performed after logarithmic transformation using the *T* test and SPSS 16.0 for Windows.

RESULTS

In our DS cohort, 10 of 14 (71%) of the children at 4 years and all 7 children (100%) at 9 years of age already had protective antibody titers according to the World Health Organization criteria

© 2011 Lippincott Williams & Wilkins

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

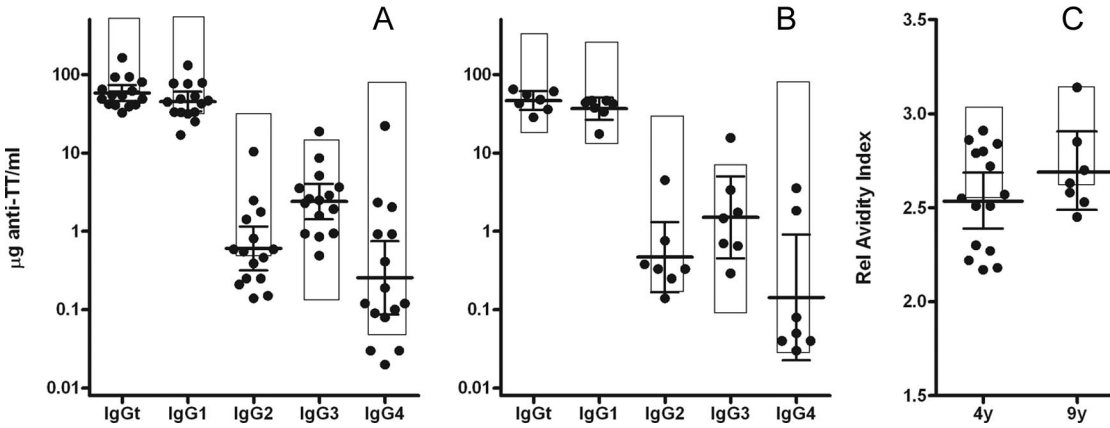

FIGURE 1. Specific anti-TT IgG and IgG-subclasses 3–4 weeks after tetanus booster vaccination at 4 years (**A**) and 9 years (**B**) of age; anti-TT IgG_1 avidity after tetanus booster vaccination at 4 or 9 years of age (**C**). Anti-TT indicates antitetanus toxoid; IgGt, total anti-TT IgG. Relative avidity index: the molarity of NaSCN required to elute 50% of TT-bound IgG_1. Closed circles: Down syndrome subjects. Horizontal lines: geometric mean Down syndrome children (95% CI); Open bars: reference group (±2SD).

before their TT-booster vaccination. All 22 DS children (100%) responded to the booster vaccination with an increase in antibody titer. Postvaccination antibody formation in DS and controls consisted predominantly of IgG_1 (about 75%), as expected.

However, postbooster vaccination anti-TT-antibody titers (Fig. 1A) and IgG_1-avidity (Fig. 1C) were significantly lower in the 4-year-old DS children as compared with AMC children: anti-TT-total IgG geometric mean titer (GMT) was 58 μg/mL (range, 32–164) in DS and 178 μg/mL (range, 72–424) in AMC ($P < 0.001$), anti-TT-IgG_1 GMT was 45 μg/mL (range, 17–130) in DS and 131 μg/mL (range, 26–380) in AMC ($P < 0.001$), anti-TT-IgG_2 GMT was 0.60 μg/mL (range, 0.14–10) in DS and 4.0 μg/mL (range, 0.62–30) in AMC ($P < 0.001$), anti-TT-IgG_3 GMT was 2.4 μg/mL (range, 0.49–19) in DS and 1.5 μg/mL (range, 0.20–17) in AMC ($P = 0.248$), and anti-TT-IgG_4 GMT was 0.26 μg/mL (range, 0.02–22) in DS and 2.0 μg/mL (range, 0.08–21) in AMC ($P = 0.005$) (Fig. 1A). IgG_1-avidity GMT was 2.5 (range, 2.2–2.9) in DS and 2.8 (range, 2.6–3.1) in AMC children ($P = 0.002$). The fold increase of post- versus prebooster anti-TT-IgG1 was not significantly different between DS and AMC children at 4 years of age.

