Analysis of T-Cell Repopulation After Allogeneic Bone Marrow Transplantation: Significant Differences Between Recipients of T-Cell Depleted and Unmanipulated Grafts

By Etienne Roux, Claudine Helg, Florence Dumont-Girard, Bernard Chapuis, Michel Jeannet, and Eddy Roosnek

We have studied the repopulation of the T-cell compartment in 27 patients transplanted with bone marrow from an (HLA)-identical sibling. Significant differences were found between recipients of unmanipulated and T-cell depleted grafts. Analysis of the T cells by a method based on amplification of minisatellite DNA regions showed that without depletion >99.9% of the clones responding to a mitogenic stimulus after transplantation were of donor origin. In contrast, when the graft had been depleted with Campath-1M plus complement, a significant part of the T cells cloned during the first weeks after transplantation comprised of recipient T cells that had survived the preconditioning. This mixed population of low numbers donor and recipient T cells (19 ± 31/mm³ at day 14) expanded rapidly (predominantly CD8+ T cells) during the first 2 months, without a significant change of the ratio of recipient/donor T cells. In 11 of 17 evaluable patients a late wave (>9 months) of donor T cells occurred. As a consequence, T-cell chimerism changed in favor of donor T cells and the CD4/CD8 ratio that had been reversed (<0.5) after the first expansion, normalized (1.5 ± 0.5). Analysis of the T-cell receptor repertoire showed that in recipients of a T-cell depleted graft, the recipient as well as the donor T cells that repopulated the peripheral T-cell pool during the first month, were the progeny of a limited number of precursors. Because without depletion, when larger numbers of donor T cells had been cotransfused with the marrow, the repertoire was much more diverse, these data show that immediately after transplantation, the peripheral pool is repopulated primarily through expansion of circulating T cells.

FUNCTIONAL RECOVERY of the immune system after bone marrow transplantation (BMT) is slow, and during the first year patients can be subject to a variety of infections. In particular T-cell immunity seems to be affected and, despite the presence of almost normal numbers of T cells, antiviral responses may be impaired for a prolonged period of time. One of the reasons why T-cell immunity is restored with greater difficulty than other components of the immune system is that the capacity of T cells to respond to foreign antigen, ie, to discriminate between self and nonself is learned early in life through thymic selection and is completed by building up memory against the encountered pathogens. Because the peripheral T-cell pool in which this T-cell memory resides will be eliminated by the pretransplant conditioning and because the diminished function of the thymus after marrow transplantation might not allow to regenerate a new naive repertoire, it is not certain that T-cell immunity will recover completely. Therefore, a substantial part of the T-cell responses after BMT will remain dependent on immunity transferred by mature donor T cells that are cotransfused with the marrow or possibly on recipient T cells that have survived the conditioning.

The intense conditioning regimens given to leukemic patients to be transplanted with an allogeneic BM serves two purposes. Firstly, it will eradicate the residual leukemic cells that might have survived earlier chemotherapy. Secondly, it will ablate the recipient hematopoiesis, providing immunosuppression and space to allow marrow engraftment. This process usually abrogates all autologous BM activity and therefore, without recurrent disease, all hematological lineages will be regenerated by the graft and be of donor origin exclusively. T cells do survive intensive conditioning better than other hematological lineages. Usually, such surviving recipient cells do not expand after transplantation with an unmanipulated marrow. However, when the transplantation is performed with a T-cell depleted graft, recipient T cells proliferate and form incomplete mixed chimeras. The fact that a few surviving recipient T cells can expand to form a significant part of the peripheral pool suggests that in the BM transplanted patient, favorable conditions exist for T-cell expansion. If these conditions such as the availability of space and the absence of a well-functioning thymus apply to the recipient T cells, it is likely that they also apply to the donor T cells that were confused with the BM.

It is not known to what proportion T cells are reconstituted through peripheral expansion or through thymic production. To address this question, we studied T-cell reconstitution in patients receiving unmanipulated and T-cell depleted allografts. By determining the recipient/donor origin of the cells by polymerase chain reaction (PCR) amplification of minisatellite DNA regions we were able to follow the development of donor T cells as well as the fate of the BM-independent recipient T cells. Our data suggest that during the first year after transplantation, T cells are essentially derived from transfused donor and surviving host T cells. Because these cells originate from two limited pools of T cells, the T-cell repertoire might be restricted, possibly explaining the absence of an adequate T-cell response early after BMT.

