International Journal of Cell Cloning 8:283-292 Suppl 1 (1990)

Human GM-CSF In Vivo: Identification of the Target Cells and of Their Kinetics of Response

M. Aglietta[a], F. Bussolino[b], W. Piacibello[a], F. Aprá[a], F. Sanavio[a], A. Stacchini[a], C. Monzeglio[c], F. Carnino[c], F. Gavosto[a]

[a]Clinica Medica A, Dipartimento di Scienze Biomediche ed Oncologia Umana, [b]Dipartimento di Genetica, Biologia e Chimica Medica, Universitá di Torino, Torino, Italy; [c]Divisione di Ginecologia C, Ospedale S. Anna, Torino, Italy

Key Words. Growth factors • GM-CSF • Hemopoiesis • Neutrophils • Progenitors • Cell kinetics

Abstract. Granulocyte-macrophage colony-stimulating factor (GM-CSF) was given for three days (8 μg/kg/day) to 14 subjects who had solid tumors and normal hemopoiesis. The treatment induced a rapid 3- to 5-fold increase in the number of circulating neutrophils, eosinophils and monocytes. Lymphocytes, platelets and reticulocytes were unmodified during treatment. Activation of circulating neutrophils during GM-CSF treatment was demonstrated by a significant, increased release of neutrophil-derived platelet-activating factor after stimulation with N-formyl-methionyl-leucyl-phenylalanine, tumor necrosis factor-alpha or phagocytosis.

The granulomonocytosis was dependent on increased bone marrow production of mature cells. Using the thymidine suicide technique, we observed that GM-CSF more than doubled the percentage of granulocyte-macrophage and megakaryocyte colony-forming units (CFU-gm and CFU-meg) and erythroid burst-forming units (BFU-e) in the S phase of the cell cycle. However, at the level of morphologically recognizable cells with autoradiography, we observed that GM-CSF increased the labeling index of the granulo-monopoietic cells, whereas that of the erythroblasts was unchanged. These data suggest that in accordance with in vitro observations, GM-CSF exerts its activity through all granulo-monopoietic lineages, whereas other cytokines (erythropoietin, thrombopoiesis-stimulating factors) may be needed to fully exploit the proliferative stimulus of GM-CSF on BFU-e and CFU-meg.

After treatment discontinuation, the proliferative activity drops to values lower than before treatment, suggesting a period of relative refractoriness of marrow progenitors to the cytocidal effect of cell cycle-specific antineoplastic agents. This hypothesis is under evaluation in a controlled clinical trial where GM-CSF is given prior to chemotherapy.

Correspondence: Dr. M. Aglietta, M.D., Clinica Medica A, Via Genova 3, 10126 Torino, Italy.

Received October 12, 1989; accepted for publication October 12, 1989.

0737-1454/90/$2.00/0

Introduction

Human granulocyte-macrophage colony-stimulating factor (GM-CSF) is a glycoprotein composed of 127 amino acids and coded by a gene situated on the long arm of chromosome 5 [1, 2]. It is produced by several types of cells in response to a variety of stimuli, including interleukin 1 (IL-1), which stimulates its production by endothelial cells and the thymic epithelium, and interferon-γ, which augments its production by monocytes and T lymphocytes [3-7]. The recent demonstration that GM-CSF and IL-1 are released by mast cells in response to activation of the IgE receptor is a significant finding, since it suggests new hypotheses concerning the mechanisms involved in hypersensitivity reactions [8].

In vitro, GM-CSF lengthens the survival of hemopoietic progenitors. It also stimulates the proliferation of all myeloid progenitors, both multipotent (granulocyte, erythroid, macrophage, monocyte colony-forming units; CFU-gemm) and committed (granulocyte-macrophage and megakaryocyte colony-forming units; CFU-gm and CFU-meg, respectively; and erythroid burst-forming units; BFU-e) [9-19]. GM-CSF receptors are maintained throughout the granulocyte and monocyte differentiation process [20]. GM-CSF sustains the production of mature granulocytes and monocytes by CFU-gm and also activates the mature cells [9-14].