After booster vaccination at 9 years of age, anti-TT-antibody total IgG, IgG_1, and IgG_3 GMT (Fig. 1B) fall within the adult reference range, but anti-TT-IgG_2 GMT and anti-TT-IgG_4 GMT remained significantly decreased: anti-TT-IgG_2 GMT was 0.47 μg/mL (range, 0.14–4.5) in DS and 2.3 μg/mL (range, 0.47–24) in Ad-REF ($P = 0.011$), anti-TT-IgG_4 GMT was 0.14 μg/mL (range, 0.03–3.5) in DS and 1.4 μg/mL (range, 0.09–28) in Ad-REF ($P = 0.015$). IgG_1-avidity (Fig. 1C) is still significantly decreased: GMT was 2.7 in DS and 2.9 (range, 2.7–3.2) in Ad-REF ($P = 0.006$). The fold increase of post- versus prebooster anti-TT-antibody titers was not significantly different between DS children at 9 years of age and Ad-REF.

Conform previous observations in healthy controls,[6] there was no correlation between the titer and the avidity of anti-TT-IgG_1 postvaccination in the DS children.

DISCUSSION

We found a decreased anti-TT humoral immune response in a cohort of 22 DS children: IgG and IgG-subclass titers are lower at 4 years of age, and—despite normalization of IgG, IgG_1, and IgG_3 titers—avidity maturation remains impaired at 9 years of age. These data show that DS children have lasting difficulties with specific anti-TT-antibody formation, although "protective levels" for TT are being reached.

We compared our data to other possibly immunocompromised patient cohorts from the literature. Patients with decreased numbers of helper-T-lymphocytes such as children with partial DiGeorge syndrome[10] and HIV^+ children[11] and adults[9] mount a protective response but with lower mean IgG anti-TT-antibody titers. There are no pediatric data on antibody avidity, but IgG_1-avidity in HIV^+ adults was normal after TT-booster vaccination.[9,11] The decreased TT-specific IgG_1-avidity levels despite repeated booster vaccination are suggestive of a subtle impairment of the selection process of memory B-cells in the germinal center in DS, which cannot be overcome by repeated TT-booster vaccination and maturation of the DS immune system with aging. This is reminiscent of patients with common variable immunodeficiency in whom insufficient somatic hypermutation leading to reduced generation of high-affinity antibodies has been described, associated with an increased frequency of severe respiratory tract infections.[12,13]

Clinicians need to be aware of this ongoing deficiency in antiprotein antibody quality in DS.

REFERENCES

1. Murphy M, Epstein LB. Down syndrome (trisomy 21) thymuses have a decreased proportion of cells expressing high levels of TCRalpha, beta and CD3. A possible mechanism for diminished T cell function in Down syndrome. *Clin Immunol Immunopathol*. 1990;55:453–467.
2. Notarangelo LD, Fisher A, Geha RS, et al. Primary immunodeficiencies: 2009 update. *J Allergy Clin Immunol*. 2009;124:1161–1178.
3. Verstegen RH, Kusters MA, Gemen EF, et al. Down syndrome B-lymphocyte subpopulations, intrinsic defect or decreased T-lymphocyte help. *Pediatr Res*. 2010;67:563–569.
4. De Hingh Y, van der Vossen PW, Gemen EF, et al. Intrinsic abnormalities of lymphocyte counts in children with down syndrome. *J Pediatr*. 2005; 147:744–747.
5. Kusters MA, Verstegen RH, Gemen EF, et al. Intrinsic defect of the immune system in children with Down syndrome: a review. *Clin Exp Immunol*. 2009;156:189–193.
6. Jol-van der Zijde CM, van der Kaaden M, Rümke HC, et al. The antibody response against tetanus toxoid: a longitudinal study in healthy infants and adults. In: Chapel HM, Levinsky RJ, Webster AD, eds. *Progress in Immune Deficiency III*. London: Royal society of medicine services Ltd; 1991:238–240.

