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The Role of GM-CSF in Infection

Summary: GM-CSF is a hemopoietic growth factor with substantial effects on the proliferation of neutrophils, eosinophils and monocytes/macrophages. Its physiologic role in infection is still poorly understood. The gene for GM-CSF is constitutively transcribed in cells substantial for antiinfectious response. Various cells are activated and induced by TNF and IL-1 to synthesize GM-CSF. No systemic GM-CSF levels can be detected in patients with infection. It is likely that GM-CSF plays its physiological role in the immediate vicinity of the cells by which it is secreted. GM-CSF functionally activates neutrophils, monocytes/macrophages and eosinophils. It may augment T-cell proliferation and function. GM-CSF is effective in mice infected with *Staphylococcus aureus* or *Salmonella typhimurium.* Its effect in infectious disease in man should be explored.

Zusammenfassung: *GM-CSF als Mediator bei Infektionen.* GM-CSF ist ein hämatopoetischer Wachstumsfaktor mit proliferationsfördernder Wirkung auf die neutrophile, eosinophile und monozytäre Reihe. Seine physiologische Rolle bei Infektionen ist bisher nur sehr unvollständig bekannt. Das Gen für GM-CSF wird konstitutiv in einer Reihe Zellen mit substantieller Bedeutung für die Infektionsabwehr exprimiert. Verschiedene Zellen werden durch TNF und IL-1 als zentrale Mediatoren entzündlicher Reaktion aktiviert und zur GM-CSF-Expression induziert. Bei Patienten mit Infektionen können keine systemischen GM-CSF Spiegel nachgewiesen werden. Es ist wahrscheinlich, daß GM-CSF seine physiologische Rolle in der unmittelbaren Umgebung der Zellen spielt, durch die es sezerniert wird. GM-CSF aktiviert Neutrophile, Monozyten/Makrophagen und Eosinophile funktionell. Es könnte eine zusätzliche Rolle bei der T-Zell-Proliferation und -Funktion spielen. *In Vivo* ist GM-CSF bei Mäusen bei Infektionen mit *Staphylococcus aureus* oder *Salmonella typhimurium* aktiv. Der Effekt vom GM-CSF sollte bei Infektionen beim Menschen untersucht werden.

Introduction

Many factors contribute to the antiinfectious barrier of the human body. Physical activity, performance, the integrity of the skin and mucosa, and the balance of benign microbial colonization are part of the global antiinfectious shield. Specific antiifectious defense is provided by the immune system. The interaction of its elements and their regulation is still poorly understood.

Some dramatic new insights haven been revealed with the discovery of a growing number of cytokines and their receptors. Powerful tools for highly effective and specific manipulation of immune response are emerging. Against this background we will review the physiologic and the potential therapeutic role of granulocyte-macrophage colony-stimulating factor (GM-CSF) in infection.

Discovery, Biochemical and Biological Characteristics

Systems for the detection of clonal growth of bone marrow cells *in vitro* were developed more than 25 years ago [1–3]. On this basis and with advancing biochemical and molecular biological methods a group of glycopeptides stimulating hematopoietic cells has been discovered.

GM-CSF was first purified from mouse lung-conditioned medium in 1977 [4]. However, spurious quantities of material did not allow sequencing and cloning. GM-CSF was purified in 1984 [5] from the supernatant of a HTLV I infected T-cell line and subsequently cloned [6,7]. The gene for GM-CSF has approximately 2.5 kilobase pairs. It is located on the long arm of chromosome 5 in the immediate vicinity of the gene for IL-3 [8]. The GM-CSF gene has four exons and three introns encoding a protein of 144 amino acids. Biologically active GM-CSF is generated after cleavage of 17 NH_2-terminal amino acids. It can be expressed in yeast, *Escherichia coli,* or CHO cells. The activity of the material decreases with glycosylation. Optimal expression is attained in yeast with substitution at position 23 with leucin for arginin [9]. Recombinant human GM-CSF has a molecular weight of 14 to 30 kD [10]. Its properties are similar to those of the natural material. An overview on the biological activities of GM-CSF is outlined in Table 1.

GM-CFS supports the clonal growth of neutrophils, eosinophils, and monocytes/macrophages [11]. It cannot support alone the maturation of neutrophil colonies from bone marrow under serum-free conditions [12]. GM-CSF has an *in vitro* effect on megakaryocytic precursors [13] together with other factors such as erythropoietin or IL-3 [14]. GM-CSF-dependent colony formation is inhibited by IFN alpha and gamma [15].

