

Vaccination

Efficacy of donor vaccination before hematopoietic cell transplantation and recipient vaccination both before and early after transplantation

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Summary:

Allogeneic hematopoietic cell transplantation is followed by humoral immunodeficiency. We evaluated whether antibody levels can be improved by recipient vaccination on day -1 and 50 and whether the levels can be further improved by donor vaccination on day -20. A total of 85 patients were randomized or assigned to one of the following strategies of immunization with *Streptococcus pneumoniae* polysaccharides, *Haemophilus influenzae* polysaccharide-protein conjugate, tetanus toxoid (protein recall antigen) and hepatitis B surface antigen (protein neo-antigen): (1) donor on day -20, recipient on days -1, +50 and +365 (D₋₂₀R_{-1,50,365}); (2) donor nil, recipient on days -1, +50 and +365 (D_NR_{-1,50,365}); or (3) donor nil, recipient on day +365 (D_NR₃₆₅). For *H. influenzae* and tetanus, IgG levels after grafting were the highest in the D₋₂₀R_{-1,50,365} patients, intermediate in the D_NR_{-1,50,365} patients and the lowest in the D_NR₃₆₅ patients. For *S. pneumoniae* and hepatitis B, antibody levels appeared to be similar in all three patient groups. The results suggest that for polysaccharide-protein conjugate antigens or protein recall antigens, recipient immunization on days -1 and 50 improves antibody levels and that donor vaccination on day -20 further improves the levels. In contrast, neither recipient immunization on days -1 and 50 nor donor immunization on day -20 appears to be efficacious for polysaccharide antigens and poorly immunogenic protein neo-antigens.

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antibody immunity predisposes transplant recipients to infections, primarily those due to *Streptococcus pneumoniae* or *Haemophilus influenzae*.^{3–6} Antibody levels can be improved either by the administration of immunoglobulin or by vaccination. The administration of immunoglobulin may decrease the rates of some infections at the time of immunoglobulin administration, but may hamper reconstitution of antibody immunity and thus increase infection rates after the immunoglobulin has been discontinued.⁶ The vaccination can improve pathogen-specific immunity both at the time of vaccination as well as years after vaccination. However, vaccination during the first year after transplantation leads to only minor increases in specific antibody levels. Moderate to marked increases in specific antibody levels during the first year after transplant were achieved when both the donor and the recipient were immunized 7–10 days before transplantation and the recipient was boosted at 3 and 6 months after transplantation.^{7,8} Presumably this happened because large numbers of antigen-specific lymphocytes were generated in the donors and because these lymphocytes (transferred with the graft) or their progeny proliferated and differentiated upon encounter with the antigen injected to the recipient 7–10 days before transplant and at 3 and 6 months after transplant.

We set out to compare the following three vaccination strategies: (1) donor vaccination on day -20 (20 days before transplant) with recipient vaccination on days -1, +50 and +365 (D₋₂₀R_{-1,50,365}); (2) no donor vaccination and recipient vaccination on days -1, +50 and +365 (D_NR_{-1,50,365}) and (3) a conventional strategy of no donor vaccination and recipient vaccination only late post transplant (on day +365, D_NR₃₆₅) as presently recommended.⁹ The rationale for immunizing the donors on day -20 instead of days -10 to -7 was to allow for the generation of an even greater number of antigen-specific lymphocytes to be transferred with the graft.¹⁰ The rationale for immunizing the recipient on day -1 instead of days -10 to -7 was to maximize the boosting effect of the antigen as theoretically a significant fraction of the antigen administered on days -10 to -7 could be metabolized or eliminated by the day of transplant (day 0). Only one early post transplant booster (on day 50 instead of at both 3 and 6 months) was given for practicality, as our patients typically return to the referring

Immune deficiency follows allogeneic hematopoietic cell transplantation and lasts for more than 1 year.^{1,2} Deficient

physician around 3 months and return to us for evaluation only at 1 year post transplant. The reason for comparing the $D_{N}R_{-1,50,365}$ strategy with the $D_{N}R_{365}$ strategy was to evaluate the benefit of recipient vaccination on days -1 and $+50$ in the absence of donor vaccination. This is important for marrow or blood stem cell grafting from unrelated donors and cord blood grafting, as in these settings donor vaccination is impractical or impossible. The reason for comparing the $D_{N}R_{-1,50,365}$ strategy with the $D_{-20}R_{-1,50,365}$ strategy was to evaluate the added benefit of donor vaccination to the recipient pretransplant and early post transplant vaccination. This is important for the setting of marrow or blood stem cell transplantation from related donors. Firstly, if there was no added benefit, there would be no reason for exposing the donors to the potential side effects of the vaccines. Secondly, if there was a substantial added benefit, this might decrease the enthusiasm for using grafts depleted of T or B cells as the grafted T or B cells likely mediate the benefit.

The ability to transfer adoptively antibody immunity and the magnitude of antibody responses to vaccination after transplant depends on the chemical structure of the antigen and the number of encounters with the antigen before transplantation.^{7,8,10–19} To study a variety of antigens, we used *S. pneumoniae* capsular polysaccharides as a prototype of polysaccharide antigens, *H. influenzae* capsular polysaccharide conjugated to a carrier protein as a prototype of polysaccharide–protein conjugate antigens, tetanus toxoid as a prototype of protein recall antigens, and hepatitis B surface antigen (HBsAg) as a prototype of protein neo-antigens for most patients.

Methods

Study design

Recipients of transplants from HLA-matched-related donors and their donors were asked to participate in a randomized comparison of immunization against *S. pneumoniae*, *H. influenzae*, tetanus and hepatitis B of the recipient on days -1 , 50 and 365 ($D_{N}R_{-1,50,365}$) vs the recipient on days -1 , 50 and 365 post transplant plus the donor pretransplant (on arrival for pretransplant evaluation; the actual median day was -20 , range -47 to -14) ($D_{-20}R_{-1,50,365}$). Recipients of transplants from HLA-matched-related donors who did not participate in the randomized study but agreed to multiple research blood draws were immunized with the same vaccines per our standard practice on day 365 and not on day -1 or 50 ($D_{N}R_{365}$) (Table 1). The goal was to answer the primary question of the efficacy of donor vaccination pretransplant ($D_{-20}R_{-1,50,365}$ vs $D_{N}R_{-1,50,365}$) in a randomized way, and the secondary question of the efficacy of recipient vaccination on days -1 and 50 ($D_{N}R_{-1,50,365}$ vs $D_{N}R_{365}$) in a nonrandomized way. As a control antigen, polio vaccine was given to all patients on day 365. The study was approved by the Institutional Review Board and all patients signed informed consents.

Table 1 Vaccination schedules^a of patients randomized to vaccination strategy $D_{-20}R_{-1,50,365}$ vs $D_{N}R_{-1,50,365}$ or assigned to vaccination strategy $D_{N}R_{365}$

	Donor vaccination		Recipient vaccination	
	Day -20	Day -1	Day 50	Day 365
$D_{-20}R_{-1,50,365}$	Yes	Yes	Yes ^b	Yes
$D_{N}R_{-1,50,365}$	No	Yes	Yes ^b	Yes
$D_{N}R_{365}$	No	No	No	Yes

^aFor *S. pneumoniae*, *H. influenzae*, tetanus + diphtheria and hepatitis B vaccines. Polio vaccine was given to all patients per our standard practice only on day 365.

