The effect of granulocyte-macrophage colony stimulating factor (rGM-CSF) on macrophage function in microbial disease

THOMAS C. JONES

Clinical Research Consultants, 24 Webergasse, 4058 Basle, Switzerland

The haematopoietic growth factor, GM-CSF, has well-documented stimulatory effects on monocyte and macrophage functions. These effects include enhanced proliferation of their progenitor cells, increased endocytosis and metabolism of mature cells, increased function as antigen-presenting cells, and increased inhibition or killing of intracellular fungi, bacteria, protozoa and viruses. The major effect of GM-CSF on monocytes and macrophages is to enhance phagocytic and metabolic functions, including increased synthesis of molecules toxic to microbes, and to release other proinflammatory cytokines. This results in inhibition and/or killing of *Candida albicans*, *Aspergillus*, *Cryptococcus*, *Pneumocystis*, *Leishmania*, *Mycobacteria*, as well as other intracellular pathogens. GM-CSF also enhances the intracellular effectiveness of antiviral and antibacterial drugs. Viral replication may be increased in activated cells, therefore, when GM-CSF is used, a combination with appropriate antiviral drugs is recommended. Several reports in patients of successful management of microbial diseases which depend on macrophage function are now reviewed. These reports support the clinical value of GM-CSF in the management of patients with cancer and chemotherapy related monocyte/macrophage dysfunction and presumed or documented microbial disease.

Keywords: macrophage; monocyte; GM-CSF; fungi; virus; bacteria.

INTRODUCTION

The effects of the pro-inflammatory, haematopoietic growth factor, GM-CSF, on macrophage function has been well recorded during the past decade [1–3]. The most important effects include monocyte/macrophage activation with the resulting increase in functions such as endocytosis, metabolism and cytokine secretion, haematopoietic progenitor cell proliferation, and antigen-presenting cell migration and stimulation. Each of these functions have a role in the response to microbial infection. The effect most utilized is that of progenitor cell proliferation and release from the bone marrow after chemotherapy-induced bone marrow dysfunction [4–6]. The primary effect when cytokines are used in this way is a reduction in the duration of neutropenia in these patients. By so doing, the long recognized association between neutropenia and infection is addressed [7], and the frequency of infection thereby reduced [4–6].

Although much less studied in randomized, clinical trials, the effects of GM-CSF on the function of monocyte/macrophages against microbial pathogens have been well described in *in vitro* models, animal experiments and in several uncontrolled trials in humans. The information is summarized here, the sections include a review of antifungal action of cytokine-stimulated macrophages, a summary of both stimulatory and in-

*To whom correspondence should be addressed at: Fax 0041616934002; e-mail 100767.711@compuserve.com

hibitory effects of cytokines on viral, bacterial or protozoal replication in macrophages, a review of the effects of GM-CSF on incorporation and action of antibiotics against intracellular microbes, and a summary of the clinical data which support the use of cytokines for macrophage activation. The special role of the macrophage as an antigen-presenting cell will be mentioned, however, the major activity in this regard is the use of GM-CSF as a vaccine adjuvant, which is summarized in other papers of this special issue.

ANTIFUNGAL ACTIONS OF CYTOKINE STIMULATED MACROPHAGES

GM-CSF affects the phagocytosis rate, protein and RNA synthesis, and antimicrobial killing functions of macrophages [2,8]. When these actions are studied in cell systems including monocytes/macrophages and various fungi, inhibition of fungal growth is observed. Smith et al. have demonstrated increased cytotoxicity of human monocytes from peripheral blood and macrophages isolated from the lamina propria from human intestine against Candida albicans after the cells were treated with GM-CSF [9,10]. In studies done with peripheral blood monocytes, Liehl et al. showed inhibition of Candida albicans colony formation when mononuclear cells were treated with GM-CSF, but not G-CSF [11]. Wang et al. [12] also showed increased function of peripheral blood monocytes from humans after stimulation in vitro with GM-CSF, IL-3 or M-CSF. Alveolar macrophages isolated from rats showed increased killing of Cryptococcus neoformans after treatment with GM-CSF [13]. Recently, hyphae of Aspergillus fumigatus were shown to be damaged by monocytes stimulated with GM-CSF [14]. These in vitro studies of monocytes or macrophages from different tissue sources have each confirmed the potential role stimulation of these cells plays in the inflammatory response to fungal infections.