In long-term bone marrow cultures the effect of GM-CSF and G-CSF on primitive clonogenic precursors has been studied by co-cultivation of light density normal human

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Table 1: Biologic activity of GM-CSF (modified [8]).

In vitro
Stimulation of proliferation
Hemopoietic progenitors
Leukemic cell lines and AML cells
BFU-E
Monocytes/macrophages
Lymphocytes?
Endothelial cells
Enhancement of function
Neutrophils
Survival and protein synthesis
Inhibition of migration
Oxidative metabolism
Degranulation
Cytokine secretion
IgA-mediated phagocytosis
Uptake and destruction of parasites, bacteria
Cytotoxicity (ADCC)
Changes in cell surface receptors
Arachidonic acid, and leukotriene release synthesis of platelet activating factor
Eosinophils
Survival
Cytotoxicity
Leukotriene synthesis
Basophils
Histamine release
Macrophages
Cytokine expression
Killing of parasites
Surface receptor antigen expression
Tumor cell killing
Adherence
Oxidative metabolism
Langerhans cells
Maturation, viability and function
Lymphocytes?
In vivo
Increased hematopoiesis, eosinophilia
Lowering of serum cholesterol
Myeloproliferative syndrome (retrovirus constructs)
Blindness and muscle infiltration in transgenic mice

marrow fibroblast feeders engineered by retroviral infection to constitutively produce one of these growth factors. G-CSF had a pronounced action on primitive adherent progenitors while GM-CSF had an effect on terminally differentiating cells. Both cytokines were strongly synergistic in this setting [16].

Long-term cultures of CD34+ cells from bone marrow contain clonogenic cells for one week under serum-free conditions. This time can be prolonged by two to three weeks with IL-1 or IL-6, and by three to four weeks with GM-CSF. A maximal expansion of these cells is achieved with a combination of IL-1 α or IL-6 up to eight weeks [17]. The proliferation inducing effect of GM-CSF and IL-3 on higly purified CD34+ hemopoietic stem cells is enhanced by TNF α, while TNF α alone has no stimulating effect [18]. In contrast, the weak effect of G-CSF on a subpopulation of CD34+ cells is inhibited by TNF α [18]. The growth of highly purified peripheral CD34+ stem cells has been studied utilizing several hemopoietic growth factors [19]. Colony formation is most effectively supported by IL-3, followed by GM-CSF. G-CSF was not effective. A combination of GM-CSF and IL-3 is not synergistic [19].

The effect of GM-CSF is not confined to myelopoiesis in some experiments. GM-CSF potentiates the growth of mouse thymocytes together with IL-1. The effect is dependent on an expression of TNF α and IL-2 [20].

Generation of GM-CSF in Response to Infection

Macrophages and activated T lymphocytes play a key role in the response to infection. TNF α and IL-1 are secreted from macrophages activated by antigen. Elevated TNF α serum levels have been demonstrated with septic shock in animal experiments [21–24] and in man [25–27]. TNF α and IL-1 have been found in the serum after experimental challenge with endotoxin [28]. Elevated TNF α serum levels have been detected in parasitic infections such as leishmaniasis and malaria [29].

TNF α and IL-1 β induce hemopoietic growth factors in the bone marrow stroma. NF-GMa is a nuclear transcription factor binding to a sequence in the promotor region of the genes of hemopoietic growth factors such as GM-CSF, G-CSF, IL-3 and Il-5 [30,31]. TNF induces NF-GMa in embryonic fibroblasts *in vitro* [31]. NF-kappa B is a second transcription factor for hemopoietic growth factors dependent on TNF α [31,32]. However, no functional data have been published to support its role in GM-CSF gene expression [8].

The GM-CSF gene is constitutively transcribed in monocytes, endothelial cells and fibroblasts [33,34]. Several other cells synthesize GM-CSF after activation: T lymphocytes, B lymphocytes, macrophages, mast cells, fibroblasts, endothelial cells, mesothelial cells, and osteoblasts [8]. Keratinocytes [35] and L428KSA Hodgkin-derived cells [36] synthesize GM-CSF. IL-1, IL-6 and TNF α enhance the transcription of GM-CSF, G-CSF and M-CSF in cultures of endothelial cells [37–39].

