

formation was published in an erratum in the February issue of the *Journal* [2]. This is an obvious error, because the study, funded by Merck & Co., used the Merck & Co. product RECOMBIVAX HB/H-B-VaxII. The Discussion section discussed only the use of RECOMBIVAX HB/H-B-VaxII as a 2-dose regimen. It was not our intent to suggest that the Twinrix product did not stimulate a good immunologic response. We regret any confusion that this may have caused for the reader, and we certainly regret the concerns that it has caused for SmithKline Beecham and Merck & Co.

Sharon Frey,<sup>1</sup> Ron Dagan,<sup>2</sup> Yaffa Ashur,<sup>3</sup>  
Xiao Q. Chen,<sup>4</sup> Jose Ibarra,<sup>5</sup> Herwig Kollaritsch,<sup>7</sup>  
Mark H. Mazur,<sup>6</sup> Gregory A. Poland,<sup>8</sup> Keith Reisinger,<sup>9</sup>  
Emmanuel Walter,<sup>11</sup> Pierre Van Damme,<sup>12</sup>  
Jean H. Braconier,<sup>13</sup> Ingrid Uhnoo,<sup>14</sup> Martin Wahl,<sup>15</sup>  
Mark M. Blatter,<sup>9</sup> Dennis Clements,<sup>11</sup>  
David Greenberg,<sup>2</sup> Robert M. Jacobson,<sup>8</sup>  
S. Ragnar Norrby,<sup>13</sup> Mina Rowe,<sup>3</sup> Daniel Shouval,<sup>3</sup>  
Sue S. Simmons,<sup>11</sup> Jan van Hattum,<sup>4</sup>  
Solveig Wennerholm,<sup>13</sup> Jacqueline O'Brien Gress,<sup>10</sup>  
Ivan Chan,<sup>10</sup> and Barbara Kuter<sup>10</sup>

<sup>1</sup>Division of Infectious Diseases and Immunology, Saint Louis University Health Sciences Center, St. Louis, Missouri; <sup>2</sup>Pediatric Infectious Disease Unit, Soroka University Medical Center and Ben-Gurion University, Beer-Sheva, and <sup>3</sup>Liver Unit, Department of Internal Medicine, Hadassah University Hospital, Jerusalem, Israel; <sup>4</sup>Department of Gastroenterology, University Hospital Utrecht, Utrecht, The Netherlands; <sup>5</sup>Future HealthCare, Fresno, and <sup>6</sup>Los Robles Regional Medical Center, Thousand Oaks, California; <sup>7</sup>Institute for Specific Prophylaxis and Tropical Medicine, University of Vienna, Vienna, Austria; <sup>8</sup>Department of Medicine, Department of Pediatric and Adolescent Medicine, Mayo Clinic and Foundation, Rochester, Minnesota; <sup>9</sup>Primary Physicians Research, Pittsburgh, and <sup>10</sup>Merck & Co., Inc., Blue Bell, Pennsylvania; <sup>11</sup>Department of Pediatrics, Duke Vaccine Unit, Duke University Medical Center, Durham, North Carolina; <sup>12</sup>Department of Epidemiology and Community Medicine, University of Antwerp, Antwerp, Belgium; <sup>13</sup>Department of Infectious Diseases and Medical Microbiology, University of Lund, Lund, <sup>14</sup>Department of Medical Sciences, Section of Infectious Diseases, University Hospital, Uppsala, and <sup>15</sup>Department of Infectious Diseases, SUlÖstra, Göteborg, Sweden

## References

1. Abraham B, Parenti D. Antibody production in response to hepatitis B surface antigen in a combination hepatitis A/hepatitis B vaccine. *J Infect Dis* 2000;182:1005 (this issue).
2. Frey S, Dagan R, Ashur Y, et al. Interference of antibody production to hepatitis B surface antigen in a combination hepatitis A/hepatitis B vaccine. *J Infect Dis* 1999;180:2018–22 (erratum: *J Infect Dis* 2000;181:814).

Reprints or correspondence: Dr. Betsy Abraham, Director, Vaccines, SmithKline Beecham, 1250 S. Collegeville Rd., U.P. 4215, Collegeville, PA 19426 (betsy\_abraham-1@sbphrd.com).