MATERIALS AND METHODS

Patients. The clinical characteristics of the 27 patients studied, conditioning regimen, and GvHD prophylaxis are described (see Table 1). Six patients (UPN 24 to 29) received unmanipulated grafts...
whereas 21 received a T-cell depleted marrow using the mononuclear antibody Campath-1M and complement.²⁰

Flow cytometric sorting and T-cell cloning. Peripheral blood (PB) cells were collected at various times post-BMT from day +3 to day +2194. Granulocytes and mononuclear cells were separated on Ficoll-Hypaque (Ficoll-Hypaque; Pharmacia, Uppsala, Sweden) density gradient centrifugation. For each recipient of a T-cell depleted graft (except for UPN 36 and 38), cell fractionation into CD4, CD8, CD14, CD16, and CD19 cell populations was performed by flow cytometric cell sorting as described.¹⁶ At least 2 × 10⁴ CD4, CD8, CD14, CD16, and CD19 cells were collected for DNA extraction. Mononuclear T cells were obtained from limiting dilution cultures (0.3 cells/well) after stimulation with phytohemagglutinin and irradiated allogeneic mononuclear cells.²¹ DNA was extracted either from a mixture of three different clones (recipients of undepleted grafts) or from each clone individually.

Analysis of donorgene recipient origin of cells. High molecular weight DNA was prepared from nuclei solubilized in Triton X-100 (Fluka, Buchs, Switzerland). The nuclei were incubated in presence of 0.5 mg/mL proteinase K for 2 hours at 65°C either at a concentration of 3 × 10⁶ cells/100 μL or, when the number of cells was below 3 × 10⁶, in a total volume of 10 μL. After 1 minute of centrifugation, one μL of the supernatant (≥180 ng of DNA) was used for PCR amplification using the primers 33.1, 33.6, YNZ 22, and Apo B primers as described.¹³,¹⁴ In brief, DNA was amplified in 20 μL of a buffer of 50 mmol/L KCl, 10 mmol/L Tris-HCl pH 8.8, 1 mmol/L MgCl₂ (33.1 and YNZ 22)/1.25 mmol/L MgCl₂ (33.6 and Apo B), 100 mg/mL bovine serum albumin (BSA), 0.2 mmol/L dNTPs, 40 ng primers and 0.5 U Taq DNA polymerase (Boehringer, Mannheim, Germany). After amplification, the samples were fractionated on 1% agarose gels and transferred onto a Nylon membrane. Hybridizations were performed for 3 hours in 5 × SSPE (1× SSPE = 10 mmol/L sodium-phosphate, 13 mmol/L potassium phosphate, 1 mmol/L, EDTA), 0.1% sodium dodecyl sulfate (SDS) at 35°C in presence of 10 ng labeled internal oligoprobe (p33.1: 5'-GGGTGGGAAGTGGAG-3', pApo B: 5'-GACAGACTAGGGTCGAGGAGG-3', pYNZ: 22: 5'-CAGAAGCAATGGCGCTTGGAG-3', pApO B: 5'-CGAGGTATTTGATCTCAGG-3'). Two washings (37°C with 2 × 10⁴ P-L = 0.1 mol/L sodium chloride, 0.05 mol/L sodium trichloride, pH 7.0, 0.1% SDS for 10 minutes), the blorts were dried and exposed at ~70°C with intensifying screens. In all experiments, blank samples (without DNA) were amplified to control for DNA carryover. The percentage of recipient DNA was evaluated by comparison with samples containing different ratios of donor- and recipient DNA (before transplantation). The calibration curves obtained takes into account the size-dependent efficacy of PCR amplification and ensures that the conditions used are quantitative. The level of detection of the minor population was routinely between 1% and 5%.²⁶

