Administration of Recombinant Human Granulocyte-Macrophage Colony-Stimulating Factor After Chemotherapy Regulates the Expression and Secretion of Monocyte Tumor Necrosis Factor (TNF) and TNF Receptors p55 and p75

By Marc A. Williams, Ioannis Kouroumoussis, Denise Syndercombe-Court, Louise Hendry, Adrian C. Newland, and Stephen M. Kelsey

Monocyte expression and secretion of tumor necrosis factor (TNF) and TNF receptors (TNF-R) p55 and p75 was studied in patients receiving granulocyte-macrophage colony-stimulating factor (GM-CSF) after intensive chemotherapy. TNF expression and secretion of biologically active TNF was increased at regeneration compared with that of patients who had received chemotherapy alone. This effect persisted for several weeks after cessation of growth factor therapy. GM-CSF restored the responsiveness of monocytes to bacterial lipopolysaccharide (LPS), which appeared to be diminished after chemotherapy alone. Expression and secretion of TNF-R p55 and p75 by monocytes was augmented by GM-CSF therapy in association with the increase in TNF protein. We propose that GM-CSF administration after chemotherapy restores the normal responsiveness of monocytes to a secondary stimulus such as LPS and primes monocytes to respond to LPS with increased expression and secretion of TNF and TNF-R.

© 1995 by The American Society of Hematology.

MATERIALS AND METHODS

Reagents. Recombinant Escherichia coli-derived human GM-CSF (rhGM-CSF; Leucomax) was a gift from Sandoz (Frimley, UK). This factor was used at a final concentration of 5 ng/mL in all in vitro assays because this concentration induced optimal expression and secretion and tumoricidal activity of TNF in pilot experiments. Purified mouse monoclonal anti-TNF neutralizing antibody (IgG1) and fluorescein isothiocyanate (FITC)-conjugated mouse monoclonal antihuman TNF (Fluorokine) were obtained from R&D Systems (Oxford, UK). Isotype (IgG1) control antibody was obtained from Dako Ltd (High Wycombe, UK). Recombinant human TNFβ with a specific activity of approximately 2 × 10^6 U/mg protein, monoclonal rat antihuman TNF receptor (p80), and monoclonal mouse antihuman TNF receptor (p60) were obtained from Genzyme Ltd (West Malling, Kent, UK). Phycoerythrin (PE)-conjugated goat-anti-mouse F(ab')2, IgG were obtained from Dako Ltd (Slough, UK). Enzyme-linked immunosorbent assay (ELISA) kits for TNF quantitation were obtained from Cistron Biotechnology Ltd (Eurogenetics Europe Ltd, Hampton, Middlesex, UK). ELISA kits for the quantitation of soluble TNF-R (p55 and p75) were obtained from R&D Systems Ltd. Endotoxin (E coli LPS 055:B5) was obtained from Sigma (Poole, Dorset, UK). All reagents were tested for contaminating endotoxin by the B-Toxate Limulus amoebocyte assay (Sigma; Poole, Dorset, UK).

Patients and control subjects. Twenty patients were recruited for the study (14 men [median age, 50.2 years; range, 42 to 57 years] and 6 women [median age, 51.5 years; range, 43 to 59 years]) who presented with a nonmyeloid malignancy (18 myeloma and 2 lymphoma) in first or second plateau phase or complete remission. These patients were treated with a single intravenous injection of cyclophosphamide at 4 g/m² followed by 5 μg of rhGM-CSF (E coli-derived nonglycosylated polypeptide; Leucomax from Sandoz/Schering Plough, Camberley, Surrey, UK) per kilogram of body weight administered as a daily subcutaneous injection over 10 to 12 days to mobilize peripheral blood granulocytes. All patients who

From the Department of Haematology, The London Hospital Medical College, Whitechapel, London, UK.

Submitted February 13, 1995; accepted August 1, 1995.

Supported by a research grant from Sandoz Pharma (Basel, Switzerland) and The London Hospital Medical College Research Support Committee.

Address reprint requests to Marc A. Williams, Department of Haematology, The London Hospital Medical College, Turner Street, Whitechapel, London E1 1BB, UK.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1995 by The American Society of Hematology.

