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The Effects of rhGM-CSF on Macrophage Function

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Markers of monocyte/macrophage activity are numerous, and offer an insight on the potential effects of recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF). These include anti-microbial effects, anti-tumour effects, inflammation and wound healing enhancing effects, antigen presenting effects and effects on the haematopoietic microenvironment. The actions of rhGM-CSF on monocytes/macrophages relevant to enhanced antimicrobial activity include increased phagocytosis, increased oxidative metabolism, increased numbers of Fc receptors, release of chemotactic factors and stimulation of mechanisms for killing intracellular viruses, fungi, bacteria and protozoa. Markers of macrophage anti-tumour activities can be divided into two types: those associated with antitumour antibodies, antibody-dependent cell cytotoxicity (ADCC) and those associated with synthesis and secretion of cytolytic substances by activated macrophages. In addition, rhGM-CSF has actions on fibroblasts and kerotinocytes consistent with an important role in wound healing. Moreover, the clinical use of rhGM-CSF to enhance antibody responses in conditions of inadequate immunisation is currently being investigated. Furthermore, rhGM-CSF appears to have a role in stimulating haemopoietic recovery following cytotoxic chemotherapy or radiation damage to bone marrow tissue.

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INTRODUCTION

AS THE MEDICAL COMMUNITY begins the pragmatic process of reversing 'neutropenic states' with cytokines, one must examine with increased intensity the cascade or network of effects which are being initiated by this therapy. Recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) has been shown to induce haematopoietic progenitor cells in the myeloid and erythroid lineages to proliferate and to stimulate mature monocytes/macrophages and neutrophils. This review focuses on the effects of rhGM-CSF on monocyte/macrophages and the resulting potential secondary events which occur as the network of immunological, haematopoietic and inflammatory interactions are readjusted.

The primary events associated with the interactions between rhGM-CSF and monocytes include formation of a GM-CSF receptor complex on the cell surface, increased phosphorylation of proteins on serine and tyrosine residues, expression of (or changes in) cell surface receptor and adhesion molecules, 'activation' (as measured by increased superoxide anion generation, phagocytosis, migration inhibition), and synthesis and/or release of inflammatory mediators and cytokines such as interleukin-1 (IL-1), tumour necrosis factor (TNF) and prostaglandins. These events lead to accumulation of cells at sites of inflammation, anti-microbial and anti-tumour actions and regulation of continued haematopoietic events. This network of interactive processes, often referred to as 'host defence', may lead to inappropriate effects on the host (i.e. fever, myalgia, hypotension) if there is excessive dysregulation or to the resolution of potentially serious disease processes (i.e. infection or malignancy) if the network of interactions is modulated correctly.

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The markers of monocyte/macrophage activity which are observed during administration of rhGM-CSF will be reviewed according to potential anti-microbial effects, anti-tumour effects, inflammation and wound healing enhancing effects, antigen presenting effects and effects on the haematopoietic microenvironment.

ANTI-MICROBIAL EFFECTS

In addition to increasing the numbers of neutrophils, eosinophils and monocytes by its effect on the kinetics of bone marrow haematopoietic cells [1], rhGM-CSF alters the function of monocytes and macrophages [2] with consequential anti-microbial actions. The actions of rhGM-CSF on monocytes/macrophages which may be relevant to enhanced anti-microbial activity include increased phagocytosis, increased oxidative metabolism, increased numbers of Fc receptors, release of chemotactic factors and stimulation of mechanisms for killing intracellular viruses, fungi, bacterial and protozoa [3]. One example in patients of enhanced phagocytosis was the rapid fall in platelets in a patient with auto-immune thrombocytopenia; most likely due to enhanced removal of partially damaged platelets [4]. Coleman *et al.* [5] also showed increased phagocytosis by tissue macrophages, and Heidenreich *et al.* [6] showed both increased phagocytosis and pinocytosis by macrophages. The best described model systems of anti-microbial effects include the activation of macrophages to inhibit *Candida albicans* [7, 8] Trypanosoma [9] and Leishmania [10, 11].

In the study of activation of human monocytes to kill *Candida albicans* by Smith *et al.* [7], monocytes were purified from the blood of healthy donors and then mixed *in vitro* with [^{3}H]leucine-labelled *Candida albicans*. The cytotoxicity of increasing numbers of monocytes was shown by a dose-dependent release of [^{3}H]leucine from the damaged *Candida*

albicans. The cytotoxic effect was shown to be due to release of superoxide dismutase and catalase.