T-cell repertoire analysis. The methodology is based on the CD3 size spectratyping method described by Gorsky et al²² that uses the size heterogeneity of the hypervariable CD3 region to evaluate the T-cell diversity. In brief, 5 × 10⁸ PB mononuclear cells (PBMC) were purified by density-centrifugation and total RNA was extracted and converted into cDNA by reverse transcriptase (GIBCO-BRL, Gaithersburg, MD) in a volume of 20 μL with oligo-

---

**Table 1. Patients: Clinical Characteristics, Chimerism, and Clinical Outcome**

<table>
<thead>
<tr>
<th>UPN</th>
<th>Sex</th>
<th>D iag</th>
<th>Conditioning Regimen</th>
<th>GVHD*</th>
<th>Prophylaxis</th>
<th>TCD</th>
<th>R/T Cells</th>
<th>% Rec</th>
<th>R/T Cells at Day5</th>
<th>D-T Cells at Day5</th>
<th>GVHD a/c</th>
<th>Clinical Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>M</td>
<td>ALL</td>
<td>TBI, CTX, VP18</td>
<td>1</td>
<td>-</td>
<td>0/52</td>
<td>1/25</td>
<td>50</td>
<td>145</td>
<td>1/25</td>
<td>-</td>
<td>2/-</td>
</tr>
<tr>
<td>25</td>
<td>M</td>
<td>ALL</td>
<td>TBI, CTX</td>
<td>2</td>
<td>-</td>
<td>0/52</td>
<td>1/25</td>
<td>50</td>
<td>145</td>
<td>1/25</td>
<td>-</td>
<td>2/-</td>
</tr>
<tr>
<td>26</td>
<td>M</td>
<td>LYM</td>
<td>TBI, CTX</td>
<td>2</td>
<td>-</td>
<td>0/52</td>
<td>1/25</td>
<td>50</td>
<td>145</td>
<td>1/25</td>
<td>-</td>
<td>2/-</td>
</tr>
<tr>
<td>30</td>
<td>M</td>
<td>ALL</td>
<td>TBI, CTX</td>
<td>2</td>
<td>-</td>
<td>0/52</td>
<td>1/25</td>
<td>50</td>
<td>145</td>
<td>1/25</td>
<td>-</td>
<td>2/-</td>
</tr>
<tr>
<td>21</td>
<td>F</td>
<td>ALL</td>
<td>TBI, TLI, CTX, DAU</td>
<td>3</td>
<td>-</td>
<td>5/20</td>
<td>1/25</td>
<td>50</td>
<td>145</td>
<td>1/25</td>
<td>-</td>
<td>2/-</td>
</tr>
</tbody>
</table>

Abbreviations: ALL, acute lymphoblastic leukemia; LYM, lymphoma; CML, chronic myelogenous leukemia; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; TBI, fractionated total body irradiation; CTX, cyclophosphamide; VP18, etoposide; THIO, thiotepa; TLI, total lymphoid irradiation including the spleen; DAU, daunomycin; BU, busulfan; CYT, cytarabine; Rel, relapse; CCR, continuous complete remission; TCD, T-cell depletion; imm. th., immunotherapy; ND, not done.

*1 = methotrexate + cyclosporine A; 2 = 1 + methylprednisolone; 3 = T-cell depletion with Campath-1M and complement + cyclosporine A; 4 = 3 + methylprednisolone.

† R, recipient; T, T-cells; all, the following lineages were tested: granulocytes, monocytes, T, B and NK cells.

‡ D, donor; Number of recipient's total mitogen responsive T-cell clones established during the first month post BMT.

§ Data are expressed as mean ± SD when three or more samples were tested, otherwise the mean of two experiments or the value of one is depicted.

---
Mixed chimerism is caused by recipient T cells that expand only in recipients of a T-cell depleted BM. After BMT, recipient hematopoietic cells are usually eradicated by the combination of the cytotoxic preconditioning regimen and the allogeneic effect of the graft. However, the removal of mature T cells from the graft weakens this allogeneic effect and recipient cells can survive. In particular when an efficient depletion method is used, a rather strict correlation is found between persistence of recipient cells, ie, mixed chimerism and T-cell depletion. Table 1 shows the clinical cells was observed only in the patients who had received an unmanipulated donor. Clearly, the persistence of recipient hematopoietic cells was observed only in the patients who had received a Campath-1M depleted graft. Except for a short period immediately after transplantation, no recipient cells were detected in any of the six patients who had received an unmanipulated marrow. Only in two patients (UPN 40 and 42), all lineages such as granulocytes, monocytes, B cells and natural killer (NK) cells were of mixed origin. In all others (17 of 19), recipient cells were confined to the T-cell lineage. The mixed origin of the T-cell compartment, already apparent during the first weeks after transplantation, was caused by recipient T cells that had survived the conditioning regimen and had kept their potential to proliferate. Indeed, a significant part of the T-cell clones that responded to a mitogenic stimulus during the 30 days after BMT was of recipient origin (Table 1). The proportion of recipient clones could be as low as 5% (UPN 30), but whenever the donor T cells had been removed from the graft, they were clearly detectable. In contrast, when a patient was grafted with an unmanipulated graft, only one recipient T-cell clone was found among the 1,385 clones analyzed. These findings strongly suggest that only in a limited number of cases (2 of 27), mixed chimerism is caused by a production of hematopoietic cells by the marrow of the recipient. Usually, the recipient marrow activity will be ablated and the leukocytes of recipient’s origin will be limited to the T-cell lineage because these cells are relatively radio resistant and long-lived so that, in contrast to other lineages, their presence can be independent of recruitment from the BM.

The initial ratio of (clonable) recipient/donor T cells determines the evolution of T-cell chimerism during the first year after BMT. After transplantation, the number of peripheral T cells increases from 19 ± 31/mm³ at day 14 to 650 ± 560/mm³ after 90 to 150 days (Table 1). During this period, the (BM-independent) recipient T cells repopulated the peripheral pool with kinetics comparable to the donor T cells. Independent of the percentage of recipient T cells that varied from 5 to 100, the recipient/donor ratio remained constant once it had been established during the first month after transplantation. This is shown in Fig 1, where the ratio...
of flow-sorted recipient/donor T cells sampled from 2 months to 4 years after transplantation is plotted against the initial (<30 days) ratio of clonable T cells. Clearly, the balance between recipient and donor T cells remains unchanged during the first year after transplantation (Fig 1A and B) and it is only in the second year that it changes in favor of donor T cells (Fig 1C). This lack of a significant advantage for the donor T cells during the first year suggests that like recipient T cells, these T cells initially increase in number through peripheral expansion rather than through recruitment from the BM.

The BM independence of the recipient T cells as well as of the donor T cells was most evident by the sometimes very fast repopulation of the T-cell compartment. T cells could reach a level of 2,000 to 3,000 cells/mm³ within 2 months after transplantation, sometimes even before the take of the myeloid lineage (UPN 25) to decrease to stable levels (300 to 700 cells/mm³) thereafter. This overshoot of mainly CD8⁺ T cells was observed in half (UPN 24 to 26) of the recipients of an unmanipulated graft (Fig 2) and in half of the recipients of a T-cell depleted graft in whom the majority of clonable T cells were of donor origin (UPN 30, 34, 37, and 38). In the latter patients the recipient T cells expanded with only marginally slower kinetics than the donor T cells (Fig 3). In the patients in whom recipient T cells initially outnumbered the donor T cells, the repopulating CD8⁺ cells were of recipient origin (Fig 4). In such cases (UPN 39 to 43) the recipient T cells peaked later, reaching a maximum (1,000 to 2,000/ mm³) after 2 to 8 months to diminish slowly thereafter (Fig 4).

A late wave of donor T cells changes the balance between recipient and donor T cells. Whereas the first T cells that repopulated the peripheral pool were of both recipient and donor origin, the late wave of T cells, observed in 2 of 2 evaluable recipients of an unmanipulated (Fig 2) and in 9 of 15 evaluable recipients of a T-cell depleted graft, comprised predominantly donor T cells. This increase in the number of donor T cells (Figs 2, 4, and 5) changed not only the total number, but also the ratio of recipient/donor T cells, because no further increase of recipient T cells occurred after they had reached their maximal numbers between 2 to 6 months after transplantation. Although the donor T cells started to replace the recipient T cells (Fig 4), this process was usually so slow (Figs 3 through 5), that in the two patients with the longest follow-up (UPN 8 and 9), significant numbers of recipient T cells were still present even 7 years after BMT. In contrast to the first expansion of predominantly CD8⁺ T cells, this late wave comprised mainly of CD4⁺ T cells. As a consequence the CD4/CD8 ratio that had been reversed (<0.5) after the first expansion, normalized (1.5 ± 0.51) and T-cell chimerism changed in favor of donor T cells.

T-cell depletion limits the T-cell repertoire complexity early after BMT. The presence of large numbers of recipient T cells originating from a limited number of precursors that had survived the preconditioning, illustrates the enormous potential of T cells to repopulate a host. The fact that during the first weeks after transplantation the number of clonable donor T cells is in the same order of magnitude as the number of clonable recipient T cells (Table 1), suggests that the number of transfused donor T cells with the potential to expand, might be limited also. Direct evidence that after T-cell depletion the peripheral pool is repopulated from a limited number of T-cell precursors only, was obtained by comparison of the TCR-diversity in recipients of T-cell depleted grafts with the one in recipients of unmanipulated marrows. To determine the TCR-complexity in the respective patients, we used the spectratyping method that measures the size heterogeneity of the CDR3 region of the TCR Vβ-chains that is caused by the random addition or
Fig 3. Overshoot of T cells in a recipient of T-cell depleted graft (UPN 37). The maximum number of T cells was observed at day 39 ± 8 (UPN 30, 34, 37, and 38) with a mean CD4/CD8 ratio of 0.32 ± 0.14. (A) The T-cell peak consisted of recipient (■) and donor T cells (◆). (B) Percentage of mixed chimerism in mononuclear cells (○) and granulocytes (●). (C) Numbers of granulocytes (●), lymphocytes (○), and monocytes (■).

Fig 4. Dominance of recipient T cells in a patient with a high percentage of recipient T-cell clones during the first 3 weeks post BMT (UPN 41). The highest number of recipient T cells (UPN 39 to 43, mean CD4/CD8 ratio of 0.32 ± 0.15) was observed between 2 to 8 months after transplantation. (A) Recipient (■) and donor T cells (◆). (B) Percentage of mixed chimerism in mononuclear cells (○) and granulocytes (●). (C) Numbers of granulocytes (●), lymphocytes (○), and monocytes (■).
T-CELL REPOPULATION AFTER ALLOGENEIC BMT

Fig 5. A late wave of donor T cells in a recipient of a T-cell depleted graft (UPN 31). (A) The number of recipient T cells (■) remains stable after the second month whereas the donor T cells (▲) increase progressively (UPN 8, 9, 31-33, 35, 38, 41, and 42, onset day 446 ± 162) with a concomitant change in CD4/CD8 ratio (0.9 ± 0.61 during the first 3 months, 1.6 ± 0.5 >500 days). The late wave of donor T cells was observed in 11 of 17 of the evaluable patients. (B) Percentage of mixed chimerism in mononuclear cells (○) and granulocytes (●). (C) Numbers of granulocytes (●), lymphocytes (○), and monocytes (●).

DISCUSSION

During the first year after BMT, patients are highly susceptible to infections. This immune deficiency is particularly severe for T-cell dependent responses and despite the normal numbers of T cells present from the third month on, patients rarely respond to vaccinations during the first year. Although it has been impossible to attribute the decreased responsiveness to one specific T-cell defect, a variety of abnormalities after quantitative recovery have been described. During the first year, the CD4/CD8 ratio is reversed because the CD8+ T cells repopulate the peripheral pool at a higher rate than the CD4+ do. Furthermore, many cells express cell surface markers that are usually expressed by a minority of T cells only. These markers such as class II, CD25, CD45RO, and CD56 represent activation/homing molecules that are characteristic for activated T cells. These anomalies appear not to be typical for allogeneic BMT, as they are also observed in other patients in whom the peripheral T-cell pool has been eliminated. After autologous BMT, T cells repopulate the peripheral pool in a similar fashion without restoring T-cell immunity directly. Furthermore, the same reversed CD4/CD8-ratio's and the lack of expression of CD45RA, the isoform expressed by naive T cells emerging from the thymus have been reported after intensive chemotherapy. In particular, the high expression of CD45RA appears to be the hallmark of a T-cell compartment that regenerates in the absence of a functioning thymus. Therefore, these findings indicate that, once the peripheral T-cell pool has been eliminated, the first wave of T cells is thymic independent. Consequently, the majority of T cells must originate from the small numbers that have survived the cytoreductive treatment or, in the case of BMT, from a combination of surviving cells and T cells that were coinfused with the graft.

