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Immunization with *Haemophilus influenzae* Type b Conjugate Vaccine in Children Given Bone Marrow Transplantation: Comparison with Healthy Age-Matched Controls

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Forty-seven patients (age range, 7 months–18 years) with malignant (38 cases) and nonmalignant (9 cases) disorders given an allogeneic or an autologous bone marrow transplantation (BMT) were immunized with *Haemophilus influenzae* type b (Hib) polysaccharide–diphtheria toxoid conjugate vaccine administered in a single dose at different time points after transplantation. Results were compared with those of 13 healthy children matched for age and sex who received the same immunization schedule. Serum and saliva samples for measurement of total IgG subclass and specific antibody levels were obtained from patients and healthy controls before and 3 weeks after vaccination. Twenty-five of the 47 patients (53%) had a specific anti-Hib IgG response, while an effective IgA and IgM response was mounted by 23 (49%) and 11 (23%) children, respectively. In the control group, 13 of 13 subjects mounted a specific IgG antibody production ($P < 0.005$ in comparison to the patients' response rate), while an IgA and IgM response was demonstrated in 12 (92%; $P < 0.01$ compared to transplanted patients) and 7 (54%; $P < 0.05$ in comparison to BMT recipients) children, respectively. Lapse of time from BMT to immunization was the most important factor predicting antibody response, as proved by an effective increase in prevaccination specific IgG levels in the majority of patients vaccinated after 2 years from transplant. Our data demonstrate that BMT recipients have a reduced capacity to mount an antibody response to polysaccharide antigens compared to normal controls, even when a protein-conjugated vaccine is employed. Since time after transplant is the major factor influencing the recovery of immune reactivity to polysaccharide antigens, the ontogeny of the B cell repertoire seems to follow a predetermined sequential program of development.

KEY WORDS: Bone marrow transplantation; polysaccharide conjugate vaccine; *Haemophilus influenzae* type b; immunization; IgG subclasses.

INTRODUCTION

Infections due to *Streptococcus pneumoniae* and *Haemophilus influenzae* type b (Hib) occur with a relatively high incidence and severity in infants and children younger than 2 years of age. In fact, these subjects are often unable to mount an adequate production of antibodies to capsular polysaccharide (CP) antigen, a major virulence factor of these bacteria (1). Since antibodies directed toward CP play a pivotal role in the defense against encapsulated bacteria (1–3), the relatively late development of humoral response to this antigen occurring during the physiological ontogeny of the immune system in childhood accounts for the increased incidence of infections observed in young children (4).

Besides children, during the process of age-dependent acquisition of antipolysaccharide antibody production, also bone marrow transplantation (BMT) recipients have an increased risk of infections due to encapsulated bacteria (5,6). This is hardly surprising as the reconstitution of the immune system after BMT can be considered a recapitulation of the physiological process of ontogeny of the immune system occurring during the first years of life (7–10).

As is the case with healthy infants, active immunization against CP has been proposed also for BMT recipients (11). Unfortunately, since the ability to display an effective antibody response to CP is slow to develop after BMT (7–9), immunization with pure polysaccharide

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vaccines has produced satisfactory results mainly when performed after a long time interval after transplantation (12, 13).

The poor immunogenicity of polysaccharide vaccines has recently been demonstrated to be improved by covalently linking polysaccharides to a protein carrier (14). In particular, covalently linking CP to a carrier molecule elicites a T cell-dependent response with development of memory cells (15), and this can overcome the inability of young children and other particular groups of patients to respond to polysaccharide vaccines alone. Hib CP–protein conjugate vaccines have been demonstrated to be immunogenic and antibodies produced after immunization with these vaccines are effective in protecting young children against Hib infection (16–18).

