# CYTOKINES IN THE TREATMENT OF INFECTION

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## Introduction

It is now well accepted that host defense mechanisms, both cellular and humoral, are regulated by a family of cytokines, interleukins and colony-stimulating factors (CSFs). Control of antibody production and humoral immunity including antigen processing, T-cell and B-cell responses and the proliferation of plasma cells are under the control of Interleukin-2 (IL-2) through IL-17. Naturally occurring diseases and gene "knockout" experiments in mice clearly indicate the essential roles for some of these factors and their receptors in maintaining humoral immunity [1]. Similarly, cellular immunity, largely mediated through T-cells, is under the control of the interleukins, and mediated through specific cell surface receptors for these factors. Although our understanding of interleukins in humoral and cellular host defense mechanisms is advancing rapidly, clinical applications of interleukins to modify *in vivo* lymphocyte mediated responses is still largely at the investigative stage. Interesting potential clinical applications include the use of IL-2 to treat HIV infected individuals [2], the use of autologous IL-2 expanded cells for prevention of cytomegalovirus infections in bone marrow transplant patients [3], and potential applications of IL-10 to modify the suppressor/helper functions of lymphocytes in a variety of inflammatory and infectious diseases [4]. The interferons, well characterized cytokines produced in response to many viral infections, can also be used pharmacologically to modify the course of hepatitis B, hepatitis C, and some other infections as outlined in recent reviews [5,6].

The supply, deployment and function of the phagocytic cells of the host defense system is also under the control of cytokines [7]. The occurrence and outcome for most bacterial, fungal and parasitic infections depends upon the availability and function of these cells, i.e., neutrophils, eosinophils, monocytes and the fixed mononuclear phagocytes found in the spleen, liver, lungs and other tissues. These cells are critical for prevention of infection from micro-organisms found normally on body surfaces, as well as for protection from more invasive and pathogenic organisms. The cytokines regulating phagocyte development and function now have several practical applications for the prevention and treatment of infections.

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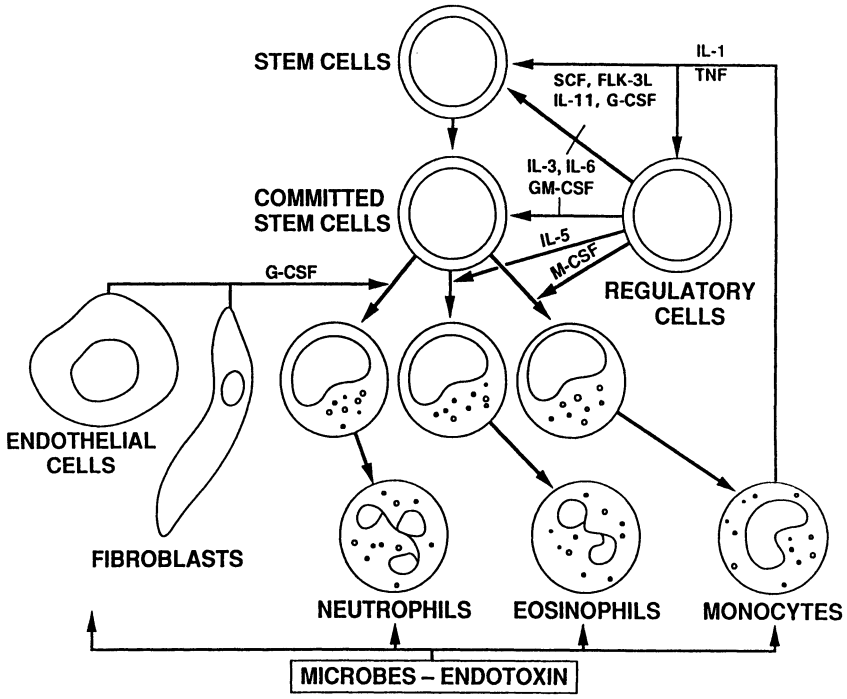


*Figure 1.* Model for the regulation of neutrophil, eosinophil and monocyte production in infectious diseases. At sites of infection, monocytes and tissue macrophages produce long range factors such as interleukin-1 (IL-1) and tumour necrosis factor (TNF). These factors activate haematopoietic stem cells and other regulatory cells of the marrow (T lymphocytes and stromal cells) to produce the factors stimulating the early phases of haematopoiesis. Microbes and endotoxin also increase the production of G-CSF, M-CSF and IL-6, which increase production and blood levels of neutrophils, monocytes and esosinophils respectively.

