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Magnitude and quality of antibody response to a combination hepatitis A and hepatitis B vaccine☆

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Abstract

Interference between antibodies generated by a combination hepatitis A and B vaccine was investigated by evaluating the quantity and quality of anti-hepatitis A virus (HAV) and anti-hepatitis B surface antigen (HBs) antibodies generated by Twinrix® (Hepatitis A Inactivated and Hepatitis B (Recombinant) Vaccine). The magnitude of the immune response was determined by a retrospective analysis of eight clinical trials, completed during stepwise development of *Twinrix*. The functionality of anti-HAV was evaluated by comparison of routine ELISA results with neutralization assays and was further characterized by defining the epitope-specificity of binding. Functionality of the anti-HBs response was not tested because a validated assay was not developed at the time this study was conducted. Results of all analyses demonstrated that the combination vaccine induced high antibody titers against hepatitis A and B and a functional anti-HAV response, with no evidence of immune interference to either viral antigen.

Keywords: Hepatitis A vaccine; Hepatitis B vaccine; Combination vaccine; Antibody response

1. Introduction

Vaccination against hepatitis A and hepatitis B is safe and highly effective in preventing disease. Two monovalent vaccines for each disease are currently marketed in the US, Havrix® (Hepatitis A vaccine, Inactivated) by GlaxoSmithKline and Vaqta® by Merck and Co. for hepatitis A; and Engerix-B® (Hepatitis B (Recombinant) Vaccine) by GlaxoSmithKline and RECOMBIVAX-HB® by Merck and Co. for hepatitis B. The hepatitis A vaccines are licensed for use in individuals 2 years of age and older, while the hepatitis B vaccines

☆ The clinical trials were reviewed by the institutional review boards at each of the investigational sites in accordance with the provisions of the Declaration of Helsinki as amended in 1989 in Hong Kong. Each volunteer gave written informed consent before enrollment.

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are licensed in different dosages and schedules for all ages.

As the number of recommended vaccines on the market increases, combination vaccines are becoming more and more important as a means of conveniently offering additional opportunities for vaccination, reducing the number of visits to the clinic for immunizations and decreasing patient discomfort. All these factors are expected to increase compliance. In addition, for some diseases, such as hepatitis A and hepatitis B, there is sufficient overlap in the epidemiology to warrant the use of a combination vaccine. The development of combination vaccines is thus an important public health measure, which has the potential to increase vaccine coverage and help break transmission of vaccine-preventable infectious diseases.

A critical element in the development of a combination vaccine is the demonstration of the lack of immune interference, a complex phenomenon whereby the immune response to one or more antigens in the vaccine is hampered by any of the vaccine component(s). We have assumed two indicators to evaluate the immune response to combination vaccines: the quantity of the antibodies induced, and the functionality of the antibodies. In previous studies on the immunogenicity of GlaxoSmithKline's combination hepatitis A and B vaccine Twinrix® (Hepatitis A Vaccine Inactivated and Hepatitis B (Recombinant) Vaccine), the magnitude of the anti-hepatitis A (HAV) and anti-hepatitis B surface antigen (HBs) antibody responses were similar to those obtained previously with either monovalent vaccine alone (André, 1990; André et al., 1992; Thoelen et al., 1999; Van Damme et al., 2001). Furthermore, several prospective, randomized, trials of *Twinrix* and corresponding monovalent vaccines showed a comparable magnitude of anti-HAV and anti-HBs responses to *Twinrix* as compared with *Havrix* and *Engerix-B* administered concurrently (Ambrosch et al., 1994; Czeschinski et al., 2000; Joines et al., 2001). However, the functional quality of either response was not explored.

The present analysis addresses two objectives: confirmation of the similarity of the magnitude of the antibody response to *Twinrix* and the monovalent vaccines *Havrix* and *Engerix-B* immediately following the vaccination series, and demonstration of the functionality of the antibody response to *Twinrix* as compared with the monovalent vaccine *Havrix*, by examining the neutralizing ability and epitope-specificity of the vaccine induced antibodies. Although a protective epitope on HBsAg has been described (Iwarson et al., 1985; Waters et al., 1991), functionality of the anti-HBs response could not be evaluated, because a validated assay was not developed at the time the present study was conducted.

