Blood cell redistribution in the lung after administration of recombinant human granulocyte-macrophage colony-stimulating factor

E. Gatti*, G.V. Scaglioni*, G. Ferrari*, L. Mutti*, E. Pozzi*, A. Stacchini**, L. Fubini**, M. Aglietta*


ABSTRACT: Granulocyte-macrophage colony-stimulating factor (GM-CSF), in addition to being a haematopoietic growth factor, has been shown to stimulate in vitro the production of interleukins 1, 6 and 8 (IL-1, IL-6 and IL-8), tumour necrosis factor-α (TNF-α) and GM-CSF by polymorphonuclear cells (PMNs), alveolar macrophages (AMs), fibroblasts and endothelial cells of the lung, and the growth and differentiation of resident alveolar macrophages.

The aim of this study was to establish whether recombinant GM-CSF (rhGM-CSF), administered subcutaneously at a dose of 5 µg·kg⁻¹ for 3 days in five patients with unresectable non-small cell lung cancer before starting chemotherapy, induces an increase in the alveolar cell count, and whether these cellular lung variations may be related to increases in the above-mentioned cytokines.

In the bronchoalveolar lavage fluid (BALF) total cell count, polymorphonuclear cells, neutrophils, and alveolar macrophages increased significantly in comparison with the baseline, and the extent of variation of the BAL cell count was considerably greater than that of the circulating leucocytes. The mean levels of all the cytokines increased, but a significant difference with respect to the basal condition was observed only for IL-6 and IL-8. After rhGM-CSF treatment, significant correlations were found between neutrophil counts and the levels of IL-6 and IL-8.

In conclusion, rhGM-CSF administration induces a cellular expansion in the lung, and the neutrophil increase appears to be related to increased levels of IL-8.

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The granulocyte-macrophage colony-stimulating factor (GM-CSF) is a member of a family of glycoproteins which promotes proliferation, maturation and functional activities of haematopoietic progenitor cells [1–3]. The in vitro effects of GM-CSF administration are based mostly on studies in patients with advanced cancer receiving recombinant human GM-CSF (rhGM-CSF) [4–6]. They include effects on peripheral blood (increase in neutrophils, eosinophils and monocytes and transient decrease in platelets); on bone marrow (increase in cellularity and in the proportion of promyelocytes, myelocytes and in eosinophil precursors); on serum biochemistry and on leucocyte function (increase of surface CD11b expression [7, 8] and production of platelet-activation factor [9]).

The first dose of rhGM-CSF may be followed within 3 h by a characteristic reaction involving transient flushing, tachycardia, hypotension, muscle-skeletal pain, nausea, vomiting, dyspnoea, arterial oxygen desaturation, reduction of carbon monoxide transfer factor, and increased alveolar-arterial oxygen gradient [10]. The reaction occurs with doses higher than 1 µg·kg⁻¹ and is more commonly observed after intravenous than after subcutaneous administration [6, 10].

Shortly after rhGM-CSF treatment, leucopenia, with disappearance of circulating neutrophils, eosinophils and monocytes [6, 10] occurs; concurrently, as observed by radionuclide leucocyte scan, rhGM-CSF induces a leucocyte sequestration in the lung [11, 12]. In vitro studies have demonstrated that GM-CSF is a primary endogenous regulator of the neutrophil recruitment [13–15] and stimulates polymorphonuclear cells (PMNs) to produce interleukin-1 (IL-1) [16] and interleukin-8 (IL-8) [17], a potent chemotactic factor for neutrophils [18]. It also increases the release of tumour necrosis factor-α (TNF-α) [19, 20], IL-1 [20] and interleukin-6 (IL-6) [21] from cells of the monocyte-macrophage lineage. TNF-α [22], like GM-CSF, induces the adhesion of neutrophils to the endothelial surface [23], and stimulates alveolar macrophages to produce IL-8 [24] and lung fibroblasts and vascular endothelial cells to release GM-CSF [25], respectively.

IL-1 and TNF-α, in turn, induce IL-6 release [26] by monocytes, macrophages, T- and B-lymphocytes and fibroblasts. Furthermore, rhGM-CSF induces the growth...
and the differentiation of resident alveolar macrophages [27–29]. The increased lung concentration of leucocytes in the presence of high levels of rhGM-CSF may be strictly related to cytokine release.