## Cytokine regulation of phagocyte production and function

All types of phagocytes derive from the common haematopoietic stem cells and production of phagocytes is governed by haematopoietic growth factors, some of which are called colony-stimulating factors. From the discovery of these factors in the 1960s to the present, much interest has focused on *in vivo* and *in vitro* studies of how these factors are affected by inflammation, infection and exposure of cells or whole organisms to bacterial lipopolysaccharide (LPS) or endotoxin [8,9]. For instance, the discovery of the key regulatory cytokine for neutrophil production, granulocyte colony-stimulating factor (G-CSF), came from experiments in mice

showing that this factor, in contrast to granulocyte macrophage colony-stimulating factor (GM-CSF), was found in high concentrations in serum or plasma from mice soon after injection of LPS [10,11].

Our current general understanding of the role of cytokines and colony-stimulating factors in influencing phagocyte production is illustrated in Figure 1. There are several key features of this schema:

1. Production of mature cells is governed by "early-acting" factors and "late-acting"" factors. The factors affecting the early phases of haematopoiesis, e.g., IL-3, IL-11, SCF and FLK-3 ligand, influence production of erythrocytes and platelets, as well as leukocytes. Receptors for these cytokines are not present on mature cells. In this schema, tumour necrosis factor (TNF) and IL-1 may serve as long range regulators interacting with these factors in stimulating early haematopoietic cell proliferation in inflammatory conditions [12].

2. Some cytokines have both early and late effects, such as G-CSF and GM-CSF. Both immature and mature cells of the neutrophilic series have receptors for both of these cytokines. Monocytes and eosinophils have GM-CSF receptors [13].

3. The late-acting factors, G-CSF, M-CSF and IL-5 specifically govern the late phases of neutrophil, monocyte and eosinophil production respectively. Levels of G-CSF and M-CSF, as well as IL-6, are increased with inflammation and decreased as inflammation resolves [14,15].

4. Maintenance of the integrity of the phagocytic system undoubtedly involves the cooperation of all of these cytokines but redundancies may exist both for protective and for evolutionary reasons [16].

## Cytokine responses in infections

Acute bacterial infections or injection of LPS induces the expression of several well characterized cytokines, including tumour necrosis factor, IL-1 and IL-6 [17-19]. The time course for these acute phase reactants is well described. Parallel studies of colony-stimulating factor responses with acute infection and after LPS administration have shown that G-CSF and M-CSF, but not GM-CSF, increase with infections or after LPS administration, both in animals and in man [15,20]. In comparison to baseline levels, the degree of increase in G-CSF levels for patients in septic shock and after high dose LPS administration is quite large [21,22]. In clinical studies the increases in G-CSF correlate with the severity of infection, the presence of bacteremia, specifically Gram-negative bacteremia, and reductions in renal function [15]. After acute infection, G-CSF fall back to normal within a few days [23].

Because serum or plasma levels of the cytokines governing haematopoiesis are very low or undetectable with current assay systems, it has been difficult to identify conditions which cause deficiencies of the factors. Gene deletion or "knockout" experiments, however, clearly show that deficiencies of G-CSF result in neutropenia with increased susceptibility to infection [24]. A similar G-CSF-deficiency state has been produced in dogs through repeated administration of recombinant human G-CSF, with resulting production of cross-reacting antibodies to canine G-

CSF [25]. A neutropenic state can also be induced by infusion of anti-G-CSF antibodies [25]. It is not yet known, however, if any clinical conditions associated with neutropenia are due to a deficiency of G-CSF or any other haematopoietic growth factor. Interestingly, animals made deficient in GM-CSF through embryonal stem cell disruption are not neutropenic; they have proteinaceous accumulations in the lung, resembling the disease alveolar proteinosis [26].

