

T cell depletion

A large-scale method for T cell depletion: towards graft engineering of mobilized peripheral blood stem cells

PR Gordon¹, T Leimig¹, I Mueller¹, A Babarin-Dorner¹, MA Holladay¹, J Houston¹, G Kerst², T Geiger¹ and R Handgretinger¹

¹Division of Stem Cell Transplantation, St Jude Children's Research Hospital, Memphis, TN, USA; and ²Children's University Hospital, University of Tuebingen, Germany

Summary:

We have investigated the feasibility and efficacy of large-scale T cell depletion from granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood stem cells (PBSC). The method is based on the use of a CD3 antibody conjugated to magnetic microbeads and magnetic activated cell sorting (Clinimacs). A total of eight large-scale experiments were performed. In four experiments, CD3⁺ T cells were depleted from PBSC obtained from volunteers mobilized with G-CSF whereas, in four experiments, T cells were depleted from PBSC from stem cell donors, in which the CD34⁺ stem cells had been removed for allogeneic transplantation by positive selection prior to T cell depletion. The mean number of processed mononuclear cells (MNCs) was 3.3×10^{10} (range 1.5×10^{10} – 5.1×10^{10}) with a mean T cell proportion of 35.8% (range 16.7–64.0%). After T cell depletion, the percentage of contaminating T cells was 0.15% (range 0.01–1.01%) with a mean log₁₀ depletion of 3.4 (range 2.8–4.1). The mean recovery of CD3-negative MNCs after depletion was 76% (range 52–100%). The mean recovery of CD34⁺ stem cells in the four evaluable experiments was 82% (range 75–92%). *In vitro* colony assays and *in vivo* NOD/SCID repopulation assays showed that this large-scale T cell depletion method has no negative impact on the function of the hematopoietic precursor cells. Therefore, we conclude that this T cell depletion method is a valuable tool for further graft engineering strategies involving mobilized PBSCs.

Bone Marrow Transplantation (2002) 30, 69–74.
doi:10.1038/sj.bmt.1703619

Keywords: T cell depletion; peripheral blood stem cells; allogeneic transplantation; Clinimacs

While bone marrow (BM) has been the source of hematopoietic stem cells in the past, G-CSF mobilized PBSCs are increasingly used as a stem cell source. Compared to BM, transplantation of allogeneic mobilized PBSC is associated with less regimen-related toxicity¹ and earlier hematopoietic recovery.² Although the risk of developing acute graft-versus-host disease (GVHD) is not increased after PBSC transplantation compared to bone marrow transplantation,^{3,4} the incidence of extensive chronic GVHD is high.⁵ The most effective means of prevention of acute and chronic GVHD is *in vitro* depletion of T lymphocytes from the graft.^{6,7} Most of the available T cell depletion strategies have been developed for BM and methods based on antibody/complement⁸ or other approaches⁹ are not well applicable for PBSC due to the high cell numbers to be processed.

Recently, a method for indirect T cell depletion of PBSCs based on the positive selection of CD34⁺ stem cells has been described.¹⁰ We and others have used this method in matched sibling transplants,¹¹ MUD transplants¹² and haploidentical transplantation.⁶ This indirect approach yields a T cell depletion of >5 log.¹⁰ However, there might be some disadvantages associated with the use of purified CD34⁺ cells, such as a higher incidence of engraftment failures,¹³ delayed immunoreconstitution,¹⁴ the lack of an anti-leukemic effect¹⁵ and the exclusion of CD34-negative stem cells from the graft. In order to overcome the disadvantages of CD34⁺ positive selection, we have evaluated a strategy to directly deplete T cells from PBSC.

In this paper, we describe a rapid and efficient method of T cell depletion based on the Clinimacs technology. This method allows the use of T cell-depleted PBSC and can be the basis for further graft engineering strategies.

