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Simultaneous Administration of Diphtheria-Tetanus-Pertussis-Polio and Hepatitis B Vaccines in a Simplified Immunization Program: Immune Response to Diphtheria Toxoid, Tetanus Toxoid, Pertussis, and Hepatitis B Surface Antigen

P. COURSAGET,[1]* B. YVONNET,[2,3] E. H. RELYVELD,[4] J. L. BARRES,[1] I. DIOP-MAR,[2] AND J. P. CHIRON[3]

Institut de Virologie de Tours, Facultés de Médecine et de Pharmacie, 37032 Tours, France[1]; Faculté de Medecine et de Pharmacie, Dakar-Fann, Senegal[2]; Laboratoire de Microbiologie, Faculté de Pharmacie, Tours, France[3]; and Unité des vaccins Bactèriens, Institut Pasteur Fondation, 92430 Marnes la Coquette, France[4]

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We studied the interactions of hepatitis B vaccine with other vaccines used in the World Health Organization expanded programs of immunization. Three groups of Senegalese children were vaccinated with hepatitis B vaccine (HB) alone, diphtheria-tetanus-pertussis (DTP)-polio vaccine alone, or a combination of hepatitis B vaccine and DTP-polio vaccines simultaneously. The immune responses to HBsAg, tetanus toxoid, diphtheria toxoid, and pertussis were measured after one and two vaccinations at 6-month intervals. The immune responses to the combination of HB vaccine and DTP-polio vaccines were similar to the immune responses observed after administration of each vaccine alone. In addition, no adverse reactions were noted. These experimental trials also demonstrated that with a DTP-polio vaccine containing 30Lf of tetanus and diphtheria toxoids, two doses given at 6-month intervals are sufficient to provide a satisfactory immune response. In the case of pertussis and HB vaccines; however, a third dose is necessary.

In most developing countries, hepatitis B virus infection is endemic (2, 16, 23), and attempts to prevent infection must be made very early during childhood (8, 12, 13, 24). In such areas, the simultaneous administration of multiple antigens by mobile teams of preventive health services help overcome the practical and logistical obstacles to providing complete immunization to children (9, 11, 21). Classical vaccination programs comprising a 4-week-interval schedule, with a booster dose one year later cannot be used in many developing countries. For example in tropical rural areas, among the several logistical problems encountered are limited medical personnel and the difficulty of communication.

For these reasons, several simplified schedules have been studied to enable the use of a reduced number of injections. Thus, it has been shown that with regard to diphtheria-tetanus immunizations, it is possible to provide immunization with two injections at 12-month intervals, using calcium phosphate adsorbed vaccine (6); for poliomyelitis immunization, two injections are necessary with a booster one-year later (22). A single injection of tetanus toxoid confers immunity to 98% of a population. The percentage of triple-positive children (protected against the three different types of poliovirus) rises from 0 to 81% 2 months after the first vaccinations, and then to 90%, after the booster 1 year later (22).

We investigated the interactions of hepatitis B vaccine with other vaccines used in the World Health Organization expanded programs of immunization. This study has already been made in laboratory animals by Mazert et al. (14). Moreover, no alterations in the immune responses to HBsAg and tetanus toxoid antigen were observed when the vaccines were administered simultaneously as hepatitis B (HB) vaccine and diphtheria-tetanus (DT)-polio vaccine to children in a series of three injections at 1-month intervals (7).

In this study, three groups of Senegalese children were given HB vaccine alone, DTP-polio vaccine together, or HB and DTP-polio vaccines simultaneously. The serological responses to HBsAg, tetanus toxoid as well as diphtheria toxoid and pertussis were studied.

MATERIALS AND METHODS

Study population. Children from 3 to 24 months old were chosen from villages in the Fatick region of Senegal and divided into three groups. Only children from whom a blood sample could be obtained 12 months after the first injection, were included in the final analysis. Group 1 (n = 48) received two HB vaccine doses at 6-month intervals, and a booster dose 12 months after the first dose (T12). The mean age at T0 was 11.0 months. Group 2 (n = 29) received DT pertussis-(DTP)-polio vaccine plus *Mycobacterium bovis* BCG vaccine at a first session, DTP-polio at a second session (T6), and then DTP-polio vaccine again 6 months later (T12). The mean age at T0 was 7.5 months. Group 3 (n = 108) received DTP-polio plus BCG plus HB vaccines at a first session, DTP-polio and HB vaccines at a second session (T6), and a dose similar to the second at T12. HB virus seric markers were searched for in 108 infants; however, due to a shortage of serum, other serological tests could be performed in only 73 of them (mean age 11.7 months).

Vaccines. HB vaccine (Hevac B), DTP-polio vaccine (IPAD-DTP-polio), and BCG used in this study were obtained commercially from Pasteur Vaccins. The vaccines studied were of the following composition. Hevac B was composed of purified HBsAg (5 μg/ml) inactivated with formaldehyde. Both subtypes ad and ay were mixed in the final preparation, and aluminium hydroxide was added as

* Corresponding author.

TABLE 1. Antibody levels at various times of blood sampling

Time	Vaccine	Tetanus antibodies		Diphtheria antibodies		Pertussis antibodies	
		No. positive (%)	Geometric mean titer (IU/ml)	No. positive (%)	Geometric mean titer (IU/ml)	No. positive (%)	Geometric mean titer (IU/ml)
T0	DTP ($n=29$)	3 (10.3)		5 (17.2)	0.01	0 (0.0)	
	DTP-HB ($n=73$)	6 (8.2)		38 (52.1)	0.05	4 (5.5)	
T6	DTP ($n=29$)	28 (96.6)	0.227	26 (89.7)	0.31	1 (3.4)	
	DTP-HB ($n=73$)	72 (98.6)	0.350	64 (87.7)	0.37	15 (20.5)	
T12	DTP ($n=29$)	29 (100)	1.775	29 (100)	1.34	25 (86.2)	35.2
	DTP-HB ($n=73$)	73 (100)	1.622	73 (100)	0.90	63 (86.3)	25.8

adjuvant (1). The IPAD DTP-polio vaccine contained 30 Lf of tetanus and diphtheria toxoids as well as 4 IU minimum protection of pertussis. The vaccine also contained killed poliovirus at a D antigen level of 5, 2, and 4 for types 1, 2, and 3, respectively. Calcium phosphate was added as adjuvant (18). Tetanus toxoid was prepared by formaldehyde detoxification of highly purified toxin, and diptheria toxoid was prepared by the use of pure toxin as starting material. We observed that individuals who had unfavorable reactions to diphtheria toxoids were sensitive to antigens different from the diphtheria toxin present in the vaccines. These individuals also reacted to antigens of nontoxic diphtheria strains, or detoxified culture filtrates of toxigenic strains cultivated in a medium with excessive concentrations of iron (20).

The advantage of toxoiding purified toxin is apparent because extraneous compounds present in the crude toxin can no longer be incorporated into the toxoid by the formation of intermolecular methylenic bonds (4, 15). It has been stated that conditions of detoxification of crude material often lead to covalent crosslinking of toxoid to other bacterial proteins and unrelated peptides which are present in culture filtrates. It is almost impossible to remove from such heterogeneous mixtures, substances known to elicit undesirable reactions (15). An adult-type (Td) vaccine is no longer necessary if purified diphtheria toxin is used for vaccine preparation. Undesirable side effects are absent even when high Lf concentrations are injected (30 Lf/ml), and it is thus possible to confer immunity to persons who generally cannot return for a second or a booster injection, particularly in developing countries (18).

Serological studies. Blood samples were taken the day of first injection (T0), 6 months later the time of second injection (T6), and 6 months after the second dose of vaccines (T12). HB virus seric markers (HBsAg, anti-HBs, anti-HBc) were tested for with commercial radioimmunoassays (Ausria II, Ausab, Corab, Abbott Laboratories). Antitoxin titers to tetanus and diphtheria were determined by the passive hemagglutination technique using highly purified toxins coupled to turkey erythrocytes by glutaraldehyde. The use of highly purified tetanus and crystalline diphtheria toxins helped eliminate false-positive reactions and enabled the calculation of titers according to corresponding international antitoxic units (IAU); this was verified by in vitro titration (17). Coupling with glutaraldehyde simultaneously detoxified the toxins (19). For determination of pertussis agglutinins, measurements were made using the agglutination test performed in microtiter plates.

Anti-HBs antibody titers are expressed in milli IU per milliliter (mIU/ml) and calculated by the method of Hollinger et al. (10). Tetanus and diphtheria antibody titers are expressed in units equivalent to IAU per milliliter. Pertussis agglutinin titers are expressed as the reciprocal of the serum dilution giving the positive result.

RESULTS

No evidence of general or severe undesirable side effects was observed during the study. At the time of the first vaccinations, three infants (10.3%) from group 2 (DTP-polio) and six (8.2%) from group 3 (DTP-polio + HB) showed evidence of tetanus antibodies.

At the time of second vaccination (T6), antibodies were present in 96.6 and 98.6% of groups 2 and 3, respectively (Table 1). The geometric mean titers were 0.227 and 0.350 IAU/ml for groups 2 and 3, respectively. At the time of booster injection (T12), that is 6 months after the second vaccination, all the infants from both groups 2 and 3 showed evidence of the presence of tetanus antibodies, with their geometric mean titers being 1.775 and 1.622 IAU/ml, respectively. The distribution pattern of tetanus antibody titers were also similar in infants who received either DTP-polio alone, or DTP-polio simultaneously with HB vaccine (Fig. 1).

Diphtheria antibodies were often found to be present in the infants at the time of the first vaccination; these were detected in 5 infants from group 2 (17.2%), and in 38 infants from group 3 (52.1%). Six months after the first dose, 89.7 and 87.7% of the infants from groups 2 and 3, respectively, had diphtheric antibodies, with their geometric mean titers 0.31 and 0.37 IAU/ml, respectively. Six months after the second dose (T12), all the infants were found to be positive for diphtheric antibodies, with their geometric mean titers increasing to 1.34 IAU/ml for infants in group 2, and 0.90 IAU/ml for those in group 3. For tetanus antibodies, no differences were noted in the distribution pattern of diphtheric antibodies between infants receiving DTP-polio alone or DTP-polio simultaneously with HB vaccine (Fig. 1).

At the beginning of the study, no pertussis antibodies were present in infants from group 2; they were however present in four infants from group 3 (5.5%). Six months after the first injection, pertussis agglutinins were present in 1 infant from group 2 (3.4%), and in 15 infants from group 3 (20.5%). However, 6 months after the second dose (T12), 86.2 and 86.3% of infants from groups 2 and 3, respectively, showed evidence of the presence of pertussis agglutinins. The geometric mean titers at that time, expressed as reciprocals of serum dilution were 35.2 for group 2, and 25.8 for group 3 ($2^{5.1}$ and $2^{4.7}$). No difference in antibody titer distribution patterns was observed for any of the other antigens studied (Fig. 1).

Anti-HBs antibodies were detected at the time of the first vaccination in 10 of 48 infants from group 1 (20.8%), and in 16 of 108 infants from group 3 (14.8%). Six months after the first dose (T6), 79.2 and 66.2% of the infants from groups 1

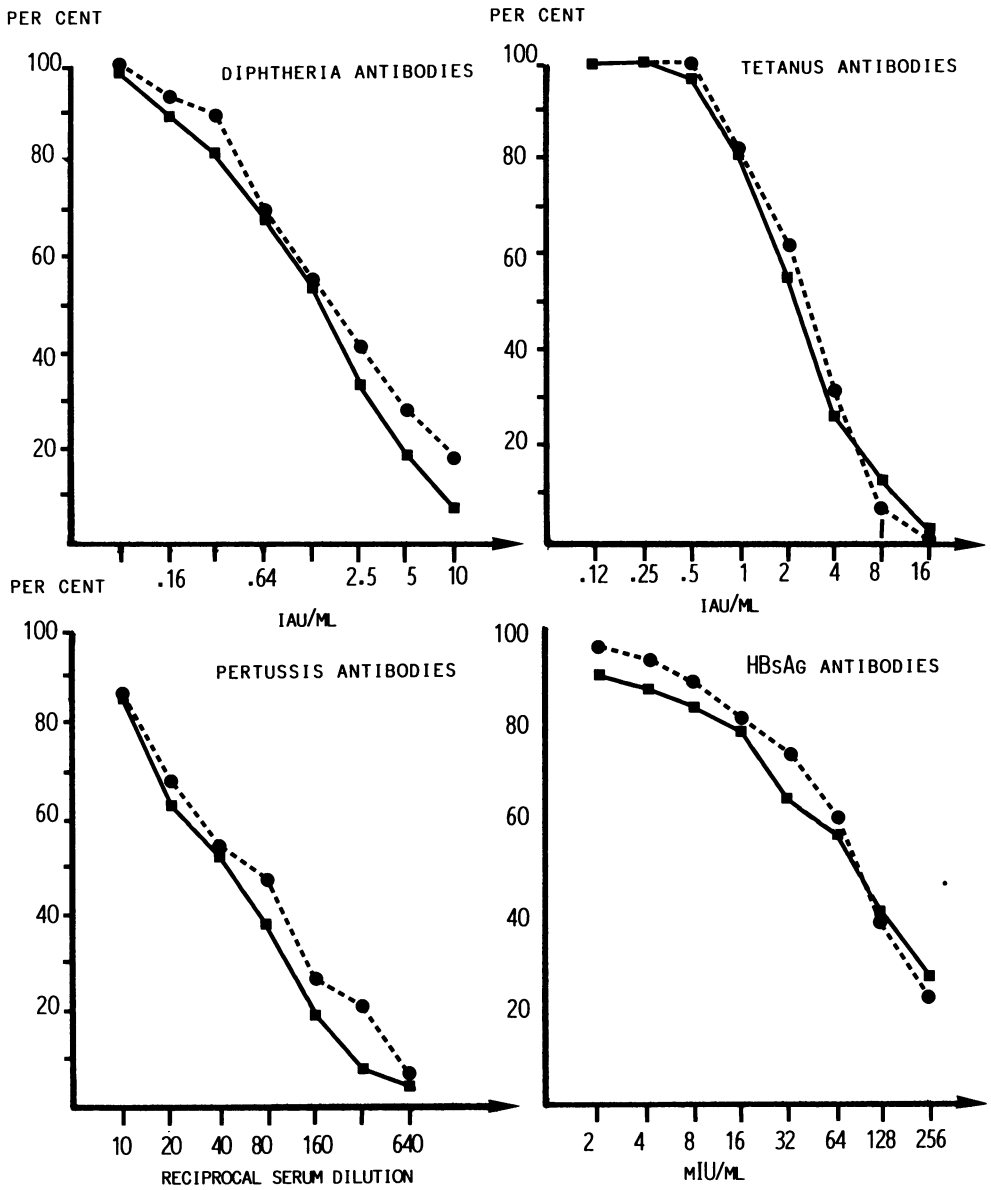

FIG. 1. Cumulative percent distribution of antibody titers against tetanus, diphtheria, pertussis, and HB surface antigen 6 months post second injection of DTP-polio vaccine, HB vaccine, or simultaneous injection of both vaccines. Symbols: ●, Vaccine alone; ■, associated.

and 3, respectively, were anti-HBs positive. Six months after the second dose (T12), 95.8% of the infants from group 1 and 89.8% from group 3 were found to be anti-HBs positive. No differences were observed in the distribution of anti-HBs antibody levels between infants receiving either HB vaccine alone or HB vaccine simultaneously with DTP-polio vaccine (Fig. 1).

DISCUSSION

Immunization of children with two sets of primary doses of DTP-polio vaccine associated with HB vaccine at 6-month intervals provide equivalent antibody responses to those observed with each of these vaccines separately. In addition, no adverse side effects occurred. These experimental trials have also shown that with a vaccine containing 30 Lf of tetanus toxoid and 30 Lf of diphtheria toxoid, two vaccinations at 6-month intervals are sufficient to provide a satisfactory immune response in terms of seroconversion and antibody titers generally obtained only after three primary series of vaccinations and a booster dose with another vaccine (3) containing 10 and 13 Lf of tetanus and diphtheria toxoids, respectively. Thus it seems that for these two antigens a booster injection is not an absolute necessity.

It has been reported that, as early as the first week of life, the immune system of an infant is capable of responding satisfactorily to all components of the DTP-polio vaccine when adsorbed toxoids are utilized (25). This is contrary to results reported earlier concerning fluid toxoids. It has equally been demonstrated that for adsorbed toxoids, passively acquired antibodies do not modify the immune response (5). Pertussis agglutinins were detected in 86.0% of the infants after the second dose of DTP-polio vaccine, but when these titers were compared to those observed in other experimental trials (3) performed with a vaccine containing

8.8 protective units, they were largely insufficient, and therefore a booster injection seems necessary. Similar results were obtained with HB vaccine. Immune responses after one dose were very low and similar to other immune responses obtained after a second dose (24). Therefore, for longer lasting protection, a booster injection is necessary.

In conclusion, the immune response to a combination of HBsAg and DTP-polio vaccines injected simultaneously has been found to be similar to that observed after administration of each of these two vaccines separately. These results thus show that HB vaccine could be introduced in WHO expanded programs of immunization in developing countries without reducing protective coverage.

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