When GM-CSF is present in vitro, it is accompanied by a rapid change in neutrophil conformation, with increased production of adhesion-promoting surface glycoproteins and an increased affinity of IgA Fc receptors [21, 22]. Several neutrophil functions are also affected by GM-CSF, including antibody-dependent cellular cytotoxicity, phagocytosis, superoxide production and nitroblue tetrazolium reduction upon challenge with N-formyl-methionyl-leucyl-phenylalanine (FMLP) [23-27], which seems to involve the recruitment of a less-responsive neutrophil subpopulation [28]. These data have given rise to the suggestion that GM-CSF is of importance in the regulation of myelopoiesis, and have thus provided a theoretical basis for its employment in vivo.

The expectations aroused by the in vitro findings have, indeed, been confirmed by the first in vivo results. Since its noteworthy biological activity is accompanied by low toxicity, GM-CSF appears to be a promising aid in the treatment of a variety of conditions [10, 11, 13, 19, 20]. The results of many of these studies of the effect of GM-CSF on primary and secondary myelopoietic disorders will be presented in this symposium.

Our own results following the administration of GM-CSF prior to chemotherapy in cases of solid tumor with no evidence of abnormal myelopoiesis [31-33] are summarized in this paper.

Results

GM-CSF target cells have been identified in subjects with normal hemopoiesis, and the extent of their response has been quantified. Our series consists

of 14 patients who received 8 μg/kg/day GM-CSF (recombinant, mammalian, glycosylated; Sandoz/Schering-Plough) for 3 days, either by i.v. perfusion (8 patients) or s.c. (6 patients). This difference in administration route had only a slight influence on the response kinetics of the hemopoietic cells and will not be considered in this paper.

The results will be discussed with reference to three points: 1) the effect of GM-CSF on peripheral cells; 2) the effect of GM-CSF on marrow cells; and 3) the effect of GM-CSF in suspension.

Effect of GM-CSF on Peripheral Cells

Administration of GM-CSF was followed by rapid leukocytosis. The leukocyte count rose from 7.9 to 15.2 × 10^9/l within 24 h, and quickly moved to 20.0 × 10^9/l by 72 h. This rise was attributable to a 3- to 4-fold increase in circulating neutrophils, eosinophils and monocytes, whereas the number of lymphocytes was unchanged (Fig. 1). Hemoglobin, reticulocyte and platelet values were also unaffected by the treatment. These data suggest that in a subject with normal hemopoiesis, an increased release into the circulation of granulocytes and monocytes predominates.

Particular attention was directed to the effect of GM-CSF on the functional status of the neutrophils. Neutrophilia is accompanied by the release of younger cells, as shown by the leftward shift of the Arneth score. The initial phase of neutrophilia is probably due to the release of marrow neutrophils, whereas the production of new neutrophils accounts for the maintenance of the phenomenon. More interestingly, neutrophils from patients during GM-CSF treatment released 2-3 times more platelet-activating factor (PAF) upon challenge with FMLP. A similar enhanced release of PAF from neutrophils is observed upon challenge with tumor necrosis factor-alpha (TNF-α) or during phagocytosis of C3 BYS opsonized baker yeast spores. Moreover, much lower concentrations of FMLP (less than half) or TNF-α (less than one-fifth) are needed to induce PAF synthesis from neutrophils. These data corroborate the view that GM-CSF, in addition to increasing the number of circulating neutrophils, is a powerful activator of neutrophils.

The physiopathological meaning of increased PAF production by neutrophils during GM-CSF treatment should be considered in the context of the relevant role of PAF in inflammatory reactions. PAF is a powerful chemotactic agent for monocytes, eosinophils and the neutrophils themselves. Moreover, it primes monocytes to release more IL-1 and TNF-α upon challenge with lipopolysaccharide and is a major mediator of eosinophil recruitment. These and other pro-inflammatory actions of PAF [34] suggest that increased PAF synthesis from activated neutrophils may greatly amplify a local inflammatory reaction.

