Clonal Dysregulation of the Antibody Response to Tetanus-Toxoid After Bone Marrow Transplantation


After bone marrow transplantation (BMT), a prolonged dysregulation of humoral immunity can be observed. In the present study, we investigated whether this is reflected in an abnormal production of specific antibodies (Ab) to the T-cell–dependent recall antigen tetanus-toxoid (TT). The study group consisted of children receiving transplants of an unmodified allogeneic graft and of adults receiving either a T-cell–depleted allogeneic or an unmodified autologous BM graft. Findings were compared with those in healthy controls. In pediatric graft recipients, who were routinely revaccinated early after BMT, the Ab response was quantitatively superior to that in adult graft recipients who did not receive early revaccination. In the majority of graft recipients, the time period after vaccination required to reach the peak level of antibodies was prolonged and the number of responding TT-specific B-cell clones was markedly decreased in comparison with controls. In controls, a low frequency of dominant B-cell clones may produce low quantities of homogeneous Ab components (H-Ab) against a heterogeneous background. However, in BM graft recipients, “overshooting” of Ab production by separate B-cell clones was observed, resulting in the development of H-Ab at a relatively high concentration. These abnormalities were present up to 10 years after BMT, irrespective of either the age of the recipient, the modulation of the graft, or the vaccination schedule used. It is hypothesized that the dysregulated Ab production is the consequence of activation of a restricted number of resting memory B cells, present in germinal centers, repopulating gradually after BMT. Our data show that routine revaccination early after BMT improves the humoral immune response. However, because of a clonally dysregulated Ab production, long-lasting qualitative defects may be present even after normalization of Ab titers.

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MATERIALS AND METHODS

Controls and Patients

This study was approved by the Institutional Review Boards on Medical Ethics at the University Hospital (BMT center for children), Leiden, The Netherlands and the Dr. Daniel den Hoed Cancer Center (BMT center for adults), Rotterdam, The Netherlands.

Controls

Sera from 20 healthy infants and children, observed from birth up to the age of 4 years, and from 20 healthy adult volunteers with a median age of 35 years (range, 25 to 45 years) were used as controls.

Pediatric Patients

Children receiving grafts of BM cells from an HLA-identical sibling between 1982 and 1989, who were cured of their original disease and who are alive and well, were included in the study (n = 38). The characteristics of these patients are given in Table 1. All received an unmodified BM graft while staying in the protective environment of a laminar flow isolator and after antimicrobial suppression of their intestinal micro-flora. By January 1, 1993, the median follow-up after BMT was 7.5 years (range, 1.0 to 11.0 years).

As we previously reported, complete chimerism was present in the majority of patients.20

Adult Patients

Recipients treated with either an HLA-identical sibling graft (n = 26) or an autologous graft (n = 9) between 1980 and 1992 who...
were alive and well were included. The characteristics of these patients are given in Table 1. In case of an allogeneic BMT, the graft was more than 2 log T-cell-depleted by albumin gradient centrifugation and E rosette sedimentation. By January 1, 1993, the median follow-up of these patients was 2.5 years (range, 0.5 to 13.0 years).

Methods

Vaccination

Controls. Twenty healthy infants and children were vaccinated according to the Dutch National Vaccination Program with diphtheria toxoid (D)-pertussis (P)-tetanus toxoid (T)-inactivated polio virus type I, II, and III (IPV) at 3, 4, 5, and 11 (n = 16) or at 14 (n = 4) months (DPT-IPV I to IV) and with DT-IPV at 4 and 9 years of age (DT-IPV V and VI; Table 2). In addition, 20 healthy adult volunteers received a DT-IPV booster (B) vaccination.

Pediatric patients. The patients and their BM donors had been vaccinated during infancy and early childhood with D(P)T-IPV vaccine, according to the Dutch National Vaccination Program. Twenty-seven of 38 patients were immunized with DT-IPV within 1 month before BMT (DT-IPV 0; Table 2). In the light of other studies, 5 BM donors also received a DT-IPV vaccination within 2 weeks before graft donation.

In the first 4 months after BMT, the recipients received 3 DT-IPV booster injections (I to III) with an interval of 1 month, starting from 6 weeks after BMT. A limited number of children received an additional injection (IV) between 6 and 24 months after BMT. In 1992, 21 children with a follow-up period of at least 3 years (range, 3 to 10 years) after BMT again received a DT-IPV injection (DT-IPV V).

