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GM-CSF: An Immune Modulatory Cytokine that can Suppress Autoimmunity

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Abstract

GM-CSF was originally identified as a colony stimulating factor (CSF) because of its ability to induce granulocyte and macrophage populations from precursor cells. Multiple studies have demonstrated that GM-CSF is also an immune-modulatory cytokine, capable of affecting not only the phenotype of myeloid lineage cells, but also T-cell activation through various myeloid intermediaries. This property has been implicated in the sustenance of several autoimmune diseases like arthritis and multiple sclerosis. In contrast, several studies using animal models have shown that GM-CSF is also capable of suppressing many autoimmune diseases like Crohn's disease, Type-1 diabetes, Myasthenia gravis and experimental autoimmune thyroiditis. Knockout mouse studies have suggested that the role of GM-CSF in maintaining granulocyte and macrophage populations in the physiological steady state is largely redundant. Instead, its immune-modulatory role plays a significant role in the development or resolution of autoimmune diseases. This is mediated either through the differentiation of precursor cells into specialized non-steady state granulocytes, macrophages and dendritic cells, or through the modulation of the phenotype of mature myeloid cells. Thus, outside of myelopoiesis, GM-CSF has a profound role in regulating the immune response and maintaining immunological tolerance.

Introduction

Colony-stimulating factors (CSF) comprising of macrophage colony-stimulating factor (M-CSF) or CSF1, granulocyte-macrophage colony-stimulating factor (GM-CSF) or CSF2 and granulocyte colony-stimulating factor (G-CSF) or CSF3, have been shown to be important for survival, proliferation, differentiation, maturation and functional activation of haematopoietic cells, including monocytes and macrophages (1). Of the known colony stimulating factors, GMCSF is capable of generating both granulocytes and macrophages

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from mouse bone marrow progenitors (2). In addition to its role as a CSF, studies have established GM-CSF's role in immunomodulation, which includes exacerbation of autoimmune arthritis through the mobilization of inflammatory macrophages (3-6). Similarly, multiple studies from our laboratory and others have shown that GM-CSF can be used to suppress a number of different autoimmune diseases in animals including Experimental Autoimmune Thyroiditis (EAT), Experimental Autoimmune Myasthenia Gravis (EAMG), Type 1 Diabetes (T1D) and colitis through the mobilization of tolerogenic dendritic cells (7-10), etc. The mechanisms underlying this pleiotropic effect of GM-CSF have not been discussed systematically.

GM-CSF is known to initiate transcription through four distinct signalling pathways, namely PI3K-Akt, ERK1/2, JAK2/STAT5 and NF-kB (11). The binding of GM-CSF to GM-CSFR leads to the recruitment and activation of Janus Kinase-2 (JAK-2) that binds to the GM-CSFRβc cytoplasmic domain and phosphorylates the signal transducer and activator of transcription 5 (STAT5) (12, 13). Once phosphorylated, STAT5 migrates to the nucleus and directs the transcription of specific genes related to cell differentiation (12, 13). GM-CSF promotes cell survival through phosphatidylinositol-3-kinase (PI3K) (14) and JAK/STAT5-Bcl-2 (15) signaling and induces cell proliferation mainly by Erk and NF-kB signaling (16, 17). However, GM-CSF also promotes inflammatory responses by signaling through ERK1/2 and NFκB activation (18). Using these multiple pathways, GM-CSF can promote growth, proliferation and differentiation of a multitude of precursors and matured cell types. Thus, in addition to its role in the differentiation of myeloid progenitors, GM-CSF has been shown to modulate the properties and functions of the more matured myeloid cells such as granulocytes, macrophages and eosinophils (19-21).

The formation of blood cells starts from haematopoietic bone marrow stem cells that are stimulated by stem cell factor (SCF) and thrombopoietin (TPO) to undergo self-renewal or differentiation into a multi-lineage committed progenitor cells known as common myeloid progenitors (CMP). GM-CSF has been found to promote the proliferation and differentiation of CMPs into the granulocytes and macrophages (GM) lineage (Fig. 1). Once formed, the GM lineage cells differentiate into the uni-lineage monocytes committed progenitors (MP) or the unilineage granulocytes committed progenitors (GP). The MP cells, when stimulated by M-CSF, give rise to monocytes while GP cells, when stimulated with G-CSF, IL-5, and SCF, give rise to granulocytes (neutrophils, eosinophils and basophils) (22). Although originally identified as a myeloid growth and differentiation factor (1), GM-CSF is now known to affect diverse cell types including lung epithelial cells, CD34+ progenitors, uterine cells, vascular endothelial cells, and fibroblasts that express its cognate receptor (GM-Rα) (23). In general, myeloid lineage cells including monocytes, macrophages, neutrophils, eosinophils, basophils and dendritic cells express GM-Rα (23). In contrast, T-cells, NK cells and B-cells have been demonstrated not to express the GM-CSF receptor, thus ruling out any direct effect of GM-CSF on lymphoid cells (23). However, by acting either directly as a growth and/or a differentiation factor of certain cell types or indirectly by affecting the phenotype of certain cells through intermediate cell types or by acting in combination with other cytokines, GM-CSF has been shown to modulate the function of multiple cell types thus affecting tolerance and autoimmunity in complex and hitherto unappreciated ways (summarized in Table-1).