^bOne $D_{-20}R_{-1,50,365}$ patient and one $D_{N}R_{-1,50,365}$ patient did not get the vaccines on day 50. On analysis, these patients were included in the $D_{-20}R_{-1,50,365}$ and $D_{N}R_{-1,50,365}$ groups respectively.

Patients and donors

Of 116 patients accrued, 85 patients were analyzed – only those available for the day -1 and 50 immunizations and for the testing of antibody levels on or after day 80. The major reason for nonavailability was death or relapse by day 80, which occurred in 28 patients. The reason for our focusing on the days 80 to 365 interval is that specific antibody levels to recall antigens fall from recipient pretransplant levels substantially only after day 80,^{15,20,21} and that during this interval a substantial rise of the antibody levels cannot be achieved by vaccination (without pretransplant vaccination). The demographic and clinical/immunological characteristics of the 85 patients are displayed in Table 2. The $D_{-20}R_{-1,50,365}$ and $D_{N}R_{-1,50,365}$ groups were balanced for all the characteristics (Table 2, footnote a), suggesting that differences in antibody levels should be due to donor vaccination pretransplant. Likewise, the $D_{N}R_{-1,50,365}$ and $D_{N}R_{365}$ groups were balanced for all the characteristics (Table 2, footnote a), suggesting that differences in antibody levels should be due to recipient vaccination on days -1 and 50.

All the patients participated also in a randomized study of marrow vs filgrastim-mobilized blood stem cell grafting and have been reported by us.^{22–24} Antibody immunity was unaffected by the type of graft, as the levels of total IgG were similar after marrow and blood stem cell transplantation,²³ the levels of antigen-specific antibodies were also similar,²⁴ and antibody responses to post transplant immunization with keyhole limpet hemocyanin and inactivated polio vaccine were also similar (Storek *et al*, unpublished). None of the patients had a preceding hematopoietic cell transplant. All patients received graft-versus-host disease prophylaxis with methotrexate (days 1, 3, 6 and 11) plus cyclosporine (daily for 6 months).²⁵

Blood was drawn for the determination of specific antibody levels in the donors pretransplant (before donor vaccination) and at 1 month after vaccination, and in the patients pretransplant (before day -1 vaccination) and on approximately days 50 (before vaccination), 80, 180, 365 (before vaccination) and 395 (1 month after vaccination). The numbers of patients studied at each

Table 2 Patient characteristics^a

	<i>D_NR₃₆₅</i> (<i>n</i> = 36)	<i>D_NR_{-1,50,365}</i> (<i>n</i> = 19)	<i>D_{-20R_{-1,50,365}}</i>
Patient age at transplant (years) – median (range)	43 (22–59)	46 (18–61)	42 (15–56)
Donor age at transplant (years) – median (range)	40 (13–65)	43 (16–51)	45 (20–63)
Patient sex			
Males	23 (64%)	13 (68%)	16 (53%)
Females	13 (36%)	6 (32%)	14 (47%)
Donor–patient histocompatibility			
HLA-A, B and DR-matched sibling	35 (97%)	19 (100%)	30 (100%)
HLA-A, B and DR-matched child			
Disease/disease stage at transplant ^b			
Good risk	17 (47%)	14 (74%)	21 (70%)
Poor risk	19 (53%)	5 (26%)	9 (30%)
Cytomegalovirus (CMV) serostatus pretransplant			
Donor+ and recipient+	11 (31%)	6 (32%)	12 (40%)
Donor– and recipient+	7 (19%)	4 (21%)	5 (17%)
Donor+ and recipient–	6 (17%)	3 (16%)	7 (23%)
Donor– and recipient–	12 (33%)	5 (26%)	6 (20%)
Unknown	0 (0%)	1 (5%)	0 (0%)
Splenectomized patients	1 (3%)	0 (0%)	2 (7%)
Conditioning ^c			
Chemotherapy only	17 (47%)	12 (63%)	20 (67%)
Chemotherapy plus TBI	19 (53%)	7 (37%)	10 (33%)
Graft type			
Marrow	19 (53%)	7 (37%)	14 (47%)
Blood stem cells	17 (47%)	12 (63%)	16 (53%)
Acute GVHD			
Grade 0–1	10 (28%)	5 (26%)	11 (37%)
Grade 2–4 ^d	26 (72%)	14 (74%)	19 (63%)
Chronic GVHD diagnosed by day 365			
None or subclinical	8 (22%)	3 (16%)	7 (23%)
Clinical limited	9 (25%)	5 (26%)	4 (13%)
Clinical extensive ^e	19 (53%)	11 (58%)	19 (63%)
Chimerism status			
Full chimera (≥90% marrow cells of donor origin by day 80)	34 (94%)	19 (100%)	27 (90%)
Unknown	2 (6%)	0 (0%)	3 (10%)
Relapse between days 80 and 365 ^f	3 (8%)	0 (0%)	4 (13%)
Death without relapse between days 80 and 365	6 (17%)	1 (5%)	4 (13%)
Glucocorticoid treatment between day 30 and 365	30 (83%)	17 (89%)	22 (73%)
Intravenous immunoglobulin given between day 30 and 365 ^g	3 (8%)	3 (16%)	2 (7%)
B-cell counts on day 80 (per μ l blood) – median (range)	2 (0–221)	9 (0–232)	8 (0–105)
B-cell counts on day 180 (per μ l blood) – median (range)	51 (0–511)	67 (4–247)	54 (2–366)
B-cell counts on day 365 (per μ l blood) – median (range)	366 (0–1351)	187 (4–637)	95 (3–1510)
CD4 T-cell counts on day 80 (per μ l blood) – median (range)	68 (3–534)	136 (20–643)	141 (43–547)
CD4 T-cell counts on day 180 (per μ l blood) – median (range)	140 (2–1295)	308 (36–918)	293 (52–743)
CD4 T-cell counts on day 365 (per μ l blood) – median (range)	219 (14–1107)	328 (79–542)	273 (16–1012)

^aFor all the characteristics, there was no significant difference ($P > 0.05$) between *D_{-20R_{-1,50,365}}* and *D_NR_{-1,50,365}* patients and between *D_NR_{-1,50,365}* and *D_NR₃₆₅* patients. The only significant differences between *D_NR₃₆₅* and *D_NR_{-1,50,365}* patients were in CD4 T-cell counts on days 80 and 180 ($P = 0.02$ and 0.01 , respectively). Significance of difference was determined by the Mann–Whitney–Wilcoxon signed rank test for patient and donor age and B and CD4 T-cell counts. For all other characteristics, the significance was determined by χ^2 test or, when appropriate, by Fisher’s exact test.

^bGood risk: chronic myelogenous leukemia in first chronic or accelerated phase, acute leukemia in first remission, refractory anemia, myelofibrosis. Poor risk: chronic myelogenous leukemia in blast crisis, acute leukemia beyond first remission, refractory anemia with excess blasts, lymphoma, multiple myeloma.