Materials and methods

Mobilization and collection of PBSCs

A total of eight large-scale experiments were performed. PBSCs were mobilized from four volunteer donors (experiments 1–4) using G-CSF (480 µg/day, Neupogen; Amgen, Thousand Oaks, CA, USA) for 4 days. A single leukapheresis was performed at day 5 using a Cobe Spectra (Cobe, Lakewood, CO, USA).

Correspondence: R Handgretinger, Division of Stem Cell Transplantation, St Jude Children's Research Hospital, 332 N Lauderdale St, Memphis, TN 38105, USA

Received 23 January 2002; accepted 4 April 2002

In experiments 5–8, allogeneic stem cell donors were mobilized as previously described.⁶ Prior to T cell depletion, CD34⁺ stem cells were positively selected from the PBSC using the Clinimacs device¹⁰ and used for transplantation. The left-over fraction was used for the further T cell depletion experiments. All PBSC donors had given written informed consent and the study had been approved by the institutional review board.

Depletion of CD3⁺ T cells

PBSCs were processed either immediately or mixed with an equal volume of autologous plasma and stored overnight at 4°C. PBSC were washed once with PBS buffer (phosphate-buffered saline supplemented with 1 mM EDTA) and incubated with the anti-CD3 antibody OKT-3 directly conjugated to magnetic microbeads (Miltenyi, Bergisch-Gladbach, Germany). In the experiments with MNC numbers $< 2 \times 10^{10}$ cells, one vial (7.5 ml) of the antibody/microbead conjugate was used. If the cell number exceeded 2×10^{10} , an additional 7.5 ml of the antibody per 2×10^{10} cells was used. Cells were then incubated under continuous agitation at room temperature for 30 min, washed twice with PBS buffer, resuspended in 300 ml buffer and then processed with the fully automated Clinimacs device (Miltenyi) equipped with LS/TS (162.01) separation columns using the program 'Depletion 2.1' according to the manufacturer's instructions. The processing time on the Clinimacs was dependent on the cell numbers with a mean of 3.2 h (range of 2.5 to 4.1 h).

Flowcytometric analysis

Prior to and after T cell depletion, cells were analyzed for CD3, CD19, CD34 and CD133 with fluorochrome-labeled antibodies (all Becton Dickinson, Mountain View, CA, USA). Flow cytometric analysis was performed using a flow cytometer (LSR, Becton Dickinson). The anti-CD3 antibody labeled with fluorescein isothiocyanate (FITC) (clone SK7, Becton Dickinson) recognizes a different epitope than the OKT-3 antibody conjugated to the magnetic microbeads. Therefore, binding of the OKT-3/microbead conjugate does not interfere with or block the binding of the anti-CD3-FITC conjugate and the residual amount of CD3⁺ T lymphocytes in the graft can reliably be determined with this method (Figure 1).

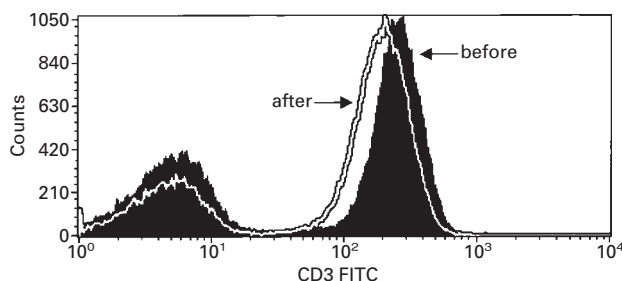


Figure 1 The presence of the OKT-3/microbead conjugate does not interfere with the binding of the anti-CD3 antibody. Mononuclear cells were stained with anti-CD3-FITC before and after labeling with OKT-3/microbeads. There is no significant interference in the binding of the anti-CD3-FITC with the OKT-3/microbeads.

Colony-forming unit (CFU) assays

CD34⁺ stem cells were isolated prior to and after T cell depletion using the CD34⁺ positive selection kit and the Variomacs device (both Miltenyi) according to the manufacturer's instructions. Purified CD34⁺ cells were plated at 5000 cells/plate in methylcellulose-based semisolid culture medium-MethoCult H4433 (Stem Cell Technologies, Vancouver, Canada). After 14 days, total numbers of colonies were counted and scored for the presence of burst-forming units-erythroid (BFU-E), colony-forming units-granulocyte/macrophage (CFU-GM) and colony-forming units-granulocyte/erythroid/macrophage/megakaryocyte (CFU-GEMM). Assays were performed in duplicate.