0006-4971/95/0611-0078$3.00/0

Blood, Vol 86, No 11 (December 1), 1995: pp 4234-4242
exhibited no other life-threatening cardiac, hepatic, renal, or microbiologic disease gave informed consent. All protocols were approved by the Local Ethics Committees. Eight patients receiving high-dose chemotherapy alone without growth factor support were used as parallel patient controls. Additionally, a total of 64 age- and sex-matched control subjects (26 men [median age, 35.5 years; range, 24 to 59 years] and 38 women [median age, 33.6 years; range, 26 to 60 years]) were studied simultaneously for each patient blood sample, such that one control subject was studied for each patient assay.

Preparation of human peripheral blood mononuclear cells (PBMCs). Blood (50 to 60 mL) in 10 IU/mL of preservative-free heparin was collected before chemotherapy (prebleed [PB]), at regeneration (R; absolute neutrophil count [ANC], >0.50 \times 10^9/L), and 1 to 3 weeks later (postregeneration [PRI]). Five milliliters of whole blood was taken for flow cytometric assays. The remainder was separated by density centrifugation over Histopaque 1077 (Sigma; d = 1.077 g/mL) for 15 minutes at 600g (18°C). Interface cells were harvested; washed four times in Hanks' Balanced Salt Solution (HBSS) without calcium and magnesium, supplemented with 10 mM L-HEPES, pH 7.40, and 2.5% fetal bovine serum (FBS; 6 minutes at 450g and 18°C); and resuspended in complete Dulbecco's modified Eagle's medium (DMEM). The resultant PBMC fraction contained less than 5% platelets using automated differential cytometry and stained positive for the following lineage-specific surface markers: CD14, 16% ± 4%; CD64, 17% ± 4%; CD3, 55% ± 6%; CD19, 9% ± 3.3%; and CD56, 22% ± 8.3%. An aliquot of the PBMC fraction at a concentration of 2 \times 10^6 cells/plate was incubated on tissue culture-treated plastic petri dishes that had been primed with 25% vol/vol heat-inactivated autologous serum, fixed cell preparations. In single-parameter fluorescence histograms (log, scale), less than 2% of the monocytes in any one sample stained positive for the appropriate negative isotype control antibody.

Cell culture. The TNF-sensitive cell lines L929 and WEHI-164 were maintained in DMEM culture medium (Sigma) supplemented with 12.50% vol/vol FBS, 2 mMol/L L-glutamine, and streptomycin and penicillin-G antibiotics. Primary cells (monocytes and whole PBMCs) were cultured in DMEM with 5% vol/vol FBS, 11 mMol/L L-glutamine, and streptomycin without antibiotics (complete DMEM). Antibiotics were not added because we had found that penicillin and streptomycin at concentrations used in cell culture applications are cytotoxic to freshly isolated primary cells. Cultures were maintained at 37°C in 5% CO2-air and 90% relative humidity. Cell lines and primary cells were greater than 95% viable using trypan blue exclusion and were maintained mycoplasma-free for the duration of the assays.

Flow cytometric quantitation of transmembrane expressed TNFs and the TNF-R (TNF-p55 and TNF-p75). Heparinized (10 IU/mL) whole blood was incubated with control diluent (HBBS [10 mMol/L HEPES in Hanks' Balanced Salt Solution] supplemented with 2 mMol/L L-glutamine and 0.5% bovine serum albumin [BSA]) with or without LPS (1 \mu g/mL) at 37°C in a shaking water bath for 50 minutes. Fifty microliters of whole blood was then removed and incubated with 50 \mu g/mL of FITC-conjugated anti-TNF or 25 \mu g/mL of anti-TNF-R antibody. Specific epitope recognition by the FITC-conjugated TNF was confirmed by preincubating aliquots of whole blood with unconjugated monoclonal anti-human TNF-neutralizing antibody. This showed that subsequent detection of transmembrane TNF was not observed and that Fc-receptor-mediated nonspecific binding was minimal, thus confirming the TNF-specific recognition by the FITC-conjugated anti-TNF. Samples stained for transmembrane TNF were lysed with FACSLyse solution (Becton Dickinson, Conley, Oxford, UK) diluted 1:10 with sterile deionized water, washed twice in HBBS (supplemented with 2 mMol/L L-glutamine, 0.50% BSA, 10 mMol/L HEPES, pH 7.4, and 0.01% sodium azide solution) at 450g and 4°C, and resuspended in the wash buffer containing 1% vol/vol formaldehyde solution. Samples stained for the p55 and the p75 receptors were washed three times in excess wash buffer at 4°C and incubated on ice for 30 minutes with excess PE-conjugated goat-antimouse F(ab')2 fragments. Stained samples were washed three times and finally resuspended in 1% vol/vol formaldehyde fixative solution.

