Immunoglobulin Levels and Monoclonal Gammopathies in Children After Bone Marrow Transplantation

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Bone marrow graft recipients suffer profound immunodeficiency during at least 3 months after transplantation. B-cell reconstitution following allogeneic bone marrow transplantation (BMT) in children was studied longitudinally by quantification of Ig (sub)classes levels in serum and by investigation of numbers and characteristics of homogeneous Ig components (H-Ig): ie, monoclonal gammopathies (MG). For the latter purpose, a sensitive immunoblotting technique capable of detecting H-Ig of a concentration as low as 0.5 μg/mL was used. Sera of 40 children grafted for a variety of diseases were investigated and followed up for 5 years. It was found that Ig (sub)classes reached normal levels from 3 months after BMT onward. The sequential increase of the different Ig isotypes was in accordance with that seen in normal ontogeny. This was especially clear following BMT for severe congenital immunodeficiency. H-Ig appeared from as early as 6 weeks after BMT in increasing numbers, beginning within IgM, IgG3, and IgG1, and afterward within other isotypes. After an initial increase of serum Ig levels, “overshooting” occurred accompanied by high frequency of H-Ig. H-Ig were still present at 5 years after BMT, when Ig levels normalized. Our data indicate that B-cell reconstitution after allogeneic BMT recapitulates normal ontogeny but in a clonally dysregulated fashion; that is, with overexpression of some clones and underexpression of others.

THE RECONSTITUTION of the immune system after allogeneic bone marrow transplantation (BMT) has been the subject of a large number of studies.1,2 Recovery of B-cell function can generally be achieved within 1 to 2 years in survivors of BMT, not suffering from chronic graft-versus-host disease (GVHD).3 With respect to levels of immunoglobulin (Ig) isotypes post-BMT, both transient decreases and persistent deficiencies associated with bacterial infections have been reported in adults.4-6 In addition, restricted electrophoretic heterogeneity of serum Ig and the appearance of homogeneous Ig components (H-Ig), that is, monoclonal gammopathies (MG) (first reported by our group in single cases of severe combined immunodeficiency [SCID] after BMT7), could be detected in up to 100% of BMT recipients depending on the sensitivity of the detection technique applied.8-10 In this study, we simultaneously investigated levels of Ig isotypes and the appearance of H-Ig within these isotypes. This was done in sera of 40 children grafted for a variety of diseases and followed up for 5 years after BMT. From our data, it may be concluded that B-cell reconstitution recapitulates normal ontogeny but in a clonally dysregulated fashion, which may last for as long as 5 years after BMT.

MATERIALS AND METHODS

Patients

Between 1974 and 1988, 102 children received allogeneic bone marrow (BM) grafts at the Leiden Department of Pediatrics for a variety of diseases. At 1 year after BMT, 67 children were alive. Forty of these children were randomly chosen and included in this retrospective study (primary T-cell deficiency or [severe] combined immunodeficiency [SCID] n = 8, severe aplastic anemia [SAA] n = 6, acute myeloblastic leukemia [AML] n = 13, acute lymphoblastic leukemia [ALL] n = 13). The relevant data concerning the patients are given in Table 1. Except for six SCID patients grafted with T-cell depleted BM from an HLA genotypically haploidentical donor, they all received a full BM graft. Thirty-six donors were younger than age 16 years, and four donors were adults (parents). All patients were transplanted within the protective environment of a laminar flow isolator and following antimicrobial suppression of their intestinal microflora.17 Low-dose cotrimoxazole and penicillin prophylaxis was given, respectively, for 150 days and 1 year after BMT. Ig suppletion up to about 3 months post-BMT was given in 14 patients. On January 1, 1992, the median follow-up of the children of the study was 7.2 years (range 1.0 to 17.2).

Approval was obtained from the Institutional Review Board for these studies. Informed consent was provided according to the Declaration of Helsinki.

