Journal of Hematology
volume 83 – number 5 – May 1998

Haematologica 1998; 83:385-391 original paper

Effect of PEG-recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF) on growth and differentiation of the HEL cell line

LUCIA CATANI, LUIGI GUGLIOTTA, MARIAROSA MOTTA, PIERLUIGI TAZZARI,* STEFANO BARAVELLI, SANTE TURA
*Istituto di Ematologia e Oncologia Medica "L. e A. Seràgnoli", University of Bologna and *Servizio di Immunoematologia e Trasfusionale, S. Orsola Hospital, Bologna, Italy*

Abstract

Background and Objective. **Thrombopoietin has been established as the major regulator of megakaryocyte and platelet production. In this study we evaluated the effects of PEG-recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF), a pegylated and truncated form of thrombopoietin, on the growth and differentiation of the HEL cell line. As a model system we chose the pluripotent HEL line that acquires multiple markers of the megakaryocyte/platelet phenotype following treatment with phorbol esters, and, more importantly, expresses the receptor for thrombopoietin (Mpl receptor) at its cellular surface.**

Design and Methods. **The effect of PEG-rHuMGDF on HEL proliferation/differentiation was evaluated in a liquid culture assay.**

Results. **Peg-rHuMGDF do not increase the proliferative capacity of HEL cell but, in parallel experiments, HEL cells showed a more mature and differentiated pattern after exposure to the cytokine. Our results show that PEG-rHuMGDF, at the optimal doses of 100-150 ng/mL, is able to induce: 1) morphological changes with the formation of cytoplasmic protrusions; 2) increased ploidy as demonstrated by cytofluorimetric analysis; 3) increased expression of megakaryocyte markers, including glycoprotein IIb-IIIa and the platelet-specific alloantigen (PI^{A1}).**

Interpretation and Conclusions. **These findings show that HEL cells represent a useful model to investigate the differentiative properties of thrombopoietin in the megakaryocyte compartment.**

Key words: thrombopoietin, PEG-rHuMGDF, HEL cell line, proliferation, maturation

Megakaryocytopoiesis is a developmental continuum beginning with the commitment of an undifferentiated, pluripotent hematopoietic stem cell into the megakaryocytic lineage. Stem cell commitment is followed by a traverse through two broad compartments. The first compartment contains progenitor cells while the second compartment consists of maturing precursor cells. The end result of this complex process is the formation of mature megakaryocytes, each capable of releasing several thousand functional platelets.[1-4] The regulation of these events has been shown to be influenced by a variety of positive, albeit not necessarily lineage specific, growth factors including interleukin-3, granulocyte-macrophage colony-stimulating factor, interleukin-6, interleukin-11, leukemia inhibitory factor and erythropoietin.[5,6]

Recently, great excitation has been generated in the field of megakaryocytopoiesis by the discovery of thrombopoietin.[7-16] This molecule, which has recently been identified and cloned, shows a potent thrombopoietic activity both *in vitro* and *in vivo*. *In vitro* studies document that thrombopoietin acts through the Mpl receptor to promote proliferation and maturation of megakaryocyte precursors.[17,18] Additional experimental evidence has demonstrated that thrombopoietin plays an important role for platelet formation and release. Platelets derived from human $CD34^+$ cells in a liquid culture system are functional.[17] Furthermore, when purified or recombinant proteins were injected into recipient animals or patients with advanced cancer, they increased the number, size and ploidy of megakaryocytes and raised the platelet count.[20,21]

The availability of continuous cell lines with megakaryoblastic or mixed megakaryoblastic/erythroid features has afforded an important tool and a valuable model to study megakaryocyte development and useful information has been obtained from the resulting studies.[22-29] Furthermore, cell lines are especially interesting for the megakaryocytopoiesis because the low number of megakaryocytes in the bone marrow has hampered the study of this lineage. We therefore decided to investigate whether PEG-rHuMGDF, a pegylated and truncated form of thrombopoietin, is able to influence the proliferation and differentiation of the human erythroleukemia cell line (HEL), since

Correspondence: Lucia Catani, MD, Istituto di Ematologia e Oncologia Medica "L. e A. Seràgnoli", Ospedale S. Orsola, via Massarenti 9, 40138 Bologna, Italy. Phone: international + 39-51-390413 • Fax: international + 39-51-398973 • E-mail: seragnol@kaiser.alma.unibo.it

these cells express significant amounts of Mpl receptor. The present study suggests that HEL cells may be a useful model for investigating the mechanisms of action of thrombopoietin on the megakaryocyte compartment.