NOD/SCID repopulation assays

After T cell depletion, CD133⁺ progenitor cells were isolated using the CD133 antibody/microbead conjugate and the Clinimacs device (both Miltenyi) according to the manufacturer's instructions. The mean purity of the CD133 cells was 94% (data not shown). NOD/SCID mice (NOD.CB17-Prkdc^{scid}/J) were irradiated with 300 cGy using a ¹³⁷Cs source (JL Shepherd, CA, USA) and injected with various numbers of CD133⁺ stem cells into the lateral tail vein. Mice were killed after 8 weeks and the bone marrow was harvested by flushing femora and tibiae. Human engraftment was determined by double-staining the BM cells with a directly labeled anti-human CD45 antibody and an anti-murine CD45 antibody (both Becton Dickinson). The presence of human cells in the mouse bone marrow was analyzed using flow cytometry.

Results

Depletion of CD3⁺ T lymphocytes and CD34⁺ recovery

A total of eight large-scale experiments were performed. The mean mononuclear cell count prior to T cell depletion was 3.3×10^{10} (range 1.5 – 5.1×10^{10}). The mean recovery of CD3-negative MNCs was 76% (range 52–100%). The mean percentage of T cells prior to depletion was 35.8% (range 16.7–64.0%). After depletion, the mean percentage of remaining T cells was 0.15% (range 0.01–1.01%). Thus, the mean log₁₀ T cell depletion was 3.4 (range 2.8–4.1). The automated processing time on the CliniMACS ranged from 2.5 to 4.1 h, dependent on the processed cell numbers. These data are summarized in Table 1. In Figure 2, a representative flow cytometric analysis of two experiments before (a, b) and after T cell depletion (c, d) is shown.

In four out of the eight large-scale experiments in which PBSCs from volunteer donors were used, the recovery of CD34⁺ stem cells after T cell depletion could be evaluated. The T cell depletion was associated with minimal loss of hematopoietic precursors, since the mean recovery of CD34⁺ stem cells was 82% (range 75–92%). These data are shown in Table 2.

Functional *in vitro* and *in vivo* assays

In order to rule out that this T cell depletion method has any negative impact on the biological function of stem cells, *in*

Table 1 Number of mononuclear cells (MNC) before and recovery of CD3-negative MNCs after T cell depletion, percentage of CD3⁺ T lymphocytes before and after T cell removal and log depletion

| Experiment | No. of MNC ($\times 10^{10}$) | MNC recovery (%) | % CD3 ⁺ T cells | | Log ₁₀ depletion |
|------------|---------------------------------|------------------|----------------------------|-------|-----------------------------|
| | | | before | after | |
| 1 | 1.6 | 76 | 64.0 | 1.01 | 2.8 |
| 2 | 3.1 | 66 | 36.0 | 0.10 | 3.1 |
| 3 | 5.1 | 67 | 32.0 | 0.04 | 3.3 |
| 4 | 5.0 | 52 | 16.7 | 0.03 | 3.2 |
| 5 | 3.6 | 86 | 28.3 | 0.03 | 3.2 |
| 6 | 4.1 | 77 | 25.7 | 0.02 | 3.4 |
| 7 | 2.1 | 100 | 57.0 | 0.01 | 4.1 |
| 8 | 1.5 | 82 | 27.0 | 0.01 | 3.7 |
| Mean | 3.3 | 76 | 35.8 | 0.15 | 3.4 |