GM-CSF seems to play its role in the cellular interaction between stroma and hemopoietic cells in the microenvironment. It may play a role in the attraction of mature monocytes and granulocytes and their activation in sites of infection [40]. In consequence there are no data for a systemic shedding of the factor during infection. No detectable GM-CSF levels have been found in patients with acute myeloid or lymphocytic leukemia after induction chemotherapy, with infection, or with regeneration [40,41]. Only two patients with AML had measurable levels of GM-CSF at the start of chemotherapy, decreasing thereafter [41]. There were no elevated GM-CSF levels in patients with lymphadenopathy, ARC and AIDS as compared to normal controls [42].

Effects Induced by GM-CSF

GM-CSF exerts pleiotropic effects on mature granulocytes, macrophages, eosinophils and on stroma cells.

Induction of Cytokines

GM-CSF induces the expression of G-CSF in bone marrow stroma cells. Adherent stroma cells depleted from macrophages synthesize a granulocyte/macrophage stimulating activity *in vitro* containing prevalently G-CSF [43]. GM-CSF stimulates the transcription of M-CSF in monocytes [11].GM-CSF induces the production of TNF and IL-1 [44]. GM-CSF and IL-3 induce TNF-mRNA in monocytes. GM-CSF activates monocytes to produce IL-1, G-CSF and TNF [40]. Some of these actions may be part of amplifying and feedback mechanisms.

Neutrophil Granulocytes

GM-CSF has both direct and indirect effects on human neutrophils [5, 45, 46]. Direct effects include inhibition of neutrophil migration, degranulation, receptor expression and effects on the cytoskeleton and cell shape [5, 45–52]. GM-CSF also induces altered response due to secondary stimuli. Responses modulated include increased superoxide generation, Ca^{++}fluxes and leukotriene B_4 synthesis [45, 47, 48, 53–57].

GM-CSF almost doubles the life span of neutrophils. It enhances the attachment of neutrophils to monolayers of human umbilical vein endothelial cells by direct action upon the neutrophil [58].

GM-CSF affects the granulocyte adhesion by down-regulation of surface adhesion molecules. LAM-1 (leukocyte adhesion molecule-1) is one of the molecules involved. Neutrophils, eosinophils, monocytes and bone marrow derived myeloid precursor cells as CFU-GM and BFU-E are LAM-1 positive. LAM-1 disappears from the cell surface of all cells except lymphocytes after incubation with GM-CSF [59]. For some time the loss of LAM-1 is accompanied by an up-regulation of CD11b [60], a molecule involved in the aggregation of neutrophils. The same effect is induced by TNF but not by G-CSF. Thus, changes of aggregation and motility of cells may be explained [59].

Using synthetic peptides of GM-CSF with truncated amino and carboxy termini, a region between amino acids 14 and 24 has been found to be essential for neutrophil attachment. There is an individually heterogeneous response to GM-CSF. The level of response to GM-CSF does not depend on the receptor number. However, a positive correlation between the response to GM-CSF and the level of response to tumor necrosis factor suggests a link between the responses to these two cytokines [58].

In another study TNF α, GM-CSF, IL-1 and TNF β, but not IL-3, specifically stimulated neutrophil adherence to basement membranes of stratified squamous epithelial cells pretreated with autoantibodies specific for the basement membrane matrix protein, type-VII collagen. Neutrophil adherence and stimulation was autoantibody- and cytokine-concentration-dependent. TNF and GM-CSF were the most potent stimulators effective at concentrations of less than 0.067 ng/ml. Neutrophil adherence appeared to be dependent, in part, on the low-affinity neutrophil receptor for IgG, Fc(gamma)RIII. GM-CSF alone or in combination with other cytokines may contribute to immune-mediated inflammation and tissue injury by stimulating immune adherence of neutrophils to tissue-bound autoantibodies and immune complexes [61].

Stimulation of granulocytes with GM-CSF *in vitro* is followed by an enhanced expression of the complement receptors CD35 (CR1) and CD11b (CR3), while the low affinity Fc-gamma receptor CD16 (FcRIII) is down-regulated. A marked increase in CD35 and CD11b expression and a substantial decrease, or even loss of CD16, were found in granulocytes from patients undergoing GM-CSF therapy before, during and after treatment. These changes occurred extremely rapidly after onset of therapy [62].

GM-CSF and G-CSF induce an enhanced O_2^-production and membrane depolarization after stimulation with chemotactic peptides. A direct O_2^-secretion is also induced by GM-CSF, but not by G-CSF [63, 64]. GM-CSF enhances the cytotoxicity of neutrophils against *E. coli* [65]. Pre-incubation of neutrophils with GM-CSF increases the neutrophil killing of *Torulopsis glabrata.* It also increases the oxygen-dependent respiratory burst in response to opsonized fungi. Besides these priming effects, GM-CSF is a weak stimulus of the neutrophil respiratory burst and degranulation. The priming and stimulatory effects of GM-CSF have been observed at 10–1,000 U/ml with an optimal concentration of 100 U/ml [66].