The Journal of Infectious Diseases 2000;182:1005–6

© 2000 by the Infectious Diseases Society of America. All rights reserved.  
0022-1899/2000/18203-005\$02.00

## Lack of Evidence of *Borrelia* Involvement in Alzheimer's Disease

**To the Editor**—The etiology of Alzheimer's disease (AD), the most prevalent cause of dementia in the elderly, is unknown. Various published reports have either supported or contradicted the possibility that *Borrelia* organisms have a role in the etiology of AD [1–5]. Here we present the results of our investigation, using polymerase chain reaction (PCR) analysis, of evidence of *Borrelia* infection among patients with AD.

Brain specimens from 15 patients with AD and 15 age- and sex-matched controls were obtained from the Johns Hopkins University Alzheimer's Disease Research Center (Baltimore; National Institutes of Health grant AG 05146). The pathological diagnosis of AD followed the recommendations of the Consortium to Establish a Registry for Alzheimer's Disease [6]. The mean ages of patients with AD and controls were 79 years (range, 56–93 years) and 79 years (range, 59–93), respectively. Autopsies were performed  $\leq$ 19 h after death. Specimens were stored at  $-80^{\circ}$  C.

DNA was extracted from a 50-mg piece of tissue from each specimen using the QIAamp Tissue Kit (Qiagen, Valencia, CA). For amplification of *Borrelia* DNA, 1 pair of oligonucleotide primers was selected (5'-[biotin]-GGCGGCACACTTAACA-CGTTAGCTT-3' and 5'-GCGAAGGCGAACTTCTGGGTC-AA-3') that produces a 153-bp product from the 16S ribosomal gene from the *Borrelia* genus. The 16S gene primers are able to amplify *B. burgdorferi sensu stricto*, *B. garinii*, *B. afzelii*, *B. andersonii*, *B. tanukii*, *B. parkeri*, *B. turdi*, *B. hermsii*, and *B. anserina*. An internal control (IC), constructed by using the *Borrelia* primers extended on their 3' ends to amplify a section of the pBR322 vector, was included in each amplification reaction tube. An IC probe binding sequence was selected, and the length of the amplified product was designed to be  $\sim$ 50 bp longer than the *Borrelia* amplicon.

The PCR analysis was performed in a mixture of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3.0 mM MgCl<sub>2</sub>, 200  $\mu$ M deoxynucleoside triphosphates, 2.5 U Taq polymerase, 0.4  $\mu$ M of each *Borrelia* primer, 500 copies of IC, 25  $\mu$ g/mL isopropylalcohol, 2.5 mg/mL bovine serum albumin, and 10% glycerol in a total volume of 50  $\mu$ L. After adding 5  $\mu$ L of the extracted sample, the reaction tubes were incubated in a thermal cycler. An initial denaturation period of 4 min at 95°C was followed by 35 cycles of 95°C for 30 s, 69°C for 30 s, and 72°C for 1 min. Incubation concluded with a final extension period of 5 min at 72°C.

*Borrelia* PCR analysis was performed in triplicate on the extracted DNA from the frontal, temporal, and occipital lobes of 10 patients with AD and 14 controls and was performed in sextuplicate on extracted DNA from 5 patients with AD and 1 control. We inoculated 18 samples, from guinea pig brains, with *B. burgdorferi* strain LP3 as positive controls, and we used

14 samples from noninoculated guinea pig brains as negative controls. All samples were assayed in a blinded fashion.

Detection of the *Borrelia* and IC amplification products was performed using the DELFIA (dissociation-enhanced lanthanide fluoroimmunoassay) time-resolved fluorescence hybridization assay (Perkin-Elmer Wallac, Gaithersburg, MD), as described elsewhere [7]. Probe sequences were as follows: *Borrelia*: 5'-GATGCACACTTGGTGTAAATCAAAAAG-3'; IC: 5'-GC-GATGCTGTCGGAAACG-3'.

To assess the sensitivity and reliability of our PCR assay, the *Borrelia* PCR target region was cloned into the pCR 2.1 vector. A dilution series was performed, with the addition of 1750 ng human genomic DNA per reaction, and the target region was then amplified, using our PCR assay. Time-resolved fluorescence was measured to determine positive amplification reactions. The quantification of  $\beta$ -actin genes has been used to calculate that each cell in the trigeminal ganglia contains 15.6 pg of DNA [8]. Therefore, we calculated that our PCR sensitivity was at least 2 *Borrelia* genomes per 112,000 cells.

When our PCR method was used, no patients with AD or controls were positive for *Borrelia* species in the brain. Therefore, no brain sample in either group was positive by PCR analysis for *Borrelia* organisms, with a 95% confidence interval of 0%–20%. All 18 samples from guinea pig brains inoculated with *B. burgdorferi* were positive, whereas all 14 samples from noninoculated guinea pig brains were negative.