Adult patients. As far as could be evaluated, all adult BM graft recipients and their donors had received routine D(P)T-IPV vaccinations in infancy and childhood. In addition, most male patients had received a DT-IPV injection at the start of their military service. None of the adult patients was vaccinated shortly before BMT and none received revaccination after BMT until the occurrence of an outbreak of poliomyelitis in The Netherlands (November 1992). At that time, all patients received three DT-IPV vaccinations at monthly intervals and one vaccination 6 months later. The response to the second vaccination (DT-IPV II) was investigated.

Serum Samples

Serum samples were obtained after informed consent before and at a median of 3 weeks (range, 2 to 4 weeks) after vaccination, with the following exceptions. From children of the control group receiving DPT-IPV IV at 14 months of age (n = 4), the postvaccination serum sample was taken 4 weeks later; from those immunized at 11 months of age (n = 16), serum was taken at 4 months after vaccination. Cord blood samples were available from all healthy infants included in the study. Sera from healthy adults were obtained at 2 and 4 weeks and at 3, 6, and 12 months after the booster. From pediatric graft recipients, serum samples were also obtained at 3 and 6 months after DT-IPV IV. From adult graft recipients, only one serum sample, taken 4 weeks after the second DT-IPV vaccination, was available. All sera were kept frozen at −20°C until analysis.

Serum Analysis

Quantification of IgG anti-TT antibodies. The concentration of IgG anti-TT antibodies was measured by enzyme-linked immunosorbent assay (ELISA), as has previously been described. The concentration was expressed in arbitrary units (AU) per milliliter using a reference serum for standardization. The Ab response in healthy children after DT-IPV VI at 9 years of age was not investigated in this study. Data on IgG anti-TT levels after vaccination of 8 pediatric graft recipients, serum samples were also obtained at 3 and 6 months after DT-IPV IV. From adult graft recipients, only one serum sample, taken 4 weeks after the second DT-IPV vaccination, was available. All sera were kept frozen at −20°C until analysis.

Definition of response to TT. In healthy infants (controls), a primary Ab response to TT was scored as positive if the postimmunization IgG titer was at least twice the preimmunization titer and reached >0.2 AU/mL. In pediatric and adult controls and in the pediatric patients a secondary Ab response was considered positive in case of an increase of the postimmunization IgG titer to 125% of the preimmunization titer, reaching at least 1.0 AU/mL. In two cases, newly arising TT-specific homogeneous Ab components (H-Ab) were detected in the absence of an increase of the Ab titer.
Controls

<table>
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<th>III</th>
<th>IV</th>
<th>V</th>
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<td>19</td>
<td>20</td>
<td>19</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>Age* Median</td>
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<td>4 mo</td>
<td>5 mo</td>
<td>12 mo</td>
<td>4 yr</td>
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<td>14-20 wk</td>
<td>6-24 wk</td>
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Pediatric patients

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<th>III</th>
<th>IV</th>
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<td>38</td>
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<td>9 mo</td>
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<td>14-20 wk</td>
<td>6-24 wk</td>
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Adult patients

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<tbody>
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<td>Period post-BMT Median</td>
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</tr>
<tr>
<td>Range</td>
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</table>

Abbreviation: -, before BMT.

*The Dutch National Vaccination Program also provides vaccination at 9 years of age. The response to this immunization was not investigated.

0-V, D(PT/IPV) vaccination number in healthy infants and children, and in BMT recipients. B, DT-IPV booster vaccination in adult controls.

finding was interpreted as an indication for a positive TT response. In the adult patients, a preimmunization sample was not available. In these patients, an Ab response was defined as positive if the postimmunization titer was \( >1.0 \text{ AU/mL} \).

**TT-specific immunoblotting.** To investigate the heterogeneity of IgG anti-TT and the occurrence of H-Ab, we applied agar gel-electrophoresis of serum samples according to Wieme, followed by TT antigen-specific immunoblotting (WABL) onto nitrocellulose membranes coated with 10 \( \mu g/mL \) (3 \( \mu g/cm^2 \)) of TT. The patterns were developed using Ig isotype-specific monoclonal antibodies (MoAbs: Nordic Immunological Laboratories, Tilburg, The Netherlands), as described earlier. The serum samples (diluted 1:250) were investigated for TT-specific IgG patterns together with a positive and a negative control (Fig 1A; these human MoAbs were kindly provided by Dr W.P. Zeijlemaker, CLB, Amsterdam, The Netherlands). In 7 selected patients with H-Ab at a relatively high concentration (0.2 to 2 \( \mu g/mL \)) in the first year after BMT, we investigated whether the H-Ab present at 3 to 10 years after BMT were still produced by the same B-cell clones. This investigation was performed by TT-specific WABL and by isoelectric focusing (IEF) followed by TT antigen-specific immunoblotting (FABL) for the characterization of the IgG subclass and light chain type of the H-Ab.