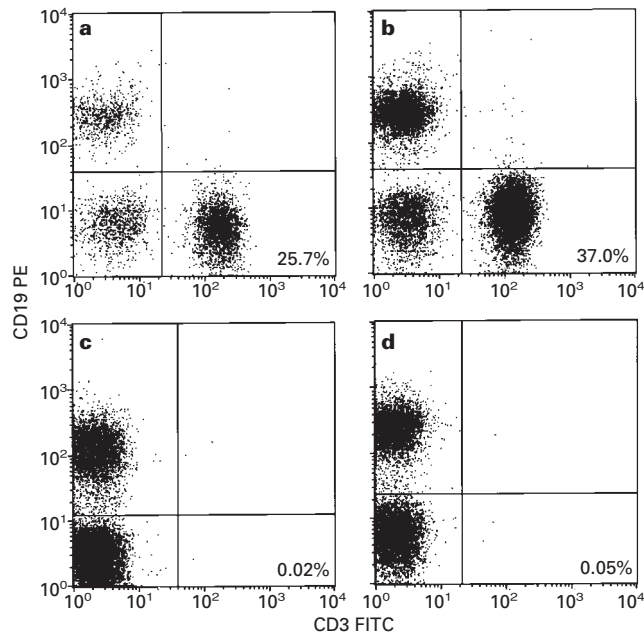


Figure 2 Two representative flow cytometric analyses of the proportion of T and B cells in mobilized peripheral stem cells before (a, b) and after (c, d) T cell depletion.

Table 2 Number of CD34⁺ stem cells before and after T cell depletion and percentage recovery of CD34⁺ cells

| Experiment | No. of CD34 ⁺ cells ($\times 10^6$) | | % Recovery of CD34 ⁺ stem cells |
|------------|--|-------|--|
| | Before | After | |
| 1 | 16 | 12 | 75 |
| 2 | 186 | 172 | 92 |
| 3 | 270 | 208 | 77 |
| 4 | 95 | 77 | 81 |
| Mean | 142 | 117 | 82 |

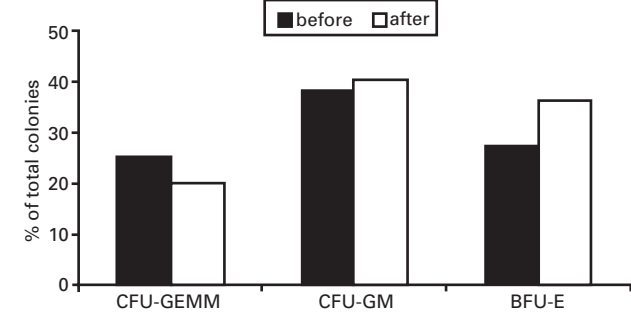


Figure 3 Percentages of colonies before and after T cell depletion. CD34⁺ cells were isolated before and after T cell depletion and 5000 CD34⁺ cells were plated. Colonies were scored and differentiated after 14 days. The average number of colonies per 5000 CD34⁺ stem cells before and after T cell depletion was 322 and 327, respectively. The data represent the average colonies of two experiments.

in vitro colony-forming assays and *in vivo* NOD/SCID repopulating assays were performed in the large scale experiments 1–4. For the colony assays, CD34⁺ stem cells isolated from the PBSC prior to and after T-depletion were plated and colonies were scored after 14 days. There was no nonspecific loss of colony-forming units. In addition, there was also no difference before and after T depletion in the composition of colonies (Figure 3). Due to the limitations of the *in vitro* CFU assay for the evaluation of primitive hematopoietic progenitors, we have additionally investigated the NOD/SCID repopulating activity of the mobilized stem cells after T cell depletion. Therefore, CD133⁺ stem cells were isolated after T cell depletion and various amounts were injected into NOD/SCID mice. Mice were killed after 8 weeks and the engraftment of human cells was assessed in the bone marrow of the mice by staining with human anti-CD45 antibodies. In all experiments, a high engraftment potential of the CD133⁺ stem cells after T cell depletion could be observed. These data are summarized in Table 3.