Materials and Methods

HEL cell line

HEL cells were cultured in RPMI 1640 (Gibco, Grand Island, NY, USA) with 10% fetal bovine serum in 24 multiwell plates (Falcon, Oxnard, CA, USA), at an optimal cell density of 0.8×10^6/mL.

Growth factor

PEG-rHuMGDF is a pegylated and truncated form of thrombopoietin; it was kindly provided by Amgen (Thousand Oaks, CA, USA).

Incubation with PEG-rHuMGDF

HEL cells were cultured in RPMI alone (without FBS) for 12-14 hours before each experiment. The cells (0.8×10^6/mL) were then cultured in RPMI plus 10% FBS plus various concentrations of PEG-rHuMGDF for 6 days without medium change. Increasing doses of the cytokine were used: 10, 50, 100, 150, 200 ng/mL. Every 24 h cells were harvested and characterized as follows.

Further experiments were then done where HEL cells (0.8×10^6/mL) were cultured in RPMI plus 10% FBS with or without PEG-rHuMGDF at the same doses as described above. In this case, every 2 to 3 days up to 16 days of culture, HEL cells were demipopulated by removal of half the culture volume, which was replaced with fresh media with or without MGDF. Every 3 days, cells were checked for viable cell count.

Viable cells

Viable cell counts were examined by using Trypan blue dye exclusion.

Detection of surface markers

The surface of the HEL cell line was analyzed for the presence of the glycoprotein IIb-IIIa (GP IIb-IIIa), Mpl receptor, the platelet-specific alloantigen (PI^{A1}) and the glycophorin-A. GP IIb-IIIa detection was done using fluorescein-isothiocyanate-conjugated (FITC) anti-GP IIb-IIIa (CD41a; Coulter). Mpl receptor was monitored using a mouse monoclonal antibody (Genzyme, Cambridge, MA, USA) followed by rabbit anti-mouse (RAM-Fl) IgG covalently linked to fluorescein (Dako). PlA1 was monitored using a human alloantibody (anti-PI^{A1}; 1:20 dilution) directed against platelet membrane GP IIIa and derived from a PI^{A1}-negative woman alloimmunized against PI^{A1} by pregnancy.[29] Binding of this antibody was tested by an indirect immunofluorescence technique using a fluorescein-isothiocyanate-conjugated goat anti-human IgG γ chain (Biosource International, Camarillo, CA, USA). Staining was performed in 100 µL of PBS containing 0.1% BSA at 4°C for 30 minutes. Non specific fluorescence was assessed by using isotype-matched control antibodies and GAM-Fl. HEL cells were also characterized with glycophorin-A MoAb (Becton Dickinson) followed by a polyclonal goat anti-mouse IgG covalently linked to fluorescein (GAM-Fl, Becton Dickinson). The samples were analyzed by flow cytometry (FACScan, Becton Dickinson, San Josè, CA, USA). Furthermore, for each antigenic analysis, the mean intensity of fluorescence (MIF), expressed as the ratio of sample mean channel/control mean channel, was measured.

DNA labeling technique and flow cytometric analysis

HEL cells were washed in PBS and the pellets were fixed in 2 ml cold 70% ethanol and stored at 4°C. The cells were then centrifuged, washed in PBS and resuspended in 0.4 ml PBS and treated with RNAase (1 mg/mL; Type I-A; Sigma, St Louis, MO, USA) for 1 h at 37°C. Propidium iodide (PI; Sigma; 50 ug/mL in PBS) was then added to each sample and, after gentle mixing, samples were incubated in the dark at 4°C for 30 minutes and then measured. FACScan (Becton Dickinson) equipped with an argon ion laser tuned to a 488 wavelength, 15 mW output has been used for analysis. For each sample up to 10,000 events were collected. The DNA content of the cells was determined by the Lysis II analysis software (Becton Dickinson).

Morphological analysis

For determination of megakaryocyte morphology, HEL cells were mounted onto poly-L-lysine coated glass slides by cytocentrifugation (Shandon, Sewickley, PA, USA). Slides were May-Grünwald Giemsa stained and cells were scored for their light microscopy morphology.

Statistical analysis

The data were expressed as means ± the standard error of the mean (SEM). Two tailed Student's t-test was used for statistical comparison.