In contrast to earlier studies (Ambrosch et al., 1994; Czeschinski et al., 2000; Joines et al., 2001) where the magnitude of antibody responses were described in single, individual studies, this analysis is the first comprehensive and systematic overview of the step-wise clinical development of a combination vaccine, involving a relatively large number of subjects (413 subjects analyzed for anti-HAV and 583 subjects analyzed for anti-HBs). The purpose of the deliberate and step-wise development (see Section 2.1) was to confirm that combining monovalent vaccines did not result in interference of the generation of antibodies to either antigen.

2. Materials and methods

2.1. Design of clinical development for Twinrix

The overall clinical development plan for *Twinrix* was prospectively designed to consist of four steps which would allow us to better understand the sources for potential differences in the magnitude and functional quality of the antibody responses to the monovalent vaccines *Havrix* and *Engerix-B* as compared with the response to the combination vaccine *Twinrix*. These steps were: the administration of: either *Havrix* or *Engerix-B* alone, *Havrix* and *Engerix-B* concurrently in opposite arms, *Havrix* and *Engerix-B* mixed in one syringe, and the combination vaccine *Twinrix* (see Tables 1–3 for a visual display). Analysis of the magnitude and functional quality of the antibody responses was retrospective.

2.1.1. Data sources and trial selection

Data from eight clinical trials were selected for the retrospective analysis from the database of

Table 1
Trial designs, vaccines administered and subjects analyzed in clinical trials selected for retrospective analysis

Clinical trial	Trial design	Number of subjects evaluated for immunogenicity (month 7 post-vaccination)				
		Havrix	*Engerix-B*	*Havrix* + *Engerix-B* in opposite arms	*Havrix* + *Engerix-B* syringe-mixed	*Twinrix*
HAV-058	Double-blind, comparative, randomized trial of *Havrix* (two lots)	87	–	–	–	–
HBV-163	Double-blind, comparative, randomized trial of *Engerix-B* (three lots)	–	33	–	–	–
HBV-B-017	Single-blind, randomized trial of *Engerix-B* vs. comparators	–	30	–	–	–
HBV-102	Double-blind, comparative, randomized trial of *Engerix-B* (three lots)	–	85	–	–	–
HBV-141	Double-blind, comparative, randomized trial of *Engerix-B* (three lots)	–	115	–	–	–
HAV-067	Open, randomized, comparative trial of *Havrix*, *Engerix-B* administered alone or concurrently in opposite arms	37	31	36	–	–
HAB-019	Open, randomized comparative trial of *Twinrix*, *Havrix* and *Engerix-B* administered concurrently in opposite arms or as a syringe mixture	–	–	42	43	45
HAB-028	Double-blind, randomized, comparative trial of *Twinrix* (three lots)	—	—	—	—	123
Total		124	294	78	43	168

GlaxoSmithKline in Rixensart, Belgium based on all the following criteria: trials with *Havrix* or *Engerix-B* with the same antigenic content as in *Twinrix*; trials with *Havrix* or *Engerix-B* administered on the same three-dose schedule as *Twinrix*; multiple trials conducted by the same investigators who studied *Twinrix*, *Havrix* and *Engerix-B*, thus aiming to obtain a demographically and ethnically comparable study population; and trials conducted relatively recently (1987–1994), during which time there was no data suggesting that the epidemiology of either hepatitis A or B at the investigating sites had changed. All subjects who had complied with clinical trial procedures and were thus included in the According-to-Protocol (ATP) analyses are presented and analyzed here. There was no further selection of any subset among these subjects.

2.1.2. Design of individual clinical trials

All trials were prospectively designed (see Table 1 for design of trials and numbers of subjects analyzed per trial). Subjects in all trials were healthy males or females above 17 years of age without serological evidence of previous infection with HAV or HBV, as determined by screening tests for anti-HAV, anti-HBs and anti-HBc at study entry. Eligibility criteria and methods of data collection were the same in all studies. Inclusion and exclusion criteria for each trial were similar; however, history of past disease or prior immunization was dependent on the candidate vaccine under investigation.

All clinical trial protocols were approved by the local or institutional Ethics Review Committees and were conducted in accordance with the Declaration of Helsinki as amended at the time of clinical trial conduct, and with the Good Clinical Practice guidelines operative at the time of clinical trial initiation. All study subjects or, if appropriate, their parents or guardians gave their written informed consent prior to study entry.