There have been relatively few studies of the response of fungal infections in animal models. In one study in mice, the use of GM-CSF led to significantly enhanced survival during 15 days associated with clearing of Candida albicans from the liver and spleen, but not from the kidney [11]. Mayer et al. [15] studied GM-CSF in neutropenic mice and showed prolonged survival in comparison to controls after infection with Candida albicans, as well as Pseudomonas and Staphylococcus. Mandujano et al. gave GM-CSF to mice for 7 or 14 days beginning 4 weeks after lymphocyte depletion and infection with Pneumocystis carinii [16]. Histologic examination of lung tissue showed a significant decrease in the intensity of infection, a significant increase in TNF-alpha secretion by al-

veolar macrophages, and a reduced inflammation score (which did not reach statistical significance) in the GM-CSF treated animals compared to controls.

Based on these in vitro and animal model studies there is good reason to be optimistic that beneficial responses in humans during fungal infection would result from treatment with GM-CSF. One of the major questions is whether inflammation mechanisms are sufficiently impaired or misdirected in various clinical conditions to be benefited by the use of exogenous GM-CSF. It is clear that the first steps required in management of a patient with neutropenia and fungal infection are that they have been given sufficient antifungal antibiotics and that they have a sufficient number of circulating neutrophils. Since GM-CSF can influence both the number of neutrophils and the intracellular concentrations of antibiotics, it will be very difficult under in vivo conditions to determine when the antifungal effects are due to the cytokine action on cellular defense mechanisms and when the result is due to changes in circulating cell numbers or enhanced antibiotic action. These questions could be resolved by very large comparative trials with G-CSF, which can exert only secondary cytokine effects on macrophages, and by measuring cellular antibiotic levels during clinical trials. For practical and cost reasons, such studies are not likely to be done, and if they were they would be severely complicated by the heterogeneity of very ill patients with a diversity of underlying conditions leading to the fungal infection. It is anticipated that little new data will emerge in the next few years concerning this point, so decisions will have to be made on whether to consider use of GM-CSF in a patient with suspected fungal infection based on the above cited data, on the few clinical observations reported below, and on the information that will evolve from personal experience with cytokine use.

EFFECTS OF CYTOKINES ON BACTERIAL, PROTOZOAL OR VIRAL REPLICATION IN MACROPHAGES

It is now well recorded that cytokines, particularly IFN-gamma and GM-CSF, can inhibit the intracellular replication of bacteria or protozoa which rely on the intracellular microenvironment for their proliferation. Organisms such as Mycobacteria, Salmonella, Listeria, and Leishmania utilize macrophages in tissue as a part of their life cycle. Bermudez and his colleagues [17, 18] have studied the effects of cytokines on the replication of Mycobacterum avium in macrophages in vitro taken from intestine, as well as from liver and spleens

of mice. Using macrophages from the intestine they showed that GM-CSF, TNF-alpha and IFN-gamma significantly inhibited the intracellular growth of *Mycobacterium avium complex* (MAC), whereas M-CSF did not [17]. They also showed that the presence of TGF-beta or IL-10 supported proliferation of these organisms since antibodies against these cytokines led to inhibition of organism growth. Further, they have shown that the peripheral blood monocytes of patients treated with GM-CSF demonstrate enhanced microbicidal effects against MAC *ex vivo* compared to cells from untreated patients [18]. Denis has shown similar mycobacteriostatic effects of GM-CSF against *Mycobacterium tuberculosis* [19].

The protozoa, *Leishmania donovani*, is also inhibited in its proliferation in murine macrophages by GM-CSF [20]. In similar studies using *Leishmania amazonensis* both GM-CSF and IFN-gamma inhibited the growth of the organism in macrophages *in vitro*, and the combination of the two was found to be synergistic [21]. In other studies, TGF-beta was shown to promote replication of this organism *in vitro* [22]. Both IFN-gamma and GM-CSF have been found to be useful adjuncts in the management of visceral leishmaniasis [23,24].

