



Short Communication

Effect of Granulocyte Colony Stimulating Factor Treatment on *ex Vivo* Cytokine Production by Blood Cells of Patients after Chemotherapy or Radiotherapy

TERESA KAMIŃSKA¹, ANNA DMOSZYŃSKA², IWONA HUS², ADAM WALTER CRONECK² and MARTYNA KANDEFER-SZERSZEŃ^{1*}

¹ Department of Virology and Immunology, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland,

² Department of Hematology, University Medical School, Jaczewskiego 8, 20-095 Lublin, Poland

Abstract. We explored *ex vivo* alterations in the cytokine release of stimulated blood cells taken from 8 patients with hematological malignancies who, after chemotherapy or radiotherapy developed leukopenia, and were treated for 3–7 days subcutaneously with granulocyte colony stimulating factor (G-CSF), daily, dose of 5 µg/kg of body weight. Blood was also taken from 8 healthy controls not treated with G-CSF and from patients before and 24 h after last dose of G-CSF and *ex vivo* treated with interferon (IFN) inducers: Newcastle disease virus (NDV), phytohemagglutinin (PHA), concanavalin A (Con A) and with tumor necrosis factor (TNF) inducer – lipopolysaccharide (LPS). Blood cells of patients before G-CSF treatment exhibited *ex vivo* a low ability to produce IFN-γ in comparison to controls. After G-CSF therapy a significant increase in IFN-α production ability was detected. We conclude that G-CSF treatment for 3–7 days does not only increase the number of white blood cells (WBC) and neutrophilic granulocytes but also modify the host response of patients with hematological malignancies to microbial infections.

Key words: recombinant human colony stimulating factor; interferons; tumor necrosis factor.

Introduction

Myelosuppression, predominantly neutropenia, is a frequent finding in patients receiving cytotoxic chemotherapy in hematological malignancies, and is often dose-limiting. Infections, caused by endogenous and nosocomial microorganisms, are the most common cause of death in leukopenic patients receiving cytotoxic chemotherapy or radiotherapy.

Granulocyte colony stimulating factor (G-CSF) promotes proliferation and maturation of neutrophils. It has been produced as a recombinant product in *Escherichia*

coli with the specific activity and spectrum of activities similar to native protein. A major use for recombinant human colony stimulating factor (rhG-CSF) is ameliorating neutropenia, which follows cytoreductive chemotherapy^{5, 8}. In addition to altering the kinetics of progenitor-cell growth and differentiation, G-CSF may regulate the survival and function of mature blood cells, especially neutrophils^{6, 15}. It can also influence the cytokine profile in sera of patients^{3, 17}. After G-CSF treatment of healthy volunteers the increase in plasma levels of tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), IL-8 and soluble TNF-α receptor (sTNF-αR), IL-1

* To whom all correspondence should be addressed.

receptor antagonists (IL-1Ra) was observed. Production of cytokines and cytokine receptors was probably caused by spontaneous release from an increased number of neutrophils and monocytes^{11, 13}.

When a low dose of endotoxin was given, additional increase in the plasma levels of cytokines and cytokine receptors was observed in healthy volunteers 2 or 12 h after G-CSF treatment¹²⁻¹⁴. However, when endotoxin was given *in vivo* 24 h after G-CSF treatment or when blood cells were taken from subjects 24 h after treatment and induced *ex vivo* with lipopolysaccharide (LPS), reduction in IL-8 and attenuation in TNF- α production was observed^{7, 12}. These results indicate that G-CSF is not only an inducer of cytokine production but it can also modify cytokine production after induction with bacteria or substances of bacterial origin.

In this paper we were interested in whether G-CSF treatment of patients with hematological malignancies, who after chemotherapy or radiotherapy developed significant leukopenia, can exert a late (24 h after last dose of G-CSF) modulatory effect on IFN and TNF production in blood cells. Three to 7 days after G-CSF treatment, at a daily dose of 5 $\mu\text{g/kg}$ of body weight, patients' blood was withdrawn and blood cells were exposed *ex vivo* to different stimuli. To cover a broad spectrum stimuli of host defence against infections, challenge virus, mitogens and LPS were used in *ex vivo* experiments.

