**References**


**Phenotypic changes in neutrophil granulocytes after G-CSF administration in patients with acute lymphoblastic leukemia under chemotherapy**

**Maria-Angelica Zarco,** *Josep-Maria Riba,*

**Neus Villamor,** *Avelina Balmes,* **Alvaro Urbano**

**Espinosa,* Evarist Feliu**

Departments of Hematology, *Hospital Universitari Germans Trias i Pujol, Badalona, Universidad Autónoma de Barcelona* and *Hospital Clinic, Barcelona, Spain*

**Phenotypic changes in neutrophil granulocytes (NG) after G-CSF have been scarcely studied. Using flow cytometry, we analyzed the changes of CD11b, CD14, CD33, CD71, HLA-DR, CD10, CD16 and CD15 on NG after G-CSF treatment in 6 patients with ALL receiving**
Intensification chemotherapy and in 10 control subjects. After G-CSF we found: expression of HLA-DR, a higher expression of CD11b, CD71 and CD14, a decrease in CD10 positivity, and fluorescence intensity in CD15 and CD16. After administration of G-CSF, the NG of patients with ALL express an immature phenotype as well as markers of proliferation.

In addition to the increase in the number of neutrophil granulocytes (NG), functional and phenotypic changes have occurred after G-CSF treatment. However, latter changes have been scarcely studied. The aim of this study was to analyze these changes in ALL patients receiving G-CSF to enhance NG recovery after intensification chemotherapy.

Six adult patients with ALL receiving intensification chemotherapy (PETHEMA ALL-93 protocol) were studied. The patients received 5 µg/kg G-CSF (Filgrastim®, Amgen-Roche) by subcutaneous injection once a day from the day after the end of chemotherapy to the day in which NG count was over \(1\times10^9/L\) (median 9 days after the last day of chemotherapy, range 5-13) in two consecutive determinations. Ten cycles of chemotherapy were evaluated. Neutrophil phenotype (CD11b, CD14, CD33, CD71, HLA-DR, CD10, CD16 and CD15) was analyzed within 24 hours following the last G-CSF administration by double immunofluorescence technique, and cell surface membrane antigens were detected by quantitative flow cytometry using a FACScan™ (Becton Dickinson, San José, CA, USA). The number of cells counted for this analysis was \(15\times10^3\). Acquisition gates were set to exclude dead cells and aggregated material. Forward versus side scatter display was used to define granulocytes. For each MoAb the percentage of positive cells was established using the Lysys II software from Becton Dickinson. The mean intensity of fluorescence of CD15 and CD16 was also studied.

There were no differences in NG surface antigenic expression among the 10 control subjects and the 10 studies performed to the 6 ALL patients before G-CSF administration (Table 1). Table 1 also shows the antigenic changes observed before and after treatment with G-CSF. The most outstanding feature was the HLA-DR expression on NG (Figure 1) in ALL cases after G-CSF treatment compared to controls and to the same studies before G-CSF treatment. Other changes included a higher expression of CD71, CD14 and CD11b (although the latter was not statistically significant), a decrease in the CD10 expression and in CD15 and CD16 fluorescence intensity.

G-CSF has been employed in ALL\(^4\) and has produced a reduction in the duration of the period of neutropenia, with no influence on either CR or relapse rates. The most outstanding finding in this study was the confirmation of the marked HLA-DR expression on NG after G-CSF administration. Such a finding was previously observed in a patient with chronic idiopathic neutropenia under G-CSF treatment.\(^5\) The

<table>
<thead>
<tr>
<th>CD14</th>
<th>CD71</th>
<th>HLA-DR</th>
<th>CD10</th>
<th>CD11b</th>
<th>CD15</th>
<th>CD16</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Controls (n=10)</td>
<td>1±0.5</td>
<td>0.3±0.4</td>
<td>0.2±0.2</td>
<td>53.2±20.2</td>
<td>98±1.3</td>
<td>94±53</td>
</tr>
<tr>
<td>ALL before G-CSF (n=6, 10 episodes)</td>
<td>1.2±0.9</td>
<td>0.4±0.7</td>
<td>0.3±0.4</td>
<td>39.6±15.3</td>
<td>96±1.9</td>
<td>102±48</td>
</tr>
<tr>
<td>ALL after G-CSF (n=6, 10 episodes)</td>
<td>5.1±1.8</td>
<td>10.6±6.0</td>
<td>4.5±1.2</td>
<td>0.4±0.5</td>
<td>93±4.8</td>
<td>125±46</td>
</tr>
</tbody>
</table>

Results are expressed as mean±standard deviation. *p=0.001; *p=0.01, Student t-test; MI = mean intensity.
marked expression of HLA-DR together with the decrease in CD10 and decrease in the mean intensity of fluorescence of CD15 and CD16 on neutrophils, suggest the presence of NG with immaturity features in patients receiving G-CSF for neutropenia secondary to intensification chemotherapy. An alternative significance for increased HLA-DR expression could be a higher proliferating activity of NG after treatment, also confirmed by the higher CD71 expression.

Several changes in the expression of neutrophil antigens following G-CSF administration (i.e. increased CD11b, CD66b, CD64, CD18, CD35, CD32, CD13, CD16 and CD45) have been observed, but this expression usually returns to normal after several hours.\(^1,6\)

Some of these changes indicate an enhanced adherence and phagocytic capacities. Our study confirms that NG after G-CSF administration also express the CD71 antigen, indicative of their active proliferation.\(^9\)

We also observed an increase in CD14 expression in NG of ALL patients after G-CSF administration, as previously mentioned,\(^7,10\) and such a receptor may be important for achieving efficient response to infections caused by Gram-negative bacteria. Different from other studies in which normal human volunteers treated with G-CSF have been employed,\(^1,2\) our control subjects did not receive G-CSF. However, the phenotypic profile of NG in control subjects was the same as that of ALL patients before chemotherapy, strongly suggesting that the phenotypic changes observed in the same ALL patients after G-CSF administration were due to the effect of this cytokine on NG. The results of our study suggest that after G-CSF administration there is not only an increase in NG number and an enhancement of the functional properties,\(^11,12\) but also that these NG carry features of immature phenotype. The biologic significance of this feature remains to be ascertained.

**Key words**

Acute lymphoblastic leukemia, G-CSF, neutrophil granulocytes, surface antigens

**Correspondence**

J.M. Ribera, Hematology Department, Hospital Universitari Germans Trias i Pujol, carretera del Canyet s/n 08916 Badalona, Spain. Phone: international +34-3-4651200 ext. 486 • Fax: international +34-3-3954206.

**Acknowledgments**

Supported by a grant (94/0639) and 97/1049 from Fondo de Investigaciones Sanitarias, a grant from Amgen-Roche and a grant (P-EP/97) from the Josep Carreras International Leukemia Foundation.

**References**


**Treatment of chronic myeloid leukemia in relapse after umbilical cord blood transplantation**

**Vinko Bogdanić,* Igor Aurer,* Boris Labar,* Vlasta Hitrec,* Damir Nemet,* Miranda Mrsić***

*Division of Hematology, Department of Medicine, and *Cytogenetic Laboratory, Department of Pediatrics, University Hospital Rebro and Medical School, Zagreb, Croatia

Umbilical cord blood (UCB) is increasingly used as a source of hematopoietic progenitor cells for allogeneic transplantation. Donor-derived buffy coat cells are considered optimal treatment for leukemia relapses after transplantation of allogeneic bone marrow. Experience with relapses after UCB transplants are sparse. Here we report a girl who received an UCB transplant for chronic myeloid leukemia, relapsed after three years,