In order to investigate the biological peculiarities of the immune response to a conjugate vaccine in paedlatric BMT recipients, we carried out a prospective study on patients given allogeneic or autologous marrow transplantation and vaccinated with an Hib polysaccharide–diphtheria toxoid conjugate (PRP-D). In particular, we evaluated whether the use of a single dose of conjugated vaccine could induce an effective serum and secretory antibody response against CP antigens. Results for this cohort of patients were compared with those obtained in age-matched healthy controls.

PATIENTS AND METHODS

Forty-seven patients (27 males and 20 females) with malignant (37 cases) and nonmalignant (10 cases) disorders, given either an allogenic (38 patients) or an autologous (9 children) BMT at the Department of Paediatrics of the Universities of Pavia and Bologna between January 1986 and May 1994 and surviving in complete hematological remission for at least 6 months after transplant were enrolled in this study. For patients given an allogeneic transplant, the donor was a compatible sibling in 31 cases, an HLA-identical unrelated volunteer in 3 cases, and an HLA-disparate relative in the remaining 4 children. Patients' median age at the time of transplant was 9 years (range, 7 months–18 years). A conditioning regimen consisting of radiotherapy [total-body irradiation (TBI) or thoracoabdominal irradiation (TAI)] and chemotherapy was employed in 29 patients, whereas the other 18 patients were given myeloablative and/or immunosuppressive drugs.

In the 31 patients transplanted from an HLA-identical sibling, graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporin A (Cs-A) administered intravenously, starting on day −1, at a dosage of 3–5 mg/kg/day for the first 21–28 days and, subsequently, p.o. at a dose of 6 mg/kg/day for 6 further months. Four patients given allogeneic BMT from a partially matched family donor received a T cell-depleted marrow (depletion was accomplished *in vitro* using the monoclonal antibody Campath-1M plus complement), while the three patients given a matched unrelated transplant were treated, in addition to Cs-A, with short-course methotrexate (19) and the monoclonal antibody Campath-1G *in vivo* (20). Chronic GVHD (c-GVHD) was classified according to previously described criteria (21). No patient with c-GVHD was receiving immunosuppressive treatment at time of vaccination. None of the patients had been treated with polyvalent or hyperimmune immunoglobulins for at least 24 weeks before immunization, whereas 27 of the 47 patients had previously had pneumococcal vaccine (Pneumovax II; Merck Sharp and Dohme; a polyvalent pneumococcal preparation containing 25 μg of capsular polysaccharide from each of 23 serotypes). Moreover, since our immunization policy was to offer DT vaccine to all children younger than 6 years of age, with a follow-up after BMT of at least 1 year, seven children had received immunization with DT vaccine (containing 30 IU) prior to exposure to the Hib CP–protein conjugate vaccine. Further details on patient characteristics, conditioning regimens, and clinical outcome are reported in Table I.

The control population consisted of 13 healthy children matched for age and sex who received the same vaccination schedule employed in BMT recipients.

The median age at the time of vaccination for patients and controls, as well as the time interval between BMT and vaccination, is reported in Table I. In order to assess the impact of recipient age at vaccination and donor age at BMT on antibody response, patients were subdivided into three groups—age 0–5, 6–10, and over 10—whereas, since only three donors were younger than 2 years of age, donor age was analyzed as a continuous variable.

Sixteen patients were vaccinated between 6 months and 1 year after BMT, 11 between 1 and 2 years, and 20 patients received the vaccine more than 2 years after transplantation. At the time of vaccination all patients had recovered normal T cell function, as proved by the study of the proliferative response to mitogens.

The vaccine (DT; Prohibit, Berna) was administered as a single dose in a total volume of 0.5 ml into the deltoid muscle. Serum and saliva samples for measurement of total IgG subclass and specific antibody levels were obtained from patients and healthy controls before and 3 weeks after immunization.

Table I. Characteristics of the 47 Patients Enrolled in the Study[a]

Age (median, range)	9 yr (7 mo–18 yr)
Sex (M/F)	27/20
Diagnosis	
ALL	16
AML	12
MDS	7
SAA	6
Thalassemia major	2
Inborn errors	2
Solid tumors	2
Allogeneic/autologous BMT	38/9
Conditioning regimens	
TBI/L-PAM	6
TBI or TAI/Cy	23
Bu/Cy/L-PAM	13
Bu/Cy	3
Cy	2
Chronic GVHD	10
Donor's age at BMT	
<2 yr	3
>2 yr	35
Recipient's age at vaccination	
<5 yr	9
5–10 yr	11
>10 yr	27
Time from BMT to vaccination	
<1 yr	16
1–2 yr	11
>2 yr	20

[a] ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; MDS, myelodysplastic syndromes; SAA, severe aplastic anemia; TBI, total-body irradiation; L-PAM, melphalan; TAI, thoracoabdominal irradiation; Cy, cyclophosphamide; Bu, busulfan; GVHD, graft-versus-host disease.

Total IgG Subclass Determinations

IgG subclass levels were determined by radial immunodiffusion using monoclonal antibodies as described previously (22). Patients' levels were compared with age-normal percentile charts elaborated in our laboratory (22).

Specific Antibody Measurements

Specific IgG, IgA, IgM, and salivary IgA to Hib were determined by means of an ELISA method described previously (23). Briefly, microtiter plates were coated with purified Hib polysaccharide (5 μg/ml; obtained from Sclavo, Siena, Italy). After postcoating with 2% BSA in PBS, diluted samples (serum was diluted 1/100; saliva, 1/10) were added. Peroxidase-conjugated rabbit anti-human IgG, IgA, and IgM (Dako) were used. Pre- and postvaccination samples were tested at the same time. Reproducibility and specificity of the assay were verified in preliminary experiments. Results are expressed as a percentage of the pooled serum from healthy blood donors.

Given that (i) more than 90% of adults and children older than 2 years of age have been reported to respond to vaccination with at least a twofold increase in anti-Hib antibody compared with prevaccination levels (24) and (ii) all our healthy controls showed this increase in specific IgG, we considered a twofold antibody response as effective.

Specific DT IgG Determination

In order to evaluate the antibody response elicited by DT, IgG antibodies directed toward the protein carrier were detected by ELISA (Testkit Diptheria toxin EIA; In Vitro Diagnostika GmbH). Moreover, we investigated whether a protective antibody level to DT could influence the response to Hib CP-protein conjugate vaccine and we considered a titer of specific DT IgG >0.1 IU/ml safe and protective, as stated and accepted in the literature (25).

Statistical Analysis

Data were stored, analyzed, and reported with the packages STATISTICA/w (StatSoft, Inc., Tulsa, OK), and Fig.P (Biosoft, Cambridge, UK), both run on a PowerExec EL (AST, Irvine, CA) personal computer. Normal distribution of data was tested with the Shapiro–Wilk test. The Wilcoxon matched-pairs test was used for the comparisons between pre- and post-vaccination values, while the Mann–Whitney rank-sum test was employed to compare patients given BMT and healthy control subjects. Differences in response percentages were compared by the Fisher exact test or chi-square test as appropriate. *P* values lower than 0.05 were considered to be statistically significant.

RESULTS

Total Serum IgG Subclasses

Total serum IgG subclass levels were quantified before and after vaccination in all patients and the results are reported in Table II.

IgG1, IgG2, and IgG3 values were below the fifth centile of the age-related reference range in 11 (23%), 18 (38%), and 1 (2%) patients, respectively. Five of the 11 IgG1-deficient children also had a combined IgG2 deficiency, whereas one child had combined IgG1–IgG2–IgG4 deficiency. In the group of patients with IgG2 levels below the fifth centile, two had a combined IgG4

Table II. Isolated or Combined Total Serum Ig Subclass Deficiency Before and After Vaccination in Patients and Healthy Controls

	Prevaccination		Postvaccination		
	No. of patients	%	No. of patients	%	*P*
Patients (n = 47)					
IgG1 deficiency	5	11	2	4	NS
IgG2 deficiency	9	19	7	15	NS
IgG3 deficiency	0	0	0	0	NS
IgG4 deficiency	5	11	5	11	NS
IgG1–IgG2 deficiency	5	11	5	11	NS
IgG2–IgG3–IgG4 deficiency	1	2	1	2	NS
IgG2–IgG4 deficiency	2	4	2	4	NS
IgG1–IgG2–IgG4 deficiency	1	2	1	2	NS
Healthy controls (n = 13)					
IgG3 deficiency	1	8	1	8	NS
IgG4 deficiency	1	8	1	8	NS
IgG2–IgG4 deficiency	1	8	1	8	NS

deficiency and one a combined IgG3–IgG4 deficiency (2%).

Undetectable IgG4 levels (<0.006 mg/ml) were observed in 10 (21%) children, 2 of them also showing associated IgG2 deficiency and one each a combined IgG1–IgG2 and an IgG2–IgG3 deficiency.

After vaccination three of the five children with isolated IgG1 deficiency and two of the nine children with isolated IgG2 deficiency achieved normal values, while isolated IgG4 deficiency persisted in all children.

Total IgG subclass levels were below the normal range in three controls: one presented combined IgG2–IgG4 deficiency, one IgG3 deficiency, and one undetectable IgG4 levels. Immunization with Hib CP–protein conjugate vaccine was not able to normalize the IgG subclass levels in these three control children.

Specific IgG to DT

Before vaccination with Hib-DT, protective levels (>0.1 IU/ml) toward DT were observed in 18 patients given BMT (38%) and in 12 controls (92%) ($P < 0.005$). Notably, six of the seven patients who had previously been immunized with DT had protective levels. Moreover, 6 of the 16 children given Hib–DT immunization within 1 year after BMT who had not received any vaccination with DT after transplantation had protective antibody levels against DT before administration of the Hib conjugate vaccine. An antibody level greater than 0.1 IU/ml was also observed in 3 of the 8 patients and in 3 of the 16 children not previously immunized against DT and given Hib–DT between 1 and 2 years and beyond 2 years after transplantation, respectively.

After immunization with Hib-DT, eight more patients (four given vaccine in the first year and four more than 2 years after BMT) reached a protective antibody level against DT. A significantly higher increment of the antibody levels was observed in controls compared to patients given BMT ($P < 0.05$). Details on both pre- and postvaccination median and range values of the study population as well as of the controls are reported in Table III and Fig. 1.

Specific Serum IgG, IgA, and IgM to Hib

Twenty-five of the 47 patients (53%) had a twofold or greater increase in specific anti-Hib IgG, while an effective IgA and IgM response was mounted by 23 (49%) and 11 (23%) children, respectively. In particular, 10 patients displayed a response of all Ig isotypes, 13 children produced anti-Hib specific IgG and IgA, 2 patients showed only specific IgG, and 1 had only an IgM response to vaccination. Details on the magnitude of the antibody response for the different Ig classes observed in patients are reported in Table III.

In the control group a twofold or greater increase in specific IgG levels was observed in 13 of the 13 subjects ($P < 0.005$ in comparison to the patients' response rate), while an IgA and IgM response was demonstrated in 12 (92%; $P < .01$ compared to transplanted patients) and 7 (54%; $P < 0.05$ in comparison to children given BMT) children, respectively. The child who did not mount an IgA response was documented to have partial IgA class deficiency. The increase in postvaccination IgG levels was significantly greater in the control population compared to the patients studied. For further details see also Table III.

Time elapsed between transplant and immunization was the most powerful variable predictive of response. In particular, whereas 17 of 20 (85%) children immunized

Table III. Comparison Between Patients' and Controls' Response to Vaccination[a]

	Patients (n = 47)		Controls (n = 13)		
	Median	(Range)	Median	(Range)	P
IgG anti-HIB (%)					
Prevaccination	25	(2–125)	35	(6–101)	NS
Postvaccination	65	(2–385)	202	(83–287)	<0.005
Fold increase	1.98	(0.37–24.45)	6.19	(1.80–21.33)	<0.005
IgA anti-HIB (%)					
Prevaccination	11	(3–72)	20	(6.0–92.0)	NS
Postvaccination	57	(5–706)	211	(10.0–517.0)	<0.05
Fold increase	1.67	(0.43–79)	8.43	(1.00–86.17)	0.058
IgM anti-HIB (%)					
Prevaccination	63	(8–179)	89	(20.0–206.0)	<0.05
Postvaccination	85	(3–400)	169	(39.0–400.0)	<0.05
Fold increase	1.25	(0.3–11.11)	2.09	(0.059–5.09)	NS
S-Ig anti-HIB (%)					
Prevaccination	137	(19–642)	74.00	(31.0–564.0)	<0.05
Postvaccination	114	(13–800)	78.00	(8.0–438.0)	<0.05
Fold increase	0.94	(0.05–7.25)	1.00	(0.06–5.09)	NS
IgG anti-DT (IU/ml)					
Prevaccination	0.07	(0.01–5.00)	0.30	(0.05–11.60)	<0.005
Postvaccination	0.13	(0.03–5.00)	3.60	(0.29–17.20)	<0.00005
Fold increase	1.17	(0.53–140.70)	2.90	(0.97–351.02)	<0.05
IgG1 (mg/ml)					
Prevaccination	5.50	(1.70–10.80)	6.30	(4.50–16.00)	<0.05
Postvaccination	5.30	(2.10–11.70)	5.11	(4.40–14.00)	NS
Fold increase	1.00	(0.51–2.11)	0.89	(0.30–1.25)	<0.05
IgG2 (mg/ml)					
Prevaccination	1.25	(0.23–3.60)	1.60	(0.60–3.90)	NS
Postvaccination	1.37	(0.24–3.40)	1.50	(0.60–5.90)	NS
Fold increase	1.00	(0.42–1.91)	1.00	(0.54–1.65)	NS
IgG3 (mg/ml)					
Prevaccination	0.62	(0.13–1.52)	0.47	(0.11–0.94)	NS
Postvaccination	0.62	(0.13–1.78)	0.39	(0.09–0.88)	<0.05
Fold increase	1.00	(0.68–1.48)	0.92	(0.50–1.13)	<0.05
IgG4 (mg/ml)					
Prevaccination	0.18	(0.005–1.70)	0.37	(0.005–3.10)	NS
Postvaccination	0.18	(0.005–1.88)	0.36	(0.005–3.10)	NS
Fold increase	1.00	(0.64–1.50)	1.00	(0.56–1.94)	NS

[a] Mann–Whitney rank-sum test was used for the comparison between patients' and controls' levels.

with Hib-DT after 2 years from transplant presented a twofold or greater increase in prevaccination IgG specific antibody levels, only 5 of 11 patients (45%) receiving vaccination within 1 to 2 years after BMT and 3 of 16 children (19%) vaccinated in the first year after transplantation showed an effective IgG response ($P <$ 0.0005; see also Table IV). Figure 2 shows the correlation between time interval from BMT to vaccination and the increase in specific IgG antibody levels ($r = 0.72$, $P < 0.0001$).

A similar influence of time between transplantation and vaccination on antibody response was observed also for specific anti-Hib IgA and IgM production, even though for the latter class differences did not reach statistical significance (see also Table IV).

Type of transplantation, patient sex, recipient age subdivision, original disease, conditioning regimen employed, and occurrence of chronic GVHD did not affect the probability of specific anti-Hib response. Likewise, children given previous antipneumococcal vaccine or with a protective anti-DT IgG level did not have a higher probability to mount a specific antibody response. Notably, the presence of IgG2 subclass deficiency before immunization predicted a lack of anti-Hib IgA and IgM antibody response.