**Assessment of WABL patterns.** Overall patterns and presence of H-Ab were analyzed independently by two investigators (E.J.A.G. and M.J.D.v.T.). The patterns were scored as either heterogeneous or of restricted heterogeneity (Fig 2). Besides the number of H-Ab, the concentration of H-Ab was assessed semiquantitatively as high (>0.5 \( \mu g/mL \)), medium, or low (<0.1 \( \mu g/mL \)), using the positive control sample diluted in phosphate-buffered saline (PBS) as a reference (Fig 1B). The limit of sensitivity for the detection of TT-specific H-Ab by WABL ranges from 5 to 50 ng/mL, depending on the intensity of the pattern of the heterogeneous antibodies.

**Statistical evaluation.** Response percentages and frequencies of H-Ab were compared with \( \chi^2 \) tests on 2 \( \times \) 2 tables, and 95% confidence intervals for the difference of the percentages were calculated. The titer values were analyzed using the Student’s \( t \)-test.

**RESULTS**

**Quantification of IgG Anti-TT Antibodies**

**Controls.** After the first vaccination, 45% of the infants responded to TT (Table 3). The infants who responded had a lower preimmunization Ab titer in the cord blood and in the prevaccination sample than did the nonresponders (\( P < .001 \) and \( P < .01 \), respectively; data not shown). This finding indicates that, in the presence of passively acquired maternal IgG anti-TT antibodies, the actual Ab production by the infant is delayed. After further vaccinations, the cumulative response rate reached 95%. Infants at 12 months of age, children at 4 years of age, and adults all mounted an Ab response.

![Table 2. Vaccination Protocols in BMT Patients and Controls](image)

![Fig 1. TT-specific immunoblotting. (A) Specificity of TT-specific immunoblotting (WABL). Abbreviations: H-Ab a TT, human IgG1 MoAb against tetanus toxoid (concentration, 0.2 \( \mu g/mL \)); H-Ab a HB, human IgG1 MoAb against hepatitis B surface antigen (concentration, 20.0 \( \mu g/mL \)); Ig, blotting on noncoated membrane (signal intensity is related to protein concentration); TT, blotting on TT-coated membrane (only signal of TT-specific MoAb is obtained with increased intensity); BSA, blotting on BSA-coated membrane (no signal is obtained). (B) Sensitivity of WABL and semiquantitative determination of homogeneous antibodies (H-Ab) at low (A), medium (B), and high (C) concentration. The values from 0.02 to 1.0 indicate the concentration range of MoAb against TT in micrograms per milliliter.](image)
After the first vaccination, at 7 weeks after BMT, 30% of the pediatric recipients responded. There was no statistically significant difference between responders and nonresponders with respect to prevaccination Ab titers, substitution with gammaglobulins, or treatment with cyclosporin A. Two revaccinations at 11 and 15 weeks after BMT resulted in a cumulative response rate of 75% of the graft recipients. Although this finding was not investigated systematically, we observed that the maximum Ab titer was reached at 4 weeks or even later after immunization and not at 2 weeks as in adult controls after booster vaccination.

Revaccination between 6 and 24 months after BMT resulted in a 100% response rate and a significantly increased GMT (Table 3). There was no difference between patients with \( n = 6 \) or without \( n = 17 \) preceding acute GVHD. Booster vaccination at 3 to 10 years after BMT resulted in a 100% response rate and a GMT similar to that in controls. Follow-up investigations showed a half-life of Ab titers comparable to that in adult controls (data not shown).

**Adult patients.** There were no significant differences with respect to the percentage of responders between adult patients who received a TCD allogeneic graft \( (4 \text{ of } 26) \) and those who received an autologous graft \( (4 \text{ of } 9) \), nor was there a difference between patients vaccinated 0.5 to 2 years \( (6 \text{ of } 17) \) and those vaccinated more than 2 years after BMT \( (12 \text{ of } 18) \). Also, the GMT was not different between these subgroups. Ten adult patients \( (30\%) \) had an IgG anti-TT titer after two DT-IPV immunizations that may not have been protective, ie, less than 0.1 AU/mL.