These data all support the importance of the balance of cytokines in determining whether an organism is in a microenvironment conducive to growth or inhibition. When type IT-cell responses (IFN-gamma, GM-CSF, IL-12, TNF-alpha) are dominant growth is inhibited. When type II responses (IL-10, IL-4, TGF-beta) occur the organisms have enhanced proliferation. How best to apply exogenous cytokines during progressive disease in humans is, of course, the key question that still needs to be answered. It is likely that the degree of immunosuppression and its duration (for example, an AIDS patient has a prolonged alteration in immune status; a chemotherapy treated patient a shorter period depending on the underlying malignancy), will dictate the best cytokine to use and for how long. It is also likely that when one is using a cytokine such as GM-CSF for immune enhancement against an intracellular infection, the dose and frequency of administration will be quite different from when the drug is used to induce a rapid circulating neutrophil response.

The proliferation of virus within macrophages stimulated with cytokines has a completely different pattern. For example, Perno *et al.* demonstrated that HIV replicated better in cytokine activated peripheral blood monocytes than in monocytes cultured for a short period without cytokines. The cytokines that induced viral replication were those that are known to activate monocytes in culture, GM-CSF, IL-3 and M-CSF [25]. A similar enhancement of influenza virus in monocytes has been shown following GM-CSF

stimulation [26]. To demonstrate this effect the control cells must be in a 'resting' state, confirming that the replication is associated with cell activation. This is consistent with the fact that viral replication is dependent on the metabolic function of the cell and this is enhanced by cytokines for which there are receptors on the cell. The relevance of these observations to the use of cytokines in patients has been debated extensively with two tentative conclusions: (1) It is preferable to ensure that a patient is receiving appropriate antiviral drugs at the time of stimulation by cytokines to achieve macrophage activation. As is described below, this activation has a marked effect on the incorporation of antiviral and antibacterial antibiotics into the cell; an observation that encourages the use of cytokines and antimicrobials together. (2) The level of macrophage activation already present in tissue is likely to be well above that which was required in the control cells against which the virus promoting effect was compared *in vitro*. Thus, it has not been surprising that there is no evidence to support the danger of the use of cytokines in patients with unidentified and, therefore, untreated viral infections.

Two of the viruses of particular interest during the management of cancer patients are cytomegalovirus (CMV) and HIV. Both of these viruses can replicate in macrophages [27,28] and both are treated with antiviral drugs. Clinical benefit during use of GM-CSF in these patients has been observed [28–30].

EFFECTS OF GM-CSF ON THE INCORPORATION AND ACTION OF ANTIBIOTICS AGAINST MICROBES

Macrophages provide a protected environment for microbes against extracellular concentrations of antibiotics because of limitations of endocytosis, membrane permeability and cellular mechanisms which promote secretion of drugs [31]. Several different lines of evidence reveal that cytokine activation of macrophages allows higher intracellular and functionally more active concentrations of certain drugs. This concept has already been shown in malignant cells in which concentrations of Ara-C were higher in the GM-CSF treated cells than in the controls [32]. In the study in which GM-CSF was shown to permit increased HIV virus replication in monocytes, a detailed evaluation of the effects of anti-viral drugs was also done [24]. The viral load was reduced in cells treated with doses of AZT 10–100 times lower when used in combination with GM-CSF than in those cells treated with AZT alone. Similar effects were seen for other reverse transcriptase inhibitors, but not for other classes of antiviral drugs. In studies by

Bermudez et al. [33], the number of M. avium organisms in the liver and spleen of infected mice was determined after treatment of the animals for 14 days with the antibiotics Amikacin or Azithromycin, or the cytokine GM-CSF alone, or in combination. Only the combinations of GM-CSF with either Amikacin or Azrithromycin led to significant (50–100 fold) reductions in the number of tissue bacteria.