## Studies of the effects of CSF administration in normal human subiects

The physiological effects of CSF administration to normal subjects has provided an important basis for trials utilizing these factors for the treatment of infections in man. Normal volunteers given G-CSF (30 μg or 300 μg/day) daily for l4 days had increases in neutrophil production approximately seven-fold [27]. Marrow studies showed an increase in early precursor cell proliferation and hastening of the transit of cells through the marrow post-mitotic pool into the blood [27]. In addition, G-CSF affects primary granule formation, increases the leukocyte alkaline phosphatase scores, primes cells for an enhanced metabolic burst as reflected by chemiluminescence assays, enhances superoxide production and enhances the killing of bacteria and fungi [28]. Surface expression of CD-14 and CD-64 are also increased, indicating that the cells produced in response to G-CSF may have enhanced interactions with foreign cells and bacterial products [28-31]. When G-CSF is administered to healthy volunteers *in vivo* it initially induces increased expression of CD-llbl8, but longer administration (5 days) causes down-regulation of this expression. There is also increased and then decreased expression of LAM-1 (leukocyte adhesion molecule-1, or L-selectin) [28]. G-CSF prolongs the life of neutrophils both *in vitro* and *in vivo* through suppression of the process of apoptosis, or programmed cell death [27,32]. Thus, G-CSF causes neutrophilia, both in pharmacologically and naturally-occurring inflammatory states. In addition, this cytokine mobilizes neutrophils prepared to focus their armamentarium of antibacterial substances at sites of infection.

Similar studies of GM-CSF show that it also stimulates neutrophil production and increases marrow and blood eosinophils and monocytes [33,34, Dale et al, unpublished observations]. In a dose of 250 μg/m2 given for 14 days in a similar protocol to that used for the above studies with G-CSF, it was observed to be somewhat less potent in inducing neutrophilia than G-CSF.

Other effects are similar, however, in priming neutrophils for enhanced metabolic response to exposure to bacteria and foreign particles. In normal subjects, as well as most clinical trials, GM-CSF has many more associated adverse effects than G-CSF.

## Preclinical applications of cytokines for the treatment of infections

### Clinical Applications

Beginning in the late 1980s several investigators began using haematopoietic cytokines for the treatment of animals with experimental infections. These pre-

clinical studies have involved mice, rats, guinea pigs, rabbits, dogs and primates, and include animals with normal haematopoiesis as well as those with drug or radiation-induced myelosuppression [28]. Models studied include sepsis, both in neonates [35-37] and adults [38-40], pneumonia [41,42], burns [43,44], and intramuscular infections [45]. The largest number of studies have involved the use of G-CSF because of its availability and its potent effects on neutrophil formation. Several general principals have been learned from these experiments. These are:

1. The neutrophilia which develops in most experimental infections is not the maximal response. G-CSF administration to haematologically intact animals with infections will generally further elevate blood neutrophils.

2. The timing of treatment is important. The most favorable results are observed when treatment precedes infection or follows soon after infection. With G-CSF, GM-CSF or other haematopoietic cytokines, the blood neutrophil count rises for several days after beginning the drug to reach a new plateau level. The effect of these cytokines on the quality, as well as the quantity of cells produced undoubtedly also affects outcome.

3. Most experiments involve the use of cytokines with antibiotics, but favorable effects also have been observed in animals treated with G-CSF without concomitant antibiotic administration. It is not yet clear if antibiotics which are concentrated intracellularly, such as rifampin, chloramphenicol, azithromycin, etc., are more effective than drugs such as the penicillins and cephalosporins, which are not actively taken up by phagocytes.

4. Increasing the body' s production of neutrophils and the supply of cells available to migrate to a site of infection increases the tissue inflammatory response. In addition, some studies have shown that the clearance of organisms from tissues is enhanced through the use of G-CSF to increase the neutrophil supply.

5. In studies with G-CSF the induction of neutrophilia and an increase in the neutrophil response is not associated with tissue injury. For example, in animals with pneumonia, G-CSF treatment did not directly cause lung injury or the acute respiratory distress syndrome (ARDS) [46-48]. Thus, preclinical trials suggested that the use of G-CSF for treatment of infections would be both safe and efficacious.

## Clinical trials

Most of our information on the use of cytokines for the treatment of infections comes from studies of G-CSF and GM-CSF. In patients with neutropenia due to cancer chemotherapy or bone marrow transplantation, these cytokines can accelerate neutrophil recovery, minimizing the duration of severe neutropenia, and reduce the occurrence and severity of infections [49]. In this regard, the results of studies are not uniform, in part related to the relative low frequency of documented infections in some of these studies. In patients with congenital, cyclic or idiopathic neutropenia, clinical trials have shown that G-CSF increases blood neutrophils and reduces the occurrence of fever, oropharyngeal inflammation, and infections [50]. To attain these benefits, daily or alternate day therapy is required. Long term treat-

ment of these patients with G-CSF has provided continuing benefit without loss of efficacy in almost all patients. Antibody formation to G-CSF or changes in marrow responsiveness has generally not occurred. In a few patients with congenital neutropenia, however, an underlying propensity for conversion to myelodysplasia and acute myeloid leukemia has been recognized and the frequency and significance of these findings is under continued investigation. GM-CSF has been investigated as a long term treatment in a few patients, but is generally not used because of its side effects. No other haematopoietic cytokines have thus far been proven to be useful for the treatment of acute or chronic neutropenia.