Materials and Methods

Human subjects and study design. This study included 8 patients, 7 of them underwent chemotherapy and 1 radiotherapy (Table 1) in the Department of Hematology, Medical University School of Lublin. Three patients suffered from acute myeloid leukemia, 1 with acute lymphoid leukemia, 2 non-Hodgkin's lymphoma, 1 multiple myeloma and 1 Hodgkin's disease. Informed consent was obtained from each patient. The study was approved by the Ethics Committee of Medical University School in Lublin.

Recombinant human G-CSF (Neupogen, Hoffman LaRoche) was given as a subcutaneous bolus injection of 5 $\mu\text{g/kg}$ of body weight for 3–7 days. The time of the patients' treatment depended on the changes in neutrophil count in blood. Eight healthy volunteers were also included in the study as a control group not treated with G-CSF.

Whole blood assay. Heparinized blood samples (20 U/ml of Heparinum, Polfa) collected before and 24 h after the last dose of G-CSF injection were diluted with Eagle's MEM supplemented with 2 mM L-glutamine, penicillin 100 U/ml, streptomycin 100 $\mu\text{g/ml}$, to obtain the density of leukocytes of 2×10^5 cells/ml, and induced by Newcastle disease virus (NDV) 5 TCID₅₀/leukocyte, phytohemagglutinin (PHA, Sigma) and concanavalin A (Con A, Sigma) 50 $\mu\text{g/ml}$ and LPS (*E. coli* 0111: B4, Sigma) 10 $\mu\text{g/ml}$ to IFN or TNF production as described in detail¹.

Table 1. Clinical characteristics of patients

Sex/age	WBC before/after G-CSF ($10^9/\text{l}$)	PLT ($10^9/\text{l}$)	Hgb/Ht (mmol/l/l)	Diagnosis	Treatment
M/44	0.9/1.2	35.0	4.65/0.22	centroblastic/centrocytic lymphoma st. IVB	2-CDA
M/58	0.6/0.6	20.0	5.15/0.25	AML-M1 acc. FAB	FRB+Ara-C
F/27	0.5/2.5	15.0	6.89/0.33	AML-M1 acc. FAB	FRB+HiAra-C
F/34	0.6/0.3	8.0	4.96/0.24	AML-M4 acc. FAB	MITO+HiAra-C
M/18	2.9/8.1	24.0	7.87/0.37	Hodgkin's disease (MC) st. III B	radiotherapy of supraphrenic lymph nodes (dose 2400 rd/t)
M/61	2.1/2.5	183.0	6.08/0.30	multiple myeloma st. III	VMBCP
M/26	1.0/16.3	25.0	6.51/0.32	immunoblastic lymphoma st. IVB	Pro-MACE-Cyta-BOM
M/20	1.1/0.4	35.0	9.0/0.43	ALL-L1 acc. FAB	MITO+HiAra-C

Abbreviations: WBC – white blood cells; Hgb – hemoglobin; Ht – hematocrit; PLT – platelets; AML – acute myeloid leukemia; ALL – acute lymphoblastic leukemia; MC – mixed cellularity; 2-CDA – 2-chlorodeoxyadenosine; FRB – fludarabine; Ara-C – cytosine arabinoside; HiAra-C – high doses of cytosine arabinoside; VMBCP – vincristine, melphalan, carmustine, cyclophosphamide, prednisone; Pro-MACE-Cyta-BOM – cyclophosphamide, adriamycin, etoposide, procarbazine, cytosine arabinoside, bleomycin, vincristine, methotrexate, leukovorin; MITO – mitoxantrone; FAB – French/American/British classification.