The addition of rhGM-CSF to the cultures (500 μ/ml) resulted in a significant increase in monocyte cytotoxicity toward the *Candida albicans* at each dose of monocytes tested. This correlated with stimulation of oxygen-reactive intermediates by the monocytes. Monocytes have also been shown to increase destruction of ingested Leishmania *in vitro* after addition of rhGM-CSF to the culture system [10, 11]. This is similar to effects on activation of monocytes of gamma interferon which were demonstrated previously.

At present, these potential host defence activities have only been realised as clinical benefits in the resolution of infection in a few patients where rhGM-CSF was requested for 'compassionate use' [12, 13]. It has also been used in combination with amphotericin B in systemic fungal infections in neutropenic patients [14]. 8 neutropenic patients with disseminated mycoses (6 who had failed amphotericin B), received rhGM-CSF plus amphotericin B. 5 patients had candidiasis, 2 had aspergillosis and 1 had trichosporinosis. 4 of the 5 candidiasis patients and the patient with trichosporinosis showed resolution of their infections and neutropenia, and 1 of the aspergillosis patients showed a partial response. In light of the *in vitro* data on the effects of GM-CSF stimulated monocytes on *Candida albicans,* this clinical observation is particularly relevant. In patients with visceral leishmaniasis reversal of neutropenia and rapid control of the leishmanial infection has been seen (Badero R, personal communication).

The role of rhGM-CSF in various infectious diseases has been reviewed recently [15]. At present, the addition of monocyte/macrophage activation to the restoration of neutrophil function induced by rhGM-CSF must be looked upon as a potential benefit in certain primarily obligate intracellular infections; however, this remains to be clearly documented. The other interesting aspect of the use of rhGM-CSF in infectious diseases derives from the demonstration of the importance of TNF as an anti-microbial agent, and that rhGM-CSF stimulates TNF synthesis and release [16]. A recently published example of TNF-related anti-microbial mechanism is that of tuberculosis [17]. The potential for cytokines to increase this factor in macrophages at sites of inflammation may allow the recognised toxicity of the systemic use of TNF to be bypassed, thus achieving the desired goal of control of the infectious process. This is an example of the requirement for careful regulation of cytokine therapy, since TNF may be a significant factor in the unwanted cascade of events recognised as the septic shock syndrome [18].

ANTI-TUMOUR EFFECTS

The markers of macrophage anti-tumour activities are divided into two types; those associated with anti-tumour antibodies, antibody-dependent cell cytotoxicity (ADCC), and those most probably associated with synthesis and secretion of cytolytic substances by activated macrophages. ADCC can be demonstrated after rhGM-CSF stimulation of all the myeloid cells, but it is particularly potent after activation of macrophages. The tumour cell lysis is triggered by the presence of IgG antibodies (not IgA or IgM) and complement is not involved in the cytolytic process.

Cytolytic activity has been shown to be expressed against tumour cell lines (melanoma cells) in culture. The cell cytolysis occurred at the same concentrations of rhGM-CSF at which myeloid cell proliferation occurs [19]. Cell lysis of leukaemic cells has been shown after stimulation of neutrophils with rhGM-CSF [20]. Although the use of rhGM-CSF in cell cycling is still controversial [21,22], one has the potential for malignant cell destruction by these neutrophil or monocyte cytolytic mechanisms. When macrophages are stimulated to synthesise and secrete cytolytic substances tumour lysis is also observed. A major part of this activity has been attributed to the secretion of TNF, since antibodies against this molecule block the process [23]. Of particular note, the activity of rhGM-CSF is a 'priming' function since a second signal was required (endotoxin) in order to achieve full cytotoxicity. This occurs via the role of rhGM-CSF to induce the mRNA of TNF in monocytes, the endotoxin then induces release of the TNF protein. This priming activity has been reported for a number of the neutrophil and monocyte functions increased by rhGM-CSF [2]. Since not all of the cytotoxic activity was blocked by TNF antibodies, it remains likely that other mechanisms of monocyte cytotoxicity exist as well [23].

