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ROLE OF GM-CSF IN AUTOIMMUNITY, INFLAMMATORY DISEASES, ADJUVANT TO VACCINE AND MAJOR SIDE EFFET

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ABSTRACT : Granulocyte-macrophage colony-stimulating factor (GM-CSF) is an important hematopoietic growth factor and immune modulator. During inflammation, GM-CSF can be secreted by several different cell types, including epithelial cells and leukocytes, and is a critically important cytokine that can drive both innate and adaptive immune responses. GM-CSF assembles and activates its heterodimer receptor complex on the surface of myeloid cells, initiating multiple signalling pathways that control key functions such as cell survival, cell proliferation, and functional activation. Till date, GM-CSF has been studied extensively in murine models and human clinical trials, alone and as adjuvant therapy. This knowledge provides opportunities for the development of new therapies and understands the action of these cytokines in haematological malignancy and chronic inflammation etc.

KEYWORDS : GM-CSF, Autoimmunity, Inflammatory diseases, Adjuvant.

INTRODUCTION

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is an important hematopoietic growth factor and immune modulator. GM-CSF was first identified in mouse lung tissue-conditioned medium following lipopolysaccharide injection into mice by its ability to stimulate proliferation of mouse bone marrow cells *in vitro* and generate colonies of both granulocytes and macrophages. Since then, several biological effects have been attributed to GM-CSF, including hematopoiesis, response to inflammation and infection, and functional enhancement of mature effector cells in antigen presentation and cell-mediated immunity[1].

GM-CSF stimulates multipotent progenitor cells depending on its concentration, the proliferation of macrophage progenitors at the lowest doses, followed by granulocyte, erythroid, eosinophil, megakaryocyte, and multipotent progenitors[2]. GM-CSF may play a pivotal role in various human inflammatory diseases including rheumatoid arthritis,

inflammatory renal disease, and inflammatory lung disorders. GM-CSF has been utilized in the clinical management of multiple disease processes. Most recently, GM-CSF has been incorporated into the treatment of malignancies as a sole therapy, as well as a vaccine adjuvant[3]. The subsequent production into a recombinant form (rHuGM-CSF; sargramostim) has led to a number of potential clinical uses of GM-CSF, including enhancement of hematopoietic recovery after chemotherapy and bone marrow transplant, treatment of infectious diseases, and use as an antitumor therapy and vaccine adjuvant[4]. So; in the present study, an attempt was made to compile the knowledge about the granulocyte-macrophage colony-stimulating factor.

GM-CSF THERAPEUTICS

A wide variety of cells can produce GM-CSF. Major sources of GM-CSF are T and B cells, monocyte/macrophage endothelial cells, and fibroblasts. Neutrophils, eosinophils, epithelial cells, mesothelial cells, Paneth cells, chondrocytes, and tumor cells can also produce GM-CSF[5]. The production of GM-CSF is stimulated by various factors, including TNF, IL-1, toll-like receptor agonists, and prostaglandin E2[6,7]. GM-CSF promotes the survival and activation of macrophages, neutrophils, and eosinophils, as well as dendritic cell (DC) maturation. GM-CSF can polarize macrophages into M1-like inflammatory macrophages, which produce a variety of inflammatory cytokines such as TNF, IL-6, IL-12p70, IL-23, or IL-1β, and thus promote Th1-Th17 responses[8,9]. On the other hand, the association of GM-CSF and Th2 immunity is also reported in allergic airway inflammation[10]. GM-CSF is reported to have diverse functions on mature myeloid cells, including antigen presentation[11], induction of phagocytosis[12-14], enhancement of pro-inflammatory cytokine production[15], and promotion of leukocyte chemotaxis[16].

GM-CSF in Auto-immunity and inflammatory diseases

Granulocyte colony-stimulating factor (G-CSF or GCSF), also known as colony-stimulating factor 3 (CSF 3), is a glycoprotein that stimulates the bone marrow to produce granulocytes and stem cells and release them into the bloodstream. In mice, transgenic studies for GM-CSF showed that many cytokines and inflammatory mediators were found to be increased in these mice and overexpression leads to macrophage accumulation, blindness, and severe damages to various tissues. GM-CSF overexpression in the stomach leads to autoimmune gastritis[17-19]. When bone marrow cells infected with a retrovirus expressing GM-CSF were transplanted, a lethal myeloproliferative syndrome was induced[20]. In RA patients, the concentration of GM-CSF in the synovial fluid and plasma was elevated[21,22] and the administration of recombinant GM-CSF exacerbated the disease