© 2011 Lippincott Williams & Wilkins
Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

7. Philip R, Berger AC, McManus NH, et al. Abnormalities of the in vitro cellular and humoral responses to tetanus and influenza antigens with concomitant numerical alterations in lymphocyte subsets in Down syndrome (trisomy 21). *J Immunol*. 1986;136:1661–1667.
8. Hawkes RA, Boughton CR, Schroeter DR. The antibody response of institutionalized Down's syndrome patients to seven microbial antigens. *Clin Exp Immunol*. 1978;31:298–304.
9. Kroon FP, van Tol MJ, Jol-van der Zijde CM, van Furth R, et al. Immunoglobulin G (IgG) subclass distribution and IgG1 avidity of antibodies in human immunodeficiency virus-infected individuals after revaccination with tetanus toxoid. *Clin Diagn Lab Immunol*. 1999;6:352–355.
10. Davis CM, Kancherla VS, Reddy A, et al. Development of specific T-cell responses to Candida and tetanus antigens in partial DiGeorge syndrome. *J Allergy Clin Immunol*. 2008;122:1194–1199.
11. Barbi M, Biffi MR, Binda S, et al. Immunization in children with HIV seropositivity at birth: antibody response to polio vaccine and tetanus toxoid. *AIDS*. 1992;6:1465–1469.
12. Schejbel L, Marquart H, Andersen V, et al. Deficiency of somatic hypermutation of immunoglobulin G transcripts is a better predictor of severe respiratory tract infections than lack of memory B cells in common variable immunodeficiency. *J Clin Immunol*. 2005;25:392–403.
13. Andersen P, Permin H, Andersen V, et al. Deficiency of somatic hypermutation of the antibody light chain is associated with increased frequency of severe respiratory tract infection in common variable immunodeficiency. *Blood*. 2005;105:511–517.

NIJMEGEN BREAKAGE SYNDROME COMPLICATED WITH PRIMARY CUTANEOUS TUBERCULOSIS

Melinda Erdös, MD, PhD, Beáta Tóth, MSc,* Imre Veres, MD,† Mária Kiss, PhD,‡ Éva Remenyik, MD, PhD,† and László Maródi, MD, PhD**

Abstract: Nijmegen breakage syndrome (NBS) is a rare autosomal recessive chromosomal instability syndrome characterized by severe immunodeficiency, growth retardation, microcephaly, a distinct facial appearance, and a high predisposition to lymphoid malignancy. We report a 7-year-old white girl with NBS associated with cutaneous tuberculosis. The patient presented with multiple red-brown, centrally scaring plaques on the leg and had neither pulmonary nor systemic manifestation of tuberculosis. Polymerase chain reaction testing using *Mycobacterium* genus- and *Mycobacterium tuberculosis* species-specific primers confirmed the clinical diagnosis of cutaneous tuberculosis. This is the first report describing the simultaneous presentation of NBS and cutaneous tuberculosis.

Key Words: Nijmegen breakage syndrome, cutaneous tuberculosis

Accepted for publication September 2, 2010.

From the *Department of Infectious and Pediatric Immunology, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary; †Department of Dermatology, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary; and ‡Department of Dermatology and Allergology, Albert Szent-Györgyi Clinical Center, University of Szeged, Hungary.

Supported by grants from the Hungarian Research Fund (OTKA PD 72445) (to M.E.), and the TÁMOP 4.2.1./B-09/1/KONV-2010–0007 project implemented through the New Hungary Development Plan and co-financed by the European Social Fund.