Results

Effect of PEG-rHuMGDF on cell proliferation

To assess the influence of PEG-rHuMGDF on the proliferation of HEL cells, we initially examined whether increasing concentrations of PEG-rHuMGDF (10 ng, 50 ng, 100 ng, 150 ng, 200 ng/mL) had any effects on the HEL cells, when compared to cells cultured without the cytokine. Liquid culture assay of the HEL cells has been done for 6 days without medium change. As clearly demonstrated in Figure 1, PEG-rHuMGDF did not significantly influence the proliferation of the cells under such condition until day 4. Starting by day 4 of culture, PEG-rHuMGDF (100 to

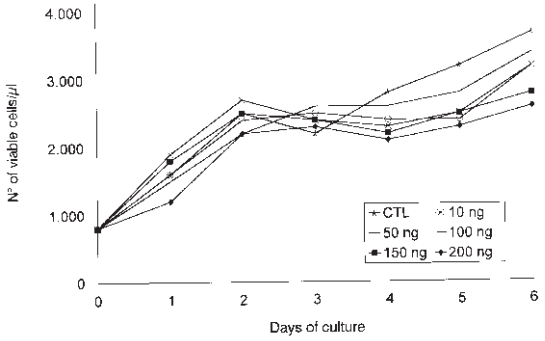

Figure 1. Counts of viable HEL cells at Trypan blue dye exclusion after incubation with PEG-fHuMGDF. Data are expressed as means of three separate exepriments performed in duplicate. SEM were contained within 10% of the means.

200 ng/mL) significantly inhibited ($p < 0.05$) the proliferation of the cells. To further confirm these data and in order to exclude that the reduction of proliferation after exposure to PEG-rHuMGDF is due to accumulation of toxic products, HEL cells were demipopulated, every 2 to 3 days up to day 16, by removal of half the culture volume which was replaced with fresh media with or without the cytokine. In this case, there was no difference at all in the proliferation between control samples and the treated cells (data not shown).

Morphology and nuclear configuration of PEG-rHuMGDF-treated HEL

The morphology of HEL cells changed after exposure to PEG-rHuMGDF. The size of the cells began to increase and cytoplasmic protrusions became prominent (Figure 2A, 2B,2C).

Ploidy distribution

The ploidy distribution of HEL cells with or without different concentrations of PEG-rHuMGDF was evaluated by flow cytometry after DNA staining with propidium iodide (Figure 3). The analysis of ploidy showed that PEG-rHuMGDF induced nuclear maturation of HEL cells with a pronounced increase of the cells > 8N, starting from day 2 of culture. The doses of 100 and 150 ng/mL were particularly effective in promoting this nuclear maturation (Figure 4). After day 4 of incubation, HEL cells, with or without PEG-rHuMGDF, failed to endoreduplicate and most cells were 2N and 4N (data not shown).

Cell-cycle analysis

We also analyzed the cell-cycle distribution of HEL cells 24, 48 and 72 hours after exposure to 150 ng/mL of PEG-rHuMGDF. This dose was chosen because in our hands it appeared particularly active for HEL differentiation and maturation. Data concerning cell cycle analysis of these cells are shown in Figure 5. After 24 hours, PEG-rHuMGDF significantly increased ($p<0.01$) the percentage of cells in G1 phase in comparison with control cells with a concomitant reduction of cells in S-phase. After 48 and 72 h of incubation, there was no difference between treated and untreated cells.

