were evaluated. Regardless of the previous variables, a preleukapheresis PB CD34+ cell concentration ≥ 40/µL was significantly related to the collection of at least 2 x 10^6 CD34+ cells/kg in a single apheresis, as previously reported. In addition to the above data, we found that to obtain a target number of 2 x 10^6 CD34+ cells/kg, PB CD34+ cell concentrations ≤ 30/µL are associated with the need for at least two leukapheresis procedures and PB concentrations ≤ 15/µL are associated with the need for at least three procedures. In conclusion, our study shows that preleukapheresis PB CD34+ cell concentration can be used to guide PBPC harvest by predicting both the total CD34+ cell yield and the number of aphereses needed to be undergone.

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CD34, mobilization, autologous peripheral blood transplantation

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References

Phenotypic changes in neutrophils after rhG-CSF administration in non-Hodgkin's lymphoma patients undergoing PBSC transplantation or conventional chemotherapy

Sir,

rhG-CSF induces several phenotypic changes in neutrophils. Increased HLA-DR expression and decreased CD10 expression have recently been described in neutrophils from some patients after rhG-CSF therapy. We evaluated these parameters in 12 non-Hodgkin’s lymphoma patients undergoing either PBSC transplantation after high-dose chemotherapy or conventional chemotherapy. The appearance of an HLA-DR-positive neutrophil subpopulation, along with a marked decrease in CD10 expression, was confirmed. However, despite this immature phenotype, rhG-CSF-induced neutrophils displayed enhanced phagocytosis and chemiluminescence.

Recombinant human granulocyte colony-stimulating factor (rhG-CSF) induces several changes in neutrophils. Recently, Zarco et al.3 described new phenotypic findings in rhG-CSF-induced neutrophils in six ALL patients undergoing chemotherapy. The appearance of an HLA-DR-positive neutrophil subpopulation, along with a decrease in the percentage of CD10+ neutrophils, appeared of particular interest.

We reviewed the clinical files of patients recently treated with rhG-CSF (Filgrastim) for whom analysis of HLA-DR and CD10 expression on circulating neutrophils before and after rhG-CSF administration was available. Twelve patients (4 females, 8 males), with intermediate and high grade non-Hodgkin’s lymphoma (NHL) were evaluated. Six patients had been treated with autologous peripheral blood stem cells (PBSC) transplantation after high-dose chemotherapy, and neutrophils had been studied before the conditioning regimen and after engraftment (i.e. neutrophils >0.5 x 10^9/L, and platelets >20 x 10^9/L), stimulated by rhG-CSF (5 mg/kg/day). The other 6 patients had been studied before the first course of chemotherapy (Promice-Cytabom) and after a five-day course of rhG-CSF (5 µg/kg/day), administered to
accelerate neutrophil recovery. Six blood donors were evaluated as controls. To evaluate the possible influence of spontaneous neutrophil recovery on HLA-DR and CD10 expression, 5 NHL patients undergoing the same conventional chemotherapy were evaluated between two therapy courses: neutrophils were studied during both the neutropenic phase (<1×10⁹/L) and when neutrophils rose to normal levels (≥1.8×10⁹/L) spontaneously. In addition, 2 patients with acute non-lymphoblastic leukemia undergoing allogeneic bone marrow transplantation were studied when engraftment was achieved.

HLA-DR and CD10 expression was evaluated by a whole blood method, using two MoAbs (clone LA243, Becton Dickinson, and clone SS2/36, Dako). The percentage of positive cells and the mean intensity of fluorescence were established using Lysis II software (Becton Dickinson). Neutrophil phagocytosis and chemiluminescence were evaluated in the PBSC transplantation group, using a flow cytometric whole blood method and an automated, computerized whole blood assay, respectively.

We found that a significant subset of HLA-DR-positive neutrophils was induced by rhG-CSF treatment, irrespective of the type of therapy. Mean values of fluorescence intensity were high, with a wide range of distribution (not shown). In contrast, CD10 expression clearly decreased (Table 1). These results appeared to be due to rhG-CSF, because normal (<1%) HLA-DR expression and conserved CD10 expression were detected both in patients after spontaneous neutrophil recovery after conventional chemotherapy, and in the two patients undergoing allogeneic bone marrow transplantation studied at engraftment (data not shown).

Despite these phenotypic changes, however, neutrophils appeared to be strongly primed both for phagocytosis and for chemiluminescence (Table 1).

We were able, therefore, to confirm the results reported by Zarco et al., in a larger series of patients undergoing different therapeutic programs. These phenotypic changes in neutrophils may be consistent with abnormal maturation of neutrophils during their accelerated bone marrow transit, as suggested previously, because CD10 expression occurs late in neutrophil maturation. The biological significance and the possible role of such phenotypic modifications on neutrophil activity are not clear, because we found that neutrophils, despite their phenotypic abnormalities, were characterized by increased phagocytic and bactericidal capacity. However, a defect of CD10 expression, which may be responsible for reduced chemotactic response of neutrophils towards fMLP, may at least in part explain the reported defect of chemotaxis after rhG-CSF administration.

Table 1. Neutrophil HLA-DR and CD10 expression, and neutrophil functions before and after rhG-CSF treatment.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=6)</th>
<th>Chemotherapy before G-CSF (n=6)</th>
<th>Chemotherapy after G-CSF (n=6)</th>
<th>PBSC transplantation before G-CSF (n=6)</th>
<th>PBSC transplantation after G-CSF (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DR %</td>
<td>&lt;1</td>
<td>0.89±0.79</td>
<td>8.37±3.37*</td>
<td>1±0.6</td>
<td>5±3.7*</td>
</tr>
<tr>
<td>CD10 %</td>
<td>50-85</td>
<td>52±13</td>
<td>52±25*</td>
<td>81.6±11</td>
<td>24.5±24.6*</td>
</tr>
<tr>
<td>Phagocytosis MFC counts/PMN/30 min</td>
<td>250-800</td>
<td>ND</td>
<td>ND</td>
<td>340±111</td>
<td>715±227*</td>
</tr>
<tr>
<td>Chemiluminescence</td>
<td>600-1600</td>
<td>ND</td>
<td>ND</td>
<td>1190±338</td>
<td>2800±993*</td>
</tr>
</tbody>
</table>

*p=0.012; †p=0.009; ‡p=0.038; ^p=0.0001; §p=0.024; °p=0.019. MFC: mean fluorescence channel.

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Key words
G-CSF, neutrophils, surface markers

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Resolution of thrombocytopenia after treatment for Helicobacter pylori: a case report

Sir,

We report the case of a 51-year-old man with idiopathic thrombocytopenic purpura who experienced a complete and lasting normalization of platelet count after antibiotic treatment for a Helicobacter pylori infection.

A 51-year-old man with previously untreated chronic idiopathic thrombocytopenic purpura (ITP) was admitted because of melena. An upper gastrointestinal endoscopy showed a small bleeding ulcer in the para-cardial region of the stomach. The patient was treated with i.v. ranitidine until endoscopically documented resolution of bleeding, and with a short course of i.v. immunoglobulins (40 g/die for 4 days), which allowed the platelet count to increase to a maximum of 162×10^9/L. Soon after a laparosplenectomy was performed without complications. The patient was discharged three days after surgery with a platelet count of 40×10^9/L. He continued to take ranitidine orally (150 mg daily) for 2 months. Six months after his splenectomy he was admitted again because of a second episode of endoscopically documented gastric bleeding manifested by melena. Over this period his platelet count never exceeded 100×10^9/L (Figure 1).

Considering that about 80% of gastric ulcers are associated with Helicobacter pylori infection1,2 and that our patient had a positive serologic test for Helicobacter (35 IU/mL, normal values less than 15 IU/mL) (Enzygnost anti-H. pylori, Boehringer, Germany) he was treated with amoxicillin (1 g three times a day) and omeprazole (40 mg daily) for 14 days. Afterwards he continued assuming oral omeprazole (20 mg daily). His platelet count, which was monitored every two weeks, started to increase at the end of antibiotic therapy and reached normal and stable values after 5 weeks (Figure 1). The first endoscopic control was performed 6 weeks after the end of treatment. No ulcers were seen, nor was Helicobacter pylori found, but a polyclonal B lymphocytic infiltration of the mucosa was detected. Three subsequent endoscopic controls with multiple biopsies were performed every three months. Histologic specimens remained negative for Helicobacter pylori and the lymphocytic infiltrate was not documented again. A serologic test for Helicobacter pylori was 20 IU/mL 6 months after the end of therapy. Helicobacter pylori infection can be associated with development of lymphoid follicles in the stomach, which may disappear after eradication of the micro-organism.3,4 This kind of infection has been hypothesized to be related with the development of gastric MALT lymphoma;5,6 its possible involvement in some autoimmune disorders is still an open question.7,8 Recently it was reported that Helicobacter pylori eradication can be followed by a significant increase in platelet count in patients with ITP and that the prevalence of such infection in patients with ITP can be high.9 Diagnosis of Helicobacter pylori infection in our case was based only on epidemiological probability and serologic data, since it was not possible to obtain histologic documentation of infection and other non-invasive tests were not available. The diagnostic accuracy of serology is, however, high10 and the infection was very probable in our patient who had a gastric ulcer and a positive serologic test. The normalization of his platelet count following antibiotic treatment was impressive: on the basis of this observation and of the recent report by Franceschi et al.9 a relationship between Helicobacter pylori infection and ITP can be hypothesized. This deserves further investigation in a larger number of patients.

Figure 1. A diagnosis of chronic ITP was established three months before the first melena. Platelet count ranged between 25 and 35×10^9/L, bone marrow was normal and a direct antiplatelet antibodies test was positive. After the first episode of melena, the patient was treated with high dose i.v. immunoglobulins and splenectomy with a transitory response. After the second melena the patient was treated with amoxicillin and omeprazole and achieved a complete and stable response.

Key words
Thrombocytopenia, helicobacter pylori

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