Fig 6. Comparison of the T-cell receptor repertoire complexity in a recipient of an unmanipulated graft (UPN 26) and in a recipient of a T-cell depleted graft (UPN 37). Blood samples were obtained at day +24 (UPN 26, 1,750 T cells/mm3) and +46 (UPN 37, 2,700 T cells/mm3) with T cells being exclusively (UPN 26) or predominantly (UPN 37) of donor origin. Spectratypes of 9 Vβ families of the recipient (R) as well as of the donor (D) are shown. Similar data were obtained from UPN 24 and UPN 34.
The capacity of mature T cell to expand and to repopulate the peripheral pool from a very low number of precursors, has been well documented. In particular when a functioning thymus is absent, transferred T cells will expand until normal numbers have been reached. The fact that this final number of T cells is independent of the numbers transferred, reflects the very precise control of the size of the peripheral T-cell pool. Therefore, at first glance, repopulation might not be so different when, after T-cell depletion, the peripheral T-cell pool has to be regenerated from a number of T cells that is much smaller than after transplantation with an undepleted marrow. If only the total number of T cells is regulated and both donor and recipient T cells expand at the same rate, T-cell depletion will only influence the ratio between these cells. Therefore, if large numbers of donor T cells are infused, the percentage of mixed chimerism will be much lower so that it might escape detection unless very sensitive methods are used.

Our data suggest that during the first year after transplantation the peripheral T-cell pool is repopulated predominantly through peripheral expansion. In patients who received a T-cell depleted graft, in whom the number of recipient T cells that had survived conditioning was of the same order of magnitude as the number of donor T cells that had survived the Campath treatment, this resulted in two parallel expanding T-cell populations of recipient and donor origin. The fact that the recipient T cells could expand without being sustained by an active BM shows that during that period the number of T-cells produced through the thymic pathway is probably insignificant. Therefore, it is likely that also the donor T cells originate from mature T cells. Although this remains to be proven formally, this assumption is supported by the finding that when CD6+ T cells are selectively depleted, the host is repopulated by T cells lacking this antigen otherwise expressed by 95% of T cells. Because during the same period, the BM does produce all other haematological lineages, this lack of production of new T cells is more likely because of a malfunctioning thymus rather than to a defect in production of T-cell precursors.

An intriguing question remains whether this peripheral expansion is dependent of a specific stimulus or whether all T cells will expand at a same rate. Without a functioning thymus, some T cells will repopulate the peripheral pool more rapidly than others. In particular intraepithelial T lymphocytes, that can differentiate extrathymically, or T cells encountering antigen might have a significant advantage over other T cells. This will contribute to distortion of the normal distribution of T-cell subpopulations that is observed after BMT, and could explain why immediately after BMT so many T cells have abnormal phenotypes. Furthermore, because many of the extrathymically differentiating T cells are CD8+ and most of the antigens encountered after BMT might be class I restricted, this could also explain why CD8+ T cells repopulate much faster than CD4+ cells.