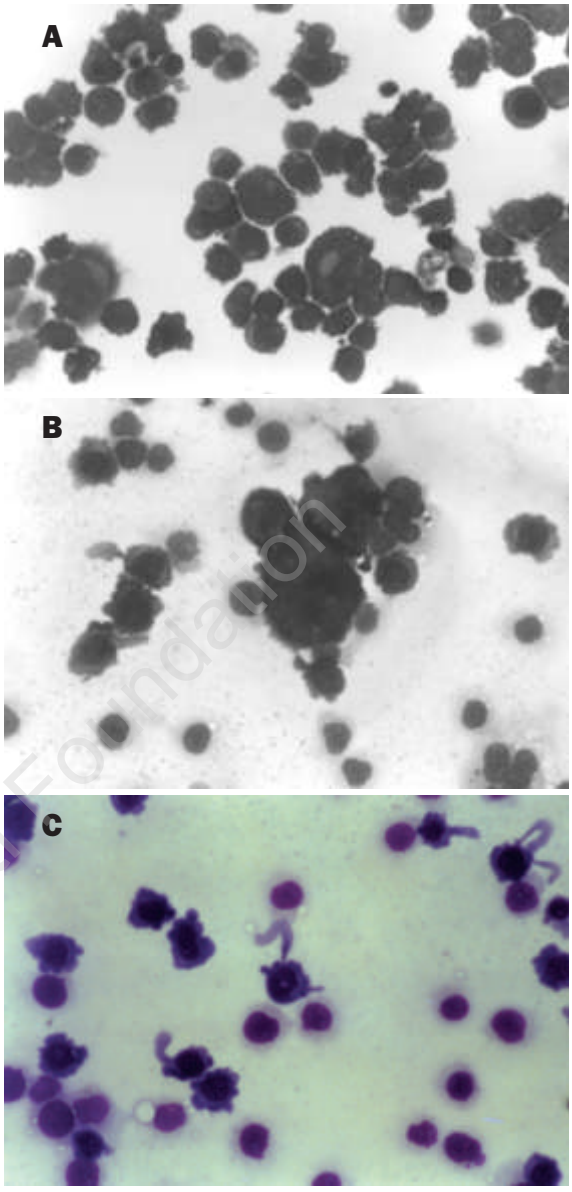

Figure 2. Morphological analysis of HEL cells stained with May-Grünwald-Giemsa after 72 hours of incubation with or without PEG-rHuMGDF. Light microscopy evaluation (×25 magnification).
A: untreated cells; B and C: HEL cells treated with 100 ng/mL of PEG-rHuMGDF. Note the presence of cytoplasmic protrusions in the PEG-rHuMGDF-treated cells.

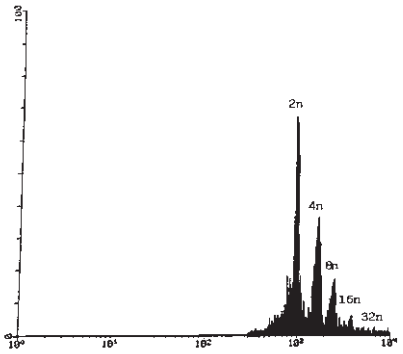

Figure 3. Flow cytometrc analysis of the DNA content in PEG-rHuMGDF-treated HEL cells after propidium iodide staining. X axis: red (propidium iodide) fluorescence intensity. Y axis: relative number of cells. A representative of three separate experiments is shown.

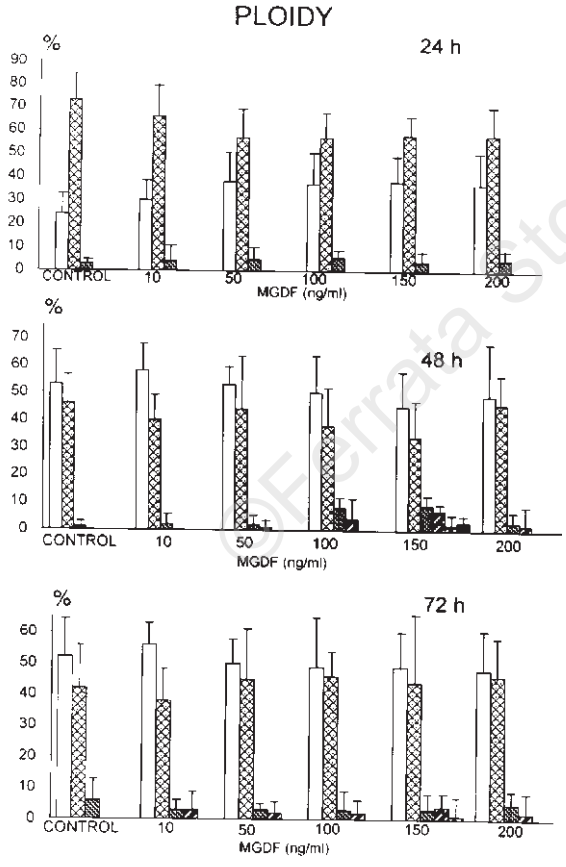

Figure 4. The ploidy distribution expressed as relative percent was evaluated by flow-cytometry analysis after incubation of HEL cells with or without PEG-rHuMGDF. The data represent the mean±SEM of three independent experiments performed in duplicate.
2N □; 4N ⬚; 8N ▩; 16N ▩; 32N ▩; 64N ■.

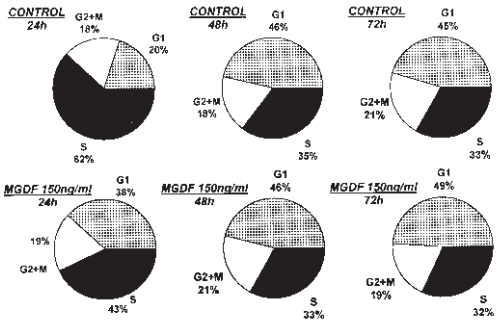

Figure 5. Cell-cycle analysis of HEL cells treated with 150 ng/mL of PEG-rHuMGDF. Data represents the mean of two experiments performed in duplicate.