Methods

Serum samples. Serum samples were taken from the BM recipient 3 weeks before BMT and longitudinally thereafter at +3, +6, and +9 weeks, +3, +4.5, +6, and +9 months, and +11, +2, +3, +4, and +5 years after BMT. A serum sample was also obtained from the BM donor at pretransplant investigation. The sera were kept frozen at –20°C until analysis.

Quantification of immunoglobulin (sub)classes. IgM, IgG, and IgA quantification was performed by single radial immunodiffusion according to Mancini in a modification as described previously.11 IgG subclass levels were determined in all samples except for those taken at +3 and +6 weeks and +4.5 months by enzyme-linked immunosorbent assay (ELISA) or dot immunobinding assay (DIBA) as previously described.12 Quantities of Ig isotypes were expressed as percentage of the mean value of age-matched controls according to the data of Cejak13 for IgM, IgG2, and IgA according to a compilation of data from the literature14 for IgG subclasses; the latter values are in agreement with age-dependent normal values as given in other publications.15 Absolute levels were considered normal if they were within ±2 SD of the mean value as derived from literature data.12,14

In six patients with SCID, daily plasma transfusions were given.

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To circumvent interference in the results, the following data of these patients were excluded from evaluation: IgM, IgA, and IgG3 levels from samples taken during plasma infusions and until 1 month after the last infusion and IgG1, IgG2, and IgG4 levels during plasma infusions and until 3 months after the last infusion (this was necessary because of differences in the catabolic rate of the different isotypes). In eight patients with acute leukemia, intravenous IgG infusions were given weekly. In these patients, data on serum IgG levels were also suppressed during IgG suppletion, and, further, IgG subclass levels were suppressed as mentioned before for patients with SCID.

Thirty-five patients received small quantities of plasma (less than 10 mL/kg/wk for about 2 months) to supply trace elements during parenteral nutrition, for which the results were not corrected. When severe immunosuppressive treatment with corticosteroids and azathioprine was given for extensive chronic GVHD (n = 6), the data on Ig isotype levels were also excluded from evaluation.

**Immunoblotting for H-Ig**. Sera from 18 of the 40 patients (SCID n = 6, SAA n = 6, acute leukemia n = 6) were investigated for the presence of H-Ig by agar gel electrophoresis according to Wieme in a modification as described earlier.28 To characterize the isotype of H-Ig, immunoblotting (IBL) of diluted serum samples after agar gel electrophoresis was applied as previously described.29 The serum dilutions and monoclonal antibodies (MoAbs) used are given in Table 2. Characterization of H-Ig was performed according to the criteria previously described.28 Only those H-Ig components were scored that were registered by two investigators independently. Using high-resolution agar gel electrophoresis, H-Ig with a concentration of about 100 μg/mL can still be detected; however, this depends on the background Ig pattern.32 The sensitivity limit for detecting H-Ig with complete characterization of Ig (sub)class and light chain isotype by IBL was between 0.5 and 5.0 μg/mL; again depending on the isotype under investigation and the background pattern.29 For IgG H-Ig, we used IgG subclass immunoblotting, which can usually detect H-Ig at a concentration as low as 0.5 μg/mL. Because of the discrepancy in the sensitivity limit between light chain and IgG subclass IBL, IgG subclass H-Ig of concentrations approximately ≤1 μg/mL could not be typed for light chains.

**Evaluation of infections**. The diagnosis of a severe invasive bacterial or fungal infection was based on clinical symptoms and confirmed microbiologically. Bacterial infections associated with profound neutropenia (n = 3) shortly after BMT or the presence of a deep-indwelling catheter (n = 4) were not taken into consideration. Only viral infections or reactivations caused by cytomegalovirus (CMV), varicella-zoster virus (VZV), and Epstein-Barr virus (EBV) were included in the evaluation. For the diagnosis of CMV infection, weekly surveillance cultures of urine and throat were performed for at least 8 weeks after BMT. A possible CMV infection was defined when the combination of a period of unexplained fever, positive CMV isolation, and a greater than twofold increase of antibody titer in the following months occurred. The diagnosis of VZV infection was based on clinical symptoms only. Possible EBV infections were confirmed late after BMT; that is, by a greater than twofold increase of antibody titer in sera routinely taken at +3, +6, and +12 months post-BMT. Infections with herpes simplex virus (HSV), mostly stomatitis occurring in the neutropenic period around the BMT, and other possible viral infections were not taken into consideration.

**RESULTS**

### Quantification of Immunoglobulin (Sub)classes

#### Donors.
Thirty-seven of the 40 donors (median age 11.6 years, range 1.3 to 60.2 years) were investigated for IgM, IgG, and IgA levels. In 31 of these donors, levels of all Ig classes were within the normal range (Fig 1). In 21 of 32 donors investigated, all IgG subclasses were normal. Selective complete deficiencies were not found (Fig 2).

#### Patients with SCID.
The findings in patients with SCID before and after BMT varied widely (an example of one patient is given in Fig 3). Before BMT, five of eight patients had been treated with intravenous Ig infusions. In one 5-month-old infant, without Ig suppletion, only a very low quantity of maternal IgG could be detected. In three children with Nezelof-type SCID,15 normal (n = 2) or even increased (n = 1) IgG levels were found at the ages of 7, 30, and 32 months, respectively. Increased IgM levels were found in three patients and below normal levels in the other five. IgA levels were normal or above normal in the patients with Nezelof-type SCID and adenosine deaminase (ADA)
deficiency. In the other four patients with SCID (T-B+), a complete IgA deficiency was observed.

At 1 year after BMT abnormal Ig levels (within one or more isotypes) were found in all eight children. At 5 years after BMT, all Ig levels were normal in six out of eight children. In two patients with SCID (T-B+), a complete and selective IgA deficiency persisted.

Patients with leukemia and SAA. In only 3 of 32 patients with leukemia and SAA, all seven isotypes tested were within the normal range before BMT (Figs 1 and 2). In one child with SAA, a complete IgA deficiency was observed. In 20 children, a decreased level of one or more Ig isotypes was found. Increases above the normal range were present in eight patients probably as a consequence of previous infections.

After BMT, Ig (sub)class levels dropped to a nadir and deficiencies developed in 27 out of 32 patients. Subsequently, the Ig levels increased in the course of time and reached the median level for age-matched controls at the following sequence. For the Ig classes: IgM (+7 months) followed by IgG (+9 months), whereas IgA levels reached the median level at 5 years after BMT (Fig 1). For IgG subclasses, the sequence was IgG1 (+5 months), IgG3 (+9 months), IgG2 and IgG4 (≥2 years). In one patient, incomplete IgA deficiency persisted at 5 years after BMT.

In compiling the data from all patients, markedly increased quantities, especially of IgM, IgG1, and IgG4, were found in the majority of cases (Figs 1 and 2), although the individual kinetic patterns were rather variable (Fig 3). In four of six patients in whom immunosuppressive treatment for chronic GVHD was given, an increase of IgM simultaneously with a decrease of IgG and IgA was noted.

Ig (sub)class deficiencies and infections. A total of 37 infections, of which 34 occurred within 12 months after BMT, were diagnosed in 22 patients (Table 3). Two children acquired an invasive bacterial infection: a septicaemia (Enterobacter sp) at 1 year after BMT, associated with IgG1 and IgG2 deficiency in one child, and a pneumonia (pneumococci) at 3 years after BMT in another child suffering from chronic GVHD associated with a deficiency of IgA and all IgG subclasses. Invasive bacterial infections did not occur when serum Ig levels were within normal ranges. Thirty-three viral infections or reactivations were diagnosed post-BMT. Twenty of these episodes were associated with Ig (predominantly IgG) deficiencies and 13 episodes occurred when serum Ig levels were within normal ranges. Vice versa, in about 70% of Ig-deficient episodes, infections did not occur (Table 3).

Immunoblotting for H-Ig

Individuals with H-Ig. Before BMT, immunoblotting was performed in the serum of 18 patients and their donors. Using Ig isotype and IgG subclass immunoblotting, one or more low-concentration H-Ig were detected in the serum of six donors (33%). Four of these might have had some immunologic abnormality; that is, one also had complete IgA deficiency, one was heterozygous for ADA deficiency, one was a familial abnormality; that is, one also had complete IgA deficiency, one was heterozygous for ADA deficiency, one was the father of a patient with SCID, and one recovered from a recent pneumonia. Low-concentration H-Ig were found also in the serum of 10 of 12 patients (88%) with leukemia or SAA. In all patients with Nezelof-type SCID, strong (ie, high-concentration) H-Ig were found, whereas in the other patients with SCID H-Ig were not detected.

Three weeks after BMT, H-Ig were present in the serum of 10 of 18 patients (56%). From 3 weeks after BMT onward, this percentage increased up to 100% at the third month after BMT (Fig 4). Follow-up investigation showed only a slight decrease in the number of patients with H-Ig: one or more H-Ig of low concentration could still be detected by immunoblotting in the serum of 13 of 18 patients (70%) at 5 years after BMT. No correlation was found between the appearance of H-Ig in a given Ig (sub)class and the serum level of the corresponding isotype (Table 4).

Number of H-Ig in the serum samples. In the serum of 6 of 18 BM donors, a mean number of 2.5 low-concentration H-Ig could be detected by heavy chain (sub)class immuno-
Fig 1. IgM, IgG, and IgA levels in children with severe aplastic anemia or acute leukemia determined longitudinally after alloge

neric BM transplantation. D: levels in BM donors; ratio: level in individual divided by median level in age-matched controls; solid line indicates the mean of the individual ratios. Months: months after BMT.

(0) Absolute level within normal range.

(0) Absolute level below normal range.

(0) Absolute level above normal range.

Fig 5). Before BMT, a mean number of five relatively strong H-Ig was found in the three patients with Nezelof-type SCID. In the serum of 10 of 12 patients with acute leukemia or SAA, a mean number of 3.6 H-Ig was present. Shortly after BMT, the mean number of H-Ig present in the serum of H-Ig-positive patients decreased. From 9 weeks onward, the mean number of H-Ig increased to a maximum of 8.2 per H-Ig-positive serum sample at 6 months after BMT. Subsequently, at 4 years after BMT, the mean number of H-Ig decreased to values as before BMT. No differences were observed between patients with SCID and leukemia or those with SAA.

The number of H-Ig that could be simultaneously characterized for both heavy chain and light chain type was consistently lower, but the changes in time of the numbers of fully characterized H-Ig after BMT showed a similar pattern as that of H-Ig characterized only for heavy chain isotypes (Fig 5).

Isotype distribution of H-Ig. In patients with SCID, at 3 to 6 weeks after BMT, the H-Ig were almost exclusively of IgM, IgG3 (peak frequency at 3 to 6 weeks), and IgG1 isotypes (peak frequency at 9 to 12 weeks) (Fig 6A). At a later stage after BMT, H-Ig of the IgG2 and IgG4 isotypes were also found but at a lower percentage. Remarkably, H-Ig with IgA isotype could not be detected. In patients with leukemia and SAA early after BMT, H-Ig of IgG2 isotype could be detected at a relatively high frequency; in addition to H-Ig of IgM, IgG3, and IgG1 isotypes at a later stage after BMT, H-Ig of IgG4 and IgA isotypes were also found at a low frequency.

As a result of differences in the sensitivity of heavy versus light chain immunoblotting, only 319 H-Ig (about 35% of the total number of H-Ig detected after BMT) could unequivocally be characterized for both heavy and light chains. Eighteen of the 34 fully characterized H-IgM were of the kappa light chain type and 16 of the lambda light chain type (ratio 1.2). For H-IgG (n = 285) these numbers were 167 kappa and 118 lambda, respectively (ratio 1.4). The kappa/lambda ratio for H-IgG in patients with leukemia and SAA after BMT showed a ratio of about 1.2 early after BMT, an increase to about 2.0 at 2 years after BMT, and a decrease to about 1.2 after that time. In patients with SCID, the kappa/lambda ratio reached a peak of 4.5 at 9 months after BMT and decreased later on to about 1.2.

DISCUSSION

An ontogenesislike sequence of Ig (sub)class recovery after BMT was reported long ago in single cases grafted for SCID.9,33,34 In the present study, we extended the investigation to a large group of children, including those grafted for SAA and acute leukemia, and confirmed this observation. The same sequence of the Ig isotype increase was reported more recently in adult patients mostly grafted for leukemia.4,6,8,35,36 However, the latter reports, focused on long-lasting Ig subclass (IgG2 and IgG4) and IgA deficiencies and their relation to infectious complications but did not investigate the longitudinal Ig isotype reconstitution systematically. Recapitulation of Ig levels according to normal ontogeny is compatible with the observation that a high proportion of peripheral B cells in the first months after BMT show the phenotype and functional characteristics of cord blood B cells.35,37-39 An ontogenetic recapitulation has also been reported after T-cell-depleted allogeneic BMT,36,37 after autologous BMT,37,40 and even following intensive chemotherapy for leukemia.41 As in adults, we also found Ig (sub)class deficiencies in children. However, these deficien-
cies were transient in the majority of cases. In this study, we did not focus on a possible relationship between Ig deficiencies and infections. The incidence of severe bacterial infections was low in our patients. There was a trend toward an increase of infections during the Ig-deficient period as was found in adults. Therefore, temporary Ig suppletion early after BMT may have a beneficial effect for BM graft recipients.

In two patients with SCID (T-B), a complete IgA deficiency persisted until last follow-up 7 years after T-cell-depleted haploidentical BMT. Persistent and complete IgA deficiency has only been reported incidentally in a case of an IgA-deficient donor and in rare cases with chronic GVHD. Levels of IgA were normal in the BM donors of these two patients with SCID. We also recently investigated lymphoid chimerism in these children using polymerase chain reaction (PCR)-amplified variable number of tandem repeat markers. One child did not receive a conditioning regimen before BMT and developed mild GVHD: T cells were exclusively of donor origin and B cells were predominantly of recipient origin. The other child received moderately intensive conditioning with cyclophosphamide and had no GVHD: T cells were predominantly of donor origin and B cells were exclusively of recipient origin. The persistence of recipient B cells after haploidentical BMT in SCID, as observed in many cases not pretreated with cytoxic conditioning, was found to be strongly associated with abnormal B-cell function after BMT. In one single-center study, a severe deficiency of all isotypes was found and required gamma globulin suppletion. In our patients, the deficiency was restricted to IgA. Investigation is still needed as to whether an inappropriate T-B cooperation, maybe as a result of HLA disparity between both subpopulations, caused the selective Ig isotype deficiency in our cases, assuming a role for T cells in the Ig isotype switching. Alternatively, an intrinsic B-cell defect next to a T-cell defect, as observed in some obligate carriers of X-linked SCID by unilateral X chromosome inactivation tests, may be present. Theoretically, both children may have inherited class III genes associated with IgA deficiency; the latter were not determined.

The kinetics of H-Ig development after BMT, and the
relation with changing Ig levels in serum, were also investigated. When using high-resolution agar gel electrophoresis with a detection limit of 100 μg/mL, a high frequency of H-Ig could be detected in the serum of 26% to 88% of BMT recipients as reported earlier by us and by others. A high frequency of H-Ig was also found in children with other primary and secondary immunodeficiencies but not in healthy children. In this study, we characterized H-Ig after BMT with a sensitive immunoblotting technique. As a consequence, not only strong, highly concentrated H-Ig but also faint, lowly concentrated H-Ig could be detected. Remarkably, in the serum of 33% of the BM donors, H-Ig of low concentration were detected. This cannot be explained by a 100-fold increase of the sensitivity of IBL as compared with conventional techniques alone, because a lower frequency was present in healthy blood donors investigated by us before with the same technique. In four out of six healthy donors with H-Ig, factors possibly predisposing for immune dysregulation were present; again indicating the sensitivity of IBL to detect this dysregulation. Even before BMT, a fairly high frequency of H-Ig has been found in the serum of the patients examined in comparison with healthy BM donors. This is in agreement with our previous observation of a relatively high frequency of H-Ig in SAA and leukemia, which may be the result of either immunosuppressive or antileukemia treatment.

### Table 3. Infections and Ig Isotype Deficiency at Time of Diagnosis of Infection

<table>
<thead>
<tr>
<th>Infectious Agent</th>
<th>Bacteria</th>
<th>Fungi</th>
<th>CMV</th>
<th>VZV</th>
<th>EBV</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infections</strong></td>
<td>40</td>
<td>40</td>
<td>16</td>
<td>30</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>IgG deficiency absent</td>
<td>2 (5%)</td>
<td>2 (5%)</td>
<td>9 (56%)</td>
<td>14 (47%)</td>
<td>10 (33%)</td>
<td>37 (22%)</td>
</tr>
<tr>
<td>IgG deficiency present</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>14 (11)</td>
</tr>
<tr>
<td>IgG1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>IgG2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>7 (6)</td>
</tr>
<tr>
<td>IgG3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>3 (2)</td>
</tr>
<tr>
<td>IgG4</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4 (3)</td>
</tr>
</tbody>
</table>

* CMV, donor and recipient seronegative in 24 cases (60%); VZV, recipient seronegative for VZV in 10 cases (33%); EBV, donor and recipient seronegative for EBV in 10 cases (33%).
† Number of patients in whom event occurred is shown in parentheses.
‡ Underscore indicates the cumulative number of patients with one or more Ig deficiencies.
§ IgG subclasses not determined in four patients.
|| IgG subclasses not determined in two patients.
The sequence of appearance of H-Ig within the different isotypes in patients with SCID after BMT was totally in accordance with the normal ontogenetic increases of these isotypes. This may be explained by the complete deficiency of lymphoid cell lineages before the immunologic recovery after BMT takes place. Other factors that might have been responsible for the differences between patients with SCID and other patients are HLA disparity and T-cell depletion of the graft in six of eight patients with SCID and the younger age of these patients. It is remarkable that H-IgA were not detected in patients with SCID even as late as 5 years after BMT. In children grafted for SAA and leukemia, H-Ig of IgG2 isotype could be detected early after BMT in contrast to what would be expected. We have still to investigate whether they are produced by plasma cells of the recipients. The observation that H-Ig of IgG1 isotypes present early and later after BMT are predominantly of donor origin as assessed by allotyping, strongly suggest that these H-Ig are produced by donor-derived B cells.

With respect to the light chain distribution of H-Ig after BMT, we found a transient predominance of kappa light chain, especially in patients with SCID. This may be the reflection of an early ontogenetic event. Follow-up investigations showed a shift to lambda–H-Ig resulting in a kappa/lambda ratio of 1.2 at 2 years post-BMT. This is lower than the kappa/lambda ratio present in heterogeneous serum Ig of healthy children and adults. Abnormal light chain ratios have also been reported in other immunologic disorders. Hammerström et al. found an extremely high relative frequency of lambda–H-Ig (kappa/lambda ratio 0.05) in BM graft recipients, which we were unable to confirm.

The frequency of H-Ig in the serum of BMT recipients

**Table 4. Immunoglobulin Levels and H-Ig at 2 Years After BMT**

<table>
<thead>
<tr>
<th>Ig Level</th>
<th>Decreased</th>
<th>Normal</th>
<th>Increased</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>1*1†</td>
<td>6/16</td>
<td>1/1</td>
<td>8/18</td>
</tr>
<tr>
<td>IgG1</td>
<td>0/0</td>
<td>8/12</td>
<td>3/6</td>
<td>11/18</td>
</tr>
<tr>
<td>IgG2</td>
<td>1/5</td>
<td>12/13</td>
<td>0/0</td>
<td>13/18</td>
</tr>
<tr>
<td>IgG3</td>
<td>1/2</td>
<td>12/16</td>
<td>0/0</td>
<td>13/18</td>
</tr>
<tr>
<td>IgG4</td>
<td>0/4</td>
<td>1/11</td>
<td>2/3</td>
<td>3/18</td>
</tr>
<tr>
<td>IgA</td>
<td>0/9</td>
<td>0/9</td>
<td>0/0</td>
<td>0/18</td>
</tr>
</tbody>
</table>

* Number of patients with H-Ig within the corresponding isotype.
† Number of patients with decreased (normal or increased) Ig level.

**Fig 4.** An example of longitudinal detection and characterization of H-Ig by immunoblotting in a patient with leukemia. At 9 weeks after BMT, multiple H-Ig appeared (three H-IgG1 lambda, one H-IgG1 kappa, and one H-IgM kappa). Follow-up investigation at 1 year showed faint H-Ig: one H-IgG1 lambda, one H-IgG2 lambda, one H-IgG2 kappa, and two H-IgM.

**Fig 5.** Mean number of H-Ig in the serum of BM donors (D) and recipients (R) before BMT and longitudinally after BMT. (i) Total number of H-Ig detected with IgM, IgA, and IgG subclass immunoblotting. (ii) Number of H-Ig characterized for both heavy and light chains.
decreased from 1 year after BMT onward. At 5 years after BMT, it equaled that observed before BMT but was still higher than in the serum of BMT donors. H-Ig in the subjects of our study were always transient and mostly of low concentration. The B-cellular clonal expansions observed in our study are quite different from what can be observed in malignant EBV-induced B lymphoproliferative syndrome (BLPS). The latter may develop in patients with a delayed recovery of T-cell function after intensive immunosuppressive treatment and nonidentical T-cell-depleted BMT. It is unlikely that EBV is important in the generation of transient low-concentration H-Ig, as seen in our study. Moreover, 7 of 18 donor-recipient couples included in this study were EBV seronegative. Serologic evidence of EBV reactivation late after BMT was obtained in only one case. The finding that in SCID mice, reconstituted with human blood mononuclear cells, H-Ig appeared also when cells from EBV-seronegative donors were used also argues against EBV as a causative agent.

In animal experiments, it was clearly shown that monoclonal gammopathies after BMT result from T < B cell dysregulations (third category of MG). In view of the transient T-cellular deficiency following BMT in humans, our data are in accordance with the existence of T < B dysregulation as the cause of H-Ig production. Our data indicate that the appearance of H-Ig is a very sensitive indicator of a disturbed T-cell regulatory function, because H-Ig could still be detected years after normalization of T-cell numbers and in vitro lymphoproliferative responsiveness. The low frequency of H-IgA and the slight preference of H-Ig for lambda light chain more than 1 year after BMT seems to be characteristic for H-Ig caused by T < B cell dysregulation. Others, using less-sensitive techniques, reported a correlation between the appearance of H-Ig and the development of GVHD; it is probable that GVHD induces the production of H-Ig in higher concentrations so that they could be detected. It is difficult to interpret our data in relation to the occurrence of GVHD, because its...
This study confirms former observations that B-cell reconstitution after BMT is a recapitulation of normal ontogeny. However, this reconstitution appears to be clonally dysregulated probably as a result of an impaired T-cell regulation. This implies that quantitative normalization of serum Ig (sub)class levels in BM graft recipients does not rule out the persistence of functional humoral immunodeficiencies; that is, with respect to specific antibody production. Normalization, or even an increase of serum Ig (sub)class levels, may be the result of a restricted number of fast-expanding Ig-secreting B-cell clones and may be accompanied by holes in the functional Ig-repertoire.

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