Interestingly, evidence from knock-out (KO) mouse studies suggest that the steady state maintenance of myeloid lineage cells depend more critically on G-CSF, M-CSF and Flt3L while GM-CSF is largely dispensable for this purpose (1). GM-CSF KO mice do not show any abnormalities in the steady state myelopoiesis (including no change in peripheral monocyte numbers) except for a small reduction in dendritic cell numbers (24-27). However, GM-CSF is able to modulate myeloid lineage populations in specialized ways. As an example, microglia, the macrophages of the brain, have been derived *ex vivo* with astrocyte-conditioned media and GMCSF (28). Additionally, microglial cells have also been shown to acquire dendritic cell phenotype in models of experimental autoimmune encephalomyelitis (EAE) (29). Likewise, GMCSF amplifies IL-3-induced differentiation of basophils from bone marrow cells (30), suggesting a possible role as a growth factor for basophil precursors. Furthermore, basophils have been shown to express MHC-II and co-stimulatory molecules and act as potent APCs to stimulate TH2 type responses both *in vitro* (31) and *in vivo* (32). It is apparent that GM-CSF has a more profound role in the modulation of immune responses beyond its role as a CSF, something that has remained under appreciated. In this review, we summarize the effects of GM-CSF on the phenotype and function of various leukocyte populations and how such effects influence the outcome of various autoimmune diseases.

Granulopoiesis and immunomodulation

G-CSF and GM-CSF are both established as granulocyte growth factors and have been approved for clinical use. The G-CSF receptor is predominantly expressed on neutrophils and drives the proliferation and maturation of neutrophils without any apparent effect on monocytes, eosinophils, or basophils (33), whereas GM-CSF can influence many myeloid cell types as discussed above (34). It is believed that G-CSF plays a homeostatic role in the maintenance of normal neutrophil count and its increase in response to infections (35, 36). Although, GM-CSF has been shown to stimulate the development and maturation of committed stem cells to neutrophils, GM-CSF knockout mice are not neutropenic (27, 37-39). Additionally, both G-CSF and GM-CSF differ in several of their unique effects on neutrophils. For example, GM-CSF exclusively causes enhanced arachidonic acid metabolism, leukotriene-B4 release and induction of IL-1 in neutrophils (Fig. 1). In contrast, G-CSF specifically causes increased release of soluble TNF receptors and IL-1 receptor antagonist proteins from neutrophils (40). In contrast, G-CSF specifically causes increased release of soluble TNF receptors and IL-1 receptor antagonist proteins from neutrophils (41, 42). Thus, both these growth factors exhibit different functional outcomes on neutrophils.

Although being dispensable for neutrophil proliferation and maturation, effects of GM-CSF on neutrophils cannot be overlooked, given its profound effects on neutrophil survival and function (43-46). Systemic or intraperitoneal administration of GM-CSF in mice leads to increased numbers of circulating neutrophils (47). The direct effects of GM-CSF on neutrophils include inhibition of migration (48), enhanced survival (49), altered cytokine production (50, 51), degranulation (52, 53) and surface phenotype (54). Additionally, GM-CSF can also modulate neutrophil response due to secondary stimuli such as increased superoxide generation, and Ca++fluxes (55-57).

Despite their abundance at the sites of autoimmune damage, the precise role of neutrophils in autoimmune disease pathogenesis remained elusive for a long time (58, 59). However, recent evidences suggest the involvement of neutrophils in various autoimmune diseases in human including rheumatoid arthritis (58, 60), systemic lupus erythematosus (61), Bullous pemphigoid (62), Epidermolysis bullosa acquisita (63), multiple sclerosis (64) and anti-neutrophil cytoplasmic autoantibodies-associated vasculitis (65). Neutrophils participate in almost every phase of autoimmune diseases such as immunization, transition and effector phase. Activated neutrophils release self-antigens upon apoptosis or while forming extracellular neutrophil trap and contribute to tissue deposition of autoantibodies. In addition, they can directly cause tissue damage through their effector function as well (66).

The distinction between G-CSF and GM-CSF has been explored in a murine model of glomerulonephritis (67). Glomerulonephritis is a complex autoimmune disorder resulting from the destruction of glomerular structure by inflammatory cells (68). Using GM-CSF −/− and G-CSF −/− mice, it was found that the absence of GM-CSF prevented the development of neutrophil related glomerular injury as well as T-cell/macrophage mediated crescentic glomerular injury. In comparison, the absence of G-CSF prevented only neutrophil related glomerular injury (67). These results establishes the contention that G-CSF regulates autoimmune conditions through the physiological turnover of neutrophils while GM-CSF affects immune regulation in a more complex manner by its secondary effect on lymphocyte activation as well.