Cell surface GP IIb-IIIa, PI^{A1} and Mpl receptor

Since different clones with a variable expression of megakaryocytic marker expression have been established, the HEL cell clone used in this study was first phenotypically characterized by indirect immunofluorescence staining and flow cytometry to ascertain the presence of megakaryocytic markers. The degree of expression of GPIIb-IIIa, PI^{A1} and Mpl receptor of HEL cells is shown in Figure 6. MIF evaluation for GP IIb-IIIa indicated that the expression of this marker significantly increased 48 hours after incubation with 100 to 200 ng/mL PEG-rHuMGDF (Table 1). These data were confirmed by the analysis of PI^{A1} expression, as shown in Figure 7. Also, PI^{A1} showed an increased expression after 48 h of incubation with PEG-rHuMGDF (100 ng/mL). Accordingly, Mpl receptor expression showed a significant reduction after 48 hours of incubation with PEG-rHuMGDF (data not shown). Glycophorin-A expression was 36% and decreased to 25% after PEG-rHuMGDF incubation.

Discussion

In this report, we have studied the effects of PEG-rHuMGDF, a truncated and pegylated form of thrombopoietin, on the growth and differentiation of HEL cell line. We chose HEL cell line because of its ability to undergo endoreduplication and to express some megakaryocytic phenotypic markers after treatment with differentiative agonists, such as phorbol esters.[30] Most importantly, this cell line express the receptor for thrombopoietin (Mpl receptor) at its cellular surface in a significant amount.[31] Due to the large numbers of cells available for study, these cell models are practical and potentially very informative.

By means of several approaches, the present paper demonstrates that PEG-rHuMGDF can stimulate

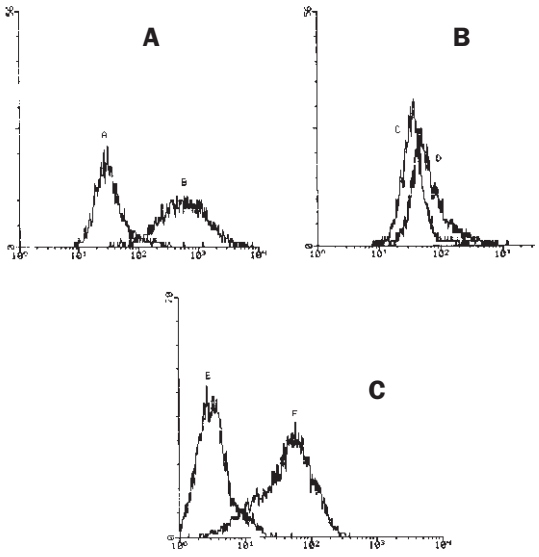

Figure 6. Flow cytometric analysis of HEL cells.
A) Staining with FITC-conjugated MoAb against GP IIb-IIIa. The A area represents the negative control while the B area represents cells treated with FITC-conjugated MoAb against GP IIb-IIIa. X-axis: logarithmic fluorescence intensity; Y-axis: relative number of cells.
B) Staining with the MoAb against Mpl receptor followed by RAM-FI. C shows the staining with an isotype-matched negative control. D represents HEL cellstreated with the anti-Mpl receptor MoAb+RAM-FI.
C) Staining with a human allo-antibody against the platelet membrane GP IIIa (PI^{A1}) followed by a fluorescein-conjugated goat anti-human IgGγ chain. E: negative control; F: positive cells labeled with the antibody against PIA1.

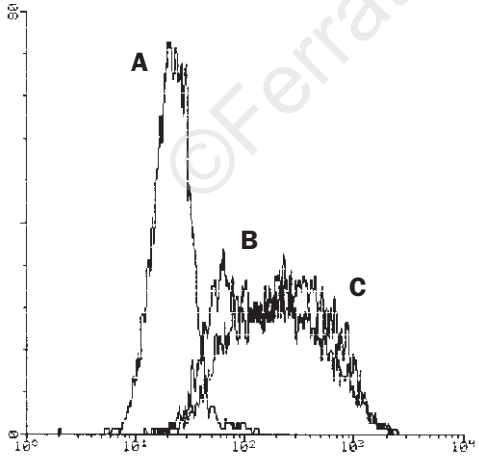

Figure 7. Cells were labeled with a human allo-antibody against platelet membrane GP IIIa (PI^{A1}) plus a fluorescein-conjugated goat anti-human IgG-γ chain.
A: negative control; B: positive control for PI^{A1} of cells unstimulated; C: positive control for PI^{A1} of PEG-rHuMGDF (100 ng/mL) treated cells after 48 hours of incubation.
X-axis: logarithmic fluorescence intensity. Y-axis: relative number of cells.