GM-CSF has been reported to partially correct deficient neutrophil phagocytosis *in vivo.* In a patient with a partial leukocyte adhesion deficiency the levels of CD11b and CD11c were 10% of controls, whereas CD11a levels were normal. Unstimulated neutrophils from this patient exhibited defective adhesion to plastic, but not to human umbilical vein endothelial cells. The adhesion to human umbilical vein endothelial cells could be further up-regulated by GM-CSF. The normal neutrophil-endothelial interaction induced *in vitro* was confirmed *in vivo* when administration of GM-CSF resulted in rapid phagocyte margination. Neutrophil migration and phagocytosis/killing were defective in neutrophils of the patient, and some improvement in phagocytosis and killing was seen following administration of GM-CSF [67].

However, GM-CSF does not only exert stimulatory effects on neutrophils. GM-CSF inhibits the chemotactic migration of neutrophils towards the tripeptide f-Met-Leu-Phe and the complement split product C5a in the Boyden chamber assay [68].

This effect may be clinically important as shown by reports on patients with continuous intravenous GM-CSF following high-dose alkylating agent chemotherapy and autologous bone marrow transplantation (aBMT). Granulocyte margination studied by ^{111}In-labeled autologous granulocytes was similar to margination before and during GM-CSF infusion. Phagocytosis of *Cryptococcus neoformans* and granulocyte hydrogen peroxide production was also without change before and during GM-CSF infusion. It was similar to patients treated with the same high-dose chemotherapy and aBMT but not receiving GM-CSF. However, migration of granulocytes to a sterile inflammatory site was markedly reduced during GM-CSF infusion as compared with baseline values [69]. There is another report on a decrease of neutrophil migration to the oral mucosa with a continuous infusion of GM-CSF after autologous BMT [70]. After discontinuation of GM-CSF, a dramatic increase of granulocytes in the oral mucosa occurred despite a fall of the neutrophil counts.

Eosinophils

GM-CSF prolongs the life span of eosinophils by 9 h [47]. It enhances the antibody-dependent cytotoxicity of eosinophils [71] and induces an enhanced expression of high-affinity receptors for IL-5 [72].

Actions on Lymphoid Cells

T-cell proliferation can be enhanced if peritoneal macrophages participating in this process are prestimulated with GM-CSF. GM-CSF synergistically enhances murine T-cell proliferation together with IL-1, but not alone [20]. GM-CSF and IL-3 synergize with IL-2 for induction of a proliferative response in human T lymphocytes with and without mitogen activation. It also potentiates the long-term growth of non-activated T cells in the presence of IL-2 [73]. There is experimental evidence that the cooperation of T cells is enhanced by induction in macrophages of IL-1 and IL-6 by GM-CSF.

Monocytes and Macrophages

Monocytes and macrophages are of substantial importance for specific immune response. They produce cytokines and augment in the presentation of antigen for the induction of humoral immune response. They are an essential part of defense against mycobacteria and parasitic infections. Many *in vitro* studies deal with these atypical infections.

Il-4, GM-CSF and IFN gamma increase the expression of HLA-DR on monocytes. IFN alpha has the same effect, but to a lesser degree. IL-4 and GM-CSF selectively increase HLA-DR and HLA-DP, but not HLA-DQ antigens. The expression of all HLA-DR, HLA-DP and HLA-DQ antigens is enhanced by IFN gamma. Combinations of IFN-gamma with either IL-4 or GM-CSF are not synergistic for the expression of any of the class II antigens on monocytes [74].

These effects may play a role in the antigen presentation by macrophages to lymphoid cells. Down-regulation of the receptors for IFN gamma on the surface of monocytes [75] may represent a pathway for negative feedback.

GM-CSF and IL-3, but not G-CSF, significantly enhance monocytic cytotoxicity after stimulation with endotoxin [76]. The Fc-dependent phagocytosis of tissue macrophages is stimulated by GM-CSF [71].

GM-CSF inhibits the intracellular growth of *Mycobacterium tuberculosis* [77] and *Mycobacterium avium* [78] in human macrophages. The effect may be clinically important because *M. avium* can survive intracellularly in the macrophages of immunocompromised patients. The effect is not due to the generation of O_2^- radicals [77].

Activation of macrophages by GM-CSF may be enhanced and amplified by feedback mechanisms: GM-CSF was found to be produced by both monocytes and large granular lymphocytes (LGL) but not by T lymphocytes from peripheral blood in response to *M. avium* [79]. GM-CSF appeared in the supernatant fluids within two days of culture of either monocytes or LGL and was produced up to seven days of incubation. Northern (RNA) blot analysis of RNA from both cell types demonstrated the expression of GM-CSF message within 24 h of stimulation [79].

Obviously GM-CSF plays an important role as a mediator for the activation of macrophages by vitamin D3. Vitamin D3 has been shown to activate human monocyte-derived macrophages to kill or inhibit intracellular growth of *M. avium* complex in a dose-dependent fashion. It was found that D3-treated macrophages produced increased concentrations of TNF and GM-CSF. There was an inhibition of D3-dependent macrophage activation by anti-GM-CSF antibody in a wide dose range of vitamin D3. Anti-TNF antibodies inhibited the macrophage function only after activation with high doses of vitamin D3 [80].

The effect of GM-CSF and TNF α on the bacteriostatic activity of macrophages on *M. avium* is additive. Enhanced killing of avirulent *M. avium* by GM-CSF is dependent on the generation of reactive nitrogen intermediates. Moreover, there is a correlation between NO_2^- generation and mycobactericidal activity of macrophages. Superoxide dismutase protected *M. avium* against macrophage effector function, seemingly by protecting the bacteria against endogenous superoxide anion. This was also apparent in macrophages from patients with chronic granulomatous disease (CGD), which were inefficient at generating reactive oxygen intermediates. Moreover, macrophages from CGD patients killed avirulent *M. avium* as efficiently as cells from normal individuals [81]).

In a mouse model the effect of GM-CSF depends on genetic resistance to mycobacteria. Infusion of GM-CSF, CSF-1 and IL-3 led to a significant, albeit rather modest,

increase in mycobacterial resistance of genetically resistant mice (A/J strain). Conversely, these CSFs dramatically increased the susceptibility of mice which lack resistance to mycobacterial infection (C57BL/6 strain). *In vitro* studies demonstrated that resident peritoneal macrophages from susceptible mice were more permissive for mycobacterial growth than cells from resistant mice. Application of CSFs on peritoneal macrophage monolayers led to increased growth, but did not change the resistance/susceptibility phenotype of isolated macrophages [82].

GM-CSF failed to normalize macrophage function in another experiment. Cultured human monocyte-derived macrophages and murine Kupffer cells showed significantly greater intracellular growth of *M. avium* complex when exposed to ethanol. This effect cannot be abrogated by GM-CSF [83].

GM-CSF exhibits further activities on the immune system. Mice chronically infected by *Trypanosoma cruzi* have a defect in their cellular immune response. It can be completely corrected by the application of GM-CSF *in vivo*. The effect seems to depend on an enhancement of the Ia antigen expression and on enhanced expression of IL-2 mRNA in accessory cells of the spleen. Furthermore, GM-CSF stimulates the production of IL-1 in peritoneal macrophages [84].

Leishmania are important worldwide pathogens for infections (e. g. Kala Azar). *Leishmania* can survive in macrophages. GM-CSF or M-CSF application can reduce intracellular parasites dose-dependently. IFN gamma potentiates the effect of GM-CSF [85]. However, GM-CSF may be important in the pathogenesis of the *Leishmania* infection itself. Murine lung-conditioned medium displays, in addition to a colony-stimulating activity on bone marrow cells, a potent growth-stimulating activity on promastigotes of *Leishmania mexicana amazonensis*. Immunoprecipitation with an antibody specific for murine GM-CSF abrogates both activities, indicating that the leishmanial growth-promoting activity is due to the presence of GM-CSF in the murine lung conditioned medium. Sequential *in vitro* passages of the parasite induce a progressive loss of sensitivity to the growth factor. Parasite forms recently collected from lesions are significantly more responsive to the growth factor than forms already adapted to grow in culture. Since it has been shown that several different microorganisms display receptors for vertebrate-like hormones and that GM-CSF is able to enhance a cutaneous leishmanial lesion, there is the hypothesis that a direct interaction between a host-derived hormone and the microorganism may play a role in the pathogenesis of the infection. This hypothesis is reinforced by the fact that GM-CSF is produced by T lymphocytes and macrophages which actively participate in the leishmanial infection [86].

The effect of GM-CSF on HIV-infected macrophages has been investigated intensively. A decreased activity of HIV reverse transcriptase with GM-CSF and interferon gamma in a HIV-1-infected monocytic cell line (U-937) has been reported [87]. However GM-CSF and M-CSF, but not G-CSF, enhance the production of HIV-1 in human monocytes [88]. The effect can be abrogated by antibodies to GM-CSF, but not by antibodies to M-CSF.

IL-3 and GM-CSF enhance the adhesion of purified human monocytes to cultured human umbilical vein endothelial cells and to plastic surfaces *in vitro*. The stimulation is biphasic with an early phase detectable by 10 min, and a late phase seen after 9 h of culture at concentrations from 6 pM to 60 pM. Both phases of stimulated adhesion were partially inhibited by a monoclonal antibody to CD18, the common β-chain of the leukocyte functional antigen family of adhesion molecules, but not by an antibody to CD11 b. The late phase of stimulated adhesion is totally dependent on *de novo* protein synthesis while the early phase is not. For the early phase adhesion receptor redistribution or conformational change may be the underlying mechanism [89].

GM-CSF in Experimental Infection

There is as yet a limited number of studies investigating the *in vivo* impact of GM-CSF in experimental infections. In transgenic mice expressing the gene for GM-CSF an excessive number of macrophages with foamy cytoplasm have been found. These cells resemble activated macrophages. They have a massively enhanced phagocytosis but fail to kill *Listeria* more efficiently than normal cells. However, the overall antibacterial activity is enhanced [90].

Recombinant murine (rm) GM-CSF has been shown to significantly protect neonatal rats from septic death due to *Staphylococcus aureus*. When rmGM-CSF was given intraperitoneally 6 h before a 90% lethal dose challenge of *S. aureus*, peak survival was observed at a dose of 30 pg/g (54% vs. 10% in animals administered saline). Blood cultures were positive for *S. aureus* in 26 of 32 saline-treated and in five of 31 rmGM-CSF-treated animals. Numbers of blood granulocytes were significantly increased 9 h after administration of rmGM-CSF but returned to control levels by 12 h. Obviously the effect of GM-CSF resulted from cell activation rather than acceleration of hemopoietic cell growth, as neutrophil storage and proliferative pools were not affected [91].

The trait of A/J mice is resistant to *S. typhimurium* due to impaired replication of the microorganism within macrophages. A/J mice were challenged with a lethal dose of *S. typhimurium* and treated with 1 µg of GM-CSF twice daily. An increased median survival time and a higher survival fraction compared to untreated controls was found. GM-CSF was most effective when given for a brief period of time (one to two days) after infection. Pretreatment of the mice or delayed treatment with GM-CSF had no effect on survival. Mice treated with GM-CSF had fewer *S. typhimurium* in the spleen and peritoneal cavity on day 4 but not on day 2 after infection.

However, GM-CSF treatment had no effect in infected C57BL/6 mice which are susceptible to *Salmonella* [92]. Murine IFN-gamma, rhG-CSF mouse GM-CSF and human IL-1 have been found to protect mice against Sendai virus infection. IFN-gamma provided protection when administered intranasally, but not by the intravenous route, several days before the infection. Intranasal administration of G-CSF one day before infection was the most effective with respect to administration route and timing. Intranasal administration of GM-CSF was found protective when given one or three days before the infection. Il-1 beta demonstrated therapeutic activity against Sendai virus infection after intranasal administration on the same day as the infection. When each of the cytokines was administered subcutaneously four times daily to cyclophosphamide-treated mice before intravenous infection with HSV, only GM-CSF showed any protective activity [93].

Conclusions about the Potential Use of GM-CSF in Infection in Man

GM-CSF plays its physiological role on the tissue level. There are no data indicating that the factor is systemically circulating during antiinfectious response. GM-CSF has been shown to stimulate granulopoiesis and monopoiesis *in vivo*. Furthermore, it exerts a broad spectrum of activating actions on granulocytes and monocytes/macrophages.

Infections may be associated with neutropenia, and depression of the neutrophil and macrophage function. In some infections, macrophages are essential for elimination of the pathogen. Against this background it is worthwhile to study the effect of GM-CSF in infections in man.

However, cell activation by GM-CSF may have some disadvantageous side effects, namely a decrease of chemotaxis, potentially enhanced adhesion to antibody-coated antigens [61] and depression of granulocyte migration [69, 79]. These potential side effects and their sequelae have to be carefully monitored. Intermittent schedules of application might be worth exploring.

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