All subjects were immunized intramuscularly in the deltoid muscle at 0, 1 and 6 months with 1 ml containing either *Engerix-B*, *Havrix* or *Twinrix* according to the clinical trial protocol. Blood samples were obtained for serological studies at five timepoints: on the day of screening (Month 0), 1 month after the first dose (Month 1) and second dose (Month 2), immediately prior to the third immunization (Month 6), and 1 month thereafter (Month 7).

2.1.3. Vaccines

In all studies, the monovalent hepatitis A vaccine used was *Havrix* (720 EL.U./ml inactivated hepatitis A antigen adsorbed onto 0.5 mg of aluminum hydroxide), the monovalent hepatitis B vaccine used was *Engerix-B* (20 μg/ml recombinant hepatitis B surface antigen adsorbed onto 0.5 mg of aluminum hydroxide); and the combination hepatitis A and B vaccine used was *Twinrix* (720 EL.U./ml of hepatitis A antigen and 20 μg/ml of hepatitis B surface antigen adsorbed onto 0.45 mg of aluminum salts as aluminum phosphate and

Table 2
Geometric mean anti-HAV titers (mIU/ml) at month 7 post-vaccination

Clinical trial	*Havrix*	*Havrix* + *Engerix-B* in opposite arms	*Havrix* + *Engerix-B* syringe-mixed	*Twinrix*
	GMT[a] (*n*)[b]	GMT (*n*)	GMT (*n*)	GMT (*n*)
HAV-058	4701 (45)	–	–	–
	3764 (42)	–	–	–
HAV-067	3994 (37)	3370 (36)	–	–
HAB-019	–	4040 (42)	6745 (43)	6514 (45)
HAB-028	–	–	–	6531 (39)
	–	–	–	6236 (43)
	–	–	–	6225 (41)

[a] GMT, geometric mean titer, measured in mIU/ml.
[b] *n*, number of subject sera assayed.

Table 3
Geometric mean anti-HBs titers (mIU/ml) at month 7 post-vaccination

Trial	*Engerix-B*	*Havrix*+*Engerix-B* in opposite arms	*Havrix*+*Engerix-B* syringe-mixed	*Twinrix*
	GMT[a] (*n*)[b]	GMT (*n*)	GMT (*n*)	GMT (*n*)
HBV-163	10358.5 (33)	–	–	–
HBV-B-017	1270.0 (28)	–	–	–
HBV-102	1378.2 (27)	–	–	–
	2601.4 (27)	–	–	–
	2053.5 (30)	–	–	–
HBV-141	5721.0 (37)	–	–	–
	5043.0 (42)	–	–	–
	8694.0 (36)	–	–	–
HAV-067	2779 (31)	3468 (36)	–	–
HAB-019	–	3799 (42)	3865 (43)	5349 (45)
HAB-028	–	–	–	7445 (39)
	–	–	–	4582 (42)
	–	–	–	5680 (41)

[a] GMT, geometric mean titer, measured in mIU/ml.
[b] *n*, number of subject sera assayed.

hydroxide). All vaccines were manufactured by GlaxoSmithKline Biologicals, Rixensart, Belgium.

2.2. Magnitude of anti-HAV and anti-HBs responses

In all assays, antibody titers were calculated in mIU/ml using a World Health Organization (WHO) reference serum against the HAV or HBs antigens. Serum samples were tested for anti-HAV antibodies using a commercial ELISA, (Enzymun, Boehringer–Mannheim GmbH, Germany) with a cutoff for seroconversion of 33 mIU/ml or, prior to the availability of the commercial assay, an inhibition ELISA developed in-house with a cutoff for seroconversion of 20 mIU/ml (Delem et al., 1993). The in-house assay had been found to be concordant with the commercial assay (Delem et al., 1996). Serum samples were analyzed for anti-HBs using a commercially available radioimmunoassay kit (Abbott Laboratories, Chicago, IL) with a cutoff for seroconversion of 1 mIU/ml. However, only anti-HBs antibody titers ≥ 10 mIU/ml were considered to be protective (Frisch-Niggemeyer et al., 1986).

Geometric mean titers (GMTs) in the anti-HAV and anti-HBs ELISAs were calculated using the log transformation of seropositive titers and then taking the anti-log of the mean of these transformed values.