Table 1. GPIIb-IIIa MIF of HEL cells treated with PEG-rHuMGDF.

	24 hours	*48 hours*	*72 hours*
Control	8.2	9.0	12.6
PEG-rHuMGDF			
10 ng/mL	4.5	8.8	8.9
50 ng/mL	4.5	10.2	10.0
100 ng/mL	7.4	18.2	9.7
150 ng/mL	4.9	15.2	8.0
200 ng/mL	5.1	12.2	9.0

The MIF of GPIIb-IIIa was evaluated in untreated cells and after exposure to different concentrations of PEG-rHuMGDF. The values represents the mean of two experiments performed in duplicate. SEM were contained within 10% of the means.

HEL cells to differentiate into more mature cells.

The effects of PEG-rHuMGDF on the proliferation of HEL cells were evaluated in a liquid assay. No effects have been documented in liquid culture assay. Only on day 6 did PEG-rHuMGDF significantly inhibit ($p < 0.05$) the cell growth. These data confirm the results obtained by Drexler and Quentemeier[32] who demonstrated that thrombopoietin does not sustain HEL proliferation.

Moreover, maturation and differentiation of HEL cells increased consistently after PEG-rHuMGDF exposure. The maturation of HEL cells was demonstrated by: 1) morphological changes, 2) increased ploidy and 3) increased expression of megakaryocyte/platelet glycoprotein markers. PEG-rHuMGDF-treated cells showed the formation of membrane blebbing. It has recently been described that thrombopoietin induces *in vitro* platelet formation by $CD34^+$ cells. In turn, platelet formation occurs via pro-platelet intermediate structures that are long cytoplasmic processes emanating from megakaryocytes cell bodies.[19]

Our data suggest that PEG-rHuMGDF induces the formation of pro-platelet-like also in HEL cells, probably by acting on cytoskeleton proteins with the induction of membrane pseudopodies. Interestingly, a thrombopoietin-dependent cell lines (HU-3/TPO, MO/e/TPO, M-MOK/TPO, TF-1/TPO) showed the same morphology (membrane blebbings) as that observed in our HEL cells after incubation with thrombopoietin.[33]

Furthermore, it is well known that megakaryocytic maturation of the nucleus involves nuclear endoreduplication, a process in which nuclear material reduplicates itself without nuclear division. Nuclear maturation was also observed after PEG-rHuMGDF treatment of HEL cells. Flow cytometry evaluation of cells labeled with propidium iodide clearly docu-

mented increased ploidy values after treatment with PEG-rHuMGDF. This effect was dose-dependent, the most effective doses of MGDF being 100 and 150 ng/mL. Accordingly, cell cycle analysis showed that PEG-rHuMGDF, at the higher doses, increases the percentage of cells in G1 phase. It is likely that at the dose of 100-150 ng/mL, PEG-rHuMGDF reduces the proliferative capacity of HEL cells, inducing, at the same time, a shift toward maturation and the differentiation pathway.

In a previous study, it has been demonstrated that the expression of megakaryocytic markers like the GP IIb-IIIa complex and the platelet-specific alloantigen (PI^{A1})[31] were markedly enhanced following the addition of the inducers DMSO or TPA to the HEL cells. Our data demonstrated that the platelet markers (GP IIb-IIIa and PI^{A1}) also significantly increased after treatment of HEL cells with PEG-rHuMGDF. Therefore, PEG-rHuMGDF also represented a potent inducer of HEL cell differentiation along the megakaryocytic lineage.

As mentioned before, a number of human hematopoietic cell lines have been examined for Mpl receptor expression. In particular, Mpl receptor was clearly detected in CMK, Dami and HEL cell lines.[32] In our experiments, the Mpl receptor expression appeared reduced after 48 h of incubation, while in the subsequent period of observation, no differences were observed between the untreated control and the PEG-rHuMGDF samples. The reduction of Mpl receptor expression after 48 h of incubation with the cytokine may suggest that the complex ligand-receptor is internalized and metabolized.