To test whether some of the above-mentioned phenomena also occur in vivo, we studied the effect of rhGM-CSF administration on the lung by evaluating: 1) the cell count determined in the bronchoalveolar lavage fluid (BALF); and 2) the TNF-α, IL-8, IL-1β, IL-6 and GM-CSF levels in the BALF.

Patients

Five male patients (median age 63 yrs; range 56–69 yrs) with unresectable non-small cell lung cancer were enrolled [30]. They had all been smokers but had stopped smoking at least 3 months before the time of the study. Recombinant GM-CSF, 5 µg·kg⁻¹, was given for 3 days by subcutaneous administration before starting chemotherapy. Each patient gave written informed consent, according to the Helsinki declaration. At the time of the study, no enrolled patient had previously undergone chemotherapy, radiotherapy, or therapy with steroids and other anti-inflammatory agents. No patient had suffered from infection in the previous 2 weeks.

Each patient was concurrently submitted to blood withdrawal and fiberoptic bronchoscopy with bronchoalveolar lavage (BAL), in basal conditions 5–7 days before the first administration and 24 h after the third administration of rhGM-CSF.

In all patients, the haematological count, alveolar cell number and the BALF levels of TNF-α, IL-8 IL-1β, IL-6 and GM-CSF were determined.

Methods

rhGM-CSF

rhGM-CSF (human recombinant, Sandoz-Schering-Plough, Basel, Switzerland; Kenilworth, NJ, USA) in purified lyophilized form was obtained by a recombinant deoxyribonucleic acid (DNA) technique.

Blood samples

Blood was collected by venipuncture at 8.00 a.m. after 12 h fasting. The blood count was performed by Technicon Hematology System H1.

Bronchoscopy and BAL

The tip of the flexible bronchoscope (Olympus BF, 1T30, Japan) was wedged into a noninvolved segment of the lung, so that the BAL, both before and after the rhGM-CSF administration, were performed in the lung opposite to the tumour, on the same segment of the middle lobe or the lingula. Aliquots of 20 mL of sterile NaCl 0.9% solution were instilled and withdrawn by gentle aspiration; a total volume of 200 mL was usually employed. All lavage fluid retrieved was passed through a double layer of sterile gauze to remove the mucus, and then centrifuged at 1,200 × g for 10 min. The supernatant was concentrated 1:10 v:v by filtering through membranes (Hamicon, YM10, Grace, Italy) which allow the passage of molecules of less than 10 kDa; the cytokines studied in the BAL were not filtered as their weight exceeded 10 kDa. The supernatant was stored in volumes of 1 mL at -20°C, until assayed. The cell pellet was washed with phosphate buffered saline (PBS) (Sigma, USA) and total cell number and differential cell count were then performed.

Cytokine assay

TNF-α, IL-8, IL-1β, IL-6 and GM-CSF were determined in duplicate by enzyme immunoassay (EIA) performed in microtiter plates without any extraction treatment. (TNF-α, EIA Medgenix Diagnostics, Belgium; human IL-8, Quantikine, British Bio-technology Ltd, UK; IL-1β, EIA Medgenix Diagnostics, Belgium; IL-6, EIA Medgenix Diagnostics, Belgium; GM-CSF EIA, Medgenix Diagnostics, Belgium). The cytokine levels were expressed as pg·mL⁻¹ of the concentrated BALF.

The principle of the different assays is based on the quantitative immunometric "sandwich" EIA technique, in which several antibodies are directed against distinct epitopes of the molecule to be determined.

Reading was performed by spectrophotometer (Multiskan Plus MK-II, Flow, Switzerland) at 450 nm wavelength. The minimum detectable concentration was estimated to be 3 pg·mL⁻¹ for TNF-α (mean value of intra-assay coefficient of variation (COV) 3.4%, and of interassay COV 9.2%); 4.7 pg·mL⁻¹ for IL-8 (mean value of intra-assay COV 7.4% and of interassay COV 9.5%); 2 pg·mL⁻¹ for IL-1β (mean value of intra-assay COV 2.8% and of interassay COV 4.5%); 3 pg·mL⁻¹ for IL-6 (mean value of intra-assay COV 5.2% and of interassay COV 4.9%); and 3 pg·mL⁻¹ for GM-CSF (mean value of intra-assay COV 4.5% and of interassay COV 9.6%).

Statistical analysis

Data are expressed as mean±standard deviation (sd). Comparisons and correlations were performed using Student's t-test for paired samples and Pearson test, respectively; p-values lower than 0.05 were considered to be statistically significant.