Cytokine assay. After incubation at 37°C for 4 h (TNF activity) or for 24–72 h (IFN activity), cytokine activity present in supernatants of blood cell cultures was assayed by biological methods as described earlier^{1, 2, 9}. Briefly, IFN was titrated on A549 cells (AT TCC CCL 1185, Rockville, MD) with vesicular stomatitis virus (VSV) as a challenge. The IFN titer was defined as a reciprocal of IFN dilution that reduced the cytopathic effect of VSV by 50%. TNF activity was titrated on L929 cells. The reciprocal of the highest dilution causing destruction of cells in 50% and compared to the standard was defined as one unit of TNF. Recombinant human IFN- α 88 (a generous gift of dr. E. Lundgren, Umea, Sweden) was used as IFN activity standard. Standard rh TNF- α , received from the Department of Bioorganic Chemistry, Center for Molecular and Macromolecular Research, Polish Academy of Sciences in Łódź, was included in each assay of TNF. IFN- α , IFN- γ and TNF- α were characterized by the neutralization test with monoclonal or polyclonal antibodies as described earlier^{1, 2, 9}.

Statistical analysis. Statistical analysis of data obtained in control (healthy persons) and experimental group was performed using Mann-Whitney test for nonparametric data. Two-tailed Wilcoxon significance test was used to statistical analysis within the experimental group before and after G-CSF treatment. Data are given as means $\pm$ SD. The level of significance was set to $p < 0.05$.

Results and Discussion

Eight patients with neutropenia after chemotherapy or radiotherapy were included in the study. Three to

seven days after treatment with daily injections of Neupogen an increase in white blood cells (WBC) count was seen in 5 from out of 8 patients. In patients, who responded to G-CSF, the percentage of neutrophils increased to 56–61% of the total number of WBC. No correlations between the type of disease or patients' age and therapy and alterations in WBC counts were seen. Three patients, who did not respond with increase in WBC count were treated thereafter with GM-CSF (data not presented). No side-effects were observed during G-CSF treatment.

TNF and IFN production, before and after G-CSF injections, was measured in blood cells induced *ex vivo* with NDV (5 TCID₅₀/cell), PHA or Con A (50 μ g/ml) or with LPS (10 μ g/ml). Blood cells taken from healthy persons served as a control of cytokine level which can be elicited by an inducing factor used in experiments. In comparison to controls, blood cells of patients with leukopenia after chemotherapy or radiotherapy, exhibited a decreased ability to produce IFN- γ after induction with PHA and Con A (Fig. 1 and 2). These differences were statistically significant at $p < 0.05$ in Mann-Whitney test, indicating that not only the number of WBC was lower, but they also possessed a low ability to produce some cytokines.

When blood of patients was taken, after G-CSF treatment, a slight increase in the ability to produce both cytokines was observed; however, because of a high standard deviation only the ability of blood cells to produce IFN after induction with NDV was statistically significant in two-tailed Wilcoxon significance test (Fig. 3). Also, TNF- α production ability was enhanced but the difference was statistically insignificant (Fig. 4).

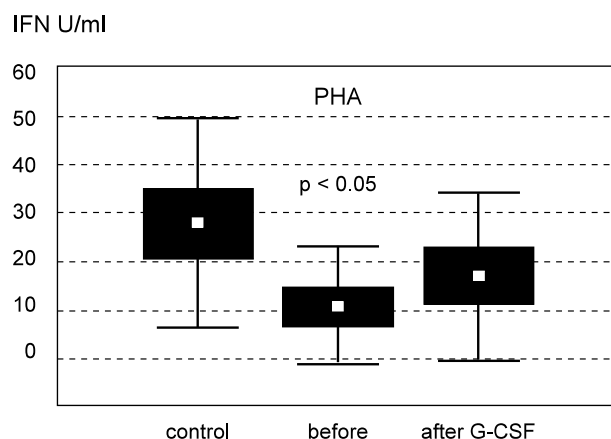


Fig. 1. IFN production by blood cells of patients treated with G-CSF. Inducer *ex vivo* PHA (50 μ g/ml). About 90% of this IFN was neutralized by monoclonal antibody E-4-18 against human IFN- γ . Statistically significant difference between control and patient's groups