Our data are contradictory with those presented by Quentemeier *et al.*[32] and by Zauli *et al.*[34] who failed to document any maturational effect of thrombopoietin on HEL cells. However, it should be underlined that Zauli *et al.* analyzed the differentiation effect of thrombopoietin after 6 days of incubation. Accordingly, the ploidy of HEL cells decreased after 6 days of culture in our experiment condition also. Our results are also in contrast with those reported by Drexler *et al*.[33] Their work, however, primarily focuses on the proliferative effect of thrombopoietin and no data are reported about morphology or ploidy of HEL cells after incubation with PEG-rHuMGDF.

In conclusion, other authors have clearly shown that thrombopoietin is able to promote megakaryocyte proliferation and differentiation of $CD34^+$ cells[7] and different continuous cell lines have been tested for TPO-proliferative/differentiative response.[30,34,35] as well as cell populations rich in megakaryocytes.[36,37] Our data clearly document that the continuous HEL cell line presents a pattern of response to PEG-rHuMGDF similar in many aspects to that of $CD34^+$ cells.[38]

This suggests that HEL cells may represent a useful model to evaluate the biochemical and molecular events involved in megakaryocyte differentiation.

Contributions and Acknowledgments

LC and LG were responsible for the design of the study. LC, MRM, PLT and SB contributed to the execution of the study. All authors contributed to the analysis and writing of the study. The technical assistance of S. Cimarelli is gratefully acknowledged.

Funding

This work was supported by CNR contract #96.00929. CT04. We are grateful to Dr. J.L. Nichol (Amgen, CA, USA) for providing PEG-rHuMGDF.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

Manuscript received October 9, 1997; accepted February 18, 1998.

References

1. Gewirtz AM. Human megakaryocytopoiesis. Semin Hematol 1986; 23:27-32.
2. Mazur EM. Human megakaryocytopoiesis and platelet production: a review. Exp Hematol 1987; 15:340-50.
3. Hoffman R. Regulation of megakaryocytopoiesis. Blood 1989; 74:1196-212.
4. Zauli G, Catani L. Human megakaryocyte biology and pathophysiology. Crit Rev Oncol Hematol 1995; 21: 135-57.
5. Debili N, Hegyi E, Navarro S, et al. *In vitro* effects of hematopoietic growth factors on the proliferation, endoreduplication and maturation of human megakaryocytes. Blood 1991; 77:2326-32.
6. Gordon MS, Hoffman R. Growth factors affecting human thrombopoiesis: potential agents for the treatment of thrombocytopenia. Blood 1992; 80:302-7.
7. Kaushansky K. Thrombopoietin: the primary regulator of platelet production. Blood 1995; 86:419-31.
8. Kaushansky K. Thrombopoietin: *in vitro* predictions, *in vivo* realities. Am J Hematol 1996; 53:188-91.
9. De Sauvage FJ, Hass PE, Spencer SD, et al. Stimulation of megakaryocytopoiesis by the c-mpl ligand. Nature 1994; 369:533-8.
10. Lok S, Kaushansky K, Holly RD, et al. Cloning and expression of murine thrombopoietin cDNA and stimulation of platelet production *in vivo*. Nature 1994; 369:565-8.
11. Kaushansky K, Lok S, Holly RD, et al. Promotion of megakaryocyte progenitor expansion and differentiation by the c-mpl ligand thrombopoietin. Nature 1994; 369:568-71.
12. Wendling F, Maraskowsky E, Debili N, et al. c-mpl ligand is a humoral regulator of megakaryocytopoiesis. Nature 1994; 369:571-4.
13. Bartley TD, Bogenberger J, Hunt P, et al. Identification and cloning of a megakaryocyte growth and development factor that is a ligand for the cytokine receptor Mpl. Cell 1994; 77:1117-24.
14. Soury M, Vigon I, Penciolelli JF, Heard J-M, Tambourin P, Wendling F. A putative truncated cytokine receptor gene transduced by the myeloproliferative leukemia virus immortalizes hematopoietic progenitors. Cell 1990; 63:1137-47.