A Becton Dickinson FACScan flow cytometer incorporating the Consort 32 LYSYS II version 1.02 software was used to assess both the percentage positivity and mean fluorescence intensity (MFI) before and after LPS stimulation. Using this technique, representative levels of percentage positivity for surface TNF in the patient and control groups were 5.3% to 46.6% for resting monocytes and 6.8% to 74.8% for LPS-stimulated monocytes. For the p55 receptor, expression ranged from 6.3% to 17.9% in the resting state and from 6.2% to 29.7% when stimulated. For the p75 receptor, expression ranged from 14.9% to 27.7% in the resting state and from 15.5% to 48.3% while stimulated. MFI for TNF expression ranged from 29.8 to 60.6 in the resting state and from 31.6 to 85.7 when stimulated with LPS. For the p55 receptor, expression MFI ranged from 25.5 to 48.1 in the resting state and from 28.2 to 69.5 when stimulated. For the p75 receptor, expression MFI ranged from 32.8 to 58.3 in the resting state and from 35.4 to 76.5 when stimulated. Spectral compensation was made for red emissions (FL2) entering the green channel (FL1) and green emissions (FL1) entering the red channel (FL2) as appropriate (range, 0.50% to 7.0%). Monocyte gates were confirmed by CD14 and CD64 expression for each sample. Data sets were recorded in real time for 1% vol/vol formaldehyde solution fixed cell preparations. In single-parameter fluorescence histograms (logy scale), less than 2% of the monocytes in any one sample stained positive for the appropriate negative isotype control antibody.

Assay of monocyte-meditated TNFs and TNF-R secretion. Monocytes at 10^6 cells/mL and PBMCs at 10^6 cells/mL were cultured as quadruplicates for 24 hours in complete DMEM. A similar quadruplicate set was set up and cultured in the presence of LPS at 1 \mu g/mL; control cultures were stimulated with control diluent alone. Supernatants were harvested after 24 hours, centrifuged at 400g for 5 minutes at 4°C, passed through a 0.22-\mu m sterile filter unit, and stored frozen at −70°C until assayed. Secreted proteins were recorded in picograms per 10^6 cells.

Assay of bioactive TNF secretion by monocytes and PBMCs. The bioactivity of secreted TNF was determined by the cytokysis of TNF-sensitive L929 and WEHI-164 target cells. Target cell monolayers were washed and resuspended to 5 \times 10^6 cells/mL. One hundred microliters of cells was added to each well of a 96-well flat-bottomed microtiter plate and allowed to equilibrate and adhere for 4 to 6 hours at 37°C before the addition of 100 \mu L of diluent, standard TNF (10 pg to 1 \mu g/mL), or supernatant containing actinomycin D (2 \mu g/mL).

After 24 hours, the plates were washed three times with warm phosphate-buffered saline (PBS) and incubated with 1% vol/vol formaldehyde and 10 mMol/L HEPES, pH 7.4, and the cells that survived the interaction with TNF were fixed for 2 minutes with 100% methanol, washed twice, and incubated with 100 \mu L/well of aqueous 1% vol/vol crystal violet solution for 20 minutes. The plate was washed thoroughly with deionized water and 100 \mu L/well 10% vol/vol glacial acetic acid was added to solubilize the stained viable cells before being read optically at a wavelength of 620 nm. Data were recorded as the percentage of specific lysis (%SL) with respect to the negative and positive controls and the TNF standard curve. The TNF specificity of this assay was confirmed by preadsorbing culture supernatants with an excess of mouse antihuman TNF neutralizing antibody for

From www.bloodjournal.org by guest on November 11, 2017. For personal use only.
2 hours at 37°C, 5% CO₂/95% air and assaying the supernatants for TNF bioactivity.