The effect of GM-CSF under inflammatory conditions is complex due to its pleiotropic activities on multiple cell populations. In one study, GM-CSF treatment of patients with Felty's syndrome (having concomitant rheumatoid arthritis) for neutropenia actually increased the severity of arthritis while enhancing neutrophil count (69). It is believed that this effect was mediated by the effect of GM-CSF on macrophages. Thus, GM-CSF's immunomodulatory role through macrophages had a more profound negative impact over its beneficial effect through granulopoiesis. Thus it has been suggested that a low dose GM-CSF that can promote granulopoiesis but not have the immunomodulatory effect may be a better alternative for therapy (70). More recently, in experimental model of Multiple Sclerosis (MS) i.e., Experimental Autoimmune Encephalomyelitis (EAE), neutrophils have been shown as the predominant circulating and CNS-infiltrating type of myeloid cells during the preclinical phase. Diminution of neutrophils has been associated with delay in disease onset, maintenance of near-normal blood/spinal-cord barrier architecture and reduced severity of EAE (64). While IL-1R type 1 has been shown to regulate the firm adhesion of neutrophils to the inflamed spinal cord vasculature (64), IL-1 induction has been reported as one of the specific effects of GM-CSF on neutrophils (36). Taken together, it is likely that IL-1 induction by neutrophils, caused by GM-CSF, is associated with the pathogenesis of multiple sclerosis. In one recent study it was demonstrated that GM-CSF treatment could exacerbate autoantibody production and skin blistering in experimental epidermolysis bullosa acquisita (EBA) through neutrophil activation. Predictively, neutralization of GM-CSF proved to be beneficial in this model (63). However, how GM-CSF affects autoantibody production is not understood.

In contrast, the CSF activity of GM-CSF has been found to be protective in autoimmune neutropenia (AIN), an antibody mediated disorder. A low dose of GM-CSF treatment is able to restore and maintain healthy neutrophil counts (71). In addition, a protective role for GM-CSF has been suggested in Juvenile systemic lupus erythematosus (JSLE) (72). It is believed that apoptotic neutrophils supply autoantigens which stimulate autoantibody production and sustain JSLE. Sera from JSLE patients were shown to cause enhanced apoptosis; GM-CSF was found to have a protective effect by preventing neutrophil apoptosis. As in other examples, GM-CSF mediated cell survival effects on mature neutrophils, outside of its role as a CSF, can have therapeutic effects in autoimmune conditions.

Apart from neutrophils, GM-CSF has also been reported to influence of other granulocyte lineage cells. It can augment eosinophil survival and phagocytosis (73, 74). Furthermore, GMCSF can also direct eosinophil priming by which they become receptive to chemotaxins and degranulating factors (75). In one study using a murine model of autoimmune gastritis, it was show that hypertrophy of gastric mucosa was associated with infiltrating eosinophils and correlated with higher GM-CSF expression in the spleen (76). In another interesting study, GMCSF was found to aggravate Murine autoimmune hemolytic anemia caused by Fc-gamma mediated phagocytosis of erythrocytes (77). While administration of Erythropoietin, the differentiation factor of erythrocytes provided complete protection from anemia, administration of GM-CSF led to rapid development of anemia. This was accompanied by the infiltration of the liver by Kupffer cells and by polymorphonuclear leukocytes. These data suggest possible a role for GM-CSF in the development of many cell populations that are not clearly understood as yet. However, these effects of GM-CSF profoundly influence the state of immune tolerance and require further studies.

Regulation of monocyte macrophage lineage and effect on autoimmunity

M-CSF and GM-CSF can both affect macrophage differentiation. M-CSF can promote the *exvivo* and the *in vivo* formation of monocyte colonies from bone marrow progenitors and unlike GM-CSF, its deficiency leads to reduced numbers of monocytes and macrophages which suggests a non-redundant role of M-CSF for monocyte development in the steady state (78, 79). In contrast, it is believed that GM-CSF regulates granulocyte and macrophage lineage cell number and activation state during immune and inflammatory conditions; however it has been shown to maintain the survival and induce the differentiation of alveolar macrophages (22, 27, 80). In vitro studies suggest that GM-CSF can act on monocytes/macrophages and promote survival and induce them to release inflammatory mediators to kill pathogenic organisms and tumors (81). In monocytes for example, GM-CSF regulates the expression of TLR2 and has been shown to induce the expression of IL-12, TNF-α, and monocyte chemoattractant protein-1 (MCP-1) by JAK2/STAT5 signaling (82, 83). Similarly, in vitro treatment of macrophage lineage cells with GM-CSF shifts the polarization to an inflammatory M1 phenotype (Fig 1) that enables them to respond strongly to stimuli (84, 85). In contrast, when M-CSF is used as the sole stimulus, it shifts monocytes into an immunosuppressive M2 phenotype (86, 87). It has been claimed that GM-CSF activates the transcription factor IRF5 to polarize monocytes to M1 phenotype, while M-CSF activates IRF4 to polarize monocytes to the M2 phenotype (88, 89).