The ability of GM-CSF to prime neutrophils in vivo to produce more PAF during phagocytosis, or when chemotactic peptides or mediators of delayed hypersensitivity are locally produced, underlines the existence of a new feedback loop between different mediators of the inflammatory response. GM-CSF treatment may cause thrombus formation and capillary leak syndrome [35-38]. So far, these toxicities have been attributed to its direct action on endothelial cells [39] and en-

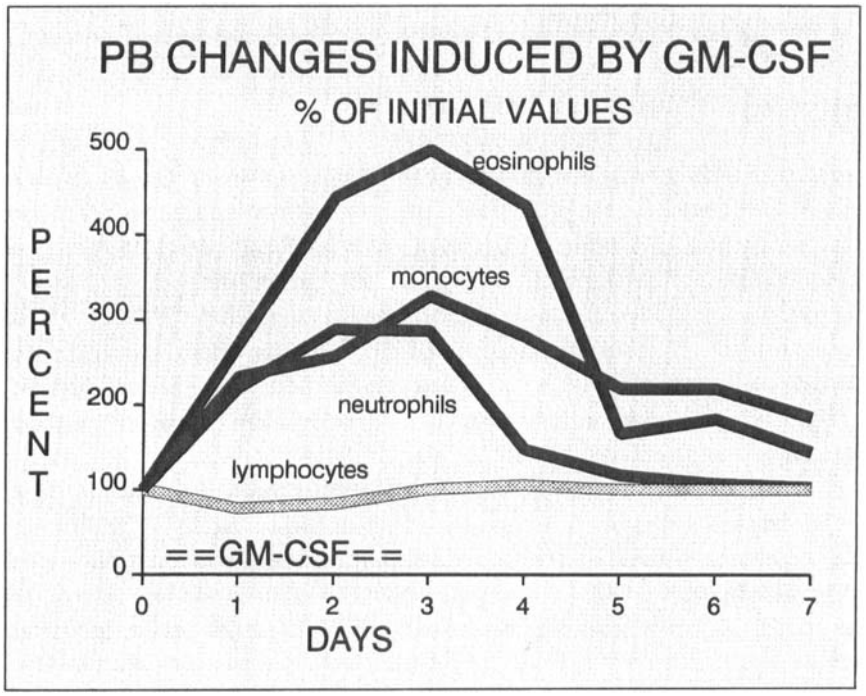

Fig. 1. Effect of GM-CSF treatment and discontinuation on leukocyte differential. GM-CSF (8 μg/kg/die × 3 days) was given to 8 subjects by continuous i.v. infusion.

hanced expression by neutrophils of the CD 11b molecule (an adhesion molecule with a role in neutrophil-endothelial cell interaction) [40]. Our findings provide an additional explanation for the origin of this toxicity. A pivotal role of PAF can be suggested, since it is a potent amplifier of platelet and leukocyte responses. Through a cascade mechanism [34], it induces the formation of large platelet thrombi (surrounded and invaded by neutrophils and then by eosinophils and macrophages). These spread over the adjoining vacuolized endothelial cells, whose cytoskeletal organization is modified by PAF [41].

Effect of GM-CSF Treatment on Marrow Cells

After 3 days of treatment with GM-CSF, there is a marked modification in marrow composition. The percentage of early granulopoietic cells (blasts + promyelocytes + myelocytes) more than doubles, and the leukoerythrogenetic ratio decreases. To further characterize GM-CSF target cells and the modifications in the proliferative status induced by GM-CSF, a series of kinetic studies was performed.

First, the percentage of progenitors in the S phase of the cell cycle was studied. During treatment, the percentage of S phase CFU-gm, BFU-e and CFU-meg more

than doubled, showing that all progenitors are a GM-CSF target. We were intrigued by the fact that the treatment produced only a granulo-monocytosis without affecting the number of erythrocytes and platelets. Therefore, the intermediate maturation steps of marrow cells were analyzed.

Autoradiographic studies showed that GM-CSF treatment increased the percentage of S phase early granulopoietic cells from 26 ± 14% to 41 ± 6%, without significantly affecting the proliferative status of erythroblasts (25 ± 12% before treatment; 30 ± 12% during treatment). The low number of megakaryoblasts (the only cell of the megakaryocyte lineage actively proliferating) did not allow a thorough investigation of the kinetic perturbations induced by GM-CSF on this compartment. The problem was analyzed indirectly: the megakaryocyte lineage was divided into four maturation steps according to morphology. During GM-CSF treatment, no changes in the compartment were evident (in contrast with the granulo-monopoietic pathway where early cells accumulate).