These data support the thesis that activated macrophages may allow higher intracellular drug concentrations. This means that those diseases such as HIV or CMV, in which cytokines are given to reverse drug-related neutropenia, may also benefit from the cellular effects of the cytokine on antibiotic efficacy. In addition, diseases such as multi-drug resistant tuberculosis and mycobacteriosis in AIDS patients may be better controlled by careful use of cytokines to potentiate antibiotic efficacy. Clinical trials exploring this use of cytokines have not yet been done. Similar studies with anti-cancer drugs are also needed.

EFFECTS OF GM-CSF ON OTHER INFLAMMATION ON IMMUNE CELLS

For completeness, it should be noted that GM-CSF has stimulatory effects on cells which are phagocytic and/or immunocompetent and which also contribute to the host response to infection. The activation by GM-CSF of neutrophils from patients with Pneumocystis carinii pneumonia to release cytotoxic metabolites has recently been reported [34], as has the activation of neutrophils against Candida albicans [35]. In addition, eosinophils are activated by GM-CSF to demonstrate increased cytotoxicity against shistosomula [36]. Recently the stimulatory effects of GM-CSF on NK cells and LAK cells has been explored [37,38]. These effects are reviewed elsewhere in this issue in regard to potential anti-tumor effects, but these cells also play roles in cytotoxicity against virus infected cells.

The most important cells in the immune response which are activated by GM-CSF are dendritic cells and Langerhans's cells [39,40]. Not only are the dendritic cells induced to proliferate in the bone marrow, their migration in tissue and activation to enhanced antigen presentation are all under the influence of GM-CSF [41]. This can have important effects during the evolution of an infectious process, particularly if these functions have been partially impaired by cancer or chemotherapy. In addition, these cells are critical in the development of anti-infective or anti-tumor vaccines. The potential for use of GM-CSF in this way is reviewed by others in this issue.

One must also be aware of the central role GM-

CSF plays in initiating a cytokine network by releasing numerous proinflammatory cytokines from the activated macrophages [1,2]. The entire cascade of infection response is mobilized. This response includes release of IL-6 which stimulates the secretion of acute phase proteins by the liver, of IL-1 which triggers the febrile and other inflammatory responses, of TNF-alpha which initiates many vascular effects, and release of G-CSF which further expands the neutrophil response. The negative side to this response must be kept in mind since these secondarily released cytokines can lead to respiratory distress syndrome (ARDS), vascular collapse, pleural, pericardial or joint inflammation. One must gauge carefully the extent of the required macrophage activation to achieve control of the infectious process, yet not exceed that level.

CLINICAL STUDIES RELEVANT TO MACROPHAGE ACTIVATION AGAINST MICROBIAL DISEASES

One of the first issues which needs to be settled is the question of whether macrophage activation by GM-CSF in the midst of sepsis would lead to catastrophic events such as shock or ARDS. This question was the focus of several clinical trials during the early development of GM-CSF. In at least three studies of the use of GM-CSF during sepsis none have shown a tendency toward aggravating or causing such reactions [42–44]. Another important issue is when to use GM-CSF in patients with a malignant clone, such as those with acute myelogenous leukemia (AML), likely to have receptors for and to be stimulated by exogenous GM-CSF. This concern has led to the recommendation that GM-CSF should not be used in patients with haematologic malignancies. More recent data have suggested that cytokines can safely be used in AML patients if the drug is used after documenting by bone marrow biopsy that successful reduction of the malignant clone after chemotherapy (usually more than 10 days after treatment) [45], and when the risk of ongoing sepsis is high and potentially fatal. Other than the previously noted potential increase in intracellular antibiotic levels, no drug–drug interactions with antibacterial or antifungal drugs have been determined which might affect the use of GM-CSF.

Bodey et al. [46] have presented the results of a small study of the use of GM-CSF with amphotericin B in the treatment of eight patients with fungal pneumonia or sepsis (five with Candidiasis, two with Aspergillus, one with a disseminated Trichophyton infection). Four of these patients were cured and two had a partial response. This was