Physiological GM-CSF concentration in the serum range from 20 to 100 pg/ml (90) and its levels rise in serum and tissues following stimulation with cytokines, antigens, microbial products or inflammatory agents such as IL-1, TNF-α or lipopolysaccharide (LPS) (90). Incidentally, and consistent with this observation, GM-CSF was first purified from the conditioned medium of lung tissue from mice following LPS injection (91). Very interestingly, GM-CSF overexpressing transgenic mice show accumulation of macrophages, blindness and a fatal syndrome of tissue damage (92). Activation of macrophages as evidenced by increased inflammatory cytokines and urokinase-type plasminogen activator were demonstrated in this mouse model (93). Similarly, adenoviral-mediated overexpression of GM-CSF in the lungs induced lung eosinophilia, macrophage expansion and fibrotic reactions (94). GM-CSF has been suggested to play a central role in promoting inflammation to aeroallergens (95) and GMCSF polymorphisms have been suggested as likely asthma determinants (96). In addition, transgenic GM-CSF overexpression in the mouse stomach led to the development of autoimmune gastritis (97). By comparison, mice treated with M-CSF every day for 4 days showed an increase in blood monocytes (98) and intra- peritoneal injection into mice increased the numbers of peritoneal macrophages. Additionally, it led to the development and differentiation of CD5+ macrophages in the peritoneal cavity (99). These findings support our notion that GM-CSF is critically involved in modulation of myeloid cells in inflammatory conditions than being required for steady state myelopoiesis. As a consequence of these modulatory functions on monocyte and macrophage populations, GM-CSF has been suggested to be a key cytokine in a variety of autoimmune diseases such as rheumatoid arthritis (RA), Crohn's disease and autoimmune gastritis (AIG) (100-102). RA is a chronic autoimmune inflammation of the synovial joints that leads finally to joint destruction and functional disability in patients (103). Several studies have shown the involvement of GM-CSF and macrophages in the pathogenesis and progression of RA (104, 105). GM-CSF as well as its receptors were detected in the tissues and synovial fluids of patients with RA (106). Further, the disease worsened following GM-CSF treatment (107, 108). In contrast, the inhibition of GM-CSF or its receptors has shown therapeutic benefit in RA patients (109, 110). In a collagen-induced arthritis model involving C57BL/6 mice, it has been reported that GM-CSF$^{-/-}$ mice showed no disease development (111). Interestingly, the humoral immune responses to collagen type II were not affected but there was a reduction in the delayed type hypersensitivity reaction. TNFα and IL-1β levels were also reduced in the joints of the affected GM-CSF$^{-/-}$ mice. In a monoarticular methylated BSA/IL-1 arthritis model it was found that the arthritis severity was lower in GM-CSF$^{-/-}$ mice than in controls (112). Other autoimmune models showed similar dependence on GM-CSF for the development of the disease e.g. glomerulonephritis (67), zymocel-induced hepatic granuloma formation (113) and EAE (114). It is believed that GM-CSF sustains inflammation by macrophages polarization into M1 inflammatory type (115).

Crohn's disease (CD) is an inflammatory bowel disease (IBD) affecting parts of the gastrointestinal tract (116). The common symptoms of CD include abdominal pain, diarrhea and weight loss; further, patients with CD are at risk of bowel cancer (116). Interestingly, GM-CSF administration was shown to have beneficial effects in CD (117). This therapeutic effect of GMCSF has been proposed to be through immune regulatory function of myeloid

populations. GMCSF mediated mucosal repair is believed to occur through the mobilization of CD11b+ monocytic population (10).

Similarly, administration of GM-CSF was found to ameliorate dextran sulfate sodium (DSS) induced colitis in mice (118). Additionally, GM-CSF$^{-/-}$ mice were more susceptible to the disease (119). In contrast, a neutralizing antibody against M-CSF inhibited DSS induced colitis in mice. These data indicate a disease promoting effect of M-CSF and a disease preventing role for GM-CSF (120). Since GM-CSF showed therapeutic effect for CD and experimental colitis, studies were conducted to evaluate if GM-CSF auto-antibodies could induce disease development. Indeed, GM-CSF autoantibodies have been identified as promoters of aggressive ilial CD (121). These results demonstrate a key role for GM-CSF as a regulatory cytokine in some autoimmune diseases such as CD through some activity outside its role as a CSF.

Multiple sclerosis (MS) and acute disseminated encephalomyelitis (ADEM) are inflammatory demyelinating autoimmune diseases of the central nervous system (CNS) (122-124). Studies have shown that both MS and EAE are mediated by Th1-type lymphocyte activation. GM-CSF has been identified as a candidate cytokine involved in EAE development. A role for GM-CSF in EAE is supported by the finding that mice deficient in GM-CSF were resistant to EAE induction following myelin oligodendrocyte glycoprotein (MOG) administration (114). The administration of GM-CSF in EAE setting or its over expression by pathogenic T cells can lead to a more severe disease (114, 125). GM-CSF is believed to affect EAE through the activation of antigen presenting cells (APCs) including microglial cells in the brain and spinal cord, as well as blood-derived macrophages and dendritic cells (126-128). The microglial cells in the healthy state are described as CD45^{low} with low levels of MHC class II (129). In contrast, perivascular macrophages showed an activated phenotype characterized by high levels of expression of both CD45 and MHC class-II (129, 130). Microglial cells were activated in the CNS, before the start of EAE, with increased infiltration of peripheral macrophages into CNS (131). These data suggested that EAE reactive T cells activate the microglial cells through the secretion of GM-CSF (132-134). Interestingly, deficiency of STAT5 in CD4+ T cells resulted in diminished development of EAE in mice (135). The loss of encephalitogenic activity of STAT5-deficient autoreactive CD4+ T cells was independent of IFN-γ or IL-17 production, but was a function of impaired expression of GM-CSF (135). It was further shown that the IL-7-STAT5 axis promotes the generation of GM-CSF/IL-3-producing CD4+ pathogenic cells (135). These results confirm the role of GM-CSF in EAE development and its production from CD4+ activated T cells is regulated by STAT5 signaling.

