Response

To the Editor:

The word “transplantation” does not necessarily imply that targeted patients will always develop prolonged cytopenia, for which treatment with G-CSF is recommended. It must be stressed that de novo hematopoietic recovery kinetics after PB SCT versus those after bone marrow transplantation or after transplants with a minimum number of hematopoietic stem/progenitor cells versus after those with more than threshold numbers should be quite different. It is clear that there are age-dependent differences in stem cell kinetics and the quantity/quality of PBSC in grafts, which certainly affect the profile of the clinical response to G-CSF. In this regard, we thank Ojeda et al for revisiting the now widely accepted clinical procedure of using G-CSF after PBSC transplantation (PB SCT) by referring to our data published in a recent issue of BLOOD. 1 In that study, we reported the results of a prospective randomized trial with 74 children who were scheduled to undergo high-dose chemotherapy followed by autologous PBSC. In the data reported by us with children and by Ojeda et al with adult patients, a 1- or 2-day earlier recovery of granulocytes did not have any meaningful practical clinical consideration. Moreover, we and Ojeda et al found that the marginal clinical benefit of an earlier recovery of granulocytes could be offset by the delayed recovery of platelets, which has been well reported by other groups, 2,3 although the exact mechanism of this phenomenon is unclear.

The quality of our multicenter study might be questioned. However, in a series of studies we have shown that morbidity and mortality related to PB SCT are essentially negligible, particularly in pediatric patients. This makes the biases that originate from differences in clinical management procedures among the participating institutes less significant. Indeed, in our intent-to-treat analysis, none of the patients suffered from serious bleeding episodes or clinically significant infectious complications. Hence, the value of extending the analysis to include minor items, such as mucositis, is questionable. All of the patients with ALL (n = 27) and neuroblastoma (n = 29) were treated uniformly according to the protocols of the Japanese Cooperative Study Group of PB SCT. Thus, it is unlikely that the quality of our multicenter trial and trivial differences in supportive care systems, if any, greatly affected the outcome of our study. We could not perform a critical cost analysis, because in Japan all medical costs related to cancer therapy for children are paid by the government, not by the family, and decisions regarding drug therapy or hospitalization depend strongly on social considerations.

There might be another pitfall with G-CSF treatment when used after transplantation, ie, the administration of G-CSF may further exaggerate cytokine dysregulation, which frequently occurs in the rapid recovery phase of hematopoiesis after PB SCT and is clinically recognized as engraftment syndrome. In addition, a sharp surge of granulocytes in the circulation may increase the trapping of cells in the microcirculation, particularly in pulmonary vessels. Consequently, the incidence and severity of pulmonary complications might be increased in adult patients, as has been reported after conventional chemotherapy. 4 Considering these points, we agree that the routine application of this expensive strategy in patients undergoing PB SCT should be seriously reevaluated in adult patients. Reconsideration of this maneuver also has important implications for the cost-effectiveness of hematopoietic stem cell transplantation.

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REFERENCES

Intracytoplasmic Detection of Cytokines in Neonatal Lymphocytes and Monocytes by Flow Cytometry

To the Editor:

In a recent publication in BLOOD, Chalmers et al1 presented data on cytokine production of lymphocytes at the single-cell level using three-color flow cytometry. A total of 6 blood samples have been investigated. Their main finding was the lower production of interleukin-2 (IL-2), IL-4, interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) by cord blood lymphocytes compared with peripheral blood lymphocytes derived from adults. The investigators concluded that the reduced incidence of graft-versus-host disease in cord blood transplantation may be due to this altered cytokine profile. Performing as well the intracytoplasmic detection of cytokines by flow cytometry on 20 cord
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The nonparametric Mann-Whitney U test.

Lymphocytes analyzed at the single-cell level by flow cytometry.