One likely explanation of these data is that other molecules (erythropoietin, thrombopoiesis-stimulating factors, transforming growth factor-β) are crucial in the regulation of thrombocytopoiesis and erythropoiesis, with the result that in normal subjects the GM-CSF stimulus cannot alter the production of terminal cells. This interpretation also helps to explain why GM-CSF has an inconstant effect as a stimulator of red cell and platelet production when administered in a scenario of altered hemopoiesis (after chemotherapy, marrow transplantation, etc.); in other words, in situations where the variability of the number of residual progenitors and of the levels of endogenous cytokines makes it less easy to predict its final effect.

Effect of GM-CSF Discontinuation

The number of circulating leukocytes rapidly falls to the pretreatment level once GM-CSF is suspended (Fig. 1). Examination of the marrow cell kinetics shows that the proliferative activity of the target cells quickly decreases to levels significantly lower than those observed prior to the administration of GM-CSF. The reasons underlying this feedback are not clear.

Three explanations are possible. First, inhibitory substances (i.e., acidic isoferritins, prostaglandins E, lactoferrin) whose action is no longer counteracted by pharmacologic levels of GM-CSF might slow the proliferation of cells. Second, the endogenous production of GM-CSF (and perhaps of other hemopoietic growth factors interacting with GM-CSF) might drop to values lower than the initial ones. Third, GM-CSF receptors might be downregulated on target cells. Whatever the biological reason for this slowing of proliferative activity, it appears likely that the 48-96 h after discontinuing the treatment with GM-CSF represent a period of partial refractoriness of bone marrow cells to the cytoreductive effect of cell cycle-specific chemotherapeutic agents. This observation, supported by data in monkeys, suggests that a short treatment with GM-CSF followed by chemotherapy (and perhaps by additional GM-CSF administration after chemo-

therapy) might reduce the hemopoietic toxicity of antineoplastic treatments. This hypothesis is at present addressed with a controlled clinical trial at our institution.

Conclusions

There is good reason to suppose that GM-CSF will become the treatment of choice for primary and secondary myelopathies. Understanding of its target cells and their kinetics of response is clearly a fundamental prerequisite for rational management, especially if several growth factors are combined with a view to reducing the doses and mimicking as closely as possible the physiological conditions of the marrow microenvironment, where hemopoietic cells are "bathed" in cytokines.

Acknowledgments

We are indebted to *Mrs. M. Rolando* for secretarial assistance. This work was supported with grants from CNR, Special Project Oncology, and from the Italian Association for Cancer Research.

References

1 Wong GC, Witek JC, Temple PA, et al. Human GM-CSF: molecular cloning of the complementary DNA and purification of the natural and recombinant protein. Science 1985;228:810-815.
2 Huebner K, Isobe M, Croce CM, Golde DW, Kaufman SE, Gasson JC. The human gene encoding GM-CSF is at 5q21-q32, the chromosome region deleted in 5q-anomaly. Science 1985;230:1282-1285.
3 Sieff CA, Niameyer CM, Mentzer SJ, Faller DV. Interleukin-1, tumor necrosis factor and the production of colony-stimulating factors by cultured mesenchymal cells. Blood 1988;72:1316-1323.
4 Segal GM, McCall E, Bagby GC Jr. Erythroid burst promoting activity produced by interleukin-1 stimulated endothelial cells is granulocyte-macrophage colony-stimulating factor. Blood 1988; 72:1364-1367.
5 Ridgway D, Borzy MS, Bagby GC Jr. Granulocyte-macrophage colony-stimulating activity production by cultured human thymic non lymphoid cells is regulated by endogenous interleukin-1. Blood 1988;72:1230-1236.
6 Piacibello W, Lu L, Watcher M, Rubin BY, Broxmeyer HE. Release of granulocyte-macrophage colony-stimulating factor from major histocompatibility class II antigen positive monocytes is enhanced by human gamma interferon. Blood 1985;66:1343-1351.
7 Piacibello W, Lu L, Williams D, et al. Human gamma interferon enhances release from phytohemagglutinin stimulated T4+ lymphocytes of activities that stimulate colony formation by granulocyte-macrophage, erythroid, and multipotential progenitor cells. Blood 1986;68:1339-1347.
8 Wodnar-Filipowicz A, Heusser CH, Moroni C. Production of the hemopoietic growth factors GM-CSF and interleukin-3 by mast cells in response to IgE receptor-mediated activation. Nature 1989;339:150-152.

