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Original Paper

Use of Granulocyte-macrophage Colony Stimulating Factor (GM-CSF) in Prevention and Treatment of Fungal Infections

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INTRODUCTION

THE EFFECTS of the pro-inflammatory, haematopoietic granulocyte-macrophage-colony stimulating growth factor (GM-CSF), on myeloid cell proliferation and function have been summarised over the past decade [1]. Monocyte/macrophage activation leads to an increase in functions such as endocytosis, metabolism and cytokine secretion, haematopoietic progenitor cell proliferation, and antigen-presenting cell migration and stimulation. The effect of GM-CSF most utilised at present has been that of progenitor cell proliferation and release from the bone marrow after chemotherapy-induced bone marrow dysfunction [2]. The primary effect when cytokines are used in this way is a reduction in the duration of neutropenia in these patients. By doing so the recognised association between prolonged neutropenia and infection is addressed, and the frequency and severity of infection thereby reduced [2, 3].

The effects of GM-CSF on the function of monocytes/macrophages against microbial pathogens have been studied in *in vitro* models, animal experiments and in uncontrolled trials in humans. This paper includes a review of antifungal action of cytokine-stimulated macrophages, a summary of stimulatory and inhibitory effects of GM-CSF on microbial replication in macrophages, a review of the effects of GM-CSF on incorporation and action of antibiotics against intracellular microbes, on antigen-presenting cells, and a summary of the clinical data which supports the use of cytokines to prevent and treat fungal infections.

ANTIFUNGAL ACTIONS OF GM-CSF AS STUDIED *IN VITRO*

GM-CSF has effects on the phagocytosis rate, protein and RNA synthesis, and antimicrobial killing functions of macrophages [1]. When these actions are studied in cell systems including monocytes/macrophages and various fungi, inhibition of fungal growth is observed. Smith and colleagues 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, reviewed in [4]. In studies done with peripheral blood monocytes, Liehl and colleagues showed inhibition of *C. albicans* colony formation when mononuclear cells were treated with GM-CSF, but not G-CSF [4]. Wang and colleagues [4] also showed increased function of peripheral blood monocytes from humans against *C. albicans* 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 [4]. Hyphae of *Aspergillus fumigatus* were shown to be damaged by monocytes stimulated with GM-CSF [4]. These *in vitro* studies of monocytes or macrophages from different tissue sources have each confirmed the role stimulation of these cells may play in the inflammatory response to fungal infections.

ANIMAL STUDIES SHOWING ANTIFUNGAL ACTIVITY OF GM-CSF

Relatively few studies of the response of fungal infections in animal models have been done. In one study in mice, the use of GM-CSF led to significantly enhanced survival over 15 days associated with clearing of *C. albicans* from the liver and spleen, but not from the kidney [4]. Mayer and colleagues [4] studied GM-CSF in neutropenic mice and showed prolonged survival in comparison to controls after infection with *C. albicans*, as well as *Pseudomonas* and *Staphylococcus*. Mandujano and colleagues gave GM-CSF to mice for 7 or 14 days beginning 4 weeks after lymphocyte depletion and infection with *Pneumocystis carinii* [4]. Histological examination of lung tissue showed a significant decrease in the intensity of infection, a significant increase in TNF-alpha secretion by alveolar macrophages, and a reduced inflammation score in the GM-CSF treated animals compared to controls.

In addition, it is now well recorded that cytokines, particularly INF 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*, *Leishmania*, cytomeglovirus and HIV utilise macrophages in tissue as a part of their life-cycle. The effect of GM-CSF on these organisms has been reviewed recently elsewhere [4, 5].

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

[4]. It is clear from several different lines of evidence 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 GM-CSF treated cells than in controls [4]. In studies by Kemper and colleagues [5] 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 azithromycin 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. No studies have been done on the effects of cytokines on intracellular concentrations of amphotericin B or azoles, however, similar mechanisms are likely to apply.

EFFECTS OF GM-CSF ON CELLS OTHER THEN MACROPHAGES OR MONOCYTES

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, as has the activation of neutrophils against *C. albicans* [4]. In addition, eosinophils are activated by GM-CSF to demonstrate increased cytotoxicity. Recently the stimulatory effects of GM-CSF on NK cells and LAK cells has been explored [4]. These effects may also be relevant to the control of fungal infections.

The most important cells in the immune response which are activated by GM-CSF are dendritic cells and Langerhans's cells [6]. 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 [6]. 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-infection vaccines [7].

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 defence 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. For practical, ethical 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 which will evolve from personal experience with cytokine use.

CLINICAL STUDIES RELEVANT TO USE OF GM-CSF TO PREVENT OR TREAT FUNGAL DISEASES

One of the first issues which had to be settled during the development of the use of GM-CSF was the question of whether myeloid cell activation by GM-CSF in the midst of sepsis would lead to catastrophic events such as shock or acute respiratory distress syndrome (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 showed a tendency toward aggravating or causing such reactions [4]. 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 and colleagues [8] has presented the results of a small study of the use of GM-CSF with amphotericin B in the treatment of 8 patients with fungal pneumonia or sepsis (5 with *Candidiasis*, 2 with *Aspergillus*, 1 with a disseminated *Trichophyton* infection). 4 of these patients were cured and 2 had a partial response. This was considered by the authors an encouraging response, particularly when the high mortality in patients with disseminated fungal infection is considered. Other single patient reports of responses of an unusual and beneficial type have been made [4]. In a study of the use of GM-CSF after autologous bone marrow transplantation (ABMT) [9], streptococcal sepsis was the most common event. In addition to these cases, in the placebo treated group there were 8 other bacterial infections (5 *Staphylococcus*, 1 *Legionella*, 1 *Fusobacterium*, 1 *Corynebacterium*) and 4 cases of fungal sepsis (2 *Candida*, 2 *Aspergillus*), a total of 12 documented infections. In the GM-CSF treated group only 2 fungal infections (both *Candida sp.*) were documented ($P = 0.004$ for all infections). It was this difference in infection rate which led to registration in the U.S.A. of GM-CSF in patients after BMT. DeWitte and colleagues recorded a significant reduction in pneumonia after allogeneic BMT when patients were treated with GM-CSF (reviewed in [4]). Dierdorf and colleagues have recently published their complete review of patients treated on a compassionate case basis with GM-CSF for neutropenia and documented pneumonia [10]. Of 68 patients treated with GM-CSF, 21 had fungal pneumonia (7 *Candida*, 8 *Aspergillus*, 4 *Pneumocystis*, 1 *Torulopsis*, 1 unidentified fungus). 23 patients had bacterial pneumonia, 2 had viral pneumonia, 1 had a protozoal infection and 21 had pneumonia of unidentified cause. In those with a significant haematological response to the cytokine, 83% survived; without a response only 14% survived. The cause of death was respiratory failure or sepsis in 2 leucocyte responders, but in 12 non-responders the cause of death was the lung infection in 7, respiratory failure in 3, and sepsis in 2. Thus, reversal of neutropenia is a major factor in survival from neutropenic pneumonia. These studies supported a correlation between a haematological response and successful

outcome of the infection. These observations have led to the recommendation that cytokines be used in patients with severe neutropenic pneumonia and/or sepsis, and that consideration be given to the use of GM-CSF specifically because of the potential advantage of macrophage activation.

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