^cAll conditioning regimens were myeloablative. The most frequent chemotherapy only regimen was IV cyclophosphamide (120 mg/kg) with PO busulfan (approximately 16 mg/kg). The most frequent chemotherapy plus total body irradiation (TBI) regimen was cyclophosphamide (120 mg/kg) with fractionated TBI (12.0–13.2 Gy). Three patients from the ‘TBI + chemotherapy’ group received fractionated total marrow irradiation (9.0 Gy, similar to upper mantle and inverted Y) instead of TBI (one *D_NR₃₆₅* patient, one *D_NR_{-1,50,365}* patient and one *D_{-20R_{-1,50,365}}* patient).

^dTypically treated with prednisone 1–2 mg/kg/day PO for 10–14 days with subsequent taper over 50 days.

^eTypically treated with prednisone (0.5–1.0 mg/kg every other day PO) plus cyclosporine (approximately 6 mg/kg every other day PO) for at least 9 months.

^fFor chronic myelogenous leukemia, the detection of bcr/abl transcript by PCR in the absence of cytogenetic or hematologic relapse was not considered a relapse. As the goal of the study was to evaluate transplant-associated immune deficiency and not malignancy-associated immune deficiency, antibody levels of patients who relapsed post transplant were evaluated only before the diagnosis of relapse.

^gTypically, 200 mg/kg weekly before day 100, 500 mg/kg monthly after day 100 until preinfusion level of 400 mg/dl has been achieved. Antibody levels in sera obtained within 2 months after the administration of intravenous immunoglobulin were not evaluated.

time-point are given in figure legends. Normal responses to *S. pneumoniae*, *H. influenzae*, tetanus and hepatitis B vaccines were evaluated in the vaccinated donors. Normal

responses to the polio vaccine were assessed in 11 healthy volunteers unrelated to the patients, median age 23 years (range, 20–41).

Vaccines

The following vaccines were used: Pnu-Immune (Wyeth/Lederle, Philadelphia, PA, USA) containing the capsular polysaccharides of 23 common pneumococcal serotypes, 0.5 ml IM; HibTITER (Wyeth/Lederle, Philadelphia, PA, USA) containing *H. influenzae* type b capsular polysaccharide conjugated to diphtheria CRM197 protein, 0.5 ml IM; Td (Wyeth/Lederle, Philadelphia, PA, USA) containing tetanus and diphtheria toxoids, 0.5 ml IM; and Recombivax HB (Merck, West Point, PA, USA) containing HBsAg, 1.0 ml IM (except for one 15-year-old patient who received 0.5 ml IM as per the manufacturer's recommendation). On day 365, all patients who were alive and in remission also received IPOL (Pasteur/Connaught, Swiftwater, PA, USA) containing formalin-inactivated poliovirus 1, 2 and 3, 0.5 ml s.c.

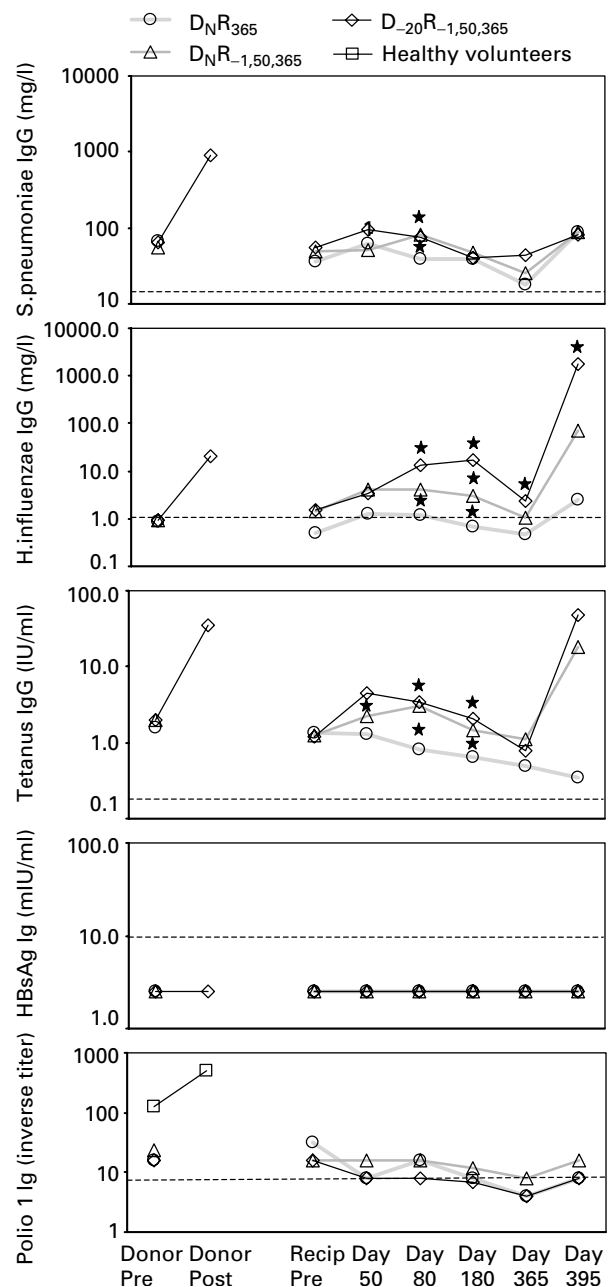
Antibody levels

Serum levels of IgG specific for tetanus toxoid, *H. influenzae* capsular polysaccharide or for a mixture of 23 common pneumococcal polysaccharide serotypes were determined by ELISA, using kits purchased from The Binding Site (Birmingham, UK). Levels of antibodies against HBsAg were determined by ELISA, using kits purchased from DiaSorin (Saluggia, Italy). Most patients and donors had undetectable anti-HBsAg pretransplant (Figure 1), suggesting that HBsAg represented a neo-antigen for most patients and donors. One $D_{N}R_{-1,50,365}$ patient was a carrier of hepatitis B; he was excluded from the analysis of post transplant anti-HBsAg levels. Levels of antibodies against poliovirus 1 were determined by a virus binding inhibition assay as described.^{26,24}

Levels of IgG against *S. pneumoniae* serotype 4, 6B, 9V, 14, 18C, 19F and 23F were determined using a flow

Figure 1 Median antibody levels for *H. influenzae* and tetanus, but not for *S. pneumoniae* or hepatitis B, are significantly higher at most post transplant time-points in the recipients vaccinated on days -1, 50 and 365 with donors vaccinated on day -20 ($D_{-20}R_{-1,50,365}$) than the recipients vaccinated on days -1, 50 and 365 with donors unvaccinated pretransplant ($D_{N}R_{-1,50,365}$) or the recipients vaccinated only on day 365 with donors unvaccinated pretransplant ($D_{N}R_{365}$). Median antibody levels for a control antigen, polio 1, are similar in all the three groups. Asterisks between circles ($D_{N}R_{365}$) and triangles ($D_{N}R_{-1,50,365}$) or between diamonds ($D_{-20}R_{-1,50,365}$) denote statistical significance ($P \leq 0.02$) of difference between the respective groups; asterisks above the diamonds denote statistical significance of difference between the $D_{-20}R_{-1,50,365}$ group and the $D_{N}R_{365}$ group. Recip Pre denotes recipients pretransplant. Donor Pre denotes donors pretransplant (prevaccination), and Donor Post denotes the $D_{-20}R_{-1,50,365}$ donors at 1 month postvaccination. As polio immunization of donors was not performed, normal pre- and 1 month postvaccination levels are instead shown for healthy adult volunteers unrelated to the patients (squares, $n = 11$). The prevaccination polio 1 antibody levels in the healthy adult volunteers may be higher than in the HCT donors because the healthy volunteers were younger (see Methods). The dashed horizontal lines denote presumed protective levels.^{52,7,28,53,26,36} The numbers of $D_{N}R_{365}/D_{N}R_{-1,50,365}/D_{-20}R_{-1,50,365}$ donors studied were 36/18/29 pretransplant (prevaccination) and 0/0/27 postvaccination, and the numbers of $D_{N}R_{365}/D_{N}R_{-1,50,365}/D_{-20}R_{-1,50,365}$ recipients studied were 36/18/25 pretransplant, 13/17/25 on day 50, 28/16/28 on day 80, 26/14/24 on day 180, 22/14/22 on day 365, and 2/13/18 on day 395.