**Biometry and statistical analysis.** All assays were prepared as duplicates or triplicates for each patient and control. Data are expressed as mean values ± SEM. Independent sample analysis between rhGM-CSF–treated patients and patients treated with chemotherapy alone were compared using the multi-parameter MANOVA (Scheffe Test) and Kruskal-Wallis two-way and three-way ANOVA (fixed effects and repeated measures) for multiple comparisons between and within groups. To test the association between TNF expression and secretion, p55 and p75 expression and secretion, and TNF secretion with TNF-R secretion in the patients and control groups, Pearson’s Product Moment Correlation was measured. Significance was set at α-values of \( P < .05 \), \( P < .01 \), \( P < .001 \), and \( P < .0001 \), as indicated. The Statistica software package (Statsoft UK Ltd, Oxford) was used for data analysis.

**RESULTS**

**Effect of GM-CSF administration on the expression and secretion of TNF.** Resting monocytes isolated from normal subjects and patients before chemotherapy exhibited low levels of transmembrane TNF. This effect could be increased by incubation with LPS ex vivo (Figs 1 and 2, \( P < .01 \)). Surface TNF levels on monocytes from patients treated with chemotherapy alone were unaltered at and several weeks after hematopoietic regeneration. However, patients treated with GM-CSF exhibited augmented monocyte TNF expression at regeneration and after regeneration compared with before chemotherapy and with patients who had received no growth factor (Figs 2A through F and 3B and C; \( P < .001 \)).

Monocytes from patients that had received chemotherapy alone responded to LPS stimulation with a minimal increase in TNF expression, unlike the response in normal controls (Fig 3). However, monocytes from patients that had received GM-CSF remained responsive to LPS at both regeneration (Fig 3B) and several weeks after regeneration (Fig 3C). TNF expression by monocytes from GM-CSF–treated patients after LPS stimulation was significantly greater than that observed for patients treated with chemotherapy alone at regeneration (\( P < .001 \)) and after regeneration (\( P < .01 \)).

Secretion of TNF from unstimulated monocytes was low or undetectable but could be augmented after LPS stimulation (Fig 4). Monocytes from patients receiving chemotherapy alone exhibited a minimal response to LPS at regeneration (Fig 4B) and after regeneration (Fig 4C), with the amount of TNF secreted being significantly less than that found before chemotherapy. By contrast, monocytes from patients receiving GM-CSF exhibited increased levels of...
GM-CSF REGULATES MONOCYTE TNF & TNF RECEPTORS

Fig 3. rhGM-CSF administration and TNF expression of monocytes studied before chemotherapy (A), at regeneration (B), and several weeks after regeneration (C). (U) Normal subjects; (W) patients treated with chemotherapy alone; (D) patients treated with rhGM-CSF. Resting (no stimulation) monocyte expression is shown on the left-hand side of the graph and LPS-stimulated monocytes on the right-hand side. LPS stimulation upregulated TNF expression in all patients before chemotherapy. Augmentation of TNF expression was observed in patients treated with rhGM-CSF at regeneration (B) and several weeks after therapy had ceased (C). The data are presented as the MFI ± SEM. *LPS upregulates TNF over resting cells at P < .01. **TNF expression greater than patients treated with chemotherapy alone at P < .05. ***TNF expression greater than that before bleeding at P < .01.

Fig 4. The effect of rhGM-CSF on TNF secretion by monocytes as quantitated by ELISA. TNF secretion was quantitated before chemotherapy (A), at regeneration (B), and several weeks after regeneration (C). (U) Normal subjects; (W) patients treated with chemotherapy alone; (D) patients treated with rhGM-CSF. Spontaneous release of TNF was minimal in patients and controls before chemotherapy and during regeneration. LPS stimulation induced a significant release of TNF before chemotherapy (A). GM-CSF–treated patients exhibited a marked augmentation in TNF secretion at regeneration (B) that persisted for several weeks after regeneration (C). Patients treated with chemotherapy alone released insignificant levels of TNF at regeneration and several weeks after regeneration. The data are presented as TNF secretion in nanograms per 10^6 cells ± SEM. *LPS significantly upregulates TNF secretion over resting levels at P < .01. **LPS upregulates TNF secretion over resting levels at P < .05. ***The observed secretion of TNF was greater than that of patients treated with chemotherapy alone at P < .001. **The observed TNF secretion was greater than the prebleed levels at P < .01.