2.3. Functional quality of anti-HAV responses

The functional quality of the anti-HAV antibody response was determined by measuring neutralizing antibodies in two ways, a viral neutralization assay and neutralizing epitope-specific antibody competition assays.

2.3.1. HAV neutralization assay

In addition to measuring the magnitude of the antibody response, as is routinely done in vaccine development, we also characterized the protective potential of the antibodies elicited to the HAV component of *Twinrix*. Sera from subjects vaccinated against *Twinrix* at 0, 1 and 6 months were tested both by ELISA for the total antibody titer to HAV, and by a viral neutralization assay for the titer of clinically relevant antibodies. The neutralization assay quantitated the inhibition of hepatitis A virus replication in cell culture by anti-HAV antibodies present in serum samples. Sera analyzed for neutralizing titers were all taken from frozen stored aliquots from Trial HAB-028,

a comparative trial of three lots of *Twinrix* (see Table 1), based on sample availability.

A predetermined amount of virus was mixed with serial dilutions of test serum sample. MRC-5 cells were then added to support viral replication (neutralizing antibodies, if present, inhibited the infection of the MRC-5 cells by virus). After a 6-day incubation to allow for multiple rounds of viral replication, the cells were frozen and thawed to extract the intracellular virus, which was then quantitated by ELISA.

The ELISA measured the amount of virus present in samples by determining how much anti-HAV antibody it bound. The virus from the neutralization assay was captured onto ELISA plates that were coated with purified IgG from a convalescent HAV-infected patient and then detected with biotinylated K3-4C8, a monoclonal anti-HAV antibody. The amount of bound biotinylated K3-4C8 was quantitated using a colorimetric enzymatic reaction based on extravidine-peroxidase and the substrate *O*-phenylene diamine hydrochloride. Samples were measured spectrophotometrically at 492 nm using a reference wavelength of 620. The neutralizing antibody titer for each sample was defined as that which caused a 50% decrease of the OD obtained with the corresponding preimmune sample.

In the viral neutralization assay, sera were tested starting at a dilution of 10. However, the serum samples were not always titrated out to endpoint. In those cases the titer was defined as $\geq$ the highest titer tested, and that value was used for calculation of the GMT. The GMT was then defined as $\geq$ the calculated value, which represents the lower limit for the true value,

2.3.2. Epitope-specific anti-HAV ELISA

To further define the epitopes recognized by the neutralizing antibodies elicited by the hepatitis A antigen component of *Twinrix* and compare them to those elicited by *Havrix* alone, a subset of sera was tested in two separate competition assays against biotinylated murine monoclonal antibodies (K3-4C8 and B5B3) that are directed against neutralizable epitopes on HAV (Stapleton and Lemon, 1987).

These assays quantitated the amount of antibodies in the test serum samples that recognized the same epitopes detected by either monoclonal antibody K3-4C8 or B5B3 (Commonwealth serum Laboratories, Australia and Biogenesis, England), using inhibition ELISAs in which the test samples competed with the monoclonal antibodies for binding to virus-coated plates. The assays are a modification of the in-house anti-HAV inhibition ELISA described previously (Delem et al., 1993). Frozen test samples chosen for this analysis were taken from a subset of 20 subjects from a single trial (HAB-019, see Table 1), ten of whom had been vaccinated with *Twinrix* and ten of whom had been vaccinated with *Havrix* and *Engerix-B* concurrently in opposite arms. The samples tested had been taken 1 month after the second injection (Month 2) and 1 month after the third injection at month 6 (Month 7).

Serial dilutions of test samples containing anti-HAV antibodies were added to microtiter plates containing bound HAV. Biotinylated monoclonal antibody K3-4C8 or B5B3 was then added to the plates, which competed with those antibodies in the test serum samples that recognized the same epitopes on HAV. The amount of biotinylated monoclonal antibodies bound to the virus on the plate was then detected spectrophotometrically as described above in the ELISA for the neutralization assay. Test samples with a majority of antibodies recognizing the K3-4C8 and B5B3 epitopes greatly inhibited the binding to the plate by the biotinylated monoclonal antibodies and resulted in a low OD value.

Cutoffs and calculations of GMT values for the epitope-specific ELISA were as described for the anti-HAV assay above. In addition, the ratio of GMTs in the epitope-specific ELISA to the total anti-HAV ELISA was calculated.