Discussion

The prevention of acute or chronic GVHD remains a major challenge in allogeneic stem cell transplantation. Immuno-

Table 3 Engraftment of CD133⁺ stem cells in NOD/SCID mice after T cell depletion

| Experiment | No. of transplanted CD133 ⁺ cells ($\times 10^6$) | No. of transplanted mice | % Engraftment ^a (range) |
|------------|--|--------------------------|------------------------------------|
| 2 | 5.0 | 5 | 40 (31–47) |
| | 2.0 | 3 | 19 (14–22) |
| | 0.5 | 1 | 9 |
| 3 | 5.0 | 4 | 50 (19–71) |
| | 1.0 | 2 | 24 (13–36) |
| 4 | 1.0 | 3 | 15 (4–33) |

^a% Engraftment denotes the ratio of the number of bone marrow cells staining positive for anti-human CD45 to the number of cells staining positive for anti-human and anti-mouse CD45.

suppressive drugs currently used for GVHD prophylaxis are associated with a number of side-effects and none of them are effective enough to reliably prevent GVHD. In the past, the reduction of T cells in bone marrow grafts has been shown to decrease the incidence of GVHD and various methods for T cell depletion have been used. However, none of these negative depletion techniques are well suited for the depletion of T cells from PBSC grafts. Mobilized PBSC are increasingly used for stem cell transplantation either due to the donor's preference¹⁶ or due to the physician's decision. In matched sibling and matched unrelated situations, the incidence of acute GVHD after transplantation of unmanipulated PBSCs is similar to unmanipulated BM. However, a high rate of extensive chronic GVHD has been observed after transplantation of unmanipulated PBSCs.⁵

In contrast to these observations, it has been shown that matched sibling,¹¹ matched unrelated¹² and three-loci mismatch haploidentical CD34⁺ positively selected stem cells^{6,7} can be grafted in the complete absence of any pharmacologic GVHD prophylaxis and without any signs of severe acute or extensive chronic GVHD. In these studies, CD34⁺ stem cells were positively selected from mobilized PBSCs either by a combination of T cell depletion and CD34-positive selection⁷ or by a one-step CD34⁺-positive selection alone.⁶ The CD34⁺ cell selection to a high purity is associated with a highly effective indirect T cell depletion of >5 log. However, the transplantation of such highly selected stem cells can be associated with a higher rejection rate, delayed immunoreconstitution or a less effective graft-versus-leukemia effect. Therefore, the transplantation of PBSCs selectively depleted of T lymphocytes would offer some advantages over the use of positively selected CD34⁺ stem cells. We have chosen the OKT-3 antibody as a T cell reagent due to its clinical availability. It is used as an immunosuppressive agent in solid organ transplants¹⁷ as well as in the depletion of T lymphocytes from bone marrow.¹⁸ The *in vitro* T cell depletion is exerted via activation of complement and complement-dependent cytotoxicity (CDC). In our setting, the *in vitro* incubation of the OKT-3/microbead conjugate might potentially result in an initial T cell activation. However, almost all of the magnetically labeled and potentially activated T cells are removed after depletion. The few remaining T cells in the graft stained with the OKT-3 antibody most likely will be destroyed by the CDC or other mechanisms *in vivo*.

The graft obtained is composed of different cell types, such as natural killer (NK) cells, monocytes, dendritic cells, and CD34-negative stem cells. The high numbers of NK cells in T cell-depleted PBSC grafts could facilitate engraftment¹⁹ or exert an anti-leukemic activity.²⁰ Monocytes and NK cells as part of the innate immune system possess anti-bacterial or anti-viral activity^{21–24} and might therefore be of importance in preventing infections in the early post-transplant phase. Moreover, recent studies have challenged the concept that all stem cells are found within the CD34⁺ subset and CD34-negative stem cells capable of long-term *in vivo* repopulation and multilineage differentiation have been identified.^{25–28} The clinical significance of CD34-negative stem cells is currently not clear, but with our described method of T cell depletion of PBSC, in con-

trast to CD34⁺ selection, this subset is retained in the graft. This might provide some further insights into its clinical significance.