TNF secretion compared with both prechemotherapy levels and with levels in patients who had received no growth factor (P < .001 for each). In this respect, administration of GM-CSF was able to restore the responsiveness of monocytes to LPS. The TNF secreted was confirmed to be biologically active by cytolyis of WEHI 164 and L929 cells (Fig 5A and B). TNF secretion, as quantified by the bioassay, was in good agreement with that obtained by ELISA.

Secretion of TNF by whole PBMCs was similar to that of purified monocytes although the levels of TNF secretion per MNC was less than those for monocytes, confirming that monocytes are the main source of secreted TNF from immune effector cells. As for purified monocytes, secretion of TNF was greatly augmented in patients receiving GM-CSF, and the apparent refractoriness to LPS was abrogated (data not shown). However, this effect was observed at regeneration only (data not shown) and did not persist after the growth factor was stopped, as was observed for purified monocytes.

Effect of rhGM-CSF administration on the phenotypic expression of the p55 TNF-R and the p75 TNF-R on monocytes ex vivo. The expression of both p55 (Fig 6) and p75 (Fig 7) was studied in control subjects and patient groups during therapy. In normal control subjects, the resting (unstimulated) monocyte p55 expression was always proportionally lower than the respective expression of p75 (P < .05). Both the p55 and p75 TNF-Rs were upregulated after LPS stimulation (P < .01).
Fig 5. Assay of soluble TNF bioactivity. Monocytes stimulated with and without LPS were assayed for the bioactivity of secreted TNF in a cytolysis assay using L929 (A) and WEHI-164 (B) as sensitive target cells. Data are represented as mean percentage of specific lysis ± SEM (error bars were omitted for clarity, but were within 15% of the mean). TNF bioactivity from resting normal control monocytes (○) and LPS-stimulated normal control monocytes (□). Unstimulated monocyte supernatants from patients receiving chemotherapy alone (+) and patients receiving rhGM-CSF therapy (×) exhibited low levels of TNF bioactivity throughout the study points. Monocytes from patients treated with chemotherapy alone (MI failed to respond to LPS, whereas LPS-stimulated monocytes from rhGM-CSF-treated patients (×) secreted high levels of TNF at regeneration and several weeks after the cessation of therapy.

At regeneration, monocytes from patients treated with chemotherapy alone exhibited levels of p55 that were similar to those observed before initiation of the chemotherapy (Fig 6B); however, monocytes from patients who had received rhGM-CSF therapy exhibited an increase in p55 expression (P < .01). Expression levels of p55 returned to baseline after regeneration, which was several weeks after the cessation of rhGM-CSF therapy (Fig 6C).

Monocytes from patients receiving chemotherapy alone exhibited an augmented expression of p55 over resting levels in response to LPS stimulation, although the response was diminished at regeneration and after regeneration. By contrast, LPS-stimulated monocytes from patients receiving rhGM-CSF exhibited significantly augmented expression levels of p55 at regeneration and after regeneration compared with resting levels (P < .01). The expression of p55 after LPS stimulation was significantly greater than that observed for patients receiving chemotherapy alone at both regeneration and even after the cessation of rhGM-CSF therapy (P < .0001). The levels of p55 in the rhGM-CSF treated patients also remained greater than the prebleed levels at both regeneration and several weeks after the rhGM-CSF therapy had ceased. The phenotypic modulation of p75 in patients receiving only chemotherapy as well as those that received rhGM-CSF therapy followed the same trends as were observed for p55 (Fig 7).