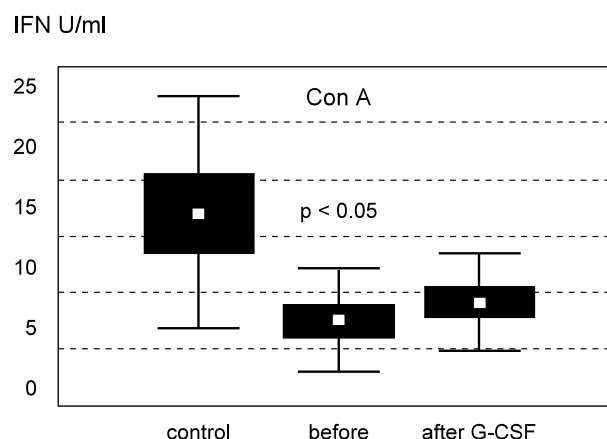


Fig. 2. IFN production by blood cells of patients treated with G-CSF. Inducer *ex vivo* ConA (50 μ g/ml). Statistically significant difference between control and patient's groups

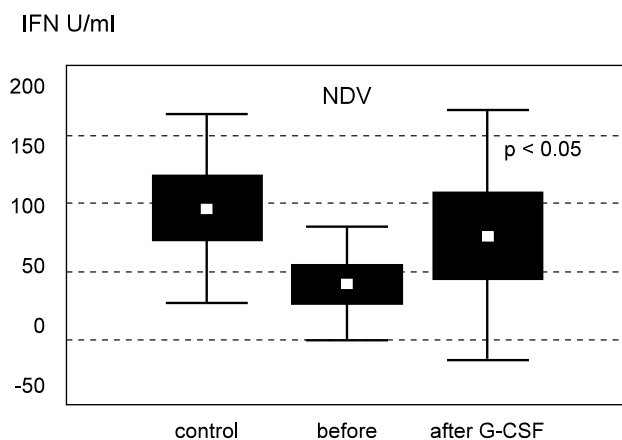


Fig. 3. IFN production by blood cells of patients treated with G-CSF. Inducer *ex vivo* NDV 5 TCID₅₀/leukocyte. About 85% of this IFN was neutralized by polyclonal antibodies against human IFN- α . Statistically significant difference within patient's group before and after G-CSF treatment

G-CSF is considered to represent a lineage-specific factor acting on polymorphonuclear cells and their precursors. However, other studies also demonstrated a significant increase in peripheral monocytes^{7, 18}. In our study we stimulated cytokine production in blood cells with fixed density of 2×10^5 WBC/ml. This method allowed to measure the potency of whole blood cell populations to produce cytokines after *ex vivo* treatment with strong inducers. NDV was chosen as a model of viral infection and inducer of predominantly IFN- α , PHA and ConA as IFN- γ inducers and LPS as the stimulus of TNF- α production^{1, 2, 4, 9, 10}.

It has been described that G-CSF treatment of healthy volunteers increased the plasma levels of TNF- α , sTNF- α R, p55 and p75, IL-1Ra¹³. When a low dose of endotoxin was given 2–12 h after G-CSF treatment additional increase in the plasma levels of cytokines and cytokine receptors were seen^{12, 13}. In contrast to that, when cytokine response of blood cells from healthy volunteers, treated with G-CSF 24 h after injection was examined, the reduction of TNF- α release after *ex vivo* induction with different stimuli was described⁷. In our experiment, however, the patients' blood cells exhibited a low ability to produce TNF- α observed before G-CSF injections in comparison to healthy controls. After G-CSF treatment this ability returned to nearly normal. It seems likely that G-CSF can modify the cytokine response by enhancing low cytokine production and inhibiting that which exceeds the physiological level.