Several studies have shown that mononuclear cells from patients treated with cytotoxic chemotherapy and rhGM-CSF have the potential for exhibiting tumorlytic properties [24-26]. In one study mononuclear cells from rhGM-CSF-treated patients required secondary stimulation *in vitro* with lipopolysaccaride of muramyldipeptide [24]. In another study, after cytotoxic chemotherapy plus rhGM-CSF, peripheral blood cells were more effective in a tumour lysis model than cells evaluated before treatment [25]. An association between GM-CSF treatment and increased secretion of TNF has also been shown [26]. Therefore, the potential for clinical benefits, through enhancing anti-tumour immunological events while reversing the neutropenic state, appear high. Measuring this benefit in actual clinical settings is more difficult since measurements of tumour regression at the cellular level or in minimal residual disease states are not standardised, and the observation of remission and relapse rates after cytotoxic chemotherapy and cytokines will require long-term treatment and several years of follow-up observation. However, such approaches are now being used to determine the anti-tumour role for rhGM-CSF. One study which has just been completed in Germany [27] explored the question of whether the use of rhGM-CSF in patients with non-Hodgkin's lymphoma could increase the number of remissions recorded after the third cycle of chemotherapy with COP-BLAM at the time of the first restaging. The co-operative lymphoma group has published that the remission rate at this time (i.e. early remission) correlates with better prolonged survival [28]. Since an effect of rhGM-CSF during three cycles on remission rate would be difficult to explain simply by allowing chemotherapy dose adherence, a potential role in enhancing host anti-tumour mechanisms emerges. An increase in early remissions was demonstrated, however, the correlation with prolonged survival requires several years of follow-up evaluation. Other studies examining the potential for rhGM-CSF to reduce the number of micrometastasis or to enhance inflammatory processes around primary tumours are in progress.

INFLAMMATION AND WOUND HEALING EFFECTS

Macrophages play an important role in the process of inflammation and the secondary signals they release may contribute to the process of wound healing. Until now most attention has focused on those negative features of the inflammatory response which are induced by cytokines. Macrophage secretion of IL-1, TNF and prostaglandins is probably responsible for the signs and symptoms such as fever and myalgias recorded in patients receiving rhGM-CSF. In addition, enhancement of levels of IL-6, acute phase proteins as well as moderate increases in TNF, were recorded in a patient with rheumatoid arthritis receiving rhGM-CSF for Felty's syndrome. The increase in those molecules coincided with a flare of the arthritic signs and symptoms [29]. This patient's illness suggests the physiological effects of rhGM-CSF, now well recorded in *in vitro* systems and in animal models, also occur in patients. However, the positive features of these molecules, which act to induce the inflammatory process, may be of greater importance. The role of the secondary cytokines in anti-microbial and anti-tumour responses has already been mentioned. In addition, it has recently been reported that prostaglandin synthesis by macrophages may down-regulate TNF release from these cells, thus serving an autoregulatory function [6]. Similarly, down-regulation of the IL-2 receptor has been described for rhGM-CSF [30]. An important effect on the formation of myofibroblasts has also been reported, although this is not a direct effect of rhGM-CSF since other cells are required in the tissue [31]. Macrophages or endothelial cells are prime candidates for the origin of the secondary messages, thus implicating cytokines and these cells in the wound healing process [32, 33]. A role for cytokines to promote more rapid resolution of skin and subcutaneous ulcers, as well as post-trauma and post-surgical settings, remains a potential area for clinical exploitation.

ANTIGEN PRESENTING EFFECTS

The important role for macrophages, dendritic cells and Langerhan's cells in antigen processing and presentation makes this process a critical measure of the enhancement of host defence and rhGM-CSF has been identified as particularly potent in this regard [34-37]. During clinical trials negative effects possibly associated with this mechanism, such as enhanced allergic responses, have been sought. The only report to date that could indicate that stimulation of antigen presentation occurs in humans has been the evidence of precipitation of thyroiditis and associated transient hypothyroidism in 2 patients with previously documented anti-thyroid antibodies [38]. Clinical use of cytokines to enhance antibody responses in conditions of inadequate immunisation is now being investigated. For example, in patients with poor responses to immunisation, such as some patients receiving hepatitis B vaccine, the use of rhGM-CSF to enhance responsiveness could provide important benefits.

EFFECTS ON THE HAEMATOPOIETIC MICROENVIRONMENT

Attention has recently focused on the role of monocytes/macrophages as vital cells in the restoration of progenitor cell activity after bone marrow damage. Bone marrow function is dependent on proper function of stroma in the microenvironment of the marrow. Monocytes contribute key factors to allow rhGM-CSF to stimulate proliferation of myeloid progenitor cells [39, 40]. The model of a complex cascade of events which involve macrophages, endothelial cells and fibroblasts as well as IL-1, TNF and the *kit* ligand has recently been presented [41]. Stimulation of this process by rhGM-CSF could be one of the more important roles for cytokine therapy after cytotoxic chemotherapy or radiation damage to bone marrow tissue.

In summary, markers of macrophage activity are numerous. Following cytokine stimulation one can anticipate clinical benefit as well as potential hazards. Careful use of these cytokines will likely provide many advantages in control of infections and malignancies, better wound healing, improved antibody responses and haematopoietic reconstitution.

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