Effect of rhGM-CSF administration on the secretion of p55 TNF-R and p75 TNF-R from monocytes ex vivo. Resting monocytes secreted low levels of p55 (Fig 8) and p75 (Fig 9), although both receptors were secreted in response to LPS stimulation. However, before chemotherapy all patients

Fig 6. Assay of monocyte p55 TNF-R expression. The expression of p55 receptor by monocytes was assessed by multiparameter flow cytometry. Resting levels, shown on the left of the graphs, were equivalent in patients and controls before chemotherapy (A) and did not alter in either patient group at regeneration (B) or several weeks after regeneration (C). Stimulation of monocytes with LPS augmented the expression of p55 in normals and controls before chemotherapy compared with resting monocytes. Patients treated with chemotherapy alone responded weakly to LPS at regeneration (P < .01) and failed to respond to LPS several weeks after regeneration. Patients treated with rhGM-CSF exhibited marked increases in membrane expressed TNF at both regeneration and several weeks after the cessation of therapy in response to LPS. In addition, the levels of p55 expressed were significantly greater than those of either normal controls or patients treated with chemotherapy alone. Levels of significance are as described in Fig 2.
exhibited significantly higher levels of spontaneous p55 secretion compared with normal controls ($P < .01$). Spontaneous secretion of p75 in both patient groups before chemotherapy was similar to that of normal controls (Figs 8A and 9A).

At regeneration and after regeneration, resting monocytes from patients treated with chemotherapy alone exhibited lower levels of p55 secretion compared with prebleed levels (Fig 8B and C; $P < .05$). By contrast, monocytes from patients who were treated with rhGM-CSF secreted similar levels of TNF-R after regeneration as before chemotherapy, such that levels were marginally greater at time points after regeneration compared with those of patients who had received only chemotherapy ($P < .01$). Monocytes from patients receiving chemotherapy alone showed reduced ability to respond to LPS at regeneration. However, responsiveness to LPS was restored in patients receiving GM-CSF.

Comparing patients receiving chemotherapy alone and those that had received rhGM-CSF therapy showed striking differences in p75 (Fig 9). Before chemotherapy, p75 secretion was similar to that observed for normal subjects. However, secreted levels of p75 were markedly attenuated at both regeneration and several weeks after regeneration in patients receiving chemotherapy alone ($P < .01$). GM-CSF therapy had a minimal effect on p75 secretion by resting monocytes.

However, the response to LPS in the two patient groups was quite different. At regeneration, monocytes from patients treated with chemotherapy alone were refractory to LPS, an effect that persisted after regeneration. In addition, levels of p75 secreted in response to LPS at both time points after regeneration were markedly less than the corresponding levels observed before chemotherapy ($P < .001$) and were significantly lower compared with the levels observed for normal patients ($P < .01$). By contrast, monocytes from patients treated with rhGM-CSF exhibited significantly greater p75 secretion in response to LPS at both time points ($P < .001$). Levels of p75 secreted by rhGM-CSF–treated patients were persistently greater than the levels observed for patients treated with chemother-
apy alone at both regeneration (~ .001) and several weeks after regeneration (~ .01).

A strong positive correlation was observed between the expression of TNF and the subsequent secretion of TNF in patients treated with rhGM-CSF (~ r = .810, ~ P < .001) that was not seen in those patients receiving chemotherapy alone at regeneration and several weeks after the cessation of rhGM-CSF therapy. A positive correlation between TNF secretion and p75 secretion in patients treated with rhGM-CSF at prebleed, regeneration, and several weeks after the cessation of rhGM-CSF therapy was also found (~ r = .663, ~ P < .01). No correlation was seen in patients receiving chemotherapy alone at either regeneration or after regeneration. There was a significant but weaker correlation between p55 secretion and TNF secretion in patients treated with rhGM-CSF therapy that was not observed in those patients receiving chemotherapy alone (~ r = .559, P < .02).

**DISCUSSION**

It has been shown in vitro that GM-CSF can increase the synthesis and expression of TNF by monocytes~26,30 and will prime monocytes for subsequent secretion of TNF in response to a secondary stimulus. GM-CSF induces rapid upregulation of TNF mRNA in human monocytes,~26,28,30 This effect facilitates increased surface expression and secretion of TNF after stimulation with bacterial LPS.~26,30 GM-CSF may also increase TNF expression directly.~24,33 Both expression and secretion of TNF are important for monocyte antitumor activity,~6,7,32 and secreted TNF may sustain an autocrine feedback loop potentiating the tumoricidal effect.~20,30,33,34

There is some evidence to suggest that the antitumor potential of monocytes is increased by administration of GM-CSF to patients.~29,31 However, the effect of GM-CSF administration on TNF and TNF-R regulation by monocytes in vivo has not been studied in detail.