activity[23]. Bone marrow adjacent to the RA joints contains an increased number of granulocyte-macrophage progenitors, colony-forming unit granulocyte-macrophages (CFU-GM), which can differentiate into granulocytes or macrophages with GM-CSF stimulation[24] and also into osteoclasts with M-CSF and RANKL stimulation[25]. Adenoviral-mediated GM-CSF gene transfer in the lung also led to severe lung eosinophilia, macrophage expansion, and fibrotic reactions[26-28]. This information has led to the hypothesis that GM-CSF may have a central role in promoting sensitization to aeroallergens in polluted air[29-30]. Interestingly, it has been suggested that human GM-CSF polymorphisms are likely asthma determinants[31].

In another study, it was found that the culture of ex vivo differentiated human MoDCs (CD14+CD33+) in the presence of GM-CSF is capable of class II-mediated prominent immune epitopes of two auto-antigens [type II collagen (CII) and cartilage gp39 (HCgp39)] observed in the inflamed synovial joints of patients with RA (collagen-induced arthritis (CIA), a mouse model of arthritis, mice with defective GM-CSF cannot develop arthritis, and using antibodies against GM-CSF results in inhibition of disease progression and a decrease in proinflammatory cytokines in the joints[32]. Similarly, in another mouse model of arthritis (in SKG mice), GM-CSF treatment increased the production of IL-1_ or IL-6 by macrophages and promoted the differentiation and augmentation of CD4+ T cells that produce IL-17 and GM-CSF. Also, administration of anti-GM-CSF was more efficient compared to anti-IL-17 in treatment and decreased disease severity[33].

There are four main signaling pathways triggered by CSF2R. After binding of GM-CSF to its receptor, Janus-kinase-2 (JAK-2) is recruited to the cytoplasmic domain of the b chain, and activation of JAK-2 occurs, which subsequently induces STAT-5 phosphorylation. This signaling pathway induces migration of STAT-5 dimers to the nucleus and promotes the transcription of various genes such as pim-1 and CIS to induce cell differentiation[34,35]. GM-CSF promotes cell survival via phosphatidylinositol-3-kinase (PI3K) and JAK/STAT-Bcl-2 signaling pathways[36]. Moreover, cell differentiation and inflammation are mediated by activation of ERK1/2 and NF-kB. Accordingly, studies have shown that GM-CSF augments the LPS-induced inflammatory response by priming of TNFalpha synthesis and also induces multipotent mesenteric mesothelial cell differentiation into macrophages through the ERK1/2 signaling pathway[37,38].

GM-CSF in crohn's disease

GM-CSF also triggers several different signalling pathways in myeloid-derived suppressor cells (MDSCs) that mainly involve the signal transducer and activator of transcription (STAT) family of transcription factors. GM-CSF is generally regarded as a cytokine with

more pro-inflammatory functions based on its activity on neutrophils and macrophages. It is likely that GM-CSF plays both protective and pathological roles[39]. GM-CSF has been proposed to be essential for a microbiota-dependent crosstalk between mononuclear phagocytes and group 3 innate lymphoid cells (ILC3s) thereby promoting intestinal homeostasis. ILC3- derived GM-CSF can promote intestinal myeloid cell homeostasis through enhancing DC and regulatory T cell function.[40,41]

GM-CSF-activated monocytes simultaneously have a regulatory potential on adaptive immunity. GM-CSF significantly induces a short-termed expression of chemokines in monocytes, which are known to attract naïve T cells, T helper 2 (Th2) cells, and/or regulatory T cells. GM-CSF may regulate the homing molecules CCR2 and CCR6 on human monocytes, which are involved in regulating several aspects of mucosal immunity, including the ability to mediate the recruitment of innate immune cells to the sites of epithelial inflammation[42].