cytometric (ImmunoArray) assay.²⁷ Briefly, all seven pneumococcal capsular polysaccharide serotypes (ATCC, Manassas, VA, USA) were covalently attached onto different microsphere sets (Luminex Corporation, Austin, TX, USA). The microsphere sets were then pooled to make up a microsphere mixture and incubated with serum samples diluted 1:400 with a 1% bovine serum albumin/PBS-based sample diluent buffer. All unbound material was then washed away using a Tween-20/PBS-based wash buffer. The quantitation of bound antibodies was performed using a polyclonal anti-human IgG conjugated with phycoerythrin (PE) and the Luminex-100 flow cytometer. Microspheres carrying a specific pneumococcal serotype were characterized by unique spectral fluorescent emission signatures after 635 nm red laser excitation of varying proportions of embedded red and infrared fluorescent dyes.



The amount of bound pneumococcal polysaccharide antibodies on each microsphere was measured by the orange fluorescence emission of PE-conjugated anti-human IgG antibodies excited with a 532 nm green laser. The amount of anti-pneumococcal polysaccharide antibody bound onto the microsphere was proportional to the measured amount of PE-conjugated anti-human IgG antibody. Using known standards (eight levels) that were calibrated against the US FDA Pneumococcal Reference Serum 89SF, standard curves for all seven serotypes were generated, and all unknown sample results were interpolated from these curves.

For statistical evaluations, levels of *S. pneumoniae*, *H. influenzae*, tetanus and HBsAg antibodies below the lower detection limit (antibody concentration of the least concentrated standard) were assigned values equaling the lower detection limit divided by 2. Levels of antibodies above the upper detection limit were remeasured using 10 and 100 times higher serum dilutions. On the rare occasion when even with the 100 times higher serum dilution the reading was higher than the upper detection limit (antibody concentration of the most concentrated standard \times 100), the level was assigned a value equaling the upper detection limit multiplied by 2.

Tetanus lymphoproliferation assay

Cryopreserved density gradient (1.077 kg/l)-separated mononuclear cells were suspended at 2×10^6 cells/ml in RPMI with 10% human AB serum, glutamine (4 mM), penicillin (100 U/ml), streptomycin (0.1 mg/ml) and amphotericin B (250 ng/ml). The mononuclear cell suspension (100 μ l) was dispensed into wells of 96 round-bottom plates. Tetanus toxoid (kind gift of Lederle Laboratories, Pearl River, NY, USA) was added to triplicate wells at a final concentration of 1.67 LFU/ml on day zero. The plates were incubated at 37°C in a humidified 5% CO₂ atmosphere for 4 days. At 18–24 h before harvest, ³H-thymidine (2 μ Ci) was added to each well. Cells were harvested using a semiautomated harvester, and counts per minute (cpm) were determined in a β scintillation counter. The mean cpm of cells exposed to the antigen minus the mean cpm of cells incubated with medium alone (Δ cpm) was calculated.

Statistics

Medians rather than means are given as the antibody levels and Δ cpm values were typically not normally distributed. Significance of differences between patient groups or donor groups at each time-point was tested by the Mann–Whitney–Wilcoxon rank sum test. Two-tailed *P*-values were used. To minimize the number of spurious findings due to multiple comparisons of antibody levels, the stringent significance level of 0.02 was used as the cutoff for declaring statistical significance.

Results

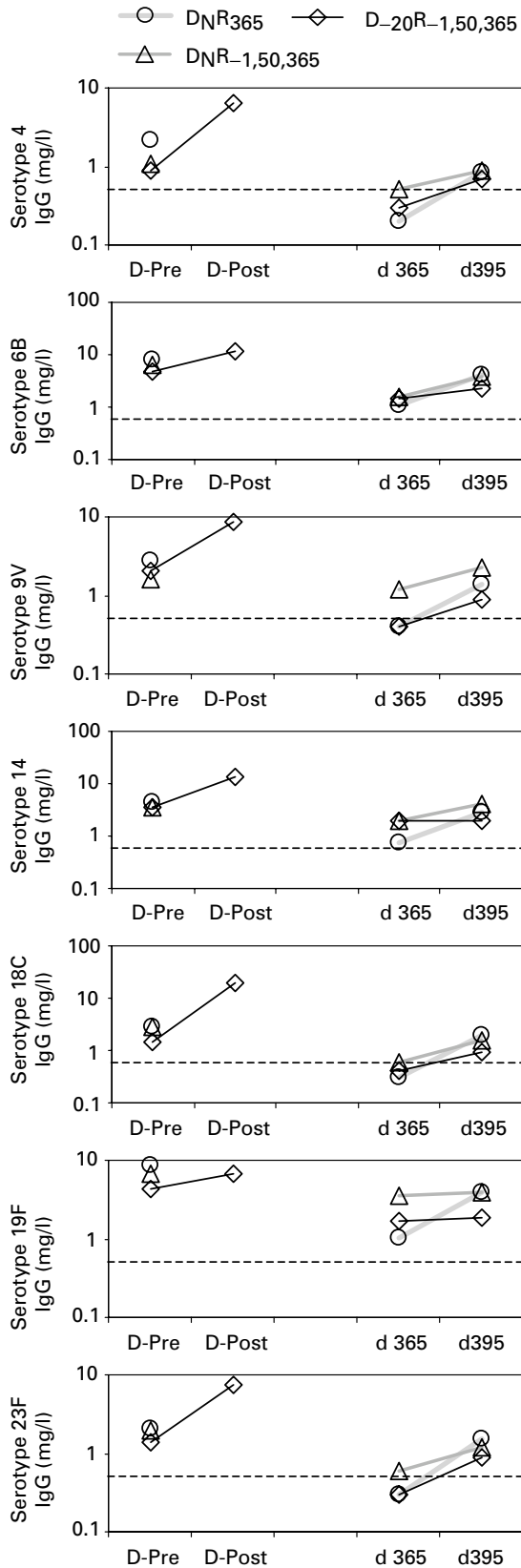
Effect of vaccination strategy on antibody levels

The effects of the donor vaccination on day –20 (determined by the comparison of patients randomized to