We have shown that expression and secretion of biologically active TNF by monocytes in patients regenerating after chemotherapy is enhanced by administration of GM-CSF. Importantly, both expression and secretion of TNF are minimal unless monocytes are exposed to an appropriate secondary stimulus such as LPS. In addition, GM-CSF therapy overcomes the refractory status of monocytes to LPS that is observed after chemotherapy and restores responsiveness to normal or supranormal levels. These observations confirm in vivo that GM-CSF will activate monocytes directly and, more importantly, will prime them to respond to an appropriate secondary stimulus. Thus, administration of GM-CSF is not associated with widespread, inappropriate TNF release, which may be highly toxic in vivo. Increased expression and secretion of TNF may augment both the antitumor and anti-infective potential of circulating monocytes and tissue macrophages after chemotherapy.

We have also observed that GM-CSF administration modulates monocyte TNF-R expression and secretion after chemotherapy. Expression of both p55 and p75 was increased at regeneration in patients receiving GM-CSF. In addition, secretion of p75 was greatly increased by GM-CSF, particularly after secondary stimulation of monocytes by LPS. Soluble TNF-R play a key role in modulating the activity of TNF under conditions of inflammation or during tumor killing.~17,19,35 The effect of low levels of TNF can be potentiated in solution by binding to soluble p75; by contrast, excess and potentially harmful levels of TNF can be neutralized by this. In this respect, it is our observation that GM-CSF restores the normal responsiveness of monocytes to LPS after chemotherapy that is of potential importance; secretion of TNF receptor p75 must be appropriate for the levels of monocyte-associated and circulating TNF and for the prevailing conditions. We have shown that, in patients receiving GM-CSF, expression and secretion of TNF correlated well with expression and secretion of TNF receptors.

It is unclear why the effect of GM-CSF administration on monocyte function should persist for several weeks after cessation of growth factor administration. The biologic half-life of GM-CSF after subcutaneous administration is 10 to 14 hours.~36 A possible explanation is the persistence of activated circulating monocytes in the blood for several weeks after GM-CSF exposure in the absence of a well-defined inflammatory site for tissue migration. In vitro and in vivo studies have suggested that GM-CSF administration may inhibit neutrophil migratory capacity.~13,38
GM-CSF REGULATES MONOCYTE TNF & TNF RECEPTORS

These observations suggest that administration of GM-CSF to patients after chemotherapy may restore or even increase the antitumor activity of the monocyte-macrophage system. No direct clinical data yet exist to support a direct antitumor role for GM-CSF, although preliminary evidence suggests that its administration may augment the immediate tumoricidal effects of chemotherapy for high-grade non-Hodgkin’s lymphomas independently of cytotoxic drug doses.36

ACKNOWLEDGMENT

The authors acknowledge Sunira Patel for technical assistance and Dr L. Side, Dr J. Cavenagh, and the Nursing Staff of the Department of Haematology of the The Royal London Hospital for arranging clinical specimens.

REFERENCES

31. Williams MA, Kelsey SM, Gutteridge CN, Newland AC: Administration of rHuGM-CSF augments monocyte tumoricidal and...
microbicidal activity in vivo following high-dose chemotherapy. Blood 84:28a, 1994 (abstr, suppl 1)


35. Slowik MR, De Luka LG, Fiers W, Pober JS: Tumour necrosis factor activates human endothelial cells through the p55 tumour necrosis factor receptor but the p75 receptor contributes to activation at low tumour necrosis factor concentration. Am J Pathol 143:1724, 1993


Administration of recombinant human granulocyte-macrophage colony-stimulating factor after chemotherapy regulates the expression and secretion of monocyte tumor necrosis factor (TNF) and TNF receptors p55 and p75

MA Williams, I Kouroumoussis, D Syndercombe-Court, L Hendry, AC Newland and SM Kelsey

Updated information and services can be found at:
http://www.bloodjournal.org/content/86/11/4234.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml