

## ORIGINAL ARTICLE

# A randomized study on donor immunization with tetanus–diphtheria, *Haemophilus influenzae* type b and inactivated poliovirus vaccines to improve the recipient responses to the same vaccines after allogeneic bone marrow transplantation

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The HLA-identical sibling donors of 111 bone marrow transplantation (BMT) recipients were randomised to receive or not to receive tetanus–diphtheria (T–d), *Haemophilus influenzae* type b (Hib), and inactivated poliovirus (IPV) vaccines 2–10 weeks before BM harvest. Fifty-three (DV+ group) recipients received the graft from a vaccinated donor and 58 (DV– group) from an unvaccinated donor. All recipients were vaccinated with the T–d, Hib and IPV vaccines at 3, 6 and 12 months after BMT. Diphtheria and Hib antibody concentrations were consistently higher in the DV+ than in the DV– group from 6 months post transplantation onwards. The differences were significant at 6 and 13 months for diphtheria and at 12 months for Hib antibody concentrations. Tetanus, PV1, PV2 and PV3 antibody levels were similar in both groups. Patients transplanted from donors with high tetanus, diphtheria and Hib antibody concentrations had higher respective antibody concentrations after BMT than those transplanted from donors with low antibody concentrations. Especially patients whose donors have low-specific antibody concentrations may benefit from donor vaccination with protein and conjugate vaccines.

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**Keywords:** BMT recipients; vaccination responses; donor vaccination

## Introduction

Myeloablative conditioning regimens before bone marrow transplantation (BMT) destroy the haematopoietic and immune systems of the recipient.<sup>1</sup> Specific T- and B-cell immune memory is largely lost. In allogeneic BMT recipients immunity to tetanus, diphtheria and polio disappears, and *Haemophilus influenzae* type b (Hib) antibody concentrations gradually decrease after the transplantation.<sup>2–11</sup> The reconstitution of the immune system originates from the cells transferred from the donor to the patient. T-cell and B-cell functional reconstitution takes 1–2 years in healthy BMT survivors and even longer in patients with chronic graft-versus-host disease (GVHD). The reconstitution of B-cell immunity from cells originating from the donor resembles the maturation of the immune system in infants.<sup>12</sup> Consequently, vaccination of patients with immunogenic protein and conjugate vaccines during the first 2–6 months after BMT has not generally induced satisfactory antibody production.<sup>11,13–15</sup>

It is generally accepted that the population, including BMT recipients, should be protected against tetanus, diphtheria and polio, although the risk of disease is low. Infections caused by *Haemophilus influenzae* have been described in BMT recipients, usually 3–12 months after the transplantation.<sup>16–20</sup> The introduction of Hib conjugate vaccines at the beginning of the 1990s has resulted in the near elimination of the Hib disease in the developed countries where children are routinely vaccinated against Hib.<sup>21,22</sup> However, some increase in the disease incidence has been seen during the last few years,<sup>23</sup> and vaccination of BMT recipients with Hib vaccines is therefore considered reasonable.

In order to investigate whether vaccination of the donor with tetanus, diphtheria, Hib and inactivated poliovirus (IPV) vaccines results in earlier and higher responses to these vaccines in the BMT recipient after the transplantation, we carried out a prospective, randomized study, comparing the responses in recipients transplanted from unvaccinated and vaccinated donors.

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## Materials and methods

### Study design

Patients and donors for the present prospective, randomized study were recruited at Helsinki University Central Hospital between June 1993 and July 2001. The donors of 162 human leukocyte antigen (HLA)-identical sibling BMT donor-recipient pairs were randomized to receive or not to receive tetanus-diphtheria (T-d), Hib, and IPV vaccines 2-10 weeks before the bone marrow harvest (Figure 1). All patients were vaccinated with the same vaccines at 3, 6 and 12 months after BMT.

### Patients

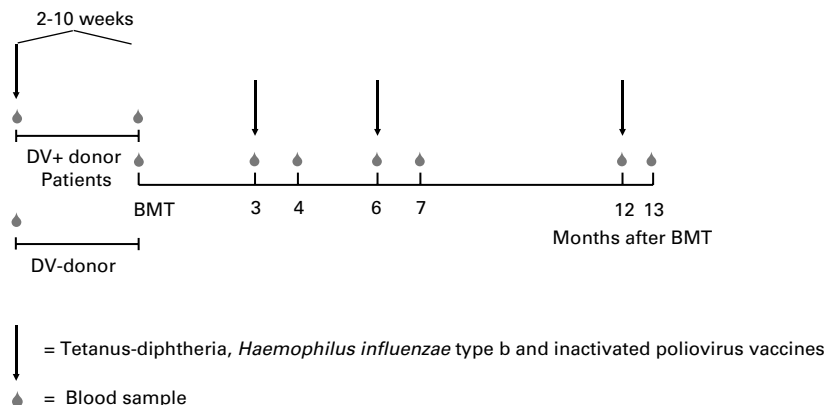
Of the 162 randomized donor-recipient pairs, 51 were not evaluable. Ten patients were not transplanted, 22 patients died after the transplantation before starting the vaccinations, 13 patients had platelets  $<40 \times 10^9/l$  at the time of the first vaccination and the vaccination program could not be started, four patients had a relapse of leukaemia before starting the vaccinations, one patient had no serum samples drawn at the scheduled time points, and one patient received intravenous immunoglobulin during the whole vaccination period. One hundred and eleven patients were immunized at the scheduled time and had serum samples available for determining the response to at least one vaccination. The characteristics of the 58 unvaccinated donor (DV-) and 53 vaccinated donor (DV+) group patients are presented in Table 1.

All 111 patients and their donors were adults ( $>18$  years). The donor and the patient were HLA-identical. The recipients with a haematological malignancy were conditioned with cyclophosphamide (60 mg/kg body weight i.v. on 2 consecutive days) and total body irradiation (total dose 12 Gy in six fractions on 5 consecutive days). Two patients were conditioned with busulphan (4 mg/kg p.o. on 4 consecutive days) and cyclophosphamide. Four patients with aplastic anaemia were conditioned with cyclophosphamide (50 mg/kg i.v. on 4 consecutive days). The bone marrow (BM) grafts were non-manipulated with the exception of the removal of red cells and plasma in cases of ABO incompatibility. The routine GVHD prophylaxis

**Table 1** Characteristics of the patients

	Donor immunization -	Donor immunization +
Total no. of patients	58	53
<i>Age of patients</i>		
Median (years)	45	43
Range (years)	20-57	19-58
<i>Gender</i>		
Male	32	28
Female	26	25
<i>Primary disease</i>		
AML	11	11
ALL	11	8
CML	11	16
MDS	11	9
Multiple myeloma	10	4
CLL or lymphoma	3	2
Aplastic anaemia	1	3
<i>Conditioning regimen</i>		
CY + TBI	56	49
CY + Bu	1	1
CY	1	3
<i>Immunosuppressive therapy</i>		
At 3 months N (%)	58	53
CsA + MP	52 (90)	41 (77)
CsA alone	2 (3)	7 (13)
MP alone	2 (3)	3 (6)
MTX ± MP	2 (3)	2 (4)
At 6 months N (%)	52	51
CsA + MP	19 (37)	17 (33)
CsA alone	27 (52)	31 (61)
MP alone	6 (12)	3 (6)
At 12 months N (%)	47	48
CsA + MP	15 (32)	11 (23)
CsA alone	7 (15)	10 (21)
MP alone	6 (13)	7 (15)
None	19 (40)	20 (42)
Acute GVHD N (%)	19 (33)	12 (23)
Chronic GVHD N (%)	25 (43)	17 (32)

Abbreviations: AML = acute myeloid leukemia; ALL = acute lymphoid leukemia; CML = chronic myeloid leukemia; CsA = cyclosporin A; MP = methylprednisolone.



**Figure 1** Schedule for serum samples and vaccinations for the donors before bone marrow harvest and for the patients before and after BMT.

after the transplantation consisted of cyclosporin A (CsA) and a short course of methotrexate (MTX) with or without methylprednisolone (MP). During the years 1993 and 1994 the BMT recipients received or did not receive, according to randomization in a study,<sup>24</sup> MP 0.5 mg/kg on days 14–20, 1 mg/kg on days 21–34, 0.5 mg/kg on days 35–48, and thereafter the dose was slowly tapered and the administration discontinued on day 110. After the year 1994, MP was given 0.5 mg/kg on days 8–14, 1 mg/kg on days 15–28, and thereafter the dose was tapered as described earlier. Due to renal insufficiency, MTX 10 mg/m<sup>2</sup> intravenously was given once a week to 4 patients instead of CsA until day 100 after the transplantation. The actual immunosuppressive treatment at the time of the vaccinations is presented in Table 1. Acute GVHD was defined according to Thomas *et al.*<sup>25</sup> and treated with MP, starting with a dose of 10 mg/kg/day. Two patients both in the DV– and DV+ group with corticosteroid-resistant acute GVHD received antithymocyte globulin as second-line treatment. Chronic GVHD was defined and graded according to Shulman *et al.*<sup>26</sup> and Sullivan *et al.*<sup>27</sup> The treatment of chronic GVHD consisted of MP and, according to individual judgement, CsA and low-dose irradiation of lymph nodes.

#### Vaccinations

In Finland, the coverage of the national childhood vaccination program for tetanus, diphtheria, poliovirus (PV) and, in persons born since the mid-1980s, for Hib, is very high, covering at least 93% of children.<sup>28</sup> The donors in the DV– group did not receive any vaccinations immediately before the bone marrow harvest. The DV+ group donors were immunized with T–d (manufactured by National Public Health Institute, Helsinki, Finland), Hib oligosaccharide mutant diphtheria toxin (CRM<sub>197</sub>) conjugate (HibTiter, Lederle – Praxis Biologicals, Pearl River, NY, USA), and inactivated trivalent PV (Imovax Polio, Pasteur Mérieux, Serums et Vaccins, France, or Polio Novum, Rijksinstituut voor Volksgezondheid en Milieuhygiene (RIVM), the Netherlands) vaccines 2–10 weeks (median 22 days, range 14–65 days) before the marrow harvest (Figure 1). One 0.5 ml dose of the T–d vaccine contained five limits of flocculation (Lf) units of tetanus toxoid and 2 Lf units of diphtheria toxoid adsorbed onto aluminium phosphate. One 0.5 ml dose of the Hib vaccine contained 10 µg of Hib capsular oligosaccharide linked covalently with 25 µg of mutant diphtheria toxin CRM<sub>197</sub>, corresponding to approximately 9 Lf units of diphtheria toxoid/dose. One 0.5 ml dose of Imovax Polio vaccine contained at least 20–4–16 and one 1.0 ml dose of Polio Novum vaccine at least 30–6–24 D antigen units for PV types 1, 2 and 3, respectively. The T–d and Hib vaccines were given i.m., PV vaccines s.c. One of the DV+ group donors had received a booster vaccination against tetanus during the preceding 5 years and she did not receive the T–d vaccine. All patients were immunized with the same vaccines at 3, 6 and 12 months after BMT. As regards the IPV vaccine, 46 of the 58 patients in the DV– group and 42 of the 53 patients in the DV+ group were immunized only by using Imovax Polio. Twelve patients in the DV– and 11 patients in the DV+ group received at least one dose

of the Polio Novum vaccine. The immunogenicity of these two PV vaccines is considered to be equal. In addition, pneumococcal and meningococcal polysaccharide vaccines were given to the donors in the DV+ group before the BM harvest and to all patients at the time of the third vaccination, at 12 months after BMT. The immune response data to these vaccines will be reported separately.

#### Serum samples

A blood sample was drawn from the unvaccinated donors (DV– donors) at the time of physical examination (Figure 1). Blood samples were collected from the donors in the DV+ group prior to the immunization and at the time of the BM harvest. Blood samples were drawn from the patients 10 days before BMT before starting the conditioning and at 3, 4, 6, 7, 12 and 13 months after the transplantation. Blood specimens taken within 2 weeks before the first and second vaccination and within 4 weeks before the third vaccination, and from 2 to 8 weeks after each vaccination were considered adequate for determining the response to that vaccination. Antibody concentrations in sera drawn from the patients who had received intravenous immunoglobulin during two months preceding a vaccination or between the vaccination and post-vaccination serum sample were not included in the analyses.

#### Serological assays

The tetanus and diphtheria IgG antibodies were determined by a double antigen EIA.<sup>29</sup> The lowest detectable antibody concentrations were 0.007 international units (IU)/ml for both tetanus and diphtheria antibodies. For statistical calculations, levels of <0.007 IU/ml were given an arbitrary value of 0.005 IU/ml. Antibody concentrations of 0.01–0.1 IU/ml are conventionally considered low positive, while concentrations of ≥0.1 IU/ml are regarded as positive. Concentrations of ≥1 IU/ml indicate good long-term protection.

The IgG antibodies to Hib were determined by EIA<sup>30</sup> using oligosaccharides derived from Hib polysaccharides conjugated to human serum albumin (HbOHA, NIBSC, Potters Bar, UK) as the antigen for coating, and serum pool lot 1983 received from Dr Carl Frasch at US FDA, Bethesda, MD, as a reference. The lowest detectable antibody concentration was 0.30 µg/ml. For statistical calculations, concentrations of <0.30 µg/ml were given an arbitrary value of 0.15 µg/ml. Two threshold concentrations were used: 1 µg/ml has been associated with long-term protection in children immunized with the Hib polysaccharide vaccine,<sup>31</sup> and 5 µg/ml has been suggested to predict protection against Hib colonization among children after vaccination with a conjugate vaccine.<sup>32</sup>

The PV antibodies were determined by a microneutralization assay.<sup>33</sup> Standard amounts (30–300 TCID<sub>50</sub> units/50 µl) of Sabin strains were used for the determination of PV1, PV2 and PV3 antibodies. PV antibody titres of ≥4 were considered protective.

### Ethical processing

The study was approved by the Ethics Committee of the Department of Medicine, Helsinki University Central Hospital. Both the donor and the recipient gave an informed consent.

### Statistical methods

Log-transformed data was used for the analysis of the antibody concentrations or titres. The results are presented as geometric mean concentrations (GMC) or geometric mean titres (GMT) with 95% confidence intervals (95% CI). The fold increases in the antibody concentrations and titres between the sera drawn prior to and 1 month after vaccination were calculated as paired differences between the post-vaccination and pre-vaccination concentrations, and the results are presented as geometric means of fold (GMF) increases with 95% CIs. The Student's *t*-test was used to compare the antibody concentrations or titres and fold responses to vaccinations between the two study groups. The antibody concentrations and titres in the DV- and DV+ patients with and without acute GVHD were compared by using the one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) *post hoc* test, when appropriate. Fisher's exact test was used to compare the frequency of individuals in the two

vaccination groups with the antibody concentrations indicated.

## Results

### Donor responses to tetanus, diphtheria, Hib and IPV vaccines

At the time of randomization the GMCs of the tetanus, diphtheria and Hib antibodies and the GMTs of the PV2 and PV3 antibodies did not differ in the DV- and DV+ donors. In the DV- group the GMT of the PV1 antibodies was 1083 and in the DV+ group 468 ( $P=0.008$ ). At the time of the BM harvest, the vaccinated donors had significantly higher specific antibody concentrations or titres than the unvaccinated ones (Table 2). The percentages of the donors with defined tetanus, diphtheria, and Hib antibody concentrations are shown in Table 3. All donors had anti-PV1, -PV2 and -PV3 titres of  $\geq 4$  both before and after vaccination with the IPV vaccine, except one donor for PV3 before vaccination. The geometric mean (GM) fold increases in the antibody concentrations or titres after vaccination were 3.7, 29.4, 29.1, 8.5, 5.5 and 17.0 to tetanus, diphtheria, Hib, PV1, PV2 and PV3 vaccines, respectively.

### Responses to vaccinations in patients

**Tetanus antibodies.** Before and after BMT the GMCs of the tetanus antibodies and the proportions of the patients with defined antibody levels were similar in the DV- and DV+ groups (Figure 2, Tables 3 and 4). After the first dose of the tetanus vaccine the tetanus antibodies did not increase either in the DV- or in the DV+ group. After the second and third vaccine doses the GM fold responses were 2.6 and 4.4 in the DV- group and 1.9 and 5.0 in the DV+ group patients.

**Diphtheria antibodies.** Before BMT the diphtheria antibody levels were similar in the DV- and DV+ group patients (Figure 2, Table 3). At the time of the second vaccine dose and thereafter there was a consistent trend

**Table 2** The geometric mean concentrations of tetanus (IU/ml), diphtheria (IU/ml), and *Haemophilus influenzae* type b (Hib;  $\mu\text{g/ml}$ ) antibodies and the geometric mean titres of poliovirus antibodies in the unvaccinated (DV-) donors at the time of medical evaluation and in the vaccinated (DV+) donors at the time of BM harvest

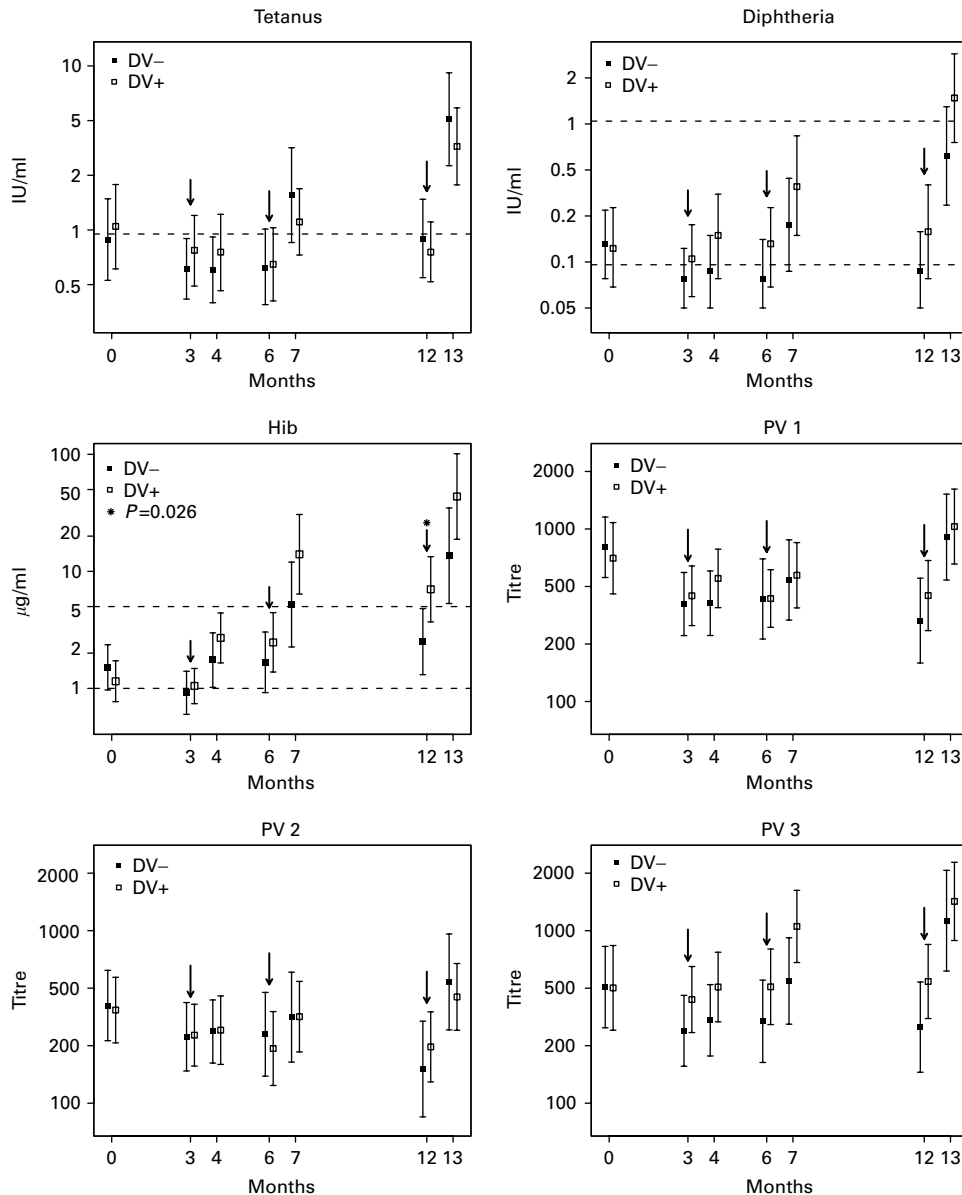
	DV- donors	DV+ donors	P
Tetanus	1.46 (0.8–2.66) <sup>a</sup>	4.81 (2.67–8.63)	0.006
Diphtheria	0.08 (0.05–0.13)	3.84 (1.88–7.86)	<0.001
Hib	1.37 (0.94–2.01)	53.4 (35.07–81.3)	<0.001
Poliovirus type 1	1083 (737–1593)	4186 (2952–5935)	<0.001
Poliovirus type 2	546 (360–827)	3333 (2429–4573)	<0.001
Poliovirus type 3	441 (243–801)	6556 (4837–8885)	<0.001

<sup>a</sup>95% confidence intervals.

**Table 3** Proportions of donors and patients with defined tetanus, diphtheria, and *Haemophilus influenzae* type b (Hib) antibody concentrations prior to BMT

Antibody concentration	DV- donors % (N=58)	DV+ donors		DV- patients % (N=58)	DV+ patients % (N=52)
		Before vaccination % (N=52)	After vaccination % (N=51)		
<b>Tetanus</b>					
$\geq 0.1$ IU/ml	88	87	94	85	90
$\geq 1.0$ IU/ml	73	75	86	59	54
<b>Diphtheria</b>					
$\geq 0.1$ IU/ml	44	54	92	59	58
$\geq 1.0$ IU/ml	10	21	71	17	19
<b>Hib</b>					
$\geq 1$ $\mu\text{g/ml}$	63	64	100	59	51
$\geq 5$ $\mu\text{g/ml}$	19	26	92	21	13

Abbreviations: DV- = donor vaccination- DV+ = donor vaccination+; Hib = *Haemophilus influenzae* type b.



**Figure 2** The geometric mean concentrations and their 95% CI of serum tetanus, diphtheria and Hib antibodies and the geometric mean titres and their 95% CI of poliovirus type 1 (PV1), PV type 2 (PV2) and PV type 3 (PV3) antibodies before and at 3, 4, 6, 7, 12 and 13 months after BMT. DV- = patients transplanted from unvaccinated donors; DV+ = patients transplanted from vaccinated donors. ↓ = T-d, Hib and inactivated poliovirus vaccines.

towards higher diphtheria antibody concentrations in the DV+ group compared to the DV- group (Figure 2, Table 4). At 6 months after BMT 24% of the DV+ group patients and 7% of the DV- group patients had a diphtheria antibody level of  $\geq 1.0$  IU/ml ( $P=0.039$ ). After the third vaccine dose, at 13 months after BMT, the GMC of diphtheria antibodies tended to be higher in the DV+ group than in the DV- group (1.45 vs 0.57 IU/ml,  $P=0.08$ , Figure 2), and a higher proportion of the patients in the DV+ group (92%) than in the DV- group (70%,  $P=0.035$ ) had a diphtheria antibody level of  $\geq 0.1$  IU/ml (Table 4). The GM fold responses to the first and second diphtheria vaccine doses were similar in both patient groups (in the DV- group 1.1 and 2.1 and in the DV+ group 1.5 and 2.8, respectively). After the third vaccine

dose there was a tendency to a higher fold response in the DV+ group compared to the DV- group, 10.0, vs 4.9 ( $P=0.07$ ).

**Hib antibodies.** The Hib antibody concentrations were similar in the DV- and DV+ group patients until 6 months post transplantation (Figure 2). After the second vaccine dose there was a trend to higher concentrations in the DV+ group compared to the DV- group. The GMC of the Hib antibodies was higher in the DV+ group than in the DV- group at 12 months after BMT, prior to the third vaccine dose (7.03 vs 2.52 µg/ml,  $P=0.03$ ), and tended to be higher at 7 months (13.99 vs 5.21 µg/ml,  $P=0.08$ ) and at 13 months (43.59 vs 13.6 µg/ml,  $P=0.06$ ) after BMT. The proportions of the patients in the DV- and DV+ groups

**Table 4** Percentages of BMT recipients with defined threshold antibody concentrations prior to and 1 month after the first, second, and third doses of tetanus-diphtheria and Hib vaccines given at 3, 6 and 12 months after BMT

Vaccination group	Antibody level	First vaccine dose		Second vaccine dose		Third vaccine dose	
		Before % (N) <sup>a</sup>	After % (N)	Before % (N)	After % (N)	Before % (N)	After % (N)
<i>Tetanus</i>							
DV–	≥0.1 IU/ml	87 (53)	86 (51)	84 (44)	90 (40)	91 (45)	95 (37)
DV+		91 (46)	84 (44)	82 (45)	93 (43)	91 (44)	97 (37)
DV–	≥1.0 IU/ml	40	39	48	70	53	89
DV+		48	46	47	61	48	81
<i>Diphtheria</i>							
DV–	≥0.1 IU/ml	38 (53)	49 (51)	52 (44)	60 (40)	47 (45)	70 (37)
DV+		52 (46)	59 (44)	58 (45)	74 (43)	61 (44)	92 (37) <sup>b</sup>
DV–	≥1.0 IU/ml	6	12	7	25	16	46
DV+		7	21	24 <sup>c</sup>	42	27	68
<i>Hib</i>							
DV–	≥1.0 µg/ml	49 (53)	61 (51)	59 (44)	70 (40)	62 (45)	81 (37)
DV+		53 (47)	64 (45)	65 (46)	80 (44)	80 (45)	87 (38)
DV–	≥5.0 µg/ml	8	22	25	48	42	65
DV+		4	36	28	64	51	82

DV– = patients transplanted from unvaccinated donors.

DV+ = patients transplanted from vaccinated donors.

<sup>a</sup>N = total number of patients with serum samples available at the indicated time points.

When the proportions of patients in the two vaccination groups with antibody levels defined were compared

<sup>b</sup>*P* = 0.035, for comparison between the DV– and DV+ groups.

<sup>c</sup>*P* = 0.039, for comparison between the DV– and DV+ groups.

with anti-Hib concentrations of ≥1 and ≥5 µg/ml were similar at all time points (Table 4). The GM fold responses to the first, second, and third Hib vaccine doses were 1.8 and 2.6, 3.1 and 5.9 (*P* = 0.07), and 5.6 and 6.9 in the DV– and DV+ group patients, respectively.

**Poliovirus antibodies.** The PV1, PV2 and PV3 antibody titres were high and similar in the DV– group and DV+ group patients in all measurements (Figure 2). All patients had PV antibody titres of ≥4 in all determinations with an exception of one DV– group patient, who had a titre of <4 to PV3 at 12 months after BMT. The GM fold responses to the first PV vaccine dose were negligible (0.92–1.2) and similar in both study groups. The GM fold responses to the second vaccine dose were 0.98 and 1.3 for PV1, 0.9 and 1.4 (*P* = 0.07) for PV2, and 1.5 and 2.2 for PV3 in the DV– and DV+ group patients, respectively. The GM fold responses to the third vaccine dose were 2.6 for PV1 in both groups, 2.9 and 1.9 for PV2, and 3.4 and 3.1 for PV3 in the DV– and DV+ group patients, respectively.

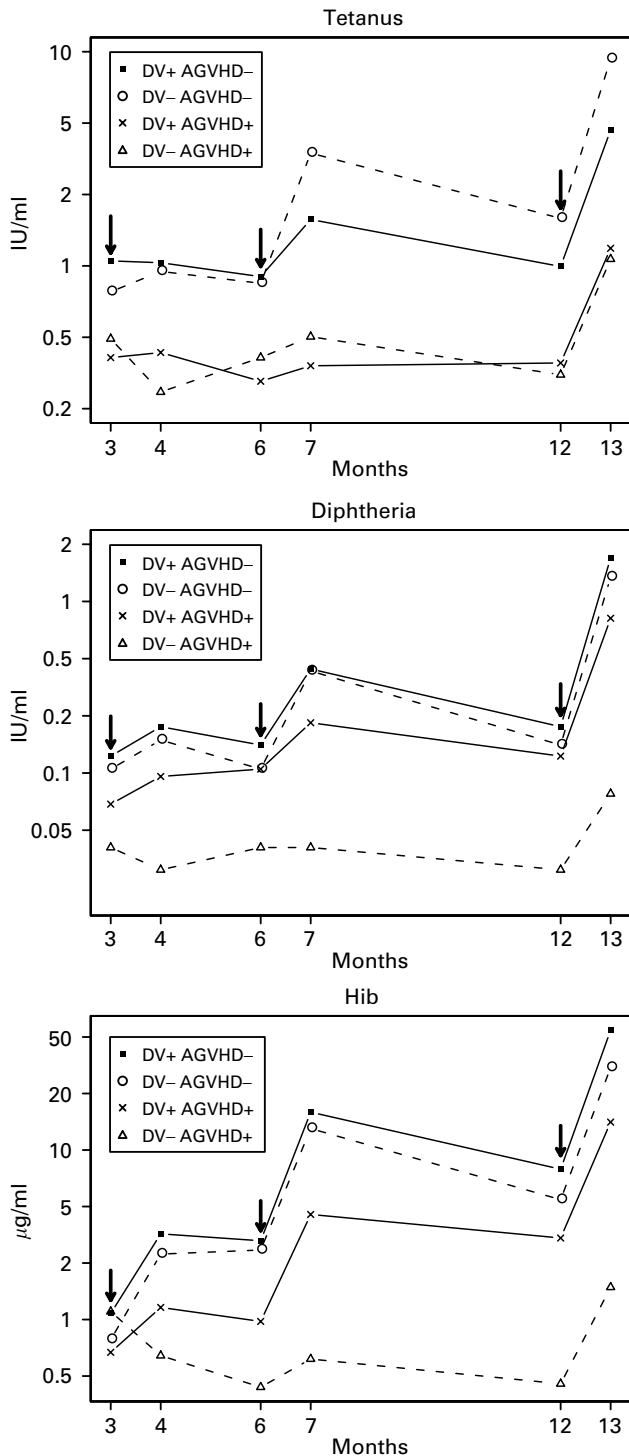
#### *The influence of donor antibody concentration on the antibody level of the patient*

Both the tetanus and diphtheria antibody concentrations were consistently significantly higher in the recipients transplanted from donors with high antibody concentrations, regardless of the donor vaccination status. For the analyses the cutoff level for tetanus was 1.0 IU/ml in both donor groups and that for diphtheria 1.0 IU/ml in DV+ donors and 0.1 IU/ml in DV– donors (data not shown).

In the DV– group the Hib antibody concentrations were higher at 13 months after BMT in the patients transplanted from donors with Hib antibody concentrations of ≥1 µg/ml than in those transplanted from donors with Hib antibody levels of <1 µg/ml (29.99 vs 2.45 µg/ml, *P* = 0.01). In the DV+ group the Hib antibody concentrations were in all donors ≥1 and in almost all donors ≥5 µg/ml.

#### *Donor vaccination and acute GVHD*

Thirty-three per cent of the DV– and 23% of the DV+ group patients had acute GVHD (Table 1). There was a tendency towards lower tetanus, diphtheria and Hib antibody concentrations in the patients with acute GVHD compared to those without (Figure 3). At 4, 7 and 13 months after BMT, one month after each vaccination, the DV– patients with acute GVHD had significantly lower GMCs of the tetanus, diphtheria, and Hib antibodies than the DV+ patients without GVHD or DV– patients without acute GVHD (for all these, *P* < 0.05). The tetanus antibody concentrations in the DV+ patients with acute GVHD were similar to those of the DV– patients with acute GVHD. The diphtheria antibody concentrations were significantly higher at 13 months after BMT (*P* = 0.03) and the Hib antibody concentrations at 12 months after BMT (*P* = 0.027) in the DV+ patients with acute GVHD compared to the DV– patients with acute GVHD, and there was a trend towards this direction beginning at 4 months post-transplantation. There were no significant differences in the PV titres between the patients with and without acute GVHD (data not shown).



**Figure 3** The influence of donor immunization and acute GVHD on the GMCs of tetanus, diphtheria and Hib antibodies after BMT. DV- = patients transplanted from unvaccinated donors. DV+ = patients transplanted from vaccinated donors. ↓ = T-d, Hib and inactivated poliovirus vaccines.

#### Donor vaccination and chronic GVHD

Forty-three per cent of the DV- group patients and 32% of those in the DV+ group had chronic GVHD (Table 1). In 24% of the patients with chronic GVHD, 6 of the 25 in

the DV- group and 4 of the 17 in the DV+ group, the disease was graded as extensive. The influence of chronic GVHD on antibody responses was analysed to vaccinations given at the time when the symptoms or signs of chronic GVHD were active or within 2 months after active GVHD had been seen.

**Tetanus and diphtheria antibodies.** Chronic GVHD had no influence on the tetanus and diphtheria antibody concentrations in any serum samples drawn from the DV- and DV+ group patients after BMT (data not shown).

**Hib.** In the DV- group patients with or without chronic GVHD the GMCs of the Hib antibodies were 0.32 and 2.27 µg/ml ( $P=0.01$ ) at 6 months, and 0.22 and 8.21 µg/ml ( $P=0.002$ ) at 7 months after BMT, respectively. At 12 months after BMT, just prior to the third vaccine dose, the GMC of the Hib antibodies tended to be higher in the DV- patients without chronic GVHD than in those with chronic GVHD (3.93 vs 1.12 µg/ml,  $P=0.06$ ). In the DV+ group, the GMC of the Hib antibodies was 1.72 µg/ml in the patients with chronic GVHD and 20.81 µg/ml ( $P=0.02$ ) in those without at 7 months after BMT. The patients with chronic GVHD received some benefit from donor vaccination with the Hib vaccine. The DV+ patients with chronic GVHD tended to have higher GMCs of the Hib antibodies than the DV- patients with chronic GVHD at 7 months (1.72 vs 0.22 µg/ml,  $P=0.07$ ) and at 12 months (4.13 vs 1.12 µg/ml,  $P=0.08$ ).

**Poliovirus antibodies.** The influence of chronic GVHD on the PV titres was seen in the DV- group patients at the time of the second vaccine dose. In the DV- group patients with or without chronic GVHD the GMTs of the PV1 antibodies were 107 and 484 ( $P=0.03$ ), and those for PV2 69 and 312 ( $P=0.04$ ) at 6 months after BMT, prior to the second vaccine dose. In the DV- patients with and without chronic GVHD the GMTs of the PV3 antibodies were 102 and 354 ( $P=0.09$ ) at 6 months and 108 and 610 ( $P=0.04$ ) at 7 months after BMT, respectively.

#### Discussion

In the present study, we prospectively randomized the HLA-identical sibling donors of BMT recipients to receive or not to receive T-d, Hib and IPV vaccines 2–10 weeks before the BM harvest. All patients were vaccinated with the same vaccines at 3, 6 and 12 months after BMT. From the second vaccination onwards, the diphtheria and Hib antibody concentrations were higher in the patients transplanted from vaccinated donors (DV+) than in those transplanted from unvaccinated donors (DV-). Furthermore, the recipients with acute GVHD were found to be a subgroup where the potential benefit from donor vaccination could be the greatest.

Only adult allogeneic stem cell transplant recipients transplanted with BM grafts without T-cell depletion were included in the present study. The previous studies comparing antibody responses in stem cell transplant recipients transplanted from vaccinated and unvaccinated

donors have included children,<sup>13–15</sup> patients transplanted with peripheral blood progenitor cells (PBPC),<sup>34</sup> and recipients transplanted with T-cell-depleted BM grafts.<sup>13,14</sup> After intensive chemotherapy CD4-positive T cells recover more rapidly in younger than in older patients,<sup>35</sup> and younger BMT recipients have better immunocompetence following BMT due to the greater capacity of the thymus to generate new T lymphocytes.<sup>36</sup> There is also evidence of better and faster immune recovery after PBPC transplantation than after BMT.<sup>37–40</sup> Our study material constitutes a homogenous population without the possible confounding effect of young age and different sources or manipulation of stem cells.

We studied the influence of vaccinating or not vaccinating the donor concurrently with several vaccine antigens of different nature. In general, the first vaccine dose given to the recipient did not induce any antibody response, but the second vaccine dose at 6 months after BMT elicited antibody responses to tetanus, diphtheria, and Hib whether the donor was vaccinated or not. However, the differences between the two study groups, that is donor vaccinated or not vaccinated, were seen only for some of the vaccine antigens used.

In contrast to a previous study on donor vaccination,<sup>13</sup> in our study the tetanus antibody concentrations increased after the second vaccine dose at 6 months after BMT in a similar manner in patients transplanted from vaccinated and unvaccinated donors. Wimperis *et al.*<sup>13</sup> observed that the patients who had been transplanted from a vaccinated donor, but not been vaccinated themselves pre-transplant, did not respond to the tetanus vaccine at 3 or 6 months after BMT. However, in their study the removal of the majority of T lymphocytes from the graft may have interfered with the antibody responses to vaccinations, since T cells have been considered necessary for B memory cell development. In the study of Molrine *et al.*<sup>14</sup> both the donor and the recipient or neither of them were vaccinated 7–10 days before transplantation. In their study the tetanus antibody concentrations increased after the second vaccine dose at 6 months after BMT in the patients transplanted from vaccinated but not in those transplanted from unvaccinated donors. Vaccination of the patient shortly before the transplantation allows the antigen retained in the recipient to stimulate the proliferation and differentiation of antigen-specific lymphocytes generated in the immunized donors and transferred with the graft. The majority of the recipients in the present study received MP routinely as GVHD prophylaxis. The influence of corticosteroids on T lymphocytes may blunt the help of the transferred T memory cells to antibody producing B cells in the patient. However, high proportions of our patients in both study groups ( $\geq 95\%$ ) achieved protective tetanus antibody levels of  $\geq 0.1$  IU/ml, in line with the results of our previous study.<sup>9</sup>