9 Pegoraro L, Avanzi GL, Lista P. Growth and differentiation factors in human hematopoiesis. Haematologica 1988;73:525-543.
10 Morstyn G, Burgess AW. Hemopoietic growth factors: a review. Cancer Res 1988;48:5624-5637.
11 Sieff C. Hemopoietic growth factors. J Clin Invest 1987;79:1549-1557.
12 Cannistra SA, Griffin JD. Regulation of the production and function of granulocytes and monocytes. Semin Hematol 1988;25:173-188.
13 Clark SC, Kamen R. The human hematopoietic colony-stimulating factors. Science 1987;236:1229-1237.
14 Metcalf D. The molecular control of cell division, differentiation, committment and maturation in hemopoietic cells. Nature 1989;339:27-30.
15 Sieff C, Ekern SC, Nathan DG, Anderson JW. Combination of recombinant colony-stimulating factors are required for optimal hematopoietic differentiation in serum deprived cultures. Blood 1989;73:688-693.
16 Caracciolo D, Shirsat N, Wong GG, Lange B, Clark SC, Rovera G. Recombinant human macrophage colony-stimulating factor (M-CSF) requires subliminal concentrations of granulocyte-macrophage (GM)-CSF for optimal stimulation of human macrophage colony formation in vitro. J Exp Med 1987;166:1851-1860.
17 Strife AC, Lambek C, Wisniewski D, et al. Activities of four purified growth factors on highly enriched human hemopoietic progenitor cells. Blood 1987;69:1508-1523.
18 Begley CG, Nicola NA, Metcalf D. Proliferation of normal human promyelocytes and myelocytes after a single pulse stimulation by purified GM-CSF or G-CSF. Blood 1988;71:640-645.
19 Sonoda Y, Yang YC, Wong GG, Clark SC, Ogawa M. Erythroid burst promoting activity of purified recombinant human GM-CSF and interleukin 3: studies with anti GM-CSF and anti IL-3 sera and studies in serum free cultures. Blood 1988;72:1381-1386.
20 DiPersio J, Billing P, Kaufman S, Eghtesady P, William RE, Gasson JC. Characterization of the human granulocyte-macrophage colony-stimulating factor receptor. J Biol Chem 1988;263:1834-1841.
21 Weisbart RH, Kacena A, Schuh A, Golde DW. GM-CSF induces human neutrophil IgA mediated phagocytosis by an IgA Fc receptor activation mechanism. Nature 1988;332:647-648.
22 Arnaout MA, Wang EA, Clark SC, Sieff CA. Human recombinant granulocyte-macrophage colony-stimulating factor increases cell to cell adhesion and surface expression of adhesion promoting surface glycoproteins on mature granulocytes. J Clin Invest 1986;78:597-601.
23 Weisbart RH, Golde DW, Clark SC, Wong GG, Gasson JC. Human granulocyte-macrophage colony-stimulating factor is a neutrophil activator. Nature 1985;314: 361-363.
24 Nathan CF. Respiratory bursts in adherent human neutrophils: triggering by colony-stimulating factors CSF-GM and CSF-G. Blood 1989;73:301-306.
25 Metcalf D, Begley CG, Johnson GR, et al. Biologic properties in vitro of a recombinant granulocyte-macrophage colony-stimulating factor. Blood 1986;67:37-45.
26 Weisbart RH, Kwan L, Golde DW, Gasson JC. Human GM-CSF primes human neutrophils for enhanced oxidative metabolism in response to major physiological chemoattractants. Blood 1987;69:18-21.
27 Vadas MA, Nicola NA, Metcalf D. Activation of antibody-dependent cell-mediated cytotoxicity of human neutrophils and eosinophils by separate colony-stimulating factors. J Immunol 1983;130:795-799.