Address for correspondence: László Maródi, MD, PhD, Department of Infectious and Pediatric Immunology, Medical and Health Science Center, University of Debrecen, Nagyerdei krt. 98, H-4032 Debrecen, Hungary. E-mail: lmarodi@dote.hu.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pidj.com).

DOI: 10.1097/INF.0b013e3181faa941

Nijmegen breakage syndrome (NBS) is a rare, autosomal recessive DNA-repair disorder characterized by severe immunodeficiency, progressive microcephaly, characteristic face, growth retardation, spontaneous chromosomal instability, and a peculiar predisposition to cancer development.[1] The syndrome is caused by mutations in the *NBS1* gene located on chromosome 8q21, which encodes for nibrin, a DNA double strand break repair protein.[2,3] The immune deficiency in affected individuals is profound and highly variable, with a tendency to progress with time.[4] In particular, defective antibody response to common pathogens and low concentrations of serum immunoglobulin isotypes and IgG subclasses are common.[4] Cellular immunity is also impaired and manifested in reduced T-lymphocyte proliferation and lymphopenia. The number of $CD3^+$ and $CD4^+$ T cells are usually decreased, whereas $CD8^+$ cell depletion is rarely seen. Infectious complications mostly affect the respiratory tract and are caused by community-acquired rather than opportunistic pathogens.

Cutaneous tuberculosis, a very rare manifestation of mycobacterial infections has recently been reported from areas with a high incidence of HIV infection and multidrug-resistant pulmonary tuberculosis. Lupus vulgaris is a characteristic clinical form of cutaneous tuberculosis which typically occurs through hematogenous, lymphatic, or contiguous spread from underlying tuberculosis, also after primary inoculation.[5,6] It may also be acquired exogenously following secondary inoculation, which is a rare complication of BCG vaccination.[7] We present here a unique association of NBS with lupus vulgaris in a 7-year-old girl with neither pulmonary nor systemic manifestations of tuberculosis.

METHODS

Measurement of serum isotypes and lymphocyte surface markers were performed by routine immunochemical and flow cytometry assays. EDTA blood from the patients and family members was obtained and genomic DNA was isolated by using GenElute Blood Genomic DNA kit (Sigma-Aldrich GmbH, Budapest). Polymerase chain reaction was performed by using 1 primer pair to selectively amplify products of the hot spot region of *NBS1* gene in exon 6. Primer sequences are available on request. Amplified segments were purified using a MICROCON YM-100 Centrifugal Filter Devices (Millipore Co, Bedford, MA). Mutational analysis was performed using the BigDye Terminator Cycle sequencing kit (Applied Biosystems, Foster City, CA) and an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA).

DNA was isolated from sections of the formalin-fixed paraffin-embedded skin biopsy samples. *Mycobacterium* DNA was detected by polymerase chain reaction, using *Mycobacterium* genus- and *Mycobacterium tuberculosis* species-specific primers.[8] To demonstrate the presence of suitable DNA, a 268 bp segment of the human β-globin gene was also amplified.

All studies were approved by the institutional review board of the Medical and Health Science Center of the University of Debrecen. Informed consent was obtained from the parents.

CASE REPORT

This 7-year-old girl was admitted to our Department for immunologic investigation because of microcephaly, mild psychomotor retardation, and chronic multiple leg scars. She was the fourth child of a nonconsanguineous Hungarian family. The pregnancy was uncomplicated and the child was delivered at term with a birth weight of 3050 g; length, 48 cm; and head circumference, 31.5 cm (<third percentile). She had received BCG vaccination on the third day of life without complication and with no history of revaccination later. The patient received medical attention because of microcephaly, failure to thrive, and dysmorphic facial features (Fig., A and B, Supplemental Digital Content 1, http://links.lww.com/INF/A607). At 5 years of age, brain magnetic resonance imaging revealed occipital hypoplasia with dilatation of the occipital horns of ventricle. She was

© 2011 Lippincott Williams & Wilkins

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

hospitalized for *Campylobacter* enterocolitis at the age of 5, and for bacterial pneumonia at the age of 7. At age 6, she presented with painless, slowly, progressively expanding, reddish, discoid plaques on both legs (Fig., E and F, Supplemental Digital Content 1, http://links.lww.com/INF/A607).