GM-CSF has also been implicated in Pulmonary Alveolar Proteinosis (PAP), a pulmonary autoimmune disorder. PAP is a potentially fatal lung disease occurring due to a defect in surfactant clearance by macrophages. Interestingly both GM-CSF$^{-/-}$ and GM-CSF receptor β subunit$^{-/-}$ mice exhibited alveolar proteinosis suggesting that GM-CSF is critical for alveolar macrophage maturation in mice (27, 80). The myeloid transcription factor, PU.1, a member of the ETS family of transcription factors, was found to mediate GM-CSF-dependent effects on alveolar macrophage differentiation (136). In humans, one form of congenital alveolar proteinosis has been shown to correlate with a defect in the expression of

the GM-CSF receptor β chain (137, 138). High levels of GM-CSF neutralizing antibody have been implicated in the acquired (idiopathic) pulmonary alveolar proteinosis (139); which can be reversed with GM-CSF (140). Thus, GM-CSF imparts either pro- or anti-inflammatory properties on monocyte-macrophage populations and help determine if they maintain, exacerbate or ameliorate autoimmune conditions.

Effect of GM-CSF on other cell populations

Lymphocytes generally do not express GM-CSF receptor (23). However in transgenic mice engineered to express a human GM-CSF receptor, it was found that administration of hGM-CSF was able to support the development of lymphocytes (141) suggesting that lymphocytes can respond to GM-CSF if they express its receptor. In addition, one study showed that GM-CSF may directly cause the expansion of Tregs (142). This study found that $Foxp3^+$ Tregs express the GM-Rα and proliferate in response to GM-CSF in the presence of other contact dependent stimuli. Further, ex vivo GM-CSF proliferated Tregs had higher suppressive capacity in preventing diabetes development than control Tregs as revealed in an adoptive transfer experiment (142).

Likewise, the expression of GM-CSF receptor on B-cells is controversial; however many studies have suggested that GM-CSF may directly influence B-cell phenotype and function including antibody production (143, 144). In one study it was demonstrated that B-cells can express both GM-CSF and its receptor and is even able to respond to GM-CSF in an autocrine manner (145). The implications of these data are not clear; it is possible that GM-CSF can modulate immune responses by regulating autoantibody production. In a recent study it was found that GM-CSF can expand IL-10 producing subset of $CD1d^{high}CD5^+$ B-cells (also called B10 cells) (146). A fusion protein comprising of GM-CSF and IL-15 has previously been shown to induce B10 cells in vitro (147). In a murine model of myasthenia gravis, these B10 cells were able to suppress Th1 type T-cell responses and prevent autoimmunity.

GM-CSF and dendritic cells in autoimmunity

The role of GM-CSF in dendritic cell development in human and mouse immune systems both *in vivo* and *ex vivo* was demonstrated almost two decades ago in a series of studies (148-151). Dendritic cells (DCs) play a pivotal role in regulating the adaptive immune response because they regulate T cell differentiation (152) by presenting antigens to naïve T cells (153, 154). While both GM-CSF and the cytokine Flt3L (Fms like tyrosine kinase 3 ligand) have been established as DC development factors ex vivo and *in vivo* (155-158), the role of GM-CSF in DC development is not clearly understood. Mice lacking GM-CSF or its receptor do not show a severe defect in DC development. In contrast Flt3L deficient mice are severely defective for all steady state DC populations (26, 159, 160). Two types of dendritic cells have been broadly defined; a) the lymphoid tissue DCs and b) migratory or tissue DCs (161-163). In the physiological steady state, lymphoid DCs, found in the spleen and other lymphoid organs consist primarily of $CD8\alpha^+$ and $CD8\alpha^-$ conventional DCs (cDCs) and $B220^+$ plasmacytoid DCs (pDCs). It has been proposed that BMDCs derived *ex vivo* with Flt3L (F-BMDCs) are phenotypical and functional equivalents of steady state DCs

(162, 164). In contrast, it is believed that *ex vivo* generated DCs derived with GM-CSF (G-BMDCs) do not have physiological counterparts in steady state.

