Allogeneic transplantation of peripheral blood progenitor cells

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With the use of the haematopoietic growth factors G-CSF and GM-CSF, haematopoietic progenitor cells can be mobilized into the blood. These peripheral blood progenitor cells (PBPC) contain the pluripotent and presumably the totipotent stem cells responsible for haematopoiesis and lymphopoiesis. Using leukaphereses, PBPCs are harvested from the blood of patients for autologous haematopoietic rescue after high dose or myeloablative cancer therapy. PBPCs have largely replaced bone marrow harvest for autologous transplantation. Allogeneic PBPCs mobilized from HLA-compatible family donors can be successfully transplanted after myeloablative therapy and can fully reconstitute haematopoiesis and lymphopoiesis [1–3].

This work followed reports of allogeneic blood cell mobilization in animals [4, 5] and in a few patients [6, 7], and on a small number of cases of treatment of poor marrow graft function [8, 9]. The first reports with clear evidence of engraftment of G-CSF mobilized allogeneic peripheral blood cells were published in 1993 [7, 8]. The allogeneic transplantation of haematopoietic progenitor cells using PBPCs is currently being studied in many centres. However, there are several problems concerning the donor and the recipient. These include 1) the long term effects of mobilization with G-CSF in normal donors, 2) the optimal G-CSF dose and schedule for PBPC mobilization, 3) the incidence of acute and chronic graft versus host disease following the transplantation of large numbers of T-cells, 4) whether the CD34+ stem cell fraction should be enriched and the T-cell numbers reduced, 5) PBPC and life-long engraftment, 6) the effect of PBPC on the graft versus leukemia effect, 7) the use of allogeneic PBPC from unrelated donors, 8) the recovery of the immune system and 9) the influence of immune reconstitution on infections.

To achieve PBPC mobilization, G-CSF has to be injected into a healthy individual for 5 to 7 days, at dosages ranging from twice daily 5 μg per kilogram body weight to once daily 16 μg per kilogram (see Table 1). The early possible side effects of G-CSF are bone pain, myalgia and dryness of the mouth in some cases. Paracetamol readily relieves the pain and can be given prophylactically. There is also leukocytosis: as observed in our own study, the number of leukocytes increased up to a mean (range) number per microliter of 46,600 (24,000–63,000) by day 4, 46,000 (26,500–74,700) by day 5, 54,000 (42,200–77,300) by day 6 and 58,000 (48,300–68,400) by day 7. So far neither we nor others have observed any clinical problems with this leukocytosis, which is of course mainly due to neutrophils [10]. The CD34+ cell counts which follow use of our own protocol are depicted in Figure 1 [11].

There is a theoretical concern about the unknown long term effects of G-CSF. However, it is unclear if such effects are to be expected. G-CSF dosages of 5–10 μg/kg result in G-CSF serum levels of approximately 30–100 μg/ml, as shown by phase I studies [12, 13]. Similar endogenous G-CSF levels are found in some neutropic patients with bacteraemia [14]. Thus the injection of 5–10 μG G-CSF/kg in normal individuals for several days may mimic conditions which are not unusual. The avoidance of general anaesthesia, autologous blood donation and painful marrow harvest

Table 1. Blood stem cell mobilization in normal donors and the numbers of CD34+ blood cells used for allogeneic transplantation; id.sib - HLA identical sibling; haplod. – HLA haplotype identical family donor; BM – bone marrow.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Type of transplant</th>
<th>G-CSF μg/kg</th>
<th>G-CSF for</th>
<th>No. of aphereses</th>
<th>CD34+ cells x 10^9/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. Link [27]</td>
<td>CD34+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N. Schmitz [1]</td>
<td>Blood, id.sib.</td>
<td>2 x 5</td>
<td>5–7</td>
<td>2–4</td>
<td>7.4</td>
</tr>
<tr>
<td>B. Speck [42]</td>
<td>Blood, id.sib.</td>
<td>5–10</td>
<td>5–6</td>
<td>1–3</td>
<td>2.2–8.1</td>
</tr>
<tr>
<td>P. Aversa [26]</td>
<td>BM, CD34+</td>
<td>10</td>
<td>5–7</td>
<td>3–5</td>
<td>7</td>
</tr>
<tr>
<td>C.</td>
<td>Blood, haplod</td>
<td>12</td>
<td>5–7</td>
<td>2–4</td>
<td>11.6</td>
</tr>
<tr>
<td>H. Link [11]</td>
<td>BM, CD34+</td>
<td>2 x 5</td>
<td>5</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td>A. Yeager [43]</td>
<td>Blood, haplod</td>
<td>10</td>
<td>6</td>
<td>2</td>
<td>17.1</td>
</tr>
</tbody>
</table>
cells per kg and ranges from 2.2 to 13.1 \( \times 10^6 \) CD34+6 read ranges from 2.2 to 13.1 \( \times 10^6 \) CD34+6. The number of allogeneic CD34 blood cells transplant-equivalent to a standard bone marrow harvest. The number of allogeneic CD34 blood cells per kilogram should suffice and be CD34+6. To maintain the same proportion of stem cells, then 2 \( \times 10^6 \) CD34+6 cells per kilogram. Thus, if one assumes that the number of lymphopoietic progenitor cells, characterized by the CD34 surface antigen [15, 16]. In our study 0.8% of cells were CD34+6 and 37% were T-lymphocytes with a ratio of 1.5:1 for T-helper (CD4) to T-suppressor or T-killer (CD8) cells. B-lymphocytes comprised 2.4%, monocytes 31%, natural killer cells 3% and granulocytes 15% of the collected cells.