**Heterogeneity and Oligoclonality of IgG Anti-TT**

**Controls.** TT-specific H-Ab were hardly detectable in cord blood and sera of infants after four DPT-IPV vaccinations (Fig 3A). After revaccination at 4 years of age or in adulthood, either one or two TT-specific H-Ab of low concentration \(<0.1 \mu g/mL\) could be observed in serum samples of about 35% of the controls, on top of an overall heterogeneous Ab response (Figs 2B and 3A).

**BMT patients.** TT-specific H-Ab of low concentration could be observed in preimmunization serum samples of about 25% of the pediatric patients before BMT (Fig 3B). After pre-BMT vaccination, this percentage was 50%, which was not significantly different from healthy children at the age of 4 years and from adults. However, after the first and second post-BMT vaccination, it increased significantly to 75% and 100%, respectively. In addition, the number of H-Ab per serum sample and the quantity of some H-Ab increased (Figs 2C and D and 3B).

After booster vaccination in the second half year after BMT, H-Ab at a medium and high concentration were found in about 50% of the patients. At follow-up investigation, 3 to 10 years after BMT, 75% of the patients still had H-Ab in their serum. In some cases, these H-Ab were of relatively high concentration, ie, greater than 1.0 \( \mu g/mL \) (Figs 2D and 3B).

In about 30% of the adult graft recipients who responded to TT vaccination, the postimmunization serum showed H-Ab. These H-Ab were also frequently at a relatively high concentration. In general, there was a strong correlation between the presence and concentration of H-Ab and the quan-
In the first year after BMT, WABL and FABL showed a quantitative decrease in the number of T-helper cells and precursors. This decrease is in contrast to the finding of a relatively high number of CD4+ CD45R0+ T lymphocytes in peripheral blood after BMT, unless this population belongs to the TH2 subset. Alternatively, the majority of T cells in the germinal centers of BMT recipients may be activated by other as yet unknown mechanisms. It is attractive to speculate that alloantigens may be involved in this activation. B cells may interact with activated T cells in a major histocompatibility complex (MHC)-unrestricted and antigen nonspecific way.
CLONAL DYSREGULATION OF THE ANTIBODY RESPONSE

Fig 3. Percentage (and 95% confidence intervals) of responders with H-Ab in IgG anti-TT. (A) Healthy pediatric and adult controls before (b) and after (a) the indicated DT-IPV vaccination (*). (B) Pediatric patients before and after BMT and adult patients after BMT. The sequential DT-IPV vaccinations are indicated (*), see also Table 2. Sera were investigated by WABL before (b) and after (a) vaccination. Note the marked difference in frequency (*P < .01, **P < .001) and concentration of H-Ab in patients compared with children after DT-IPV and adults after boostervaccination (see also Fig 2). H-Ab at a (C) low, (B) medium, and (V) high concentration, respectively. The numbers indicate the number of individuals investigated: all individuals were analyzed before (b) vaccination; only samples of responders were investigated after (a) vaccination.

and proliferate and mature to Ig-producing cells in the presence of interleukins (ie, IL-4 and IL-10). Interestingly, Ig levels in graft recipients with acute GVHD are significantly higher than in recipients without.