Specific Secretory IgA

Levels of specific secretory IgA to Hib were analyzed in 45 of the 47 patients and in all controls. A twofold increase was observed in 8 (18%) patients and in 3 (23%) of the healthy subjects (P = NS; see also Table III). Notably, two of the responder patients did not show a serum specific response, and again, the lapse of time

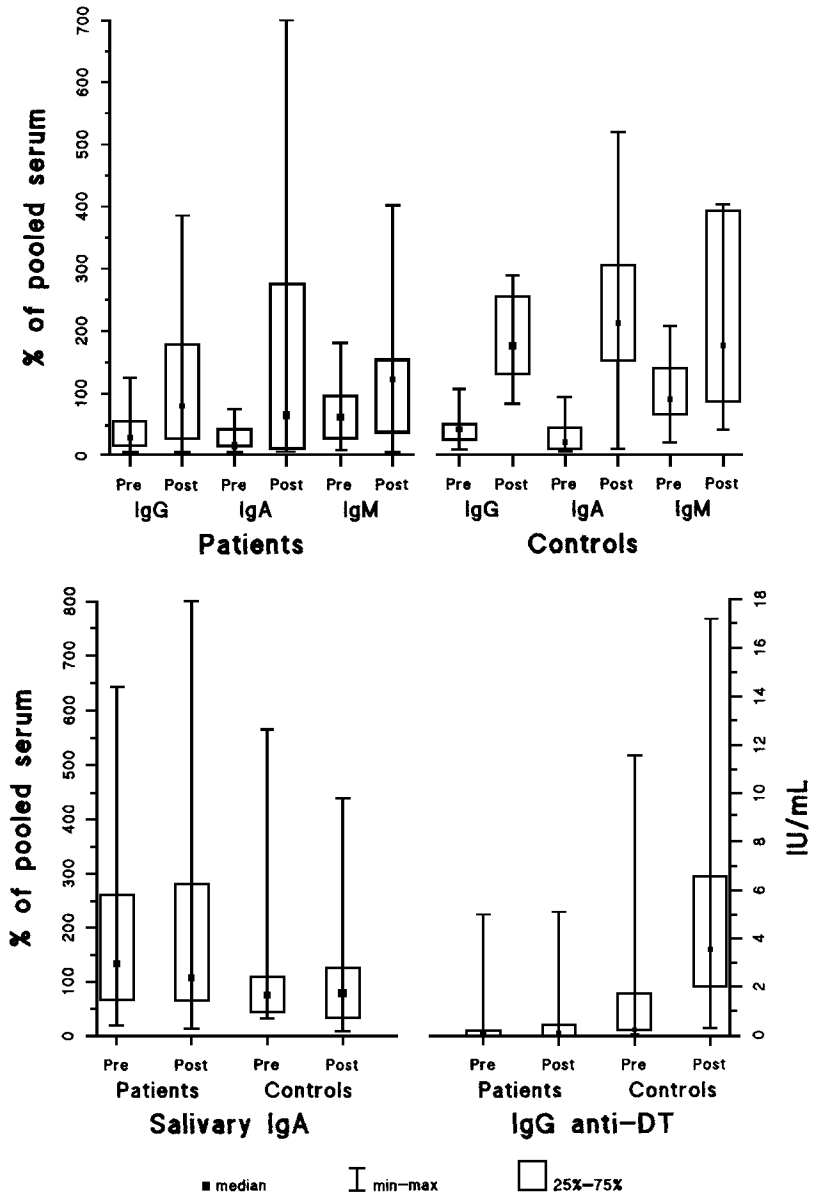

Fig. 1. Specific anti-Hib IgG, IgA, and IgM levels (top), specific anti-Hib salivary IgA levels (bottom left), and specific anti-DT IgG levels (bottom right) before and after vaccination in patients and controls.

between transplantation and vaccination was significantly correlated with the probability of response (see also Table IV).

DISCUSSION

It has been reported that the ability to respond to T cell-independent polysaccharide antigens is slow to mature after marrow transplantation (13, 26). Since conjugation with a proteic carrier has been proposed as a mean of overcoming the unresponsiveness to these antigens in both young children and BMT recipients (16–18, 27), the principal aims of our study were to evaluate whether a protein–carrier conjugate vaccine could increase the immunogenicity of a single dose of Hib CP and to identify the main variables influencing the ability to mount an antibody response towards polysaccharide antigens.

Compared to normal controls, who displayed a strong IgG response after a single dose of Hib conjugate

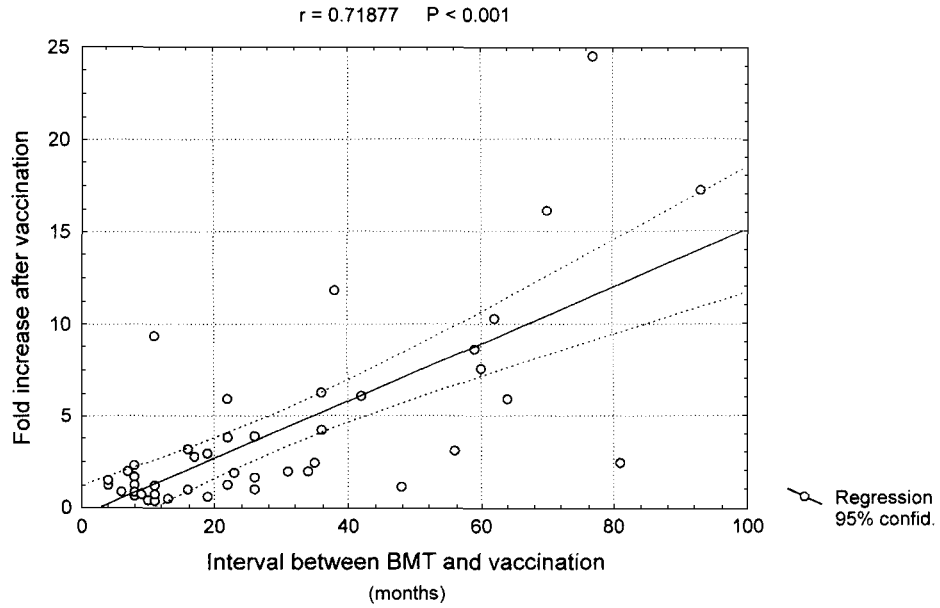

Fig. 2. Correlation between the time interval from BMT to vaccination (months) and the increase in specific IgG antibody levels ($r = 0.72$, $P < 0.001$).

vaccine, in our cohort only 53% of patients were able to mount an efficient production of this immunoglobulin class. Serum concentrations of Hib-specific IgA and IgM classes provide further support to the reduced responsiveness of BMT recipients toward the specific antigenic challenge represented by Hib polysaccharides conjugate with a proteic carrier. These findings are not entirely surprising given that patients receiving transplantation of hematopoietic stem cells experience a prolonged period of immunodeficiency. In fact, during the first months after transplantation, in these unique subjects, a response to neoantigens is absent and that to recall antigens or specific polyclonal stimuli are blunted as compared to healthy controls (7–9).

Results obtained in our cohort of patients significantly differ from those reported in other studies, where the use of a Hib conjugate vaccine was able to elicit an adequate antibody response in the majority of patients (27, 28). This discrepancy could be attributed to the vaccination schedule we chose to adopt. In fact, previously published studies documented that marrow recipients given two doses of Hib conjugate vaccine displayed a better antibody response compared with patients receiving a single dose of immunization (27, 28). It can be hypothesized that in immunocompromised patients, as well as in young children in whom the immune system is characterized by a state of immaturity, repeated injections of conjugate Hib vaccine are needed to induce a strong anamnestic response. Therefore, in view of these considerations, it seems reasonable to employ two doses of vaccine to maximize the rate of responders, particularly in BMT recipients given a vaccination within the first 2 years after transplantation.

Table IV. Percentage of Response to Hib Vaccination According to the Interval Between BMT and Immunization

	Vaccination (%)			
	Within 1 yr from BMT ($n = 16$)	From 1 to 2 yr after BMT ($n = 11$)	After 2 yr from BMT ($n = 20$)	χ^2 P
IgG	19	45	85	<0.0005
IgA	19	27	85	<0.0005
IgM	6	27	35	NS
Salivary IgA	7	0	37	<0.05

The antibody response was better in patients immunized late after graft. In particular, more than 80% of children given immunization after at least 2 years from BMT had a twofold increase in prevaccination IgG-specific antibody levels, whereas the percentages of responding subjects significantly declined with decreasing time after BMT. The correlation between lapse of time from transplantation and ability to mount a humoral response was demonstrated also by Barra *et al.* (27), whereas a recent paper by Parkkali *et al.* (24) failed to confirm this correlation. Since immune reconstitution of BMT recipients is a time-dependent phenomenon, we

speculate that efficient specific anti-Hib antibody production can take place only when a full restoration of immune response is achieved. We cannot exclude that the lack of any significant influence of other patient- or transplant-related variables (such as original disorder, type of transplant, donor employed, chronic GVHD occurrence, etc.) is due to the limited number of patients studied.

This study is the first one evaluating the production of Hib-specific secretory IgA. However, we did not find any significant difference between patients and healthy controls. Lapse of time after transplantation was the only predictive variable influencing the probability of response, similarly to what observed for serum antibodies.

Given that most studies suggest that BMT patients respond to pure or conjugate CP vaccine late after transplantation (12, 27, 28), earlier protection is advisable. An intriguing approach to obtain high Hib-specific antibody concentrations in the early phase after BMT has been demonstrated to be donors' immunization (29). In fact, Molrine *et al.* documented that vaccination of BMT donors together with posttransplant booster doses resulted in protective antibody serum levels throughout the posttransplant period (29). The immunological recovery after transplantation of haematopoietic stem cells is considered to be dependent on two distinct phenomena. In the early posttransplant period, there is an expansion of mature donor-derived lymphocytes transferred with the graft. Thereafter, *naive* lymphocytes derived from the differentiation of donor haematopoietic stem cells colonize the lymphoid organs and sustain the late immune response of recipients (30). While patients receiving a T cell-depleted transplant are at particular risk of infections, BMT recipients transplanted using donors either recently vaccinated against or immune to a certain pathogen usually have a more rapid recovery of specific T cell response than those who received bone marrow from unprimed donors (31, 32).

There is no obvious explanation for the observation that the presence of IgG2 subclass deficiency before vaccination was associated with lack of development of a mature B cell subset committed to the production of anti-Hib IgA and IgM antibody. However, it can be hypothesized that, as in patients with primary immunodeficiencies (33), a low serum IgG2 concentration can probably reflect a general immunodeficiency state and a delayed immunologic reconstitution.

It is worth noting that in three of the five children with isolated IgG1 deficiency and two of the nine children with isolated IgG2 deficiency, vaccination promoted normalization of serum levels of these subclasses. The deficiency of the IgG subclasses found in some BMT recipients could represent a transient phenomenon ascribable to the immaturity of humoral immune response. With increasing time after transplant, functional donor cells repopulate host lymphoid tissue determining the recovery of immune reactivity. It can be hypothesized that vaccination acted as a trigger favoring the activation or the maturation of B cells devoted to the specific IgG subclass production or that this recruitment could be the consequence of modification of regulatory influences in the process of B cell activation.

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