GM-CSF in Cancer

Recombinant GM-CSF has made significant contributions in the supportive care of cancer patients, owing to enhanced myeloid recovery after cytotoxic chemotherapy.GM-CSF, a potent cytokine promoting the differentiation of myeloid cells, can also be used as an immune-stimulatory adjuvant to elicit antitumor immunity[43]. In a preclinical mouse model, the mechanism of this impairment of antitumor response by GM-CSF has also been shown to increase the generation of Foxp3+ Tregs and Gr-1+ CD11b+ myeloid derived suppressor cells (MDSCs) that inhibit the function of antigen-specific T cell.[44]

In one of the study on tumor; it was found that antitumor growth was demonstrated in subcutaneously implanted colon cancer cell line overexpressing GM-CSF and its heterodimer receptors into immune-deficient nude mice, which exclude the interference of GM-CSF-induced immune response. The results showed significant smaller tumor burdens in-vivo in GM-CSF overexpressing compared with shRNA knockdowned GMCSF tumor cells. Moreover, the 5-year survival rate was increased in patients with colorectal cancer with concurrent overexpression of GM-CSF and its receptor subunits, suggesting a direct inhibitory role of GMCSF in tumor growth.[45]

GM-CSF is a potent activation and maturation cytokine in the differentiation, maturation and migration of DCs to lymph nodes. In a murine tumor model, vaccination with GM-CSF-secreting cancer cells promotes the in vivo yields, recruitment and activation of DCs. In addition, GM-CSF stimulates an increased expression of co-stimulatory molecules B7–1 and

CD1d on DCs suggesting that a lower amount of antigen would be required to induce optimal T-cell proliferation and activation of NKT cells to execute an antitumor response.[46]

GM-CSF in pregnancy

Pregnancy is associated with a transient depression of maternal cell-mediated immunity to protect the semi allogeneic embryo from rejection. Granulocyte macrophage colony-stimulating factor (GM-CSF), a lympho-haemopoietic cytokine with well-defined wide range effects covering proliferation, differentiation chemotaxis and adhesion in many cell types, plays an important role in the course of a successful pregnancy by promoting the growth and/or differentiation of the trophoblast[47]. GM-CSF blood concentrations increased during normal pregnancy significantly reduced in RSA (Recurrent abortion frequency) and that such a reduction can be efficiently reverted by IVIg treatment. In fact pregnant healthy women showed very high GM-CSF blood concentrations when compared with non-pregnant healthy women. On the contrary, pregnant RSA patients did not show any increase in the GM-CSF blood concentrations, which were therefore significantly reduced in comparison with healthy pregnant women matched for age and gestational week[48].

GM-CSF in alveolar proteinosis

The absence of GM-CSF function in vivo is associated with pulmonary alveolar proteinosis (PAP), a respiratory disease characterized by an accumulation of surfactant in the lung caused by defective alveolar macrophage function.

GM-CSF, a myelopoietic growth factor and pro-inflammatory cytokine, plays a critical role in alveolar macrophage homeostasis, lung inflammation, and immunological disease. Both administration and inhibition of GM-CSF are currently being therapeutically tested in COVID-19 clinical trials. This Perspective discusses the pleiotropic biology of GM-CSF and the scientific merits behind these contrasting approaches. GM-CSF administration in patients with COVID-19 may improve lung function by strengthening the alveolar wall and enhancing viral clearance, and this approach may thus provide particular benefit in the early stages of COVID-19. By contrast, GM-CSF or GM-CSFR blockade could be a beneficial treatment for the cytokine storm and inflammatory myeloid cell tissue infiltration associated with moderate-to-severe COVID-19[49].

GM-CSF adjuvant to vaccines

GM-CSF as a sole agent in the treatment of prostate cancer and melanoma; GM-CSF has also been shown promising as a vaccine adjuvant in whole-cell, DC, and peptide-based vaccine trials for the treatment of melanoma, ovarian, colorectal, prostate, pancreatic, renal cell, and breast cancer. The efficacy of GM-CSF has been demonstrated in controlled prospective

trials, as well as in trials with comparisons to other immune adjuvants. Granulocyte-macrophage colony-stimulating factor has been used safely and effectively as a sole agent, as a byproduct of engineered tumor cells in whole-cell vaccines, as well as an adjuvant in peptide-based and DC-based vaccines used to treat multiple malignancies. Faries and colleagues reported on a randomized assessment of 97 patients with stage II-IV melanoma treated with a whole-cell allogenic vaccine (Canvaxin™; CancerVax, CA, USA) with or without GM-CSF adjuvant[50]. Spitler and colleagues performed a Phase II trial investigating the use of recombinant human GM-CSF (sargramostim) in the adjuvant setting for patients with resected melanoma[51.] Dranoff and colleagues were able to show that vaccination with engineered GM-CSF-secreting melanoma cells augments antitumor immunity in patients with metastatic melanoma with minimal toxicities[52].