A number of findings suggest that a selective proliferation and subsequent persistence of mature CD8+ T cells and not a thymus independent maturation of T-cell precursors is the major mechanism of repopulation. Firstly, even when T cells can differentiate extrathymically, the production of T-cell precursors will remain BM dependent. Therefore, in our patients this pathway is only for the recipient T cells that are not supported by a productive BM. Secondly, the expansion of CD8+ T cells occurs too rapidly to be BM dependent. Moreover, a role for antigen is suggested by the fact that this expansion might even be accelerated when the patient suffers from viral infections. Furthermore, the transient peak of T cells observed in half of our patients is reminiscent of the expansion that is observed before antigen specific cells are clonally deleted from the periphery when tolerizing high doses of antigen are present. Thirdly, once that the expansion had occurred and some TCRs had been selected, these clones persisted for years after BMT suggesting that they were continuously stimulated by antigen. It is obvious that, if most of the T cells during the first 3 months after BMT would have been selected in some way, the normal repertoire will be diluted by cells with very limited specificities that will lead not only to the loss of memory, but also to the inability to mount primary responses.

The consequences of T-cell depletion on the overall immune status of the patient are hard to assess. Clearly, most of the immunity residing in memory donor T cells will be lost, which can result in lower early immune responses against CMV. Moreover, as we show in this report, apart from allowing recipient T cells to survive, T-cell depletion also leads to a rather restricted TCR repertoire of the initial T-cell population. It should be noted that the observed oligoclonality of T cells was neither characteristic for the recipient T cells nor, as has been observed by others, for the CD57+ population, because at the moment of blood sampling none of these populations were particularly dominant. At present, we do not know whether this limited TCR-usage during the first month after BMT has a significant influence on the further development of the T-cell repertoire. Although the repertoire appeared to diversify a few months later to become indistinguishable from the one in undepleted patients, we did not compare the TCR complexity within a spectratype band. Therefore, it remains possible that, with similar spectratype patterns, the two repertoires still differ significantly with respect to TCR diversity. Such differences could remain unnoticed clinically because the potential drawbacks of a limited T-cell repertoire might be compensated for by a faster immunological reconstitution in the absence of GVHD or its treatment.

It is not certain whether the immune system will recover completely after BMT. Obviously this will depend on the capacity of the thymus to recover, which is linked to the patients age. Indeed, CD4/CD8 ratios do seem to normalize faster in young children. However, it could be that in adults, the thymus is able to regenerate and that the late wave of T cells that we observe in half of our patients is, at least partially, thymus derived. This would explain why this late wave consists of donor T cells exclusively and why the end of the first year, when this late wave emerges, CD4/CD8 ratios normalize and naïve resting T cells reappear. Moreover, because at the same time patients start to respond to vaccinations, one could argue that because of the limited initial TCR repertoire after BMT, a substantial part of the immune responses has become dependent on the recruitment of naïve T cells.
It has been generally accepted that T-cell depletion lowers the degree of GVHD allowing the survival of recipient T cells that would otherwise be eradicated by the mature T cells in the graft. Our data allow us to speculate on a mechanism that is fundamentally different. One could argue that T-cell depletion only changes the initial ratio of recipient/donor T cells, which subsequently will lead to the presence of large numbers of recipient CD8+ T cells. Because such cells are known to be the very efficient inducers of energy in host reactive allogeneic T cells5,6 their presence could induce a state of tolerance thereby preventing the most severe forms of GVHD.

ACKNOWLEDGMENT
We thank Béatrice Cherubini and Solange Vischer for excellent technical assistance, Dominique Wohlfend for cell sorting, and Dr Nathalie Rufer for critical reading of the manuscript.

REFERENCES
11. Lum LG, Munn NA, Schanfeld MS, Storb R: The detection of specific antibody formation to recall antigens after human bone marrow transplantation. Blood 67:582, 1986
30. Roberts MM, To LB, Gillis D, Mundy J, Rawling C, Ng K, Juttner CA: Immune reconstitution following peripheral blood stem cell transplantation, autologous bone marrow transplantation and allogeneic bone marrow transplantation. Bone Marrow Transplant 12:469, 1993
38. Verdunck LF, de Gast GC: Is cytomegalovirus infection the major cause of T cell alterations after (autologous) bone-marrow transplantation? Lancet 1:932, 1984
Analysis of T-cell repopulation after allogeneic bone marrow transplantation: significant differences between recipients of T-cell depleted and unmanipulated grafts

E Roux, C Helg, F Dumont-Girard, B Chapuis, M Jeannet and E Roosnek