3. Results

3.1. Magnitude of anti-HAV and anti-HBs responses

Eight clinical trials met the criteria for inclusion in the analysis, as seen in Table 1 (Ambrosch et

al., 1992, 1994; Just and Berger, 1988; Thoelen et al., 1999; Van Damme et al., 1992, 1994). All subjects were between 17 and 51 years of age. A total of 413 subjects were analyzed for anti-HAV and 583 subjects were analyzed for anti-HBs titers.

Table 2 shows the anti-HAV GMTs for the subjects at Month 7, 1 month after completing the immunization regimen. All subjects seroconverted, regardless of whether they received *Havrix* alone, *Havrix* and *Engerix-B* concurrently in opposite arms or mixed in the syringe, or as the combined vaccine *Twinrix*. GMTs were higher for subjects receiving *Twinrix* as compared with recipients of the monovalent vaccine alone.

The corresponding anti-HBs serological data are shown in Table 3. Seroprotection rates were similar in subjects receiving the monovalent vaccine and the combination vaccine. All but one subject (1%) among the *Twinrix* vaccinees and five subjects among the *Engerix-B* vaccinees (2%) developed seroprotective titers. The anti-HBs GMTs obtained in subjects vaccinated with *Twinrix* were all above the median value of the GMTs obtained following *Engerix-B* alone (2779 mIU/ml), strongly suggesting that administration of the combined vaccine does not interfere with the development of the anti-HBs antibody response.

3.2. Functional quality of vaccine-induced anti-HAV antibodies

As seen in Table 4, a good correspondence was obtained between the magnitude of the total antibody response as measured by ELISA and the neutralizing component of the response, indicating that nearly 100% of the anti-HAV response in the *Twinrix* vaccinees was functional.

Table 5 shows that the GMT generated in recipients of *Twinrix* was slightly higher than recipients of *Havrix* and *Engerix-B* at all time points for both the total Ig response and the epitope-specific responses. The third dose at Month 6 induced a comparable increase in *Twinrix* and *Havrix* recipients for both sets of responses. The 95% confidence interval of the GMTs in the *Twinrix* compared with the *Havrix* and *Engerix-B* groups showed considerable overlap in all three assays. The ratios of the GMTs in the epitope-specific assays to the GMT in the total anti-HAV ELISA were therefore similar for the two groups. Thus, similar proportions of the overall serological response were directed at the neutralizable epitopes in the two vaccine groups. The functionality of the anti-HBs antibodies induced by *Engerix-B* or *Twinrix* was not assayed because a validated assay was not developed at the time that this study was conducted.

Table 4
Seroconversion (SC) rates and anti-HAV GMTs in anti-HAV ELISA and HAV neutralization assay

Time point	Test	Number of subjects	SC rate[a]	GMT[b]
Month 0	ELISA	124	0	–
	Neut.[c]		0	–
Month 2	ELISA	124	99.2	785
	Neut.		99.2	≥595
Month 6	ELISA	70	97.1	538
	Neut.		100	≥610
Month 7	ELISA	70	100	6264
	Neut.		100	≥5972

[a] SC, seroconversion rate, defined as percentage of samples with a titer ≥33 mIU/ml for the anti-HAV ELISA and as a titer >10 giving 50% neutralization for the neutralization assay.

[b] GMT, geometric mean titer, measured in mIU/ml.

[c] Neut., as tested by-HAV neutralization assay; ≥ actual value not determined (see Section 2).

Table 5
Total Ig anti-HAV and K3-4C8 and B5B3 epitope-specific antibody GMTs (mIU/ml) by ELISA in subjects administered *Twinrix* or *Havrix* and *Engerix-B*

Vaccine administered	Anti-HAV Ig GMT[a] (95% CI)[b]	K3-4C8 GMT[c] (95% CI)	Ratio[d]: K3-4C8 anti-HAV Ig	B5B3 GMT[c] (95% CI)	Ratio[d]: B5B3 anti-HAV Ig
Twinrix					
Month 2	1452 (1007–2092)	1540 (1090–2176)	1.06	1473 (919–2362)	1.01
Month 7	9549 (6299–14471)	8544 (5797–12589)	0.89	10004 (6569–15224)	1.05
Havrix + Engerix-B					
Month 2	941 (658–1345)	815 (479–1385)	0.87	691 (421–1133)	0.73
Month 7	5775 (3865–8629)	5461 (3508–8502)	0.95	5505 (3640–8327)	0.95