D_{–20}R_{–1,50,365} vs D_NR_{–1,50,365} strategy) and of the recipient nonrandomized comparison of D_NR_{–1,50,365} vs D_NR₃₆₅ patients) differed among the various antigens (Figure 1). For *H. influenzae* and tetanus, the median IgG levels were higher in D_{–20}R_{–1,50,365} than D_NR_{–1,50,365} patients at most post transplant time-points (statistically significant for *H. influenzae* on day 180 and for tetanus on day 50), suggesting that the donor vaccination was efficacious. The median IgG levels were higher in D_NR_{–1,50,365} than D_NR₃₆₅ patients at all post transplant time-points (statistically significant for both *H. influenzae* and tetanus on days 80 and 180), suggesting that the recipient vaccination on day –1 and 50 was efficacious. Of the 3 vaccination strategies, only the D_{–20}R_{–1,50,365} strategy was associated with median *H. influenzae* IgG levels higher than the presumed protective level (1 mg/l)²⁸ at all the post transplant time-points. In contrast to *H. influenzae* and tetanus, *S. pneumoniae* IgG levels in all the three groups were similar, except on day 80. For HBsAg, the median antibody levels in all 3 groups were below the detection limit (arbitrarily assigned a value of 2.5 mIU/ml) both pretransplant and at all the post transplant time-points, precluding comparison among the groups. However, an advantage of the D_{–20}R_{–1,50,365} group over the other two groups was unlikely because the 90th percentiles of the anti-HBsAg levels (which were above the detection limit) were similar in all three groups; they were actually lower in the D_{–20}R_{–1,50,365} group than in the D_NR₃₆₅ group on day 50, 80 and 180 (data not shown).

The differences in *H. influenzae* and tetanus IgG (D_{–20}R_{–1,50,365} > D_NR_{–1,50,365} > D_NR₃₆₅) did not appear to be attributable to differences in donor or recipient specific IgG levels pretransplant. There were no significant differences between the patient groups in the levels of recipient *H. influenzae* or tetanus IgG pretransplant or the levels of donor *H. influenzae* or tetanus IgG pretransplant (Figure 1). D_{–20}R_{–1,50,365} vs D_NR_{–1,50,365} patients and D_NR_{–1,50,365} vs D_NR₃₆₅ patients were also balanced in the following factors that could theoretically affect the antibody levels: patient age, donor age, histocompatibility, CMV serostatus, splenectomy, conditioning, GVHD prophylaxis, incidence of grade 2–4 acute GVHD or clinical extensive chronic GVHD, relapse rate, the administration of glucocorticoids and post transplant B cell and CD4T cell counts (Table 2, footnote a). Moreover, humoral immunity of the D_NR₃₆₅, D_NR_{–1,50,365} and D_{–20}R_{–1,50,365} patients was similar as there were no significant differences in antibody levels against polio 1 (control antigen – Figure 1).

The apparent lack of influence of the donor vaccination on day –20 and recipient vaccination on day –1 and 50 on post transplant IgG levels against *S. pneumoniae* could possibly be due to the fact that we determined total IgG against 23 serotypes. It is conceivable that IgG levels against certain serotypes were higher in D_{–20}R_{–1,50,365} patients compared to D_NR_{–1,50,365} or D_NR₃₆₅ patients. To evaluate this possibility, we determined days 365 and 395 levels of IgG against serotype 4, 6B, 9V, 14, 18C, 19F and 23F (Figure 2). For each serotype, there was no significant difference in the day 365 or 395 levels between D_{–20}R_{–1,50,365} and D_NR_{–1,50,365}



patients, between $D_{NR-1,50,365}$ and D_{NR365} patients, or between $D_{-20R-1,50,365}$ and D_{NR365} patients. Thus, there was no advantage of donor immunization with pneumococcal polysaccharides on day -20, and there also was no advantage of recipient immunization on days -1 and 50.

Effect of vaccination strategy on lymphoproliferation

For *H. influenzae* and tetanus, the most robust IgG response to the day 365 vaccination was observed in $D_{-20R-1,50,365}$ patients, intermediate response in $D_{NR-1,50,365}$ patients and the lowest response in D_{NR365} patients (Figure 1). This suggested that the $D_{-20R-1,50,365}$ patients had the largest number of antigen-specific lymphocytes or the most functional antigen-specific lymphocytes (either antigen-specific helper T cells ready to proliferate and help B cells or antigen-specific B cells ready to proliferate and differentiate into plasma cells), and that the D_{NR365} patients had the lowest number of or the least functional antigen-specific lymphocytes. To evaluate this hypothesis, *in vitro* lymphocyte proliferation upon stimulation with tetanus toxoid was measured on day 365 (before the day 365 vaccination). This assay measures the stimulation of tetanus-specific CD4T cells and B cells.^{29,30} As expected, the lymphocyte proliferation was significantly higher in $D_{-20R-1,50,365}$ patients than $D_{NR-1,50,365}$ patients ($P=0.03$) or D_{NR365} patients ($P<0.001$), and significantly higher in $D_{NR-1,50,365}$ patients than D_{NR365} patients ($P=0.01$) (Figure 3). Of interest, the lymphocyte proliferation in the $D_{-20R-1,50,365}$ patients was even higher than in the donors ($P<0.001$). Thus, the $D_{-20R-1,50,365}$ vaccination strategy resulted in a large number of or very functional antigen-specific CD4T cells or B cells.

Adverse events

Two patients developed a severe local reaction (possible infection) to day -1 vaccination during severe neutropenia. Both patients were from the $D_{NR-1,50,365}$ group. Both patients were given tetanus + diphtheria vaccine into the right quadriceps muscle on day -1. The first patient developed right thigh swelling on day 11. Computer tomogram showed an area of hypodensity (suggesting inflammation) in the quadriceps muscle. Fluid aspirated from that site showed Gram positive rods (possibly Clostridia) that did not grow in culture. On day 12, the site was surgically explored. Inflamed tissue was excised, and histology showed necrotizing fasciitis with mild myositis. No microorganisms were seen and cultures were

Figure 2 Median IgG levels for pneumococcal serotypes before and 1 month after vaccination in patients (days 365 and 395) and their HCT donors (D-Pre and D-Post). The levels were not significantly different ($P>0.02$) for each serotype at each time-point between $D_{NR-1,50,365}$ vs $D_{-20R-1,50,365}$ groups, D_{NR365} vs $D_{NR-1,50,365}$ groups, or D_{NR365} vs $D_{-20R-1,50,365}$ groups. The dashed horizontal lines represent the presumed protective levels of 0.5 mg/l for each serotype.^{52,7} The numbers of $D_{NR365}/D_{NR-1,50,365}/D_{-20R-1,50,365}$ donors studied were 4/7/14 pretransplant (pre-vaccination) and 0/0/13 post-vaccination, and the numbers of $D_{NR365}/D_{NR-1,50,365}/D_{-20R-1,50,365}$ patients studied were 20/7/17 on day 365, and 2/11/11 on day 395.