The number of CD34+6 cells is usually accepted as an estimate of the number of progenitor and possible stem cells [16]. It is assumed that CD34+6 cells represent pluripotent and committed haematopoietic stem cells [16–18]. Therefore analysis of CD34+6 subgroups is necessary to define the critical number of the most primitive stem cells. Those cells are not committed to lymphohaematopoietic cell lineages, which express the Thy-1 antigen, are HLA-DR negative and have other characteristic [16, 19–21].

In autologous PBPC-transplantation about 2–3 \( \times 10^6 \) CD34+6 cells per kilogram are necessary for reliable and rapid haematological recovery after myeloablative therapy [22–24]. In the autologous setting, the minimum number of CD34+6 cells needed for haematopoietic and lymphopoietic engraftment is not known. In autologous bone marrow transplantation, 2 \( \times 10^6 \) nucleated cells per kilogram body weight are sufficient for durable engraftment [25]. About 1% of these cells express the CD34 antigen [11]. This corresponds to 2 \( \times 10^6 \) CD34+6 cells per kilogram. Thus, if one assumes that the CD34+6 cells from marrow and peripheral blood contain the same proportion of stem cells, then 2 \( \times 10^6 \) CD34+6 blood cells per kilogram should suffice and be equivalent to a standard bone marrow harvest.

For patients with an HLA-identical sibling donor, the number of allogeneic CD34 blood cells transplanted ranges from 2.2 to 13.1 \( \times 10^6 \) CD34+6 cells per kg (Table 1). Current experience with the transplantation of blood stem cells suggests that 2–4 \( \times 10^6 \) CD34 cells per kilogram are enough for durable engraftment with HLA-matched related donors. Our own experience showed an accelerated haematopoietic recovery if CD34+6 selected blood cells are transplanted together with bone marrow cells [11].

In the setting of HLA-mismatched family donors, CD34-selected blood cells have been given in addition to bone marrow, in order to enhance engraftment [26]. Surprisingly, the incidence of severe acute graft versus host disease (aGvHD) was not increased in comparison to published data. However, 7 of 17 recipients were children, who have a lower risk of acute GvHD [26]. The number of additionally transplanted blood cells is given in the lower part of Table 1.

In HLA-haploidentical T-cell depleted bone marrow transplantation, the addition of large numbers of blood CD34 cells to marrow cells may overcome the anti-donor immune-response of the recipients [26]. Thus the graft failure and rejection rates can probably be decreased by a 7–10 fold increase of progenitor cells in the T-cell depleted transplant inoculum.

The transplantation of mobilized allogeneic blood cells resulted in the regeneration of granulopoiesis to more than 500 granulocytes per \( \mu l \) within 9–15 days, and thrombopoiesis was restored within 16–39 days [1–3, 25, 27]. We have transplanted CD34+6 blood cells after immunoselection. Data showing granulocyte recovery are depicted in Figure 2. The cells were selected with CD34-specific biotin-labeled antibodies, which were processed with an avidin coated column (Ceprate SC, CellPro, Bothell, WA, U.S.A.). The CD34+6 cells retained on the column were later washed out and concentrated. By this method, the CD34+6 cells concentration was increased from 1% to 60%–80%. However, 30%–50% of CD34+6 cells were lost. With this procedure, CD34+6 T-cells were reduced by 2–3 log levels. For the 10 recipients the number of CD3+6 cells was reduced from approximately \( 2 \times 10^8 \) to \( 1.2 \times 10^6 \) per kilogram body weight. T-cell reduction was used with the aim of avoiding the possible increase of severe acute GvHD caused by the transplantation of large numbers of T-cells. The critical number of T-cells for induction of acute GvHD is within the range of \( 1 \times 10^5 \)–\( 1 \times 10^6 \) cells per kilogram body weight [28–30]. This global estimate may not be adequate since it has been shown that the number of donor T-helper precursor cells may be decisive [31, 32] (Table 2). However, these data were generated from bone marrow transplantation. Furthermore, transplanting viable donor buffy coat blood cells after BMT in order to enhance the graft versus leukaemia effect significantly increased the incidence of grade II–IV acute GvHD and led to a higher rate of non-relapse deaths [33] (Table 2). Chronic (cGvHD) may develop in a large proportion of recipients of unmodified blood with large numbers of T-cells. The transfusion of donor buffy coat cells in addition to bone marrow in patients with severe...
leukocytes x 10^3/μl blood (median)