The nonspecific cell loss is low and the CD34⁺ cell recovery is excellent. The method has no negative impact on the progenitor cells, since the *in vitro* and *in vivo* biological functions of the stem cells after T cell depletion are completely maintained, as shown by the *in vitro* colony assays and by the *in vivo* NOD/SCID repopulating activity of the CD133⁺ stem cells. We have chosen the CD133 antigen because it identifies hematopoietic progenitor cells with high NOD/SCID repopulating activity.²⁹ The high number of remaining B cells in a T cell-depleted PBSC graft could potentially be associated with a higher incidence of post-transplant donor-derived EBV-associated lymphoproliferative syndromes.³⁰ This problem can be addressed, however, by preemptive therapy with adoptively transferred donor-derived EBV-specific cytotoxic T lymphocytes,³¹ anti-CD20 antibodies³² or the additional removal of B cells using an appropriate antibody conjugated to the microbeads together with the OKT-3/microbead conjugate. Currently, experiments for additional B cell depletion using an anti-CD19/microbead conjugate are underway.

Whereas in experiments 1–4 T cells have been depleted directly from untouched PBSCs, the T depletion in experiments 5–8 was performed with PBSCs from which the CD34⁺ stem cells had been positively selected prior to the T cell depletion using the Clinimacs system. These experiments were performed in order to see whether the prior CD34⁺ selection would have any impact on the subsequent T cell depletion. As shown in Table 1 (experiments 5–8), this T cell depletion method can be performed with the same efficacy as with untouched PBSCs (Table 1, experiments 1–4). These observations raise the further possibility of graft engineering. The CD34⁺ stem cells can be removed from a PBSC graft prior to the manipulation of the leftover fraction. The leftover fraction can then be further processed without the risk of loss of or damage to the CD34⁺ cells, thus offering an add-on approach to already established transplantation procedures. The T (and B) cells can be removed from the leftover fraction and the residual cells (NK cells, dendritic cells, CD34-negative precursors) can be extensively manipulated *ex vivo*. The CD34⁺ stem cells are then transplanted together with the manipulated, T (B) depleted leftover fraction. Such a possible graft engineering scenario is shown in Figure 4. All these graft manipulation steps can be performed with automated devices and are easily transformable to large-scale methods and to GMP conditions.

In summary, we have shown that T cells can be depleted from mobilized PBSCs with a high efficacy and with a high CD34⁺ recovery. The biological functions of the processed stem cells are fully maintained. Moreover, this method can be combined with positive CD34⁺ cell selection, thus allowing the manipulation of a graft without the risk of stem cell damage. This method can be the basis for further graft engineering strategies to evaluate different graft compositions and manipulations in order to improve the outcome of allogeneic stem cell transplantation.

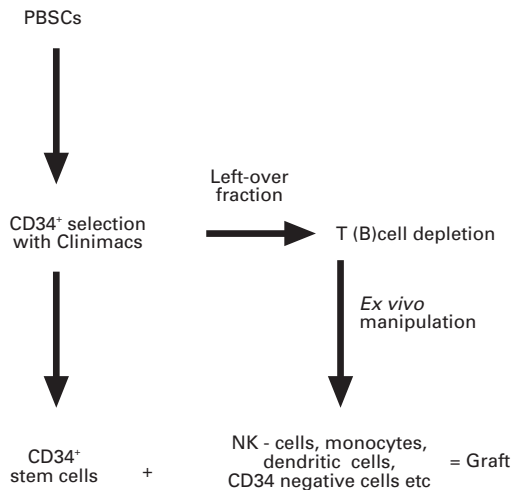


Figure 4 Proposed scheme for graft engineering of peripheral blood stem cells (PBSC). The CD34⁺ cells are purified in order to protect them from potential damage by further graft manipulation. The left-over fraction is then T (B) cell depleted. The remaining cells (natural killer (NK) cells, monocytes, CD34-negative stem cells) can then be manipulated without the risk of damaging the CD34⁺ population, eg augmentation of NK activity with interleukin 2, *ex vivo* generation of dendritic cells, expansion of CD34-negative stem cells with cytokines or others and transplanted together with the CD34⁺ stem cells.