28 Fleischman J, Golde DW, Weisbart RH, Gasson JC. Granulocyte-macrophage colony-stimulating factor enhances phagocytosis of bacteria by human neutrophils. Blood 1986;68:708-711.
29 Aglietta M, Gavosto F. CSFs, from basic science to clinical trials. Bone Marrow Transpl 1989;4(suppl 1):16-19.
30 Steward WP, Scarffe JH. Clinical trials with haemopoietic growth factors. Prog Growth Factor Res 1989;1:1-12.
31 Aglietta M, Piacibello W, Stern A, Gavosto F. Human granulocyte-macrophage colony-stimulating factor: target cells and kinetics of response. UCLA Symposia on Hemopoiesis. New York: Alan R. Liss, Inc., 1989 (in press).
32 Aglietta M, Piacibello W, Sanavio F, et al. Kinetics of human hemopoietic cells after in vivo administration of granulocyte-macrophage colony-stimulating factor. J Clin Invest 1989;83:551-557.
33 Aglietta M, Monzeglio C, Aprá F, et al. In vivo priming of human normal neutrophils by granulocyte-macrophage colony-stimulating factor: effect on the production of platelet activating factor. (submitted).
34 Braquet P, Touqui L, Shem TS, Vargaftig BB. Perspectives in platelet activating factor research. Pharmacol Rev 1987;39:97-145.
35 Antman KS, Griffin JD, Elias A, et al. Effect of recombinant human granulocyte-macrophage colony-stimulating factor on chemotherapy induced myelosuppression. N Engl J Med 1988;319:593-598.
36 Brandt SJ, Peters WP, Atwater SK, et al. Effect of recombinant human granulocyte-macrophage colony-stimulating factor on hematopoietic reconstitution after high dose chemotherapy and autologous bone marrow transplantation. N Engl J Med 1988;318:869-876.
37 Vadhan-raj S, Keating M, Lemaistre A, et al. Effects of recombinant human granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndromes. N Engl J Med 1987;317:1545-1552.
38 Groopman JE, Mitsuyasu RT, Deleo MJ, Oette DH, Golde DW. Effect of recombinant human granulocyte-macrophage colony-stimulating factor on myelopoiesis in the acquired immunodeficiency syndrome. N Engl J Med 1987;317:593-598.
39 Bussolino F, Wang JM, Defilippi P, et al. Granulocyte- and granulocyte-macrophage colony-stimulating factor induce human endothelial cells to migrate and proliferate. Nature 1989;337:471-473.
40 Socinski MA, Cannistra SA, Sullivan R, et al. Granulocyte-macrophage colony-stimulating factor induces the expression of the CD 11b surface adhesion molecule on human granulocytes in vivo. Blood 1988;72:691-697.
41 Bussolino F, Camussi G, Aglietta M, et al. Human endothelial cells are target for platelet activating factor. I. Platelet activating factor induces changes in cytoskeleton structures. J Immunol 1987;139:2439-2446.

Discussion

Broxmeyer: Did you have any idea of the birth rates you were looking at with the double-labeling that you did with the BUDR in vivo and in vitro, and do you have any idea what cells you are talking about?

Aglietta: The double-labeling was performed on light-density bone marrow cells obtained by Ficoll separation. In this particular experiment, we did not attempt to differentiate the cells on the basis of their morphology (a difficult task when immunofluorescence and autoradiography are associated).

Broxmeyer: I would agree with the data that once you stop the GM-CSF if you started with a medium cycling state and then you go up with GM-CSF, as soon as you stop you go below the values you started with. In collaboration with Dr. Vadhan-Raj of M.D. Anderson, we looked at patients with sarcomas who were treated with GM-CSF, and the earliest we have looked at after the GM-CSF treatment was two days. At that time, the cells were already below cycling, and we are going to be looking at day 1 to see if the drop off is very, very fast.

Aglietta: Yes, but it is not as fast as I would have expected. Considering the rapid decline in leukocytosis following treatment discontinuation, our original hypothesis was that the lowest proliferation was present 12 or 24 hours after treatment discontinuation, and not 72 hours as we observed.