Results

After rhGM-CSF administration, circulating leucocytes (WBC), granulocytes (PMN), neutrophils (N) and eosinophils (E) increased in all patients (fig. 1), and the difference was statistically significant compared to baseline. A statistically significant increase was observed for: WBC (mean±sd 11,030±887 vs 18,210±3,030; p=0.007); PMN (8,028±1,006 vs 15,080±2,754; p=0.005); N (7,482±961
Fig. 1. – Changes in peripheral blood cells after rhGS-CSF treatment. WBC: white blood cells (circulating leucocytes); PMN: polymorphonuclear cells (granulocytes); N: neutrophils; E: eosinophils; B: basophils; Ly: lymphocytes; Mo: monocytes; rhGM-CSF: recombinant granulocyte-macrophage colony-stimulating factor. : patient No. 1; : patient No. 2; : patient No. 3; : patient No. 4; : patient No. 5.

Fig. 2. – Changes in BALF cells after rhGM-CSF treatment. TAC: total alveolar cells; AM: alveolar macrophages; BALF: bronchoalveolar lavage fluid. For further abbreviations see legend to figure 1. : patient No. 1; : patient No. 2; : patient No. 3; : patient No. 4; : patient No. 5.
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vs 13.659±2.825; p=0.007); E (466±371 vs 1,337±297; p=0.001). Basophils (B) increased in three out of five patients, monocytes (Mo) in four out of five patients, and lymphocytes (Ly) in only one patient. No significant increase was found for: B (79±47 vs 87±22); Ly (1,939±591 vs 1,744±643); Mo (886±224 vs 1,122±217).

In the BALF, significant difference before and after rhGM-CSF administration was observed in total alveolar cell (TAC) count, granulocytes (PMN), neutrophils (N), and alveolar macrophages (AMs), but not in eosinophils (E), basophils (B) or lymphocytes (Ly) (fig. 2). A statistically significant increase was found for: TAC (mean±so 97.46±73.310 vs 205.00±124.000; p=0.018); PMN (7,980±14,050 vs 52,420±44,680; p=0.042); N (4.340±6.272 vs 20.240±13.570; p=0.016); AM (79.990±59.920 vs 142.000±74.610; p=0.009). Conversely, no significant increases were observed for: E (3.640±7.080 vs 27.000±32.570); B (not detected vs 5.180±8.538); Ly (9.520±5.677 vs 10.500±8.037). Total alveolar cell count, granulocytes, neutrophils, eosinophils and alveolar macrophages increased in all patients, whereas basophils and lymphocytes increased in three out of five patients.

To establish whether, in response to the rhGM-CSF treatment, cellular expansion occurred in the blood as well as in the BALF, the extent of variation, expressed as the ratio between the cell count determined after and before rhGM-CSF administration, was calculated (table 1). For some cell populations, the increase in the BALF was much higher than that observed in the blood. For instance, the average increase in the BALF was 48.3 fold for PMNs, 10.1 fold for neutrophils, and 2.5 fold for AMs; whereas, in the blood, PMNs and neutrophils approximately doubled, and monocytes increased 1.3 fold (table 1).

The mean levels of TNF-α, IL-8, IL-1β, IL-6 and GM-CSF, determined in the concentrated BALF 24 h after the rhGM-CSF treatment, increased with respect to the basal condition, but a statistically significant difference was observed only for IL-8 (p=0.03) and IL-6 (p=0.023) (fig. 3). No statistically significant increase after rhGM-CSF administration was found for: TNF-α (29.2±36.4 vs 47.9±42.8 pg·mL⁻¹); GM-CSF (66.9±15.4 vs 90.4±30.5

<table>
<thead>
<tr>
<th>Extent of variation</th>
<th>Blood</th>
<th>BALF</th>
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<tbody>
<tr>
<td>WBC</td>
<td>1.7 (1.5–2.1)</td>
<td>2.9 (1.5–6.9)</td>
</tr>
<tr>
<td>PMN</td>
<td>1.9 (1.4–2.3)</td>
<td>48.3 (3.6–130)</td>
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<tr>
<td>N</td>
<td>1.7 (1.2–2.3)</td>
<td>10.1 (2.6–11)</td>
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<tr>
<td>E</td>
<td>5.9 (1.7–17)</td>
<td>2.4 (1.4–3.4)</td>
</tr>
<tr>
<td>B</td>
<td>2.5 (0.4–8.1)</td>
<td>– –</td>
</tr>
<tr>
<td>Ly</td>
<td>0.9 (0.6–1.2)</td>
<td>1.5 (0.2–4.3)</td>
</tr>
<tr>
<td>Mo</td>
<td>1.3 (0.9–1.7)</td>
<td>2.5 (1.5–6.1)</td>
</tr>
</tbody>
</table>

Values are presented as mean, and range in parenthesis. For abbreviations see legends to figures 1 and 2.