GM-CSF treatment of wild type (WT) mice leads to increased DC numbers in spleen (7). This effect could be either due to a proliferative effect on DCs or a particular DC/precursor population. It is believed that DC development occurs through successive differentiation of the hematopoietic stem cell (HSC). Various precursors for different DC subtypes have been proposed; their capacity to become DCs is critically dependent on Flt3L. The Macrophage and DC precursor (MDP; $Lin^{-}c\text{-}kit^{hi}CD115^{+}CX3CR1^{-}Flt3^{+}$) can differentiate into lymphoid tissue resident conventional DCs (cDCs), plasmacytoid DCs (pDCs) and monocytes. MDPs are themselves are derived from the earlier myeloid precursor (MP). A later stage precursor, the common DC precursor (CDP; $Lin^{-}c\text{-}kit^{lo}CD115^{+}Flt3^{+}$) can differentiate into cDCs and pDCs. A further differentiated precursor called pre-CDC ($Lin^{-}CD11c^{+}MHCII^{+}SIRP\alpha^{int}Flt3^{+}$) can only generate cDCs (165). Thus, it is possible that GM-CSF merely acts as a growth factor for certain DC precursors *in vivo*, whose increased turnover leads to the observed increase in DC numbers after GM-CSF treatment. Alternatively, GM-CSF may drive the differentiation of a very specific subset of DCs *in vivo* that are phenotypically distinct from steady state DCs (164). It has been suggested that GM-CSF may be required for the development and differentiation of a special class of DCs resembling inflammatory DCs (iDCs: $CD11c^{+}CD11b^{+}MAC3^{+}$) possibly through the differentiation of $CD11b^{hi}Ly6c^{hi}$ precursors (162, 164) A potential precursor for inflammatory DCs (iDCs) (or Tip DCs; TNF-α, and inducible nitric oxide synthase secreting) (Fig. 1) has also been identified as $CD11b^{hi}Ly6c^{hi}$ monocyte precursor (162, 164).

GM-CSF can direct the differentiation of DCs from CCR2+ monocyte precursors (monocyte derived DCs: moDCs) that can promote autoimmunity through TH17 cells (166). TH17 cells are known to be involved in the pathogenesis of autoimmune reactions in both humans and mice (167, 168). It was demonstrated in an EAE mouse model that inflammatory stimuli can differentiate monocytes into Cd11c+MHXCII+ moDCs that secrete high levels of IL-1b and IL-6, leading to maintenance and differentiation of TH17 cells. MoDCs were found to be more potent in TH17 polarization than conventional DCs (cDCs). Further, GM-CSF was found to be a critical factor influencing the capacity of moDCs to promote TH17 differentiation. TH17 cells in turn secreted GM-CSF and created a feedback loop that sustained autoimmunity (166).

GM-CSF mobilized DCs have been implicated in the development and maintenance of Experimental Autoimmune Myocarditis (EAC) an autoimmune disease affecting the cardiac tissue (102, 169). It has been demonstrated that GM-CSF can act on two distinct monocyte lineage cells, CD133+ and CD11b+ to differentiate them into inflammatory moDCs. It is speculated that autoreactive TH17 cells infiltrate cardiac tissue and secrete GM-CSF causing either differentiation of inflammatory moDCs within the inflamed heart or migration of moDCs from the bloodstream (170).

In sharp contrast, we and others have used low-dose GM-CSF to modulate DC differentiation *in vivo* and effectively treat several experimental autoimmune diseases like

Experimental Autoimmune Thyroiditis (EAT), Experimental Autoimmune Myasthenia Gravis (EAMG) and Type 1 Diabetes (T1D) (7-9). Our studies showed that GM-CSF can modulate the phenotype of CD8a- DC into a semi-mature state characterized by the increased expression of co-stimulatory molecules like CD80/86 but accompanied by low expression of pro-inflammatory cytokines like IL-1b and IL-12 (171, 172). These GM-CSF modulated tolerogenic DCs led to the mobilization of IL-10 secreting regulatory T cells (Tregs) which were responsible for disease suppression (171, 172). A similar protective effect of GM-CSF has also been shown in inflammatory bowel disease (IBD) and Type 1 diabetes (T1D) (10, 173).

We have used GM-CSF derived bone marrow DCs (G-BMDCs) as an *ex vivo* model to explore the mechanism behind this GM-CSF mediated tolerance restoration/induction (Fig 2). Our studies revealed at least two different mechanisms by which GM-CSF modulates DC function to mobilize Tregs. In the first, G-BMDCs caused proliferation of Foxp3+ natural regulatory T-cells (nTregs) upon direct contact (174, 175). G-BMDC mediated proliferation of nTreg occurred through a non-canonical TCR-independent mechanism. We have shown that GBMDCs expressed two ligands critical for this function: a) the TNF-family ligand OX40L and b) the notch family ligand Jagged-1. Co-signaling initiated by OX40L/jagged-1 through their cognate receptors OX40 which is constitutively expressed on $Foxp3^+$ Tregs (176, 177) and Notch3 which is preferentially expressed on Foxp3 Tregs (178) induced nTreg proliferation in an IL-2 dependent manner (174, 175). Further, we showed that G-BMDCs could also induce adaptive (or induced) Tregs (iTregs) upon TCR ligation. G-BMDCs express high levels of TGF-β which facilitates adaptive conversion of CD4+Foxp3- T cells to become CD4+ Foxp3+ iTregs by a TCR dependent mechanism (174). We believe that the ex vivo model of G-BMDCs represents a modulatory effect of GM-CSF on DCs in physiology that enhances immunological tolerance through the Treg axis.