Figure 2. Regeneration of neutrophil granulocytes in patients after allogeneic transplantation of CD34+ blood cells; 5 patients received only cyclosporin A for prophylaxis of acute GvHD and 5 patients received both cyclosporin and methotrexate 15 mg/m² on day 1 and 10 mg/m² on days 3 and 6. For comparison, the data from 11 patients with allogeneic bone marrow transplantation are shown. All patients received 5 μg/kg G-CSF per day until the level of 500 neutrophils/μl was reached for 3 consecutive days. Recombinant human erythropoietin was given at a dose of 150 U/kg as continuous infusion from day 7 until transfusion independence for 7 consecutive days [11,27].

 aplastic anaemia resulted in an incidence of cGvHD of 68% and a mortality rate of 27%, whereas without donor buffy coat cells only 33% developed cGvHD, without lethal complications [34]. In adoptive immunotherapy for relapsed chronic myeloid leukaemia, a high incidence of acute and chronic GvHD has been reported [35,36]. One major advantage of allogeneic bone marrow transplantation is the prevention of leukemic relapse, i.e., the graft versus leukaemia effect [35]. It is exerted by donor T-cells, natural killer cells, lymphokine activated killer cells and the direct effects of cytokines. Experience from bone marrow transplantation suggests that the critical minimal number of T-cells needed to achieve this graft versus leukaemia effect is about 1 x 10^5 cells per kilogram body weight [37]. Whether this number is also valid for blood cell transplantation is so far unknown. Extrapolation to blood cell transplantation suggests it may be possible to avoid the unwanted effects of GvHD by reducing the number of T-cells to approximately 1–5 x 10^5 cells per kilogram. However, the number of T-cells given should not be lower than this if the graft versus leukaemia effect is to be preserved.

The experience of aGvHD and cGvHD with allogeneic blood cells is inconclusive. Some authors suggest a lower incidence of severe aGvHD than with bone marrow transplantation, whereas others show no difference. Data on GvHD from some recently published studies are summarized in Table 3.

We have found that giving immunoselected CD34+ cells and 1 x 10^6 CD3 cells per kilogram leads to a higher incidence of aGvHD, when cyclosporin A alone is given for prophylaxis of GvHD. Four of five patients developed grade III–IV acute GvHD and two patients died from GvHD-associated complications. The three surviving patients have chronic GvHD. Because of this we now also give methotrexate 15 mg/m² on day 1 and 10 mg/m² on days 3 and 6 after transplantation. There has been only one case of grade III aGvHD in eight patients treated so far using this protocol of cyclosporin A and methotrexate. Chronic GvHD was observed in

Table 3. Incidence of acute and chronic GvHD after allogeneic blood transplantation; n.a. not available.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Type of prophylaxis</th>
<th>T-cells x 10^5/kg</th>
<th>Acute GvHD</th>
<th>Chronic GvHD</th>
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<tr>
<td>Bensinger</td>
<td>CsA, MTX</td>
<td>385</td>
<td>36</td>
<td>32</td>
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<tr>
<td></td>
<td>CsA, Prednisolone</td>
<td></td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Bensinger</td>
<td>CsA, MTX</td>
<td>385</td>
<td>4</td>
<td>1 of 2</td>
</tr>
<tr>
<td></td>
<td>CsA, Prednisolone</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Korbling</td>
<td>CsA, Prednisolone</td>
<td>430</td>
<td>45%</td>
<td>66%</td>
</tr>
<tr>
<td>(CD34+)</td>
<td>CsA, MTX</td>
<td>1.05</td>
<td></td>
<td>2 of 5</td>
</tr>
<tr>
<td>Schmitz</td>
<td>CsA, MTX</td>
<td>330</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Speck [42]</td>
<td>CsA, MTX</td>
<td>n.a.</td>
<td></td>
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</table>
two of five evaluable patients. After transplant of unmodified blood cells, the incidence of cGVHD ranges from 32% to 66% [36, 38, 39].

In our experience, the reduction of T-cells to approximately $1 \times 10^6$ CD3-T-cells per kilogram reduces the severity of acute and chronic GvHD when cyclosporin A and methotrexate are given for GvHD prophylaxis. Further studies are needed to clarify optimal GvHD-prophylaxis. As methotrexate delays haemopoietic regeneration, prednisolone may be an alternative. Immune system recovery was assessed in our small group of patients. There are no major differences compared with bone marrow transplantation with unmodified or T-cell depleted marrow cells [27]. T-cell depletion can delay T-cell recovery after transplantation [40]. Since certain authors report an increased incidence of Epstein–Barr Virus associated lymphoma (EBV) [41] after T-cell depletion, attention must be given to this problem.

Several groups have now shown long term engraftment lasting more than one year. It must therefore be accepted that allogeneic blood cells may provide a suitable alternative to the transplantation of bone marrow cells [1–3, 20, 27].

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References

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