References

- 1 Przepiorcka D, Ippoliti C, Khouri I *et al*. Allogeneic transplantation for advanced leukemia: improved short-term outcome with blood stem cell grafts and tacrolimus. *Transplantation* 1996; **62**: 1806–1810.
- 2 Russell JA, Brown C, Bowen T *et al*. Allogeneic blood cell transplants for haematological malignancy: preliminary comparison of outcomes with bone marrow transplantation. *Bone Marrow Transplant* 1996; **17**: 703–708.
- 3 Bensinger WI, Clift R, Martin P *et al*. Allogeneic peripheral blood stem cell transplantation in patients with advanced hematologic malignancies: a retrospective comparison with marrow transplantation. *Blood* 1996; **88**: 2794–2800.
- 4 Schmitz N, Bacigalupo A, Hasenclever D *et al*. Allogeneic bone marrow transplantation vs filgrastim-mobilised peripheral blood progenitor cell transplantation in patients with early leukaemia: first results of a randomised multicentre trial of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant* 1998; **21**: 995–1003.
- 5 Zucha JM, Gooley T, Bensinger WI *et al*. CD34 cell dose in granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cell grafts affects engraftment kinetics and development of extensive chronic graft-versus-host disease after human leukocyte antigen-identical sibling transplantation. *Blood* 2001; **98**: 3221–3227.
- 6 Handgretinger R, Klingebiel T, Lang P *et al*. Megadose transplantation of purified peripheral blood CD34⁽⁺⁾ progenitor cells from HLA-mismatched parental donors in children. *Bone Marrow Transplant* 2001; **27**: 777–783.
- 7 Aversa F, Tabilio A, Velardi A *et al*. Treatment of high-risk acute leukemia with T cell-depleted stem cells from related donors with one fully mismatched HLA haplotype. *New Engl J Med* 1998; **339**: 1186–1193.
- 8 Gilmore MJ, Patterson J, Ivory K *et al*. Standardization of T-cell depletion in HLA matched bone marrow transplantation. *Br J Haematol* 1986; **64**: 69–75.
- 9 Champlin RE, Passweg JR, Zhang MJ *et al*. T cell depletion