Echoing these observations, one study found a protective effect of GM-CSF in a low dose streptozotocin (STZ) induced model of type-1 Diabetes (179). Using a human insulin promoter to drive murine GM-CSF expression in a transgenic mouse model, it was first observed that islet specific expression of GM-CSF led to higher infiltration of myeloid cells in the pancreas, predominantly consisting of F4/80+ macrophages. This was also accompanied by increase in CD11b+Cd11c+ cells in the spleen, a phenotype common to G-BMDCs (174). Periinsulitis characterized by lymphocytic infiltration was also observed in these ins-GM-CSF transgenic mice; however they were resistant to the development of hyperglycemia upon STZ treatment (179). It is speculated that the altered activation of these GM-CSF modulated myeloid cells have tolerogenic influence on T-cell activation thereby providing a protective effect.

Myeloid-derived suppressor cells (MDSCs) of granulocytic (G-MDSC-$CD11b^+Ly6C^{lo/int}Ly6G^{hi}$) and monocytic (M-MDSC-$CD11b^+Ly6C^{hi}$) phenotype are identified as myeloid progenitor cells that exhibit immunosuppressive properties (180). MDSCs have been shown to regulate immune responses during autoimmune conditions (181, 182). It has been suggested that MDSCs suppress T cell activity through the secretion of inducible nitric oxide synthase (iNOS) and arginase-1 (182). GM-CSF has been shown to not only promote the development of MDSCs from bone marrow precursors (183) but also

determine their suppressive capacities (184). Combinations of GM-CSF and G-CSF have been used in studies to generate MDSCs from bone marrow cultures. In one study, human MDSCs derived from umbilical cord precursors with GM-CSF and G-CSF was able to prevent the development of diabetes in a xenogeneic mouse model (185). These MDSCs mobilized Tregs through IDO secretion and consequently suppressed CD8+ T-cell proliferation. In another study, heterogeneous populations of MDSCs generated from bone marrow precursors with GM-CSF, G-CSF and IL-6 suppressed autoimmunity in a proteoglycan induced model of arthritis. These cells suppressed T-cell proliferation through the production of nitric oxide (186).

In summary, GM-CSF appears to act as a growth and differentiation factor for different types of non-steady state DCs that are capable of either causing or ameliorating autoimmune conditions.

Conclusion

Due to its role as a CSF, recombinant human GM-CSF (rhGM-CSF) has been primarily considered for the amelioration of acute and chronic states of neutropenia, including facilitation of bone marrow and stem cell transplantation (187-189). However, as literature suggests, GMCSF exhibits pleiotropic effects on several different cell types (summarized in Table-1). Based on this evidence, it is likely that although GM-CSF primarily induces myeloproliferation, its role in the steady state maintenance of myeloid cell populations is redundant. The cytokines G-CSF, M-CSF and Flt3L play non-redundant roles in the physiological steady state for maintenance of the granulocytic, monocytic and dendritic cell populations respectively (Fig 1). In contrast GMCSF plays a critical role under inflammatory or immunomodulatory conditions by inducing specialized cell types from precursors or by influencing phenotypes of mature cell populations. Thus GM-CSF exerts a profound effect on the state of immune tolerance as evident from studies involving a wide array of autoimmune conditions.

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Highlights

1. GM-CSF is an established growth and differentiation factor for multiple cell lineages.
2. The role of GM-CSF in steady state myelopoiesis appears to be redundant.
3. GM-CSF has an additional role in the modulation of myeloid cell functions.
4. Immune modulatory role of GM-CSF determines the outcome of many autoimmune diseases.
5. We review literature to discuss of the role of GM-CSF in immune tolerance.