On admission, her height was 97 cm; weight, 15 kg; and head circumference, 48 cm, all below the third percentile. She had a "bird-like" face characterized by a prominent midface and large ears, with a receding forehead and mandible, long philtrum, and micrognathia (Fig., A and B, Supplemental Digital Content 1, http://links.lww.com/INF/A607). She was fully cooperative and communicated adequately despite of striking retardation of physical growth and severe microcephaly. Café au lait spots and hypopigmented areas were noticed all over her body. Erythematous, indurated, scaly plaques on the right leg with areas of scarring was observed (Fig., E and F, Supplemental Digital Content 1, http://links.lww.com/INF/A607). The lesions were not painful on palpation.

RESULTS

Histopathologic examination revealed lupus vulgaris with well-developed noncaseating epithelioid granulomas, with inchoate central necrosis and some Langhans-type giant cells (Fig., C and D, Supplemental Digital Content 1, http://links.lww.com/INF/A607). No mycobacteria were identified in tissue by the Ziehl-Neelsen stain.

Molecular analysis of DNA extracted from paraffin-embedded skin specimen was positive for the *M. tuberculosis* IS6110 insertion sequence. Chest radiograph and abdominal sonography were normal. There was no history of tuberculosis and exposure to patients with tuberculosis. Screening for active tuberculosis in the family was negative.

Laboratory tests showed decreased concentrations of IgG (0.51 g/L) and IgA (undetectable) and normal IgM (1.14 g/L). Flow cytometry analysis of peripheral blood mononuclear cells disclosed decreased number of $CD19^+$ cells (4%; 0.06 G/L) and $CD4^+$ cells (24%; 0.25 G/L), and decreased $CD4^+/CD8^+$ ratio (0.55). The diagnosis of NBS was confirmed by mutation analysis of *NBS1* which revealed a c.657del5bp deletion mutation in exon 6 in both alleles, predicting a p.K219fsX234 change in the nibrin protein. Her 16-year-old sister was also diagnosed with NBS. Treatment was commenced with 400 mg/kg intravenous immunoglobulin infusions every 3 weeks. In addition, the patient was daily given rifampicin (150 mg), isoniazid (150 mg), and ethambutol (200 mg). A favorable outcome of the skin disease was observed, suggesting the beneficial effect of antimycobacterial therapy.

DISCUSSION

Cutaneous manifestations of tuberculosis are seen in less than 0.5% of cases.[9] On the basis of data available from the ESID Registry, association of NBS with lupus vulgaris has not been reported before. Lupus vulgaris is a progressive and common form of cutaneous tuberculosis and usually results from dissemination from pulmonary tuberculosis. It follows a chronic course and without treatment may slowly extend with time. Lupus vulgaris is generally under-diagnosed because of its rarity and diverse clinical manifestations, and lack of awareness of the disease. Disseminated mycobacterium infection in a patient with NBS was reported by Resnick et al.[10] This patient died at the age of 10 and had no skin manifestations.

NBS patients have defective humoral and cellular immunity which predispose them to infections. The impaired cellular immunity may play a role in the predisposition to infections by intracellular pathogens including mycobacteria. However, association of this primary immunodeficiency with tuberculosis is surprisingly rare. The case presented in this report is unique, because cutaneous tuberculosis is typically seen in patients with pulmonary tuberculosis. Cutaneous tuberculosis is extremely rare in patients having neither pulmonary nor systemic manifestation of tuberculosis. Our case is also notable because she developed lupus vulgaris, the well-organized form of the pathologic lesions seen in cutaneous tuberculosis. The clinical and pathologic lesions seen in cutaneous tuberculosis vary from scrofuloderma to lupus vulgaris. In scrofuloderma occurring mostly in patients with immunodeficiencies, histopathological examinations usually show a large number of mycobacteria in the lesions with a small to moderate number of lymphocytes in the granuloma. In contrast, lupus vulgaris is often seen in immunocompetent patients. In lupus vulgaris, lesional tuberculous bacilli are usually absent or scant, and cultures are often negative. Finally, our child had a favorable response to combined antituberculous therapy despite severe immunodeficiency.