- of bone marrow transplants for leukemia from donors other than HLA-identical siblings: advantage of T cell antibodies with narrow specificities. *Blood* 2000; **95**: 3996–4003.
- 10 Schumm M, Lang P, Taylor G *et al*. Isolation of highly purified autologous and allogeneic peripheral CD34⁺ cells using the CliniMACS device. *J Hematother* 1999; **8**: 209–218.
- 11 Beelen DW, Peceny R, Elmaagacli A *et al*. Transplantation of highly purified HLA-identical sibling donor peripheral blood CD34⁺ cells without prophylactic post-transplant immunosuppression in adult patients with first chronic phase chronic myeloid leukemia: results of a phase II study. *Bone Marrow Transplant* 2000; **26**: 823–829.
- 12 Lang P, Handgretinger R, Schumm M *et al*. Transplantation of purified peripheral CD34⁺ stem cells from unrelated donors in children: Effective prevention of GVHD. *Blood* 1999; **94** (Suppl. 1): 667a.
- 13 Kato S, Yabe H, Yasui M *et al*. Allogeneic hematopoietic transplantation of CD34⁺ selected cells from an HLA haplo-identical related donor. A long-term follow-up of 135 patients and a comparison of stem cell source between the bone marrow and the peripheral blood. *Bone Marrow Transplant* 2000; **26**: 1281–1290.
- 14 Eyrich M, Lang P, Lal S *et al*. A prospective analysis of the pattern of immune reconstitution in a paediatric cohort following transplantation of positively selected human leucocyte antigen-disparate haematopoietic stem cells from parental donors. *Br J Haematol* 2001; **114**: 422–432.
- 15 Schreiber KL, Forman J. Effect of graft-versus-host disease on anti-tumor immunity. *J Immunol* 1990; **144**: 2018–2026.
- 16 Switzer GE, Goycoolea JM, Dew MA *et al*. Donating stimulated peripheral blood stem cells vs bone marrow: do donors experience the procedures differently? *Bone Marrow Transplant* 2001; **27**: 917–923.
- 17 Hooks MA, Wade CS, Millikan WJ. Jr. Muromonab CD-3: a review of its pharmacology, pharmacokinetics, and clinical use in transplantation. *Pharmacotherapy* 1991; **11**: 26–37.
- 18 Keever-Taylor CA, Craig A, Molter M *et al*. Complement-mediated T-cell depletion of bone marrow: comparison of T10B9.1A-31 and Muronomab-Orthoclone OKT3. *Cytotherapy* 2001; **3**: 467–481.
- 19 Murphy WJ, Koh CY, Raziuddin A *et al*. Immunobiology of natural killer cells and bone marrow transplantation: merging of basic and preclinical studies. *Immunol Rev* 2001; **181**: 279–289.
- 20 Zeis M, Uharek L, Glass B *et al*. Allogeneic NK cells as potent antileukemic effector cells after allogeneic bone marrow transplantation in mice. *Transplantation* 1995; **59**: 1734–1736.
- 21 Bonig H, Korholz D, Lex C *et al*. Monocyte deactivation and its reversal in a patient with chemotherapy-induced leukopenia and severe systemic infection. *Med Pediatr Oncol* 2000; **34**: 39–42.
- 22 Sato K, Hida S, Takayanagi H *et al*. Antiviral response by natural killer cells through TRAIL gene induction by IFN-alpha/beta. *Eur J Immunol* 2001; **31**: 3138–3146.
- 23 Biron CA, Brossay L. NK cells and NKT cells in innate defense against viral infections. *Curr Opin Immunol* 2001; **13**: 458–464.
- 24 Dokun AO, Chu DT, Yang L *et al*. Analysis of in situ NK cell responses during viral infection. *J Immunol* 2001; **167**: 5286–5293.
- 25 Osawa M, Hanada K, Hamada H, Nakauchi H. Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science* 1996; **273**: 242–245.
- 26 Zanjani ED, Almeida-Porada G, Livingston AG *et al*. Human

- bone marrow CD34⁻ cells engraft *in vivo* and undergo multilineage expression that includes giving rise to CD34⁺ cells. *Exp Hematol* 1998; **26**: 353–360.
- 27 Bhatia M, Bonnet D, Murdoch B *et al*. A newly discovered class of human hematopoietic cells with SCID-repopulating activity. *Nat Med* 1998; **4**: 1038–1045.
- 28 Bonnet D. Normal and leukemic CD34-negative human hematopoietic stem cells. *Rev Clin Exp Hematol* 2001; **5**: 42–61.
- 29 de Wynter EA, Buck D, Hart C *et al*. CD34⁺AC133⁺ cells isolated from cord blood are highly enriched in long-term culture-initiating cells, NOD/SCID-repopulating cells and dendritic cell progenitors. *Stem Cells* 1998; **16**: 387–396.
- 30 van Esser JW, van der HB, Meijer E *et al*. Epstein–Barr virus (EBV) reactivation is a frequent event after allogeneic stem cell transplantation (SCT) and quantitatively predicts EBV-lymphoproliferative disease following T-cell-depleted SCT. *Blood* 2001; **98**: 972–978.
- 31 Heslop HE, Rooney CM. Adoptive cellular immunotherapy for EBV lymphoproliferative disease. *Immunol Rev* 1997; **157**: 217–222.
- 32 Faye A, Quartier P, Reguerre Y *et al*. Chimaeric anti-CD20 monoclonal antibody (rituximab) in post-transplant B-lymphoproliferative disorder following stem cell transplantation in children. *Br J Haematol* 2001; **115**: 112–118.