Fig. 3. – Changes in BALF cytokine levels after rhGM-CSF treatment. TNF-α, IL-8, GM-CSF, IL-1β and IL-6 in the concentrated BALF of the five patients studied. TNF-α: tumour necrosis factor-α; IL: interleukin; GM-CSF: granulocyte-macrophage colony-stimulating factor. □: patient No. 1; : patient No. 2; : patient No. 3; ■: patient No. 4; ◯: patient No. 5.
pg·mL⁻¹; IL-1β (15.8±17.9 vs 23.9±20.6 pg·mL⁻¹). Conversely, a significant increase was observed for: IL-8 (148.8±89.2 vs 490±259.1 pg·mL⁻¹); IL-6 (36.6±22.7 vs 154.1 vs 85.6 pg·mL⁻¹). TNF-α and IL-1 increased in three patients, GM-CSF in four patients and IL-6 and IL-8 in all patients.

To establish whether, after rhGM-CSF treatment, the variation in the BAL cellularity may be influenced by the cytokine increase, the levels of IL-6 and IL-8 were correlated with the counts of alveolar macrophages, polymorphonuclear cells, neutrophils and eosinophils. A statistically significant correlation was found only between neutrophils and, respectively, IL-6 (r=0.883; p=0.047) and IL-8 (r=0.917; p=0.028).

Discussion

As already known, rhGM-CSF administration increases circulating leucocytes. Interestingly, we observed that after rhGM-CSF administration, the response in the blood and in the lung differs considerably; in fact, the ratio of the cellular increase was much higher in the BALF than in the peripheral blood. These observations suggest that rhGM-CSF is able to induce in vivo a complex phenomena of cell redistribution in the alveolar compartment, by means of a process of recruitment and cellular metabolic activation, with sequential release of multiple cytokines, some of them with chemotactic effects.

In the lung, the most important targets of GM-CSF are the alveolar macrophages and the granulocytes. On the alveolar macrophages, as demonstrated previously by in vitro studies, rhGM-CSF induces the release of IL-1β, IL-6 and TNF-α [20, 21]. TNF-α, in turn, causes a further secretion of IL-6 [26] and IL-8 [24] by alveolar macrophages, and of GM-CSF by fibroblasts and endothelial cells [25]. On neutrophils, rhGM-CSF induces the release of IL-8 [17] and IL-1 [16]. Our results, obtained in an in vivo study in which the level of the different cytokines was determined in the BALF, seem to confirm the in vitro data.

The increased level of the cytokines observed in the BALF may be biologically relevant to induce an effect on the alveolar cellular expansion. This hypothesis is supported by our finding of a significant correlation between the neutrophil count and the levels of IL-6 and IL-8 in BALF, suggesting an IL-8-induced neutrophil recruitment to the lungs. Conversely, the increase in IL-6 is not clear. It probably does not play a role in the expansion of the alveolar macrophages or PMNs in the BALF, but it may be a sign of a nonspecific cellular activation induced firstly by GM-CSF itself and, secondly, by the other cytokines present in the alveolar compartment.

The increase in the alveolar macrophages could be sustained by two main mechanisms: the migration from the blood to the lung of cells belonging to the monocyte-macrophage lineage; and the in situ proliferation of resident macrophages [29] as an outcome of GM-CSF administration. The increase in the neutrophils could depend on their migration from the blood to the lung, as the consequence of a high CD11b expression, which increases the adhesion of granulocytes to the human endothelial cells [7, 12], and through a chemotactic process mediated by GM-CSF itself and IL-8.

Nevertheless, the role of the different cytokines is still not clearly defined. In fact, after rhGM-CSF therapy, the increased levels of the cytokines which were positively correlated with the number of alveolar neutrophils could be a consequence of the cell expansion. However, this seems unlikely because neutrophils are not known to produce GM-CSF and the number of macrophages, even though they represent the major source of TNF-α-induced IL-8 production, showed no correlation with IL-8.

In short, we may conclude that rhGM-CSF induces a cellular expansion in the alveolar compartment and the neutrophil accumulation is likely to be due to a chemotactic process mediated by IL-8.

References