Adjuvants like Granulocyte macrophage-colony Stimulating Factor (GM-CSF) have been found to improve the response rate to vaccines. This study was conducted to evaluate the efficacy of GM-CSF as an adjuvant to HB vaccine in ESRF patients who were non-responders to the usual three double dose vaccinations (primary non-responders). The seroconversion rate improved from an initial 62% (31/50) to an overall 84% (42/50) after the use of GM-CSF[53]. The use of GM-CSF could augment the immunologic response to a recombinant vaccine against the hepatitis B virus (HBV) in 80 HIV-infected patients (18-35 years old). They received a double dose (40 microg) of recombinant HBV vaccine IM at 0, 1, and 6 months and were randomized to receive either concurrent 20 microg of GM-CSF (n=40) or placebo IM (n=40) with the first vaccine dose. A significant increase in the seroconversion rate was observed after the second vaccine dose in the GM-CSF group (62% GM-CSF versus 30% control group $P<0.0074$)[54]. GM-CSF as a monotherapy, as adjuvant with or without cancer vaccines, or in combination with chemotherapy.

GM-CSF SITE OF ACTION

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a pluripotent cytokine produced by many cells in the body, which regulates normal and malignant hemopoiesis as well as innate and adaptive immunity. GM-CSF assembles and activates its heterodimeric receptor complex on the surface of myeloid cells, initiating multiple signaling pathways that control key functions such as cell survival, cell proliferation, and functional activation.

GM-CSF functions through a heterodimeric receptor composed of an a-subunit that binds GM-CSF with low affinity (GMRa) and a subunit that is shared with the receptors for interleukin-3 (IL-3) and interleukin-5 (IL-5), the b common chain (bc). The bc-subunit binds cytokine very poorly by itself (McClure et al. 2003), but converts low-affinity cytokine

binding by the a-subunit to a high-affinity interaction and is the principal signal-transducing subunit. The molecular mechanisms regulating GM-CSF receptor activation have been elusive but important insights have been revealed by the recent crystal structure of the GM-CSF receptor ternary complex (Hansen et al. 2008) and are starting to clarify cytokine receptor pleiotropy as well as the function of the related IL-3 and IL-5 receptors. These