[a] GMT, geometric mean titer, measured in mIU/ml.
[b] 95% CI, 95% confidence interval.
[c] GMTs directed against the K3-4C8 and B5B3 epitopes were determined by eptiope-specific ELISAs.
[d] The ratio of the GMTs obtained in the K3-4C8 epitope-specific ELISA to the GMT obtained in the ELISA for total anti-HAV Ig, and the ratio of the GMTs obtained in the B5B3 epitope-specific ELISA to the GMT obtained in the ELISA for total anti-HAV Ig were calculated.

4. Discussion

This analysis addresses the question of whether anti-HAV and anti-HBs responses are compromised quantitatively or qualitatively when hepatitis A and B antigens are formulated in a combination vaccine as compared with individual monovalent vaccines. Subjects used for these comparisons received either monovalent hepatitis A or hepatitis B vaccines alone, concurrently in opposite arms, together as a mixture in the syringe, or as a combination vaccine. This study confirms and extends those reported previously (Ambrosch et al., 1994; Czeschinski et al., 2000; Thoelen et al., 1999) by evaluating the magnitude of the immune response to *Twinrix* throughout its clinical development, with systematic comparisons to the monovalent vaccines administered singly, concurrently or as a mixture. In addition, this study also evaluated the functionality of the immune response.

Evaluation of the duration of the induced response is another critical parameter in the development of any vaccine. This last parameter was not a topic for evaluation in this study. However, the duration of a vaccine-induced immune response is deduced to be directly related to the magnitude of the immune response (Banatvala et al., 2000; Van Herck et al., 2000). Recently, the anti-HAV and anti-HBs antibody response to Twinrix was reported to persist for at least 5–6 years (Van Damme et al., 2001).

All vaccinees seroconverted for HAV, and the titers obtained after administration of *Twinrix* were in fact higher than concurrent administration of both vaccines or hepatitis A vaccine alone. All but one of the *Twinrix* vaccinees were seroprotected against HBV, and their titers were all in the range of 3000–4000 mIU/ml, which have been reported by others to be predictive of long-term protection up to 8 years post-booster (Gesemann and Scheiermann, 1995; Van Damme et al., 2001; Van Herck et al., 1998). In earlier studies, Ambrosch et al. found that administration of combined and syringe-mixed hepatitis A and hepatitis B vaccines at 0, 1 and 6 months resulted in significantly higher titers against both hepatitis A and B than concurrent vaccination in opposite arms (Ambrosch et al., 1994). Similarly, Czeschinski et al. (2000) reported more recently that recipients of *Twinrix* had significantly higher anti-HAV titers and higher anti-HBs titers than recipients of

either vaccine alone. Thus, several studies indicate that there is no negative effect on the magnitude of the antibody response to either the A or B component in *Twinrix* vaccinees.

Our data presented here, and recently published (Abraham and Parenti, 2000; Kallinowski et al., 2000) contrast with those obtained by others (Frey et al., 1999) who found that a combination vaccine of hepatitis A and B antigens resulted in a lower GMT and seroprotection rate to the hepatitis B component upon completion of a two-dose immunization regimen, as compared with concurrent administration of the monovalent vaccines. This discrepancy may lie in the different formulations of the two vaccines.

In the analysis presented, we also characterized the quality of the antibodies produced. We first demonstrated that the anti-HAV response generated in *Twinrix* vaccinees was functional, by showing that the antibodies generated inhibited viral infection of cells in vitro. We next demonstrated that the proportion of total antibody elicited to the hepatitis A component of *Twinrix* that was directed against the K3-4C8 and B5B3 epitopes was similar to that generated in recipients of the individual monovalent vaccines concurrently. The monoclonal antibodies which recognize these epitopes are known to have neutralizing activity, which may further indicate that the humoral response of the *Twinrix* vaccinees was functional (Stapleton and Lemon, 1987).