Considering the ability of blood cells to produce IFN it should be stressed that the low ability to respond to PHA and ConA was slightly higher after G-CSF

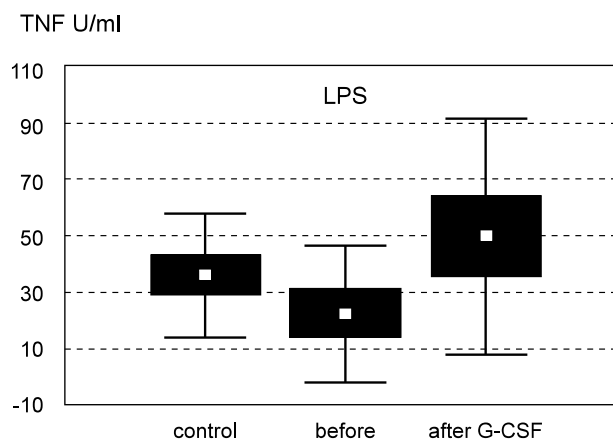


Fig. 4. TNF production by blood cells of patients treated with G-CSF. Inducer *ex vivo* LPS (10 μ g/ml). About 95% of this TNF was neutralized by polyclonal antibody against human TNF- α

treatment, but these differences were not statistically significant because of high standard deviations. However, when blood cells of the patients treated with G-CSF were infected with the virus, they produced more IFN- α than before treatment, and these differences were statistically significant.

Monocytes and B lymphocytes are the main source of IFN- α induced by a virus; however, we cannot exclude that also neutrophilic granulocytes were involved in IFN production. It has been described that neutrophilic granulocytes produce IFN- α after stimulation *in vitro* with G-CSF¹⁶. This IFN is considered as an important factor in feedback inhibitory regulation of neutropoiesis, but low levels of IFN could also "prime" blood cells *in vivo* to produce more IFN after induction *ex vivo* with such a strong inducer as NDV.

Three of the patients, examined in our study, suffered from acute myelogenous leukemia. In our previous report¹⁰ we have demonstrated the defect in IFN production ability of leukocytes of patients with acute myelogenous leukemia type M4 and M5. The results presented in this paper showed that G-CSF treatment can stimulate defective IFN production in patients with hematological malignancies, especially in response to viral infection. We suppose that such moderate influence of G-CSF on other cytokine production ability of blood cells could be beneficial for these patients.

References

1. DANILUK J. and KANDEFER-SZERSZEŃ M. (1994): Interferon production by peripheral blood cells of patients with alcoholic liver disease (ALD). Arch. Immunol. Ther. Exp., **42**, 231–238.