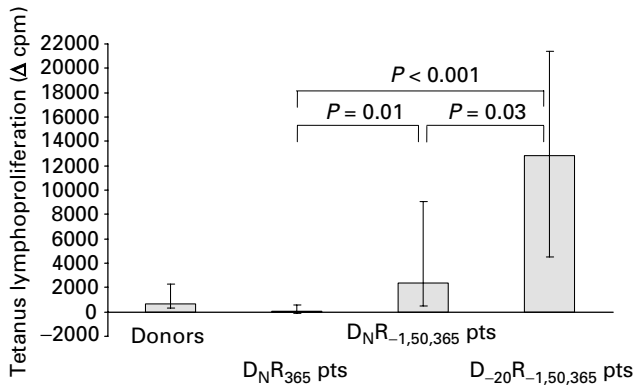


Figure 3 Median tetanus toxoid-stimulated lymphoproliferation on day 365 is the highest in the D₋₂₀R_{-1,50,365} patients, intermediate in the D_NR_{-1,50,365} patients and the lowest in the D_NR₃₆₅ patients. As a reference, donors' lymphoproliferation pretransplant (prevaccination) is displayed. The error bars span the 25th through the 75th percentiles. The assay was performed on individuals for whom cryopreserved mononuclear cells were available, i.e., that is donors, 10 D_NR₃₆₅ patients, 13 D_NR_{-1,50,365} patients and 15 D₋₂₀R_{-1,50,365} patients. The patients were representative of the whole cohorts (Table 2) regarding patient and donor age, graft type, acute and chronic GVHD, treatment with glucocorticoids and intravenous immunoglobulin (data not shown), and regarding B and CD4 T-cell counts. Specifically, median B-cell counts were 320, 187 and 79, and median CD4 T-cell counts were 258, 370 and 317 per microliter in D_NR₃₆₅, D_NR_{-1,50,365} and D₋₂₀R_{-1,50,365} patients, respectively, at 1 year post transplant.

negative. The patient was treated with hyperbaric oxygen, filgrastim and penicillin and clindamycin (in addition to prophylactic antibiotics), and recovered without long-term sequelae. The second patient developed right thigh swelling by day 5. Magnetic resonance imaging showed a subcutaneous area of probable inflammation. Clindamycin was added to prophylactic antibiotics and the swelling improved. On day 7, clindamycin was discontinued. By day 9, the swelling worsened. Clindamycin was restarted, and the swelling abated. Culture of the vaccine (another vial from the same lot) was negative. These two cases suggest that unexpected side effects of licensed vaccines may occur in the peritransplant setting.

Discussion

The major findings of this study are that for *H. influenzae* (prototypal polysaccharide-protein conjugate antigen) and tetanus (prototypal protein recall antigen), specific antibody levels after transplant can be improved by vaccinating the recipient on days -1 and 50, and that the levels can be further improved by vaccinating the donor on day -20. This suggests that immunizing the recipient pretransplant and early post transplant is beneficial even if the donor cannot be vaccinated as in the setting of cord blood transplantation or unrelated marrow or blood stem cell transplantation. Moreover, it suggests that if in the setting of T- and B-cell-replete transplantation the donor can be vaccinated, it is beneficial to vaccinate also the donor.

Disappointingly, even the D₋₂₀R_{-1,50,365} strategy did not result in a substantial rise of antibodies for *S. pneumoniae*

polysaccharides and for HBsAg (prototypal protein neo-antigen). The lack of efficacy for pneumococcal capsular polysaccharides is particularly disturbing as *S. pneumoniae* causes significant morbidity and mortality. In the 1970s and early 1980s, 9–27% transplant survivors developed an infection due to *S. pneumoniae* and up to 8% died of pneumococcal infections.^{31,32} In the 1990s, only 3–6% transplant survivors developed a pneumococcal infection (Storek and Sullivan, unpublished; Kulkarni *et al*³³), probably as a result of chemoprophylaxis with sulfamethoxazole/trimethoprim or penicillin. The percentage may rise in the 2000s due to the rapid emergence of resistant pneumococci.^{34,35} Fortunately, vaccination of the donor pretransplant and the recipient pretransplant and early post transplant with the new heptavalent polysaccharide + protein conjugate vaccine (Prevnar) appears to improve the post transplant antibody levels for the seven serotypes contained in the vaccine.² For the serotypes not contained in the heptavalent vaccine, the best prophylaxis continues to be antibiotic prophylaxis during the first year and immunization with the 23-valent polysaccharide vaccine at 1 year post transplant (see Figures 1 and 2 for the moderate efficacy of the 23-valent vaccine at 1 year post transplant).

Regarding protein neo-antigens, our inability to achieve detectable levels of antibodies against HBsAg in the D₋₂₀R_{-1,50,365} patients contrasts with the study of Wimperis *et al*¹⁰ describing detectable antibody levels to keyhole limpet hemocyanin (KLH) post transplant in all patients immunized with KLH pretransplant whose donors were immunized 3 weeks pretransplant. The discrepancy could be due to the relatively poor immunogenicity of HBsAg compared to KLH. In most healthy persons, anti-HBsAg levels become detectable (exceed the detection limit of 5 mIU/ml) only after two doses of hepatitis B vaccine,³⁶ whereas a marked rise in KLH antibodies occurs already after one dose of KLH.³⁷ Collectively, the donor vaccination at ~3 weeks pretransplant with recipient vaccination both pretransplant and early post transplant may be efficacious for highly immunogenic protein neo-antigens like KLH or protein recall antigens like tetanus toxoid, but not for poorly immunogenic protein neo-antigens like HBsAg. Repetitive donor vaccination with a poorly immunogenic protein neo-antigen pretransplant (converting the neo-antigen to a recall antigen) may circumvent this problem.³⁸

The use of antibody levels as surrogates of protection against infection is a drawback of our study. This is because the infections for which we vaccinated are relatively rare. Only one patient in our study developed recurrent pneumococcal infections (D₋₂₀R_{-1,50,365} patient) and one patient developed a *H. influenzae* infection (D_NR_{-1,50,365} patient) in the first year after transplant (see Storek *et al*²³ for the follow-up for and definition of infections). For the same reason, the Centers for Disease Control recommendations are also based on antibody levels.⁹ Up until recently only infections that are rare (*H. influenzae*) or unreported (tetanus, diphtheria, polio) after transplantation could have been possibly prevented by the D₋₂₀R_{-1,50,365} vaccination strategy. This precluded a vaccination trial with the end point of the incidence of clinical infection, as extremely

large numbers of patients would be required to document a benefit. However, with the advent of the pneumococcal heptavalent conjugate vaccine and the inactivated varicella vaccine and the documentation of their immunogenicity in transplant recipients,^{7,39} the time has come to compare the D₋₂₀R_{-1,50,365} or a similar vaccination strategy with a standard management, in a trial using the *S. pneumoniae* and *H. influenzae* conjugate vaccines and the varicella inactivated vaccine. The trial should evaluate the end point of the incidence of clinical *S. pneumoniae*, *H. influenzae* and varicella virus infections. Given the potential toxicities of the D₋₂₀R_{-1,50,365} vaccination (including vaccination site infections – see Results, last paragraph), the D₋₂₀R_{-1,50,365} or similar strategy can be recommended for routine clinical use only if a favorable risk : benefit ratio is found in the proposed clinical trial.

Transfer of antigen-specific immunity was first documented by Lum and co-workers^{16,40–43} Boosting of the transferred immunity by immunizing the donor within 1 month pretransplant and of the recipient within 1 week pretransplant was pioneered by Wimperis and co-workers^{17–19,44–47} However, the patients in the boosting studies were usually followed for only several months. Here we show that the effect of the donor vaccination pretransplant and recipient vaccination peri-transplant against a protein recall antigen or a polysaccharide–protein conjugate antigen appears to persist for at least 1 year.