In the present study donor vaccination against diphtheria induced higher responses after vaccination in the BM recipients than when the donor had not been vaccinated. The T-d vaccine and the diphtheria toxoid conjugate component of the Hib vaccine used contained altogether 11 Lf units of diphtheria toxoid. Saxon *et al.*<sup>41</sup> have shown that BM collected from donors immunized with 1.0 Lf units

of diphtheria toxoid 6–7 days before BM harvest contained B cells spontaneously producing IgG antibody to diphtheria. The relatively high Lf dose may explain the good responses from low baseline diphtheria antibody levels. In the subgroup analysis, the patients transplanted from donors with high diphtheria antibody levels achieved good diphtheria antibody responses to vaccinations after BMT. However, only 70% of the DV– recipients achieved a protective anti-diphtheria level of  $\geq 0.1$  IU/ml after the three T-d vaccine doses.

In the Hib vaccine, a polysaccharide antigen poorly immunogenic in children with an immature immune system has been conjugated with a protein, in this case to a non-toxic diphtheria toxin variant, to improve the immunogenicity. We observed higher antibody concentrations in the DV+ group than in the DV– group. The previous studies on donor vaccination with Hib conjugate vaccines<sup>14,34</sup> had a different study design in which both the donor and the recipient or neither of them were immunized pre-transplant. In contrast to our results, Molrine *et al.*<sup>14</sup> did not observe any increase in the Hib antibody concentrations after the second Hib vaccine dose at 6 months post transplantation in the unvaccinated donor group patients, which may be partly explained by a longer delay in the study of Molrine *et al.* to the extraction of post-vaccination blood samples.

Similar proportions of patients transplanted from unvaccinated and vaccinated donors, 81 and 87%, achieved IgG Hib antibody concentrations of  $\geq 1$   $\mu$ g/ml. Barra *et al.*<sup>5</sup> vaccinated recipients at time points varying between 4 and 83 months post transplant with two doses of the Hib-tetanus toxoid conjugate vaccine achieving an Hib antibody concentration of  $\geq 1$   $\mu$ g/ml in 85% of the recipients. In the studies with the same Hib conjugate vaccine as was used in the present study, similar proportions of BMT recipients (80–89%) have achieved Hib antibody concentrations of  $\geq 1$   $\mu$ g/ml with schedules containing 2–4 immunizations between 3 and 24 months after BMT.<sup>11,14,42</sup> All patients attained Hib antibody concentrations of  $\geq 1$   $\mu$ g/ml by immunizing both the donor and the patient pre-transplant and by using four Hib vaccine doses.<sup>14</sup> It is possible that only by immunizing both the donor and the patient pre-transplant and by immunizing the patient with four vaccine doses all patients achieve Hib antibody concentrations considered predictive of long-term protection.

Acute GVHD impaired the tetanus, diphtheria and Hib, but not PV antibody responses in our study until 13 months after BMT. In contrast, in a previous study of Barra *et al.*<sup>5</sup> acute GVHD did not impair the response to the Hib conjugate vaccine. The delay from BMT to the start of vaccination was shorter in the present study compared to the study of Barra *et al.* and, thus may partly explain the differences in the antibody responses between the patients with and without acute GVHD. Acute GVHD and its treatment may suppress the development of B memory cells at 3 months after BMT and impair the responses to the later vaccinations. However, in the patients with acute GVHD donor vaccination elicited higher diphtheria and Hib antibody concentrations; the specific antibody concentrations in the DV+ patients with acute GVHD, in

contrast to those in the DV– patients with acute GVHD, were not lower than in the patients without acute GVHD. The observed benefit from donor Hib conjugate vaccination, where a poorly immunogenic polysaccharide is conjugated to a protein, together with the results from the use of pneumococcal polysaccharide protein conjugate vaccines after BMT<sup>15</sup> supports the concept that this group of vaccines may be effective in immunizing patients with acute GVHD. Encapsulated bacteria, especially *Streptococcus pneumoniae*, are able to cause severe and even lethal infections in stem cell transplant recipients.<sup>43</sup> Prevention of these infections without risk of serious side effects may be more easily and economically performed by using polysaccharide conjugate vaccines than by using prophylactic antibiotics.

In the patients with chronic GVHD the Hib and PV antibody levels were lower than in those without; the differences were observed especially in the DV– group patients and at the time of the second vaccination and 1 month post-vaccination. This is in contrast to previous Hib vaccination studies, in which chronic GVHD has not influenced antibody responses to Hib conjugate vaccines.<sup>5,11,14,42</sup> The differences in the timing of chronic GVHD in relation to vaccinations and in the diversity of the manifestations of chronic GVHD between different studies may, at least partly, explain the discrepancies in the GVHD results. In line with our results, lower responses to the IPV vaccine have been described in the patients with chronic GVHD compared to those without.<sup>3,4</sup> In the long-term follow-up the patients with chronic GVHD have tended to lose immunity to PV after vaccination more rapidly than those without chronic GVHD.<sup>44</sup>

In conclusion, immunization of donors with Hib and diphtheria vaccines before the BM harvest results in higher Hib and diphtheria antibody concentrations after recipient vaccination with these same vaccines. Particularly the patients whose donors have low baseline antibody levels as well as those who are at the greatest risk for developing GVHD may benefit from donor vaccination with polysaccharide protein conjugate and protein vaccines.

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