Functionality of the anti-HBs response was not tested. Identification of a protective epitope on HBsAg has been hampered due to the lack of a cell line, which will support in-vitro growth of HBV. A putative protective epitope for humans on HBsAg has been identified by the monoclonal antibody RF1 in a protection assay in chimpanzees (Iwarson et al., 1985; Waters et al., 1991). Using this antibody it has been demonstrated in competitive radioimmunoassays that *Engerix-B* elicits antibodies that react with the RF1 epitope (Hauser et al., 1987). This vaccine contains the same HBs antigen that is used in *Twinrix*. Preliminary ELISAs have been developed to compare the sera of individuals receiving a DTPa-based combination vaccine containing an identical antigen to that in *Engerix-B* with sera of recipients of monovalent *Engerix-B* with respect to the presence of antibodies that compete with the RF1 antibody, and development of a validated ELISA is ongoing. The observation that this DTPa-based combination vaccine induces RF1-like antibodies indirectly supports the induction of a functional anti-HBs response by *Twinrix*, since both these combination vaccines, contain the same HBs antigen.

Combination vaccines are part of routine immunization programs in the US and worldwide. Combination vaccines presently available in the US include those against diphtheria and tetanus toxoid (DT and Td), DT and pertussis (DTP and DTaP), DTP or DTaP and *H. influenzae* type B (DTP-Hib, DtaP-Hib), measles and rubella with or without mumps (MR, MMR), polyvalent *S. pneumoniae* and polyvalent *N. menigitidis* (Decker and Edwards, 1999). In addition, many other combination vaccines are under clinical investigation. Varying positive and negative effects on antibody responses to the components of numerous combination vaccines, including DTP-inactivated poliovirus (IPV) $\pm$ Hib, DTP-Hib, DT(a)P-Hep B, and MMR-varicella (MMRV) have been observed in many instances (reviewed by Decker and Edwards, 1999).

Immune interference can be attributed to many different mechanisms (reviewed by Insel, 1995). Some of the physicochemical causes of immune interference in combination vaccines include effects on the ionic interactions with adjuvants, leading to adsorption or displacement of various vaccine components, and interferences due to buffers, stabilizers or other excipients of one of the vaccines with the other(s). Had *Twinrix*, a combination of an inactivated virus and a subunit vaccine, affected the immunogenicity to HAV or HBs, it would most likely have been attributed to these types of interactions. In addition, components of combination vaccines can induce Th_1 or Th_2 responses that are mutually antagonistic. In the case of live viral combination vaccines, one virus can interfere with the growth of another by inducing the release of inhibitory cytokines such as interferon. Conjugate vaccines that share a common carrier antigen can be subject to carrier-induced epitope suppression. Although its exact

mechanism is unclear, experimental models suggest that large numbers of carrier-specific B cells, high titers of carrier-specific antibody, induction of energy in hapten-specific B cells and induction of suppressor cells all play a role in carrier-induced epitope suppression.

Although immune interference is a real phenomenon, its clinical significance must be put into perspective. For some antigens, such as diphtheria toxoid, HBV and Hib, a correlation between antibody titers and vaccine efficacy has been well established, whereas for others, such as pertussis, no such correlate of protection exists (Decker and Edwards, 1999). In addition, in several cases, such as HBV, there is a generally accepted protective antibody level of ≥ 10 mIU/ml. Immune interference, which lowers the antibody titers to levels that are still well above the cutoff for seroprotection may not be of immediate clinical concern, but could have implications for long-term persistence of antibodies and the potential need for boosters (European Consensus Group on Hepatitis B Immunity, 2000). While the ability to mount an anamnestic response is of key importance for long-term protection, there is currently no reliable measure of the development of immunological memory. Although high titers of antibody following vaccination do not give a complete picture of the immune response, several mathematical models correlate high titers with long-term persistence of antibody (Ambrosch et al., 1992; Gesemann and Scheiermann, 1995; Van Herck et al., 2000) and, for hepatitis B, a good correlation exists between the robustness of the primary humoral response and the subsequent development of the memory response (Banatvala et al., 2000).

In conclusion, when administered in a three-dose immunization regimen, *Twinrix* elicits a vigorous immune response to both the hepatitis A and B components of the vaccine, which includes high titers of neutralizing antibodies to HAV, and anti-HBs GMTs that are known to be protective in the long-term. Furthermore, combining the two existing and widely used hepatitis A and B monovalent vaccines does not impair the immunogenicity of the response to either of the individual vaccine components.

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