Krantz: The data you have on the effect of GM-CSF on other progenitors has been substantiated by a large number of groups now. In fact, Epo causes proliferation of CFU-gm, and GM-CSF causes proliferation of BFU-e. Now it seems to me that there might be two possibilities for this: either a depletion of one progenitor might cause a feedback to the stem cell that eventually generates more of the others in cell cycle, or a hyperactive feedback such that a generation of new cells of one cell line might produce other cytokines that affect the other cell lines. I was wondering if you or anyone else in the audience had any thoughts or evidence about which of these mechanisms or some other mechanisms are in play?

Aglietta: Well, you are asking a very difficult question. When you inject GM-CSF, your patient's marrow cells are already under the influence of other cytokines (G-CSF, Epo, IL-3, etc.). We do not know how the levels of these cytokines are modified by the presence of pharmacological concentrations of GM-CSF, nor do we know how this treatment modifies the receptor status of the progenitors.

Broxmeyer: That may be true with GM-CSF, but it is not known whether Epo stimulates other cytokines, as far as I know. It would be interesting to know whether it does or it doesn't.

Aglietta: I personally don't have any data.

Metcalf: I am not denying that there are all sorts of indirect actions that can happen in vivo once you have injected one agent, but I think the selective response that he was describing has a much simpler explanation, and that relates to the dose and the concentration of GM-CSF used. It is well known in vitro that for GM-CSF to stimulate the eosinophil, erythroid and the megakaryocyte lineages, you need to use progressively higher concentrations. In mice you only see a stimulation of mature megakaryocytes, for example, when you use quite high doses of GM-CSF. On the contrary, granulopoietic progenitor cells are much more responsive to GM-CSF. So it is possible to have an initial one or two divisions with a concentration that is quite unable to lead to the formation of maturing progeny. So, my explanation would be that if you were giving a rather low dose you achieved a rather low concentration that was enough to elicit some changes in cycling status of the cells, but not quite high enough. If the patients have been like mice, they actually give you an accumulation of maturing progeny.

Aglietta: That might be possible. Your point is that granulocyte/monocyte progenitors are more sensitive to GM-CSF than the other progenitors.

Metcalf: It is the progeny that are the more responsive. You can change the progenitor cell, but whether or not it generates progeny, is concentration-dependent.

Aglietta: I see your point. However, our results are not fully substantiating this since day 7 CFU-gm, which are a progeny of day 14 CFU-gm, are less stimulated in vivo by GM-CSF.

Metcalf: I don't think that anyone has proved that day 14 progenitors are the progeny of day 7.

Aglietta: Well, Jacobson showed this a few years ago.

Krantz: I still don't think this answers the effect of erythropoietin.

Metcalf: No, I was careful to say at the beginning there are many responses in vivo that have to involve other mediators, and the most dramatic is the response to G-CSF, which has absolutely no documented action on erythroid precursors in vitro or on stem cells, but in vivo induces a massive splenic erythropoiesis and a 100-fold rise in stem cells. Now you have to involve other regulators in some sort of complex network response. But in this particular case, there is a very simple explanation which is unrelated to the fact that there may be other factors operating.

O'Reilly: I'd like to ask a follow-up question on that. If you are talking about G-CSF in the context of progenitors found in the marrow, that would certainly hold true, and potentially, within the context of the marrow microenvironment. But do we necessarily know that G-CSF would not have alternative effects on a different microenvironment in terms of inducing some sets of cells, for example, erythroid proliferation in the spleen? Is it possible that G-CSF, acting on the microenvironment within the stroma, might set out a wholly separate cascade of events of what happens in the bone marrow?

Metcalf: That's what I am saying. If you take purified stem cells in vitro and add G-CSF, nothing happens, but in vivo things do happen, and they happen particularly in the spleen. Therefore, it has to be some sort of indirect or combined action, and it has to be favored in the spleen. I think that what you are suggesting is probably quite true. I mean you probably might have migration, but you also seem to have a lot of action occurring with the spleen.

Griffin: There is one other thing that one might consider to explain the altered traffic of stem cells. Both G and GM might be exhibiting these effects not because of effects on the stem cells, but acting on the endothelial cells changing them with the ability of stem cells to get out of or into different compartments.

Aglietta: I agree. Indeed, the effect of G-CSF and GM-CSF on endothelial cells that we recently showed (Nature 1989; 337:471) might be a reason for the increased dismissal of progenitors during G-CSF or GM-CSF treatment.