15. Keleman E. Specific thrombopoietin cloned and sequenced - with personal retrospect and clinical prospects. Leukemia 1995; 9:1-2.
16. Suzukawa K, Satoh H, Taniwaki M, Yokota J, Morishita K. The human thrombopoietin gene is located on chromosome 3q26.33-q27, but is not transcriptionally activated in leukemia cells with 3q21 and 3q26 abnormalities (3q2126 syndrome). Leukemia 1995; 9:1328-31.
17. Debili N, Wendling F, Cosman D, et al. The Mpl receptor is expressed in the megakaryocytic lineage from late progenitors to platelets. Blood 1995; 85:391-401.
18. Drexler HG, Quentemeier H. Thrombopoietin: expression of its receptor Mpl and proliferative effects on leukemic cells. Leukemia 1996; 10:1405-21.
19. Choi ES, Nichol JL, Hokom MM, Xornkohl AC, Hunt P. Platelets generated *in vitro* from pro-platelet displaying human megakaryocytes are functional. Blood 1995; 85:402-13.
20. Farese AM, Hunt P, Boone T, Mac Vittie TJ. Recombinant human megakaryocyte growth and development factor stimulates thrombocytopoiesis in normal human primates. Blood 1995; 86:54-63.
21. Basser RL, Rasko JEJ, Clarke K, et al. Thrombopoietic effects of pegylated recombinant human MGDF (PEG-rHuMGDF) in patients with advanced cancer. Lancet 1996; 348:1279-81.
22. Martin P, Papayannopoulou T. HEL cells: a new erythroleukemia cell line with spontaneous and induced globin expression. Science 1982; 216:1233-5.
23. Ogura M, Morishima Y, Ohno R, et al. Establishment of a novel human megakaryoblastic leukemia cell line, Meg-01, with positive Philadelphia chromosome. Blood 1985; 66:1384-92.
24. Sato T, Fuse A, Eguchi M. Establishment of a human leukaemic cell line (CMK) with megakaryocytic characteristics from a Down's syndrome patient with acute megakaryoblastic leukaemia. Br J Haematol 1989; 72:184-90.
25. Greenberg SM, Rosenthal DS, Greeley TA, Tantravahi R, Handin RI. Characterization of a new megakaryocyte cell line: the Dami cell. Blood 1988; 72:1968-77.
26. Avanzi G, Lista P, Giovinazzo B, et al. Selective growth response to IL-3 of a human cell line with megakaryoblastic features. Br J Haematol 1988; 69:359-66.
27. Fugman DA, Witte DP, Jones CLA, Aronow BJ, Lieberman MA. In vitro establishment and characterization of a human megakaryoblastic cell line. Blood 1990; 75:1252-8.
28. Mouthon M, Freund M, Titeux M, et al. Growth and differentiation of the human megakaryoblastic cell line (ELF-153): a model for early stages of megakaryocytopoiesis. Blood 1994; 84:1085-91.
29. Aster RH. Platelet specific alloantigen systems: hystory, clinical significance and molecular biology. In: Alloimmunity and beyond. Ed. Nance ST. 89, 1993. p. 203-9.
30. Komatsu N, Kunitama M, Yamada M, et al. Establishment and characterization of the thrombopoietin-dependent megakaryocytic cell line, UT-7/TPO. Blood 1996; 87:4552-60.
31. Tabilio A, Rosa JP, Testa U, et al. Expression of platelet membrane glycoproteins and α-granule proteins by a human erythroleukemia cell line (HEL). EMBO J 1984; 3:453-9.
32. Quentemeier H, Zaborski M, Graf G, Ludwig WD, Drexler HG. Expression of the receptor MPL and proliferative effects of its ligand thrombopoietin on human leukemia cells. Leukemia 1996; 10:297-310.
33. Drexler HG, Zaborski M, Quentemeier H. Thrombopoietin supports the continuous growth of cytokine-dependent human leukemia cell lines. Leukemia 1997; 11:541-51.
34. Zauli G, Bassini A, Viatale M, et al. Thrombopoietin enhances the aIIb3-dependent adhesion of megakaryocytic cells to fibrinogen or fibronectin through PI3 kinase. Blood 1997; 89:883-95.
35. Bonsi L, Grossi A, Strippoli P, et al. An erythroid and megakaryocytic common precursor cell line (B1647) expressing both c-mpl and erythropoietin receptor (Epo-R) proliferates and modifies globin chain synthesis in response to megakaryocyte growth and development factor (MGDF) but not to erythropoietin (Epo). Br J Haematol 1997; 98:549-59.
36. Nagahisa H, Nagata Y, Ohnuki T, et al. Bone marrow stromal cells produce thrombopoietin and stimulate megakaryocyte growth and maturation but suppress proplatelet formation. Blood 1996; 87:1309-16.
37. Ito T, Ishida Y, Kashiwagi R, Kuriya S. Recombinant human c-Mpl ligand is not a direct stimulator of proplatelet formation in mature human megakaryocytes. Br J Haematol 1996; 94:387-90.
38. Carlo-Stella C, Tabilio A. Stem cells and stem cell transplantation. Haematologica 1996; 81:573-87.