2. DANILUK J., KANDEFER-SZERSZEŃ M. and BOROWSKA L. (1996): Tumor necrosis factor and interferon production by peripheral blood leukocytes of patients with alcoholic cirrhosis. *Arch. Immunol. Ther. Exp.*, **44**, 97–101.
3. DENECKER N. E., KULLBERG B. J., DREUTH J. P., RAEMAEKERS J. M. and VAN DER MEER J. W. (1997): Regulation of production of proinflammatory cytokines and antagonists during chemotherapy-induced neutropenia in patients with hematological malignancies. *Cytokine*, **9**, 702–710.
4. DMOSZYŃSKA A., KANDEFER-SZERSZEŃ M., BOROWSKA L., ADAMCZYK-CIOCH M., HUS I., HUS M. and ROLIŃSKI J. (1995): Spontaniczne i indukowane wytwarzanie czynnika martwicy nowotworów (TNF- α) przez leukocyty krwi obwodowej chorych na ostrą białaczkę typu M4 i M5 wg FAB. *Acta Haematol. Pol.*, **26**, 205–211.
5. FAULDS D., LEWIS N. J. W. and MILNE R. J. (1992): Recombinant granulocyte colony-stimulating factor (rG-CSF). *PharmacoEconomics*, **1**, 231–249.
6. GROOPMAN J. E., MOLINA J. M. and SCADDEN D. T. (1989): Hematopoietic growth factors. Biology and clinical application. *N. Engl. J. Med.*, **321**, 1449–1459.
7. HARTUNG T., DÖCKE W. D., GARTNER F., KRIEGER G., SAUER A., STEVENS P., VOLK H. D. and WANDEL A. (1995): Effect of granulocyte colony-stimulating factor treatment on *ex vivo* blood cytokine response in human volunteers. *Blood*, **85**, 2482–2489.
8. JOSE J. M. and ARMITAGE J. O. (1995): Clinical applications of hematopoietic growth factors. *J. Clin. Oncol.*, **13**, 1023–1035.
9. KAMIŃSKA T., KANDEFER-SZERSZEŃ M., SZUSTER-CIESIELSKA A., RZESZOWSKA G., MARKOWSKA H. and MODRZEWSKA R. (1996): Interferon and tumor necrosis factor production by peripheral blood leukocytes of patients with infectious mononucleosis. *Arch. Immunol. Ther. Exp.*, **44**, 353–357.
10. KANDEFER-SZERSZEŃ M., DMOSZYŃSKA A., BOROWSKA L., ADAMCZYK-CIOCH M., HUS M., HUS I. and ROLIŃSKI J. (1996): Interferon production by peripheral blood leukocytes of patients with acute myeloid leukemia. *Arch. Immunol. Ther. Exp.*, **44**, 119–122.
11. MICHON J. M., GEY A., MOUTEL S., TARTOUR E., MERESSE V., FRIEDMAN W. and TEILLAUD J. L. (1998): *In vivo* induction of functional Fc gamma RI (CD64) on neutrophils and modulation of blood cytokine mRNA levels in cancer patients treated with G-CSF (rMet HuG-CSF). *Br. J. Haematol.*, **100**, 550–556.
12. PAJKRT D., MANTEN A., VAN DER POLL T., TIEL-VAN BUUL M., JANSEN J., WOUTER TEN CATE J. and VAN DEVENTER J. H. (1997): Modulation of cytokine release and neutrophil function by granulocyte colony-stimulating factor during endotoxemia in humans. *Blood*, **90**, 1415–1424.
13. POLLMACHER T., KORTH C., MULLINGTON J., SCHREIBER W., SAUER J., VEDDER H., GALANOS C. and HOLSBOER F. (1996): Effects of granulocyte colony-stimulating factor on plasma cytokine and cytokine receptor levels and on the *in vivo* host response to endotoxin in healthy men. *Blood*, **87**, 900–905.
14. POLLMACHER T., KORTH C., SCHREIBER W., HERMANN W. and MULLINGTON J. (1996): Effect of repeated administration of granulocyte colony-stimulating factor (G-CSF) on neutrophil counts, plasma cytokine and cytokine receptor levels. *Cytokine*, **8**, 799–803.
15. REPP R., VALERIUS T., WIELAND G., BECKER W., STEININGER H., DEO Y., HELM G., GRAMATZKI M., VAN DE WINKEL J. G. and LANG N. (1995): G-CSF-stimulated PMN in immunotherapy of breast cancer with a bispecific antibody to Fc gamma RI and to HER-2/neu (MDX-210). *J. Hemother.*, **4**, 415–421.
16. SHIRAFUJI N., MATSUDA S., OGURA H., TANI K., KODO H., OZAWA K., NAGATA S., ASANO S. and TAKAKU F. (1990): Granulocyte colony-stimulating factor stimulates human mature neutrophilic granulocytes to produce interferon α . *Blood*, **75**, 17–19.
17. SHITARA Y., YUGAMI S., IJIMA H., SATOMATU M. and KUROUME T. (1994): Cytokine profile during high-dose rhG-CSF therapy in severe congenital neutropenia. *Am. J. Hematol.*, **45**, 58–62.
18. WILTSCHE C., KRAINER M., WAGNER A., LINKESCH W. and ZIELINSKI C. C. (1995): Influence of *in vivo* administration of GM-CSF and G-CSF on monocyte cytotoxicity. *Exp. Hematol.*, **23**, 402–406.

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