Inborn errors of immunity including interferon -γ receptor α *and* β chain deficiencies, signal transducer and activator of transcription 1 deficiency, interleukin (IL)-12 p40 deficiency, IL-12 receptor β1 chain deficiency, and nuclear factor-κB essential modulator deficiency have been proposed to predispose patients to infections with various mycobacterial species.[11–14] Intriguingly, IL-12 β1 receptor deficiency has been shown to associate mostly with *M. tuberculosis* infections but not with invasive BCG or environmental mycobacterial disease. The occurrence of *M. tuberculosis* infection in our patient should prompt research to define the expression and function of IL-12 and IL-12 receptor in NBS.

REFERENCES

1. Weemaes CM, Smeets DF, Van der Burgt CJ. Nijmegen breakage syndrome: a progress report. *Int J Radiat Biol.* 1994;66:S185–S188.
2. Saar K, Chrzanowska KH, Stumm M, et al. The gene for the ataxia-telangiectasia variant, Nijmegen breakage syndrome maps to a 1-cM interval on chromosome 8q21. *Am J Hum Genet*. 1997;60:605–610.
3. Varon R, Vissinga C, Platzer M, et al. Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. *Cell*. 1998;93:467–466.
4. Gregorek H, Chrzanowska KH, Michalkiewicz J, et al. Heterogeneity of humoral immune abnormalities in children with Nijmegen breakage syndrome: an 8-year follow-up study in a single centre. *Clin Exp Immunol*. 2002;130:319–324.
5. Kumar B, Muralidhar S. Cutaneous tuberculosis: a twenty-year prospective study. *Int J Tuberc Lung Dis*. 1999;3:494–500.
6. Wozniacka A, Schwartz RA, Sysa-Jedrzejowska A, et al. Lupus vulgaris: report of two cases. *Int J Dermatol*. 2005;44:299–301.
7. Sehgal VN, Srivastava G, Bajaj P, et al. Reinfection (secondary) inoculation cutaneous tuberculosis. *Int J Dermatol*. 2001;40:205–209.
8. Park H, Jang H, Kim C, et al. Detection and identification of mycobacteria by amplification the internal transcribed spacer regions with genus- and species-specific PCR primers. *J Clin Microbiol*. 2000;38:4080–4085.
9. Woo PN, Batta K, Tan CY, et al. Lupus vulgaris diagnosed after 87 years, presenting as an ulcerated birthmark. *Br J Dermatol*. 2002;146:525–526.
10. Resnick IB, Kondratenko I, Togoev O, et al. Nijmegen breakage syndrome: clinical characteristics and mutation analysis in eight unrelated Russian families. *J Pediatr*. 2002;140:355–361.
11. Maródi L, Notarangelo LD. Immunological and genetic bases of new primary immunodeficiencies. *Nat Rev Immunol*. 2007;7:851–861.
12. Al-Muhsen S, Casanova JL. The genetic heterogeneity of mendelian susceptibility to mycobacterial diseases. *J Allergy Clin Immunol*. 2008;122:1043–1051.
13. Dorman SE, Picard C, Lammas D, et al. Clinical features of dominant and recessive interferon gamma receptor 1 deficiencies. *Lancet*. 2004;364:2113–2121.
14. Filipe-Santos O, Bustamante J, Chapgier A, et al. Inborn errors of IL-12/23- and IFN-gamma-mediated immunity: molecular, cellular, and clinical features. *Semin Immunol*. 2006;18:347–361.

© 2011 Lippincott Williams & Wilkins

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.