The D₋₂₀R_{-1,50,365} vaccination strategy may also be explored for tumor antigens.^{47,48} Data about antitumor effect of antibodies have been accumulating. High levels of antibodies against tumor-associated antigens in nontransplant patients have been associated with low tumor progression rates^{49,50} and the administration of antibodies against tumor-associated antigens has resulted in tumor remissions. Examples include trastuzumab/anti-her2neu for breast cancer, cetuximab/anti-EGFR for colon cancer, rituximab/anti-CD20 or anti-Ig idiotype for non-Hodgkin's lymphoma, and alemtuzumab/anti-CD52 for chronic lymphocytic leukemia (reviewed by White *et al*⁵¹). Moreover, the D₋₂₀R_{-1,50,365} strategy may improve antitumor lymphocyte functions other than antibody production (eg, proliferation – Figure 3).

In summary, donor vaccination pretransplant and recipient vaccination on days –1 and 50 are likely to be of no benefit when immunizing with a polysaccharide antigen or a poorly immunogenic protein neo-antigen. Donor vaccination pretransplant (around day –20) and/or recipient vaccination on days –1 and 50 lead to high antibody levels during the first year after transplant when immunizing with a polysaccharide–protein conjugate antigen, a protein recall antigen and, possibly, a highly immunogenic protein neo-antigen. However, given the uncertainty about adverse effect (see Results, last paragraph) and about whether increased antibody levels translate into fewer infections, we recommend vaccination of donors pretransplant and recipients peri-transplant only in the setting of clinical trials. Outside of clinical trials, the Centers for Disease Control guidelines (currently recommending only post transplant vaccination)⁹ should be followed.

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References

- 1 Parkman R, Weinberg KI. Immunological reconstitution following hematopoietic stem cell transplantation. In: Thomas ED, Blume KG, Forman SJ (eds). *Hematopoietic Cell Transplantation*. Blackwell Science: Malden, 1999, 704–711.
- 2 Storek J, Witherspoon RP. Immunologic reconstitution after hematopoietic stem cell transplantation. In: Atkinson K (ed). *Clinical Bone Marrow and Blood Stem Cell Transplantation*. Cambridge University Press: Cambridge, in press.
- 3 Sheridan JF. Immunoglobulin G subclass deficiency and pneumococcal infection after allogeneic BMT. *Blood* 1990; **75**: 1583–1586.
- 4 Aucouturier P, Barra A, Intrator L *et al*. Long lasting IgG subclass and antibacterial polysaccharide antibody deficiency after allogeneic bone marrow transplantation. *Blood* 1987; **70**: 779–785.
- 5 Riches PG, Walker SA, Rogers TR *et al*. Relative deficiency of serum IgA, IgG2 and IgG4 during reconstitution following BMT: relationship to infection. *Bone Marrow Transplant* 1986; **1** (Suppl. 1): 53.
- 6 Sullivan KM, Storek J, Kopecky KJ *et al*. A controlled trial of long-term administration of intravenous immunoglobulin to prevent late infection and chronic GVHD following marrow transplantation: Clinical outcome and effect on subsequent immune recovery. *Biol Blood Marrow Transplant* 1996; **2**: 44–53.
- 7 Molrine DC, Antin JH, Guinan EC *et al*. Donor immunization with pneumococcal conjugate vaccine and early protective antibody responses following allogeneic hematopoietic cell transplantation. *Blood* 2003; **101**: 831–836.
- 8 Molrine DC, Guinan EC, Antin JH *et al*. Donor immunization with *Haemophilus influenzae* type B (Hib)-conjugate vaccine in allogeneic bone marrow transplantation. *Blood* 1996; **87**: 3012–3018.
- 9 Dykewicz CA, Jaffe HW, Kaplan JE. Guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients. *Biol Blood Marrow Transplant* 2000; **6**: 659–713, 715; 717–627; quiz 729–633.
- 10 Wimperis JZ, Gottlieb D, Duncombe AS *et al*. Requirements for the adoptive transfer of antibody responses to a priming antigen in man. *J Immunol* 1990; **144**: 541–547.
- 11 Witherspoon RP, Storb R, Ochs HD *et al*. Recovery of antibody production in human allogeneic marrow graft recipients: influence of time posttransplantation, the presence or absence of chronic graft-versus-host disease, and antithymocyte globulin treatment. *Blood* 1981; **58**: 360–368.
- 12 Guinan EC, Molrine DC, Antin JH *et al*. Polysaccharide conjugate vaccine responses in bone marrow transplant patients. *Transplantation* 1994; **57**: 677–684.
- 13 Amlot PL, Hayes AE, Gray D *et al*. Human immune responses *in vivo* to protein (KLH) and polysaccharide (DNP-Ficoll) neoantigens: normal subjects compared with bone marrow transplant patients on cyclosporine. *Clin Exp Immunol* 1986; **64**: 125–135.

- 14 Barra A, Cordonnier C, Preziosi MP *et al*. Immunogenicity of *Haemophilus influenzae* type b conjugate vaccine in allogeneic bone marrow recipients. *J Infect Dis* 1992; **166**: 1021–1028.
- 15 Parkkali T, Ruutu T, Stenvik M *et al*. Loss of protective immunity to polio, diphtheria and *Haemophilus influenzae* type b after allogeneic bone marrow transplantation. *APMIS* 1996; **104**: 383–388.
- 16 Donnenberg AD, Hess AD, Duff SC *et al*. Regeneration of genetically restricted immune functions after human bone marrow transplantation: influence of four different strategies for GVHD prophylaxis. *Transplant Proc* 1987; **19** (Suppl 7): 144–152.
- 17 Saxon A, Mitsuyasu R, Stevens R *et al*. Designed transfer of specific immune responses with bone marrow transplantation. *J Clin Invest* 1986; **78**: 959–967.
- 18 Gottlieb DJ, Cryz Jr SJ, Furer E *et al*. Immunity against *Pseudomonas aeruginosa* adoptively transferred to bone marrow transplant recipients. *Blood* 1990; **76**: 2470–2475.
- 19 Labadie J, VanTol JD, Dijkstra NH *et al*. Transfer of specific immunity from donor to recipient of an allogeneic bone marrow graft: effect of conditioning on the specific immune response of the graft recipient. *Br J Haematol* 1992; **80**: 381–390.
- 20 Ljungman P, Wiklund-Hammarsten M, Duraj V *et al*. Response to tetanus toxoid immunization after allogeneic bone marrow transplantation. *J Infect Dis* 1990; **162**: 496–500.
- 21 Ljungman P, Lewensohn-Fuchs I, Hammarstrom V *et al*. Long-term immunity to measles, mumps and rubella after allogeneic bone marrow transplantation. *Blood* 1994; **84**: 657–663.
- 22 Bensingier WI, Martin P, Storer B *et al*. Transplantation of bone marrow as compared with peripheral blood cells from HLA-identical relatives in patients with hematologic malignancies. *N Engl J Med* 2001; **344**: 175–181.
- 23 Storek J, Dawson MA, Storer B *et al*. Immune reconstitution after allogeneic marrow transplantation compared with blood stem cell transplantation. *Blood* 2001; **97**: 3380–3389.
- 24 Storek J, Viganego F, Dawson MA *et al*. Factors affecting antibody levels after allogeneic hematopoietic cell transplantation. *Blood* 2003; **101**: 3319–3324.
- 25 Storb R, Deeg HJ, Whitehead J *et al*. Methotrexate and cyclosporine compared with cyclosporine alone for prophylaxis of acute graft versus host disease after marrow transplantation for leukemia. *N Engl J Med* 1986; **314**: 729–735.
- 26 Herremans MM, Reimerink JH, Ras A *et al*. Evaluation of a poliovirus-binding inhibition assay as an alternative to the virus neutralization test. *Clin Diagn Lab Immunol* 1997; **4**: 659–664.
- 27 Lim LCL, Lal M, Patnaik M. Simultaneous detection of IgG responses against 7 pneumococcal capsular polysaccharide serotypes (4, 6B, 9V, 14, 18C, 19F, 23F) utilizing a multi-analyte immunoarray assay. First Annual Meeting of the Federation of Clinical Immunology Societies, Boston. *Clin Immunol* 2001, 151–152.
- 28 Peltola H, Kayhty H, Virtanen M *et al*. Prevention of *Hemophilus influenzae* type b bacteremic infections with the capsular polysaccharide vaccine. *N Engl J Med* 1984; **310**: 1561–1566.
- 29 Rosato MT, Jabbour AJ, Ponce RA *et al*. Simultaneous analysis of surface marker expression and cell cycle progression in human peripheral blood mononuclear cells. *J Immunol Methods* 2001; **256**: 35–46.
- 30 Schneider S, Bruns A, Moewes B *et al*. Simultaneous cytometric analysis of (auto)antigen-reactive T and B cell proliferation. *Immunobiology* 2002; **206**: 484–495.
- 31 Atkinson K, Farewell V, Storb R *et al*. Analysis of late infections after human bone marrow transplantation: role of genotypic nonidentity between marrow donor and recipient and of nonspecific suppressor cells in patients with chronic graft-versus-host disease. *Blood* 1982; **60**: 714–720.
- 32 Winston DJ, Schiffman G, Wang DC *et al*. Pneumococcal infections after human bone marrow transplantation. *Ann Intern Med* 1979; **91**: 835–841.
- 33 Kulkarni S, Powles R, Treleaven J *et al*. Chronic graft versus host disease is associated with long-term risk for pneumococcal infections in recipients of bone marrow transplants [In Process Citation]. *Blood* 2000; **95**: 3683–3686.
- 34 Whitney CG, Farley MM, Hadler J *et al*. Increasing prevalence of multidrug-resistant *Streptococcus pneumoniae* in the United States. *N Engl J Med* 2000; **343**: 1917–1924.
- 35 Tauro S, Dobie D, Richardson G *et al*. Recurrent penicillin-resistant pneumococcal sepsis after matched unrelated donor (MUD) transplantation for refractory T cell lymphoma. *Bone Marrow Transplant* 2000; **26**: 1017–1019.
- 36 Clements ML, Miskovsky E, Davidson M *et al*. Effect of age on the immunogenicity of yeast recombinant hepatitis B vaccines containing surface antigen (S) or PreS2 + S antigens. *J Infect Dis* 1994; **170**: 510–516.
- 37 Ward MM, Hall RP, Pisetsky DS. Serum interleukin-2 receptor responses to immunization. *Clin Immunol Immunopathol* 1990; **57**: 120–124.
- 38 Ilan Y, Nagler A, Shouval D *et al*. Development of antibodies to hepatitis B virus surface antigen in bone marrow transplant recipient following treatment with peripheral blood lymphocytes from immunized donors. *Clin Exp Immunol* 1994; **97**: 299–302.
- 39 Hata A, Asanuma H, Rinki M *et al*. Use of an inactivated varicella vaccine in recipients of hematopoietic-cell transplants. *N Engl J Med* 2002; **347**: 26–34.
- 40 Lum LG, Munn NA, Schanfield MS *et al*. The detection of specific antibody formation to recall antigens after human bone marrow transplantation. *Blood* 1986; **67**: 582–587.
- 41 Lum LG, Noges JE, Beatty P *et al*. Transfer of specific immunity in marrow recipients given HLA-mismatched, T cell-depleted, or HLA-identical marrow grafts. *Bone Marrow Transplant* 1988; **3**: 399–406.
- 42 Shiobara S, Lum LG, Witherspoon RP *et al*. Antigen-specific antibody responses of lymphocytes to tetanus toxoid after human marrow transplantation. *Transplantation* 1986; **41**: 587–592.
- 43 Wahren B, Gahrton G, Linde A *et al*. Transfer and persistence of viral antibody-producing cells in bone marrow transplantation. *J Infect Dis* 1984; **150**: 358–365.
- 44 Wimperis JZ, Prentice HG, Karayiannis P *et al*. Transfer of functional humoral immune system in transplantation of T-lymphocyte depleted bone marrow. *Lancet* 1986; **8477**: 339–343.
- 45 Wimperis JZ, Brenner MK, Prentice HG *et al*. B cell development and regulation after T cell depleted marrow transplantation. *J Immunol* 1987; **138**: 2445–2450.
- 46 Wimperis JZ, Brenner MK, Drexler HG *et al*. Rapid recovery of helper activity following T cell depleted allogeneic marrow transplant. *Clin Exp Immunol* 1987; **69**: 601–610.
- 47 Kwak LW, Taub DD, Duffey PL *et al*. Transfer of myeloma idiotype-specific immunity from an actively immunised marrow donor. *Lancet* 1995; **345**: 1016–1020.
- 48 Anderson Jr LD, Mori S, Mann S *et al*. Pretransplant tumor antigen-specific immunization of allogeneic bone marrow transplant donors enhances graft-versus-tumor activity without exacerbation of graft-versus-host disease. *Cancer Res* 2000; **60**: 5797–5802.

- 49 von Mensdorff-Pouilly S, Verstraeten AA, Kenemans P *et al*. Survival in early breast cancer patients is favorably influenced by a natural humoral immune response to polymorphic epithelial mucin. *J Clin Oncol* 2000; **18**: 574–583.
- 50 MacLean GD, Reddish MA, Koganty RR *et al*. Antibodies against mucin-associated sialyl-Tn epitopes correlate with survival of metastatic adenocarcinoma patients undergoing active specific immunotherapy with synthetic STn vaccine. *J Immunother Emphasis Tumor Immunol* 1996; **19**: 59–68.
- 51 White CA, Weaver RL, Grillo-Lopez AJ. Antibody-targeted immunotherapy for treatment of malignancy. *Annu Rev Med* 2001; **52**: 125–145.
- 52 Black S, Shinefield H, Fireman B *et al*. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Northern California Kaiser Permanente Vaccine Study Center Group. *Pediatr Infect Dis J* 2000; **19**: 187–195.
- 53 Gergen PJ, McQuillan GM, Kiely M *et al*. A population-based serologic survey of immunity to tetanus in the United States. *N Engl J Med* 1995; **332**: 761–766.