In summary, using a very sensitive PCR assay that is able to amplify a *Borrelia*-specific DNA target sequence from all *B. burgdorferi sensu lato* species known to cause disease in humans, we found no evidence of *Borrelia* organisms in brains of patients with AD.

Adriana R. Marques,<sup>1</sup> Susan C. Weir,<sup>2,a</sup>  
Gary A. Fahle,<sup>2</sup> and Steven H. Fischer<sup>2</sup>

<sup>1</sup>Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, and <sup>2</sup>Clinical Pathology Department, Warren Grant Magnuson Clinical Center, National Institutes of Health, Bethesda, Maryland

#### References

- MacDonald AB, Miranda JM. Concurrent neocortical borreliosis and Alzheimer's disease. *Hum Pathol* 1987;18:759–61.
- Miklosy J. Alzheimer's disease: a spirochetosis? *Neuroreport* 1993;4:841–8.
- Miklosy J, Kasas S, Janzer RC, et al. Further ultrastructural evidence that spirochaetes may play a role in the aetiology of Alzheimer's disease. *Neuroreport* 1994;5:1201–4.
- Pappolla MA, Omar R, Saran B, et al. Concurrent neuroborreliosis and Alzheimer's disease: analysis of the evidence. *Hum Pathol* 1989;20:753–7.
- Gutacker M, Valsangiacomo C, Balmelli T, et al. Arguments against the involvement of *Borrelia burgdorferi sensu lato* in Alzheimer's disease. *Res Microbiol* 1998;149:31–7.
- Mirra SS, Heyman A, McKeel D, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* 1991;41:479–86.
- Fahle GA, Gill VJ, Fischer SH. Optimal activation of isoporsalen to prevent amplicon carryover. *J Clin Microbiol* 1999;37:261–2.
- Pevenstein SR, Williams RK, McChesney D, Mont EK, Smialek JE, Straus SE. Quantitation of latent varicella-zoster virus and herpes simplex virus genomes in human trigeminal ganglia. *J Virol* 1999;73:10514–8.

<sup>a</sup> Present affiliation: MBI Fermentas, Burlington, Ontario, Canada  
Reprints or correspondence: Dr. Adriana R. Marques, Bldg. 10, Room 11N228, 10 Center Dr., Bethesda MD, 20892-1888 (amarques@niaid.nih.gov).

The Journal of Infectious Diseases 2000;182:1006–7

© 2000 by the Infectious Diseases Society of America. All rights reserved.  
0022-1899/2000/18203-0056\$02.00

### *Helicobacter pylori* Infection, *cagA* Status, and Duodenal Ulcer Disease in Children

**To the Editor**—I would like to comment on a recently published report by Queiroz et al. [1]. In this article, the authors described the significant association between *cagA*-positive status and duodenal ulcer disease in symptomatic children with *Helicobacter pylori* infection, and they also found a direct correlation between degree of inflammation and *cagA*-positive status. Interestingly, a close association between increasing age and *cagA* status, in *H. pylori*-positive children without duodenal ulceration, was reported. Unfortunately, a similar comparison in the duodenal ulcer-positive children could not be done, because all of them were *cagA* positive. Although the results of this report are consistent with those of studies in adults [2–5], the picture in the pediatric population is still unclear. On the basis of serology test results (Helicoblot 2.0; GeneLabs Diagnostics, South America), we reported that >50% of symptomatic and/or asymptomatic *H. pylori*-infected children were found to be positive for *cagA* antibody, but duodenal ulceration was found in only 9% (2 children, only one of whom was positive for *cagA* antibody) [6]. In addition, only a weak association was found between inflammatory index (assessed by the revised Sydney criteria) [7] and the presence of *cagA* antibody in symptomatic children. No association was observed between *cagA* status and age, sex, community location, or *H. pylori* density. Although the revised Sydney criteria [7] were used by Queiroz et al., intestinal metaplasia and *H. pylori* density parameters were not scored.

The association between *cagA* status and the development of duodenal ulcer disease (or between *cagA* status and the inflammatory index) in children with *H. pylori* infection is probably more complicated than we contemplate. From the epidemiological point of view, it is interesting to note that, in countries where *H. pylori* infection and *cagA*-positive status are very high, the rates of duodenal ulcer disease and gastric cancer are considerably low [8, 9]. These data suggest that other environmental factors are more crucial than *cagA* status for ulcer development. *H. pylori* infection seems to have a close association with increasing age. Older children with *H. pylori* in-