In the present study, we showed that the occurrence of H-Ab at a low concentration within an otherwise heterogeneous response is a normal phenomenon, because this type of clonal dominance was also found in healthy children after DT-IPV vaccination and in adult controls after booster vaccination. Clonal dominance has also been described in adults after influenza vaccination. However, in BM graft recipients, the restricted heterogeneity of TT-specific IgG and the development of H-Ab at a relatively high concentration has to be considered as an abnormal response. This oligoclonal Ab response was found both in children receiving grafts of unmodified BM and vaccinated before and early after BMT and in adults receiving grafts of either autologous or T-cell-depleted allogeneic BM who did not receive revaccination early after BMT. Previous studies in immune-deficient mice and in Rhesus monkeys after BMT also showed a similar response. There are at least two possible explanations for this restricted response of prolonged duration. First, it may be the result of the transfer of a limited number of memory B-cell clones from the donor. This hypothesis is in accordance with the finding of a skewed Ig VH repertoire of B cells early after BMT. Under certain conditions, booster vaccination of the BM donor 2 weeks before donation has been shown to increase the quantitative Ab response in the recipients. On careful observation of the data presented in some of those reports it can be concluded that this response was also oligoclonal. Second, it may be that only a limited number of memory B cells become activated in view of the restricted possibilities for cell-cell contact with T-helper cells, resulting from underrepresentation of the latter population in germinal centers after BMT. Results of animal experiments are in favor of the latter hypothesis because restricted heterogeneity of Ig and H-Ig develop after thymectomy and disappear again after infusion of peripheral T cells. However, the persistence of a restricted Ab response and of H-Ab of high concentration in a number of graft recipients many years after BMT, when numbers and functions of specific T cells have become normal, indicates that, in addition to abnormalities at the T-cell level, abnormalities at the B-cell level may persist. In murine graft recipients, germinal center reconstitution after BMT has been shown to be oligoclonal after antigenic stimulation. In human BMT, a delayed reconstitution of germinal centers has been described in cases with GVHD.

Our findings of an impaired specific Ab response post-BMT have clinical implications with respect to vaccination policy of BMT recipients. Previous studies showed long-lasting but transient persistence of donor immunity in non-vaccinated BMT recipients. Vaccination of the donor before graft donation, together with vaccination of the recipient early after BMT, may contribute to a quantitatively good Ab response. BM graft recipients respond to vaccinations in the following sequence: TD-recall antigens (at about 3 months), TD-primary antigens (at about 6–12 months), and T-cell-independent antigens (at about 1+ year). This sequence can be observed after BMT with either an unmodified graft, a T-cell–depleted graft, or a B-cell depleted graft. However, under the latter condition, all responses are primary because of the lack of transferred memory B-cell clones. In the present study, we showed that repeated vaccinations of children early after BMT resulted in a quantitatively enhanced specific humoral immunity at 6 to 24 months after BMT, whereas this response in adults was not yet normal at 2 years post-BMT after two vaccinations. However, we do not know whether the qualitative abnormalities, present as a clonally restricted response, will provide adequate protection. It may be that increased quantity compensates for decreased quality of antibodies in the first years after BMT.

With our very sensitive technique we have clearly shown long-lasting oligoclonality at the B-cell level after BMT. Preliminary data on the T-cell receptor repertoire, which is of relevance for antigen-specific help for B cells, do not indicate a long-lasting oligoclonality at the T-cell level.

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Fig 4. Follow-up investigation of H-Ab in IgG anti-TT in 3 patients after BMT. WABL (antigen-specific immunoblotting after agar gel electrophoresis) and FABL (antigen-specific immunoblotting after isoelectric focusing) patterns of sera from 3 children early after BMT (obtained after DT-IPV III) and 6 to 8 years later (obtained after DT-IPV V). (A) Sera of 1984 and of 1992 showed an almost identical Ab pattern of restricted electrophoretic heterogeneity and the presence of a TT-specific H-Ab at a high concentration. WABL combined with IgG subclass and light chain type immunoblotting identified this H-Ab as IgG1κ (data not shown). FABL patterns showed identity of the dominant clone. The pattern of the latter serum also shows some changes in the expression level of other clones. (B) The serum of 1986 showed a restricted pattern and an H-Ab at a high concentration. The serum of 1992 showed a more heterogeneous pattern with other dominant clones in addition to the original clone. (C) The serum of 1985 showed a restricted pattern with an H-Ab at a high concentration. The serum of 1992 showed a heterogeneous pattern. The values from 8.0 to 112.0 represent the IgG Ab titer to TT in AU per milliliter. The values from 1:25 to 1:500 indicate the dilution of the serum samples.

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REFERENCES


2. Storek J, Saxen A: Reconstitution of B-cell immunity following bone marrow transplantation. Bone Marrow Transplant 9:395, 1992


45. Lum LG, Munn NA, Schanfield MS, Storb R: The detection of specific antibody formation to recall antigens after human bone marrow transplantation. Blood 67:582, 1986


49. Rencher SD, Manif I, Heslop HE, Turner VE, Brenner MK, Hurwitz JL: Reconstitution of the T-cell receptor $\alpha\beta$ repertoire in recipients of allogeneic BMT. Bone Marrow Transplant 10:521, 1992
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