With phorbol myristate acetate and ionomycin, the production of IL-2

[MFI], with the interquartile range in parentheses. After stimulation

and the cytokine expression per cell (mean fluorescence intensity

be overestimated. We therefore emphasize that only the use of adequate

negative controls such as unlabeled anticytokine antibodies enables a

meaningful interpretation of data obtained by flow cytometric detection

intracytoplasmic cytokines. The technique of cell separation perform-

ing density gradient centrifugation (Ficoll-Hypaque) used in the study

of Chalmers et al also requires further comment. Although these

preparations provide useful information about individual cell types,

functional responses of purified cell preparations may not represent

physiological reactions occurring in vivo. De Groote et al clearly

demonstrated that density gradient centrifugation may affect cell

activation status, resulting in considerably altered cytokine production

compared with whole blood culture technique. Whole blood containing

physiological levels of regulatory cytokines may therefore more closely

approximate circulating cells in vivo and better reflect disease state

alterations. To avoid artificial modifying effects on cell function, we

preferred the whole blood assay before flow cytometric analysis of

intracytoplasmic cytokines.

Additionally, we would like to comment on the decreased production

of TNF-α by cord blood lymphocytes. This observation could be

misinterpreted as a diminished capacity of neonates to produce inflam-

matory cytokines. However, it should be kept in mind that monocytes

are a major cellular source for this cytokine. Andersson et al had

already shown that cord blood monocytes were able to produce TNF-α

at the single-cell level. Moreover, we found an increased amount of

IL-6-positive monocytes in cord blood versus adult blood (Table 2).

Because IL-6 represents a further step in the lipopolysaccharide-

induced cytokine cascade after TNF-α secretion, these results support a

well-developed inflammatory reaction in neonates.

In summary, our results based on a larger series show an even more
decreased cellular immune response in neonates, characterized by a

profoundly reduced production of IL-2 and IFN-γ. However, the ability

of neonatal monocytes to produce TNF-α and IL-6 (Table 2) suggests

that an impaired inflammatory response is not responsible for the

reduced incidence of graft-versus-host disease in cord blood transplan-
tation. To clarify these and other questions in neonatal immunology, the

method presented here has the potential to become the standard assay

for examining cytokine production at the single-cell level. Adequate

negative controls and the whole blood culture technique instead of cell

separation should be preferred to prevent misinterpretation of data.

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Table 1. Reduced Capacity of Neonatal T Lymphocytes

to Produce IL-2 and IFN-γ

<table>
<thead>
<tr>
<th></th>
<th>Cord Blood (n = 20)</th>
<th>Adult Blood (n = 34)</th>
<th>P Value*</th>
</tr>
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<tbody>
<tr>
<td>IL-2 producing cells (%)</td>
<td>17 (13-26)</td>
<td>34 (28-37)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>IL-2 expression per cell (MFI)</td>
<td>8 (7-9)</td>
<td>14 (12-15)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>IFN-γ producing cells (%)</td>
<td>4 (3-5)</td>
<td>18 (14-20)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>IFN-γ expression per cell (MFI)</td>
<td>3.5 (3-4)</td>
<td>12 (10-15)</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

Values are expressed as the median of cytokine producing cells (%) and the cytokine expression per cell (mean fluorescence intensity [MFI]), with the interquartile range in parentheses. After stimulation with phorbol myristate acetate and ionomycin, the production of IL-2 and IFN-γ is significantly lower in cord blood than in adult blood lymphocytes analyzed at the single-cell level by flow cytometry.

*Statistical differences comparing the two groups were tested by the nonparametric Mann-Whitney U test.

Table 2. Increased Production of IL-6 in Neonatal Monocytes

<table>
<thead>
<tr>
<th></th>
<th>Cord Blood (n = 20)</th>
<th>Adult Blood (n = 30)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 producing cells (%)</td>
<td>63 (57-66)</td>
<td>50 (42-60)</td>
<td>&lt;.001</td>
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<tr>
<td>IL-6 expression per cell (MFI)</td>
<td>14 (13-16)</td>
<td>9 (8-10)</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

Values are expressed as the median of cytokine producing cells (%) and the cytokine expression per cell (mean fluorescence intensity [MFI]), with the interquartile range in parentheses. After stimulation with lipopolysaccharide, the production of IL-6 is significantly higher in cord blood than in adult blood monocytes analyzed at the single-cell level by flow cytometry.

*Statistical differences comparing the two groups were tested by the nonparametric Mann-Whitney U test.