Another important application of the haematopoietic cytokines is for the treatment of neutropenia associated with HIV infection [51]. The first major clinical trial of GM-CSF was in men with HIV infection. This initial trial and numerous subsequent trials have demonstrated that HIV positive patients with neutropenia due to viral infection or as a complication of anti-viral chemotherapy or cancer chemotherapy will respond to GM-CSF or G-CSF to increase their blood neutrophil counts. This responsiveness is retained even in the late stages of HIV infection. Accumulating data suggests that G-CSF and GM-CSF affects neutrophils in HIV infected individuals by several mechanisms: enhanced production, reduced cell loss through apoptosis and improvement in neutrophil function. The use of G-CSF or GM-CSF for neutropenia in HIV positive patients remains controversial, however, because of the lack of well designed control trials establishing clinical benefit. Some recent evidence suggests that G-CSF may reduce the occurrence of bacterial infections in the most neutropenic of these patients [52]. G-CSF is used more widely than GM-CSF to maintain neutrophil levels so that HIV positive individuals can receive anti-viral chemotherapy.

**CSF treatment of infections of non-neutropenic infections**

Based upon experience with G-CSF administration for the prevention and treatment of infections in neutropenic patients and the preclinical studies in animals, the first major treatment trials of G-CSF for infections in non-neutropenic patients were begun in individuals with community-acquired pneumonia. The initial studies established that even with elevation of the blood neutrophil counts to 50 to $100 \times 10^9$/L there was no apparent adverse effect on lung function or blood oxygenation [53]. Subsequently a large randomized trial was conducted in the U.S. and Australia, testing whether G-CSF is a useful adjunct to conventional antibiotic therapy or severely ill patients with community-acquired pneumonia [54]. Patients enrolled in this trial tended to be elderly patients with other health problems predisposing them to a more prolonged hospital course, need for intravenous antibiotics and greater likelihood of secondary complications. The patients were not sufficiently ill to be classified as having septic shock. In this trial the primary endpoint was the time to resolution of fever, tachypnea, hypoxia and pneumonia by x-ray examination. Although the time to this endpoint was not decreased for the study population as a whole, the more severely ill patients in the trial, particularly those with multi-lobar pneumonia, appeared to benefit from G-CSF treatment. In

addition, the study also showed that G-CSF treatment reduced the rate of multi-organ failure, acute respiratory distress syndrome, and development of septic shock. Resolution of pulmonary infiltrates by x-ray was also more rapid in the G-CSF treated group. Comparing the G-CSF treated patients to the placebo groups, several other interesting and important observations were made. The median of three-fold increase in blood neutrophils was not associated with the worsening of pulmonary status. Neutrophil infiltration of other tissues as could be determined by clinical evaluation was not observed and there were very few side effects of this therapy. Based upon these findings, particularly the apparent benefit for the sicker individuals, further studies of G-CSF treatment in pneumonia are under way. To date there have been limited trials of GM-CSF for the treatment of infections in man. There is some promising data for adjunctive use of GM-CSF in the treatment of leishmaniasis [55-57]. Thus far, no clear conclusions have come from these trials.

## Conclusion

Normal host defense mechanisms are governed by the production of cytokines, interleukins, and the colony-stimulating factors. Recent research has delineated many important mechanisms and potential applications for cytokines for enhancing host defenses to prevent or treat infection. One of the most promising applications is in the use of the cytokines which influence haematopoiesis and the regulation and deployment of phagocytes. Among these cytokines, G-CSF is the most promising candidate, because of its unique role in regulating and maintaining the blood neutrophil count. Physiological studies, preclinical trials and clinical studies in both neutropenic and non-neutropenic patients suggest that G-CSF is useful for prevention of infections in several circumstances and may prove useful as an adjunct for antibiotics, particularly for patients with severe infections. Clinical trials are now underway testing this hypothesis.

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