Hepatitis B immunity in teenagers vaccinated as infants: an Italian 17-year follow-up study

E. Spada[1,2*], L. Romanò[3*], M. E. Tosti[1], O. Zuccaro[1], S. Paladini[3], M. Chironna[4], R. C. Coppola[5], M. Cuccia[6], R. Mangione[7], F. Marrone[8], F. S. Negrone[9], A. Parlato[10], E. Zamparo[11], C. M. Zotti[12], A. Mele[1], A. R. Zanetti[3] on behalf of the Study Group[†]

1) *Centro Nazionale di Epidemiologia, Sorveglianza e Promozione della Salute,* 2) *Dipartimento di Malattie Infettive Parassitarie e Immunomediate, Istituto Superiore di Sanità, Rome,* 3) *Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Milan,* 4) *Dipartimento di Scienze Biomediche ed Oncologia Umana, Università di Bari, Bari,* 5) *Dipartimento di Sanità Pubblica, Medicina Clinica e Molecolare, Università di Cagliari, Cagliari,* 6) *Dipartimento di Prevenzione, Servizio di Epidemiologia, ASP Catania, Catania,* 7) *Servizio di Sanità Pubblica, Epidemiologia e Medicina Preventiva, AUSL1 Agrigento, Unità Operativa di Licata, Licata, Agrigento,* 8) *UO Pediatria e Consultorio Familiare, AUSL Cesena, Cesena,* 9) *Dipartimento Prevenzione Collettiva Salute Umana, ASP Potenza, Potenza,* 10) *Area Dipartimentale di Epidemiologia e Prevenzione, ASL Napoli 2 Nord, Naples,* 11) *Dipartimento di Prevenzione, ASL 6 of Friuli-Venezia Giulia Region, Pordenone and* 12) *Dipartimento di Scienze della Sanità Pubblica e Pediatriche, Università di Torino, Turin*

Abstract

We assessed the persistence of hepatitis B surface antigen antibody (anti-HBs) and immune memory in a cohort of 571 teenagers vaccinated against hepatitis B as infants, 17 years earlier. Vaccinees were followed-up in 2003 and in 2010 (i.e. 10 years and 17 years after primary vaccination, respectively). When tested in 2003, 199 vaccinees (group A) had anti-HBs <10 mIU/mL and were boosted, 372 (group B) were not boosted because they had anti-HBs ≥10 mIU/mL (n = 344) or refused booster (n = 28) despite anti-HBs <10 mIU/mL. In 2010, 72.9% (416/571) of participants had anti-HBs ≥10 mIU/mL (67.3% in group A vs. 75.8% in group B; p 0.03). The geometric mean concentrations (GMCs) were similar in both groups. Between 2003 and 2010, anti-HBs concentrations in previously boosted individuals markedly declined with GMC dropping from 486 to 27.7 mIU/mL (p <0.001). Fifteen vaccinees showed a marked increase of antibody, possibly due to natural booster. In 2010, 96 individuals (37 of group A and 59 of group B) with anti-HBs <10 mIU/mL were boosted; all vaccinees of the former group and all but two of the latter had an anamnestic response. Post-booster GMC was higher in group B (895.6 vs. 492.2 mIU/mL; p 0.039). This finding shows that the immune memory for HBsAg persists beyond the time at which anti-HBs disappears, conferring long-term protection.

Keywords: Booster, hepatitis B, immune memory, long-term protection, vaccination
Original Submission: 4 December 2013; **Revised Submission:** 30 January 2014; **Accepted:** 9 February 2014
Editor: G. Antonelli
Article published online: 16 February 2014
Clin Microbiol Infect 2014; **20**: O680–686
10.1111/1469-0691.12591

Corresponding author: Professor A. R. Zanetti, Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Via C. Pascal 36, 20133 Milan, Italy
E-mail: alessandro.zanetti@unimi.it

*Enea Spada and Luisa Romanò have equally contributed to this study.

[†]All group members are listed in Appendix 1

Introduction

Hepatitis B virus (HBV) is a leading cause of acute and chronic hepatitis, including cirrhosis and hepatocellular carcinoma. This serious and potentially fatal infection can be prevented by vaccination, and a substantial reduction of new HBV infections, carrier rate and HB-related mortality has been observed in countries where vaccination has been implemented [1–3].

In Italy, universal vaccination of infants and 12-year-old adolescents became mandatory in 1991 [4,5]. At the end of 2003, the first infant cohort vaccinated in 1991 turned 12 years of age. In 2004, the vaccination of 12-year-olds was stopped, whereas vaccination of infants continued. Over 95% vaccination coverage was achieved in a few years with an outstanding record of safety and effectiveness [6]. To date, over 19 million young people under 32 years of age have been vaccinated. Our vaccination campaign has greatly contributed to the decline in incidence of both acute hepatitis B and acute

infection by hepatitis Delta virus (HDV), as a consequence of the biological association between HBV and HDV [7,8].

Although the highly protective efficacy of hepatitis B vaccine has been confirmed, the long-term duration of protection achieved by vaccination remains unclear [9]. Studies on long-term protection induced by hepatitis B vaccination have been mainly conducted in areas of high HBV endemicity. Some studies suggest that vaccine-induced immunity may persist for up to 20 years or longer, after complete primary hepatitis B vaccination [10–14]. Other studies suggest that the immune memory may begin to wane during the second decade after vaccination [15–19]. Moreover, long-term follow-up data are scarce in areas of low endemicity [20–24]. Thus, assessing the persistence of immunity following primary vaccination in infants and adolescents is crucial to determine whether a booster vaccination is needed later in life, when subjects may be at increased risk of HBV infection either due to lifestyle or professional exposure.

In 2003, a large multicentre study was carried out in Italy to assess hepatitis B vaccine-induced immune memory in children who were immunized as infants 10 years earlier. Despite the loss of protective concentrations (≥10 mIU/mL) of antibodies to hepatitis B surface antigen (anti-HBs) in a number of vaccinees, a strong immune memory still persisted 10 years after primary immunization, thus suggesting that no routine booster doses of vaccine were necessary for a decade [22]. In 2010, we decided to extend the follow-up of that study by recalling the children, now teenagers, with the aim of assessing the persistence of anti-HBs and immune memory 17 years after primary vaccination.

Materials and Methods

Study participants

A cohort of vaccinees immunized as infants with three paediatric doses of recombinant hepatitis B vaccine (Engerix B, SmithKline Beecham, Biological, Rixensart, Belgium) given at 3, 5 and 11 months of age was first followed-up in 2003 or 10 years after primary vaccination [22], and recalled in 2010 or 17 years after priming. At enrollment, all participants were in good health and none had a history or presented signs or symptoms of clinically overt hepatitis. Exclusion criteria also included congenital or acquired immune disorder, sensitivity or allergy to any component of the study vaccine and having received extra doses of hepatitis B vaccine between 2003 and 2010.

Participants were subdivided into two groups: group A who were given a booster dose of hepatitis B vaccine in 2003 and group B who were not boosted at that time.

Ethics

The study was approved by the Ethics Committee of the University of Milan. Before inclusion, written informed consent was obtained from each participant or, for those still under age (<18 years), from their parents or legal guardians.

Procedures and definitions

Participants were tested for anti-HBs concentrations and the presence of antibody to core antigen (anti-HBc) as a marker of HBV infection. Individuals positive for anti-HBc were tested further for hepatitis B surface antigen (HBsAg) and HBV-DNA, following the same laboratory algorithm applied in the 2003 study [22].

Individuals with anti-HBs concentrations ≥10 mIU/mL were considered protected while those with antibody <10 mIU/mL were given a booster adult dose of recombinant hepatitis B vaccine (Engerix B, 20 μg) and retested 2 weeks later.

An anamnestic response was defined as a four-fold post-booster rise in anti-HBs concentration compared with the pre-booster concentration or an increase up to ≥10 mIU/mL for those who had no detectable anti-HBs.

Individuals showing post-booster anti-HBs concentrations <10 mIU/mL were offered two additional vaccine doses at 1 and 6 months after the booster, and retested 1 month after the third dose.

A natural booster (exposure to HBV without infection) was defined as seroconversion to ≥10 mIU/mL or at least a four-fold increase in anti-HBs concentration in the sample collected in 2010 versus the sample collected in 2003 in the same subject, without revaccination and no appearance of anti-HBc antibody. To avoid differences due to the use of different kit batches, measurements of antibody concentrations in such samples were repeated in parallel on the same run.

Serological testing

HBsAg, anti-HBc and anti-HBs were detected by commercially available kits (AxSYM HBsAg, CORE and AUSAB, Abbott Park, IL, USA). The measurement range of AxSYM AUSAB was 2–1000 mIU/mL, defined by the detection limit and the maximum of the calibration curve. Samples with anti-HBs ≥1000 mIU/mL were diluted with the manual dilution protocol, according to the manufacturer's instructions, to obtain the final sample concentration. Samples below the detection limit (2 mIU/mL) of the assays were recorded as undetectable. HBV-DNA was detected by real-time PCR by the TaqMan HBV test (Roche, Branchburg, NJ, USA) with a 95% detection limit of 6.7 IU/mL (approximately 39 copies per mL).

All samples collected in 2003 were stored at −80°C and thawed in 2010 for this follow-up study.