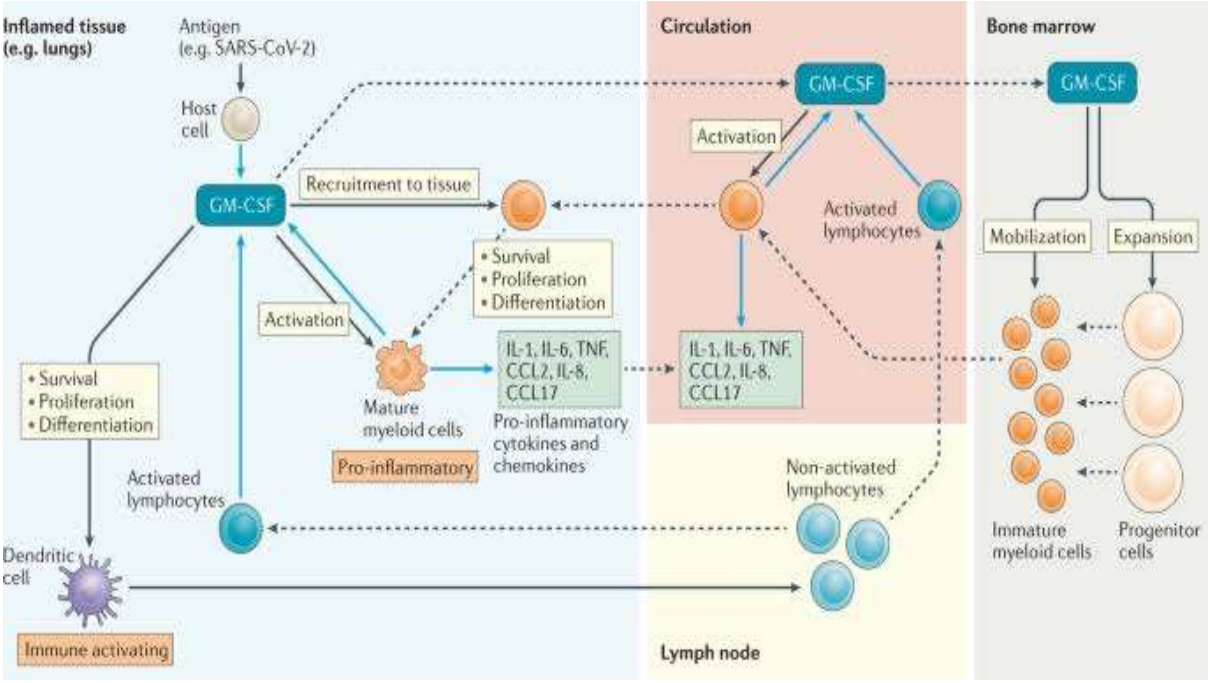

Fig. No.1 GM-CSF and Inflammation[49]

insights into GM-CSF receptor activation have clinical significance and are being harnessed to develop new treatments for hematological malignancies and inflammatory diseases. Excessive stimulation of GM-CSF receptor signaling through excess and persistent GM-CSF or abnormal downstream events has been shown to contribute to chronic inflammation[55]

GMCSF has a variety of effects on the immune system including activation of T cells and maturation of dendritic cells, as well as an ability to promote humoral and cell-mediated responses. The hematopoietic cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) has been investigated as a monotherapy, and as a component of combination therapies for melanoma. Cell surface receptors for GM-CSF have been characterized by the use of radiolabeled ligand-binding studies. High-affinity GM-CSF receptors (Kd = 30-100 pM) are widely expressed by hematopoietic cells, with mature neutrophils expressing the highest number of receptors (500-600/cell) and immature leukemic cell lines the least (20-200 receptors/cell)[56,57]. The mechanisms of signal transduction from the GM-CSF and IL-3 receptors are not well understood. In the neutrophil, GM-CSF enhances superoxide

production when the cells ar**e** triggered by agents, such as formyl methionyl leucine phenylalanine (fMLP) [58]. This priming does not occur directly as a result of increased calcium flux or activation of protein kinase C [59], but is associated with enhanced Na++in flux, release of arachidonic acid and generation of lipoxygenase products[60,61]. An expanding role for GM-CSF in regulating immune responses has been recognized based upon its activity on the development and maturation of antigen presenting cells and its capability for skewing the immune system.

GM-CSF SIDE EFFECT

GM-CSF, have dose related side effects. GM-CSF should be used in range being from 5-10 micrograms/kg/day either by 4-6 h intravenous infusion or by subcutaneous injection. At such doses, adverse effects are predominantly mild-to-moderate in nature, occur in 20-30% of patients and usually comprise fever, myalgia, malaise, and rash or injection site reaction. Early trials using very high doses of GM-CSF were often associated with marked adverse effects, which in rare cases proved severe (pericarditis and thrombosis). Similarly, a so-called "first-dose reaction", defined as a syndrome of hypoxia and hypotension after the initial but not subsequent doses of GM-CSF, was observed in certain predisposed patients following doses above 10 micrograms/kg/day. Subsequent trials have established that intravenous bolus or short infusions of GM-CSF are more likely to promote adverse effects. Certain patient groups, for example those with myelodysplastic syndrome, acute myeloid leukaemia, inflammatory disease, autoimmune thrombocytopenia or malfunctional immunological responsiveness, require careful clinical monitoring in order to avoid potential complications following the administration of GM-CSF[62].

CONCLUSION

Overall, GM-CSF plays an important role in inflammatory responses in autoimmune disease via induction of various cells and mediators. On-going and complete clinical trials targeting GM-CSF. Granulocyte-macrophage colony-stimulating factor (GM-CSF) can be viewed as a pro-inflammatory cytokine rather than as a key regulator of steady-state and systemic myelopoiesis. GM-CSF likely has a central role in the local activation, recruitment, and survival of macrophage lineage cells and PMNs, perhaps even contributing to macrophage proliferation a sites of inflammation. Granulocyte macrophage-colony stimulating factor (GM-CSF) is now best viewed as a major regulator governing the functions of granulocyte and macrophage lineage populations at all stages of maturation. There is recent evidence for a key role for GM-CSF in inflammatory and autoimmune diseases, therefore making it worthy

of consideration for targetting. Recently, the pathogenicity of GM-CSF-producing CD4 T cells in autoimmune and inflammatory diseases is clarified and gaining increasing attention.

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