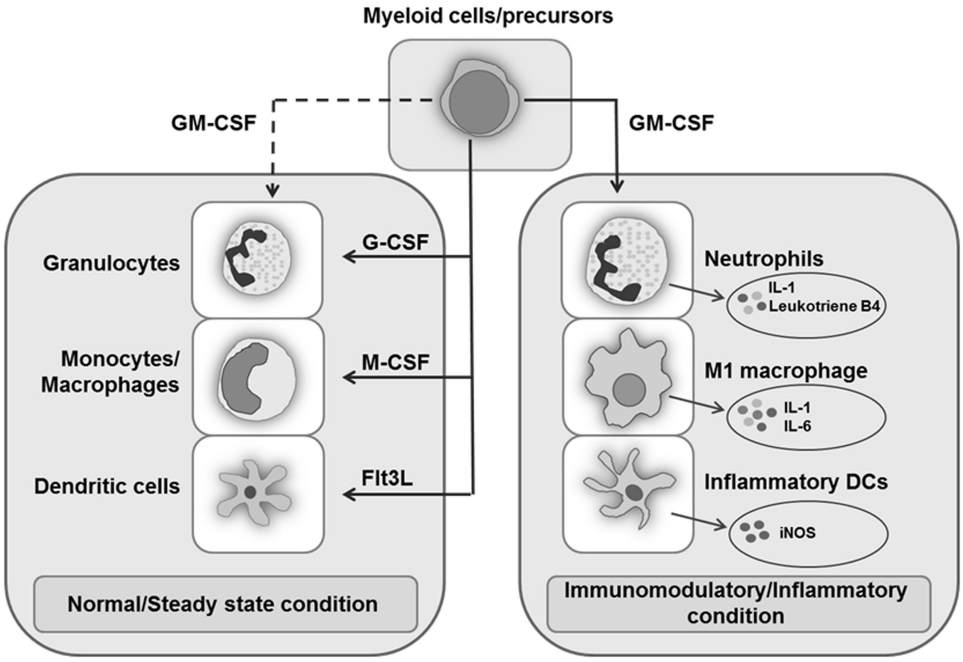

Fig. 1. The two distinct roles of GM-CSF: myelopoiesis and immune modulation

In the physiological steady state, granulocyte, monocyte/macrophage and dendritic cell populations are maintained by G-CSF, M-CSF and Flt3L respectively in a non-redundant manner. While GM-CSF is capable of myeolopoiesis, under inflammatory conditions it can modulate the phenotype of myeloid precursors or mature myeloid cells that determine the balance between tolerance and autoimmunity.

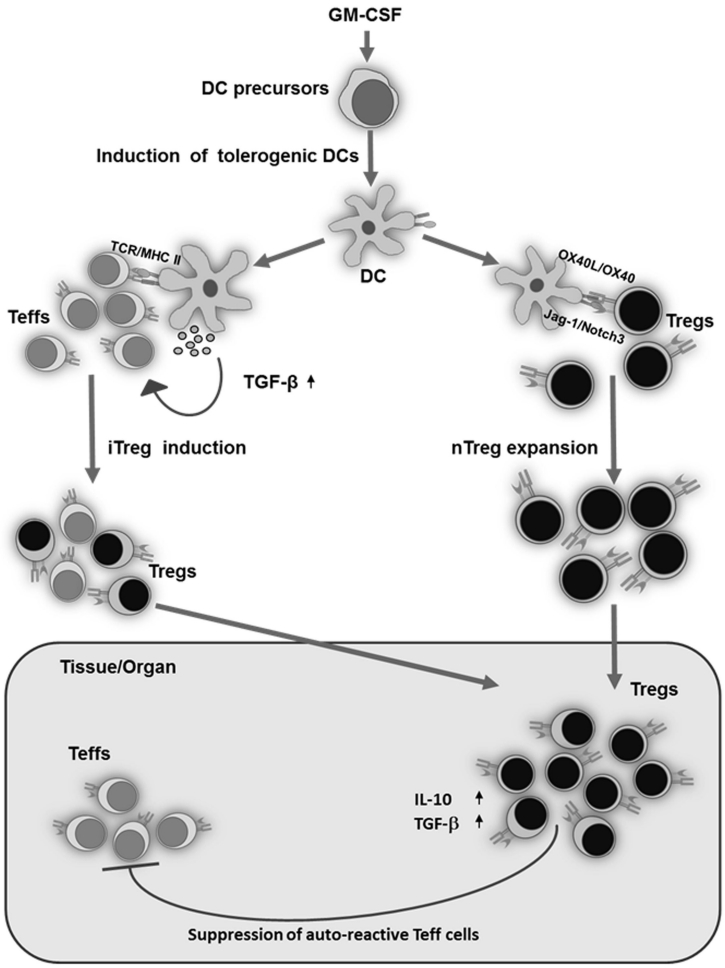

Fig. 2. GM-CSF induces tolerance through the mobilization of Tregs

GM-CSF differentiates bone marrow precursosrs into tolerogenic dendritic cells that can 1) induce adaptive Tregs through secretion of TGF-β and 2) cause proliferation of natural Tregs through co-signaling by OX40L and Jagged-1.

Table-1
GM-CSF in inflammation and autoimmunity

Role of GM-CSF in different autoimmune diseases	Main responsive cell type	Proposed Mechanism
GM-CSF administration exacerbates rheumatoid arthritis (RA) (108). Inhibition of GM-CSF function shows therapeutic benefits (109, 110).	Macrophages	Multiple possibilities have been suggested: polarization of macrophages into M1 inflammatory type (115), increasing numbers of infiltrating cells in the joints (112).
GM-CSF administration ameliorates Crohn's disease (CD) (117).	Monocytes	GM-CSF improves intestinal barrier integrity by augmenting innate immune responses (117). This may occur through immunoregulatory function of GM-CSF responsive CD11b monocyte (10).
Administration of GM-CSF exacerbates experimental autoimmune encephalomyelitis (EAE) (114, 125).	Macrophage/microglia/ Dendritic cells (DCs) (Indirect effect on CD4+ T cells)	GM-CSF may sustain EAE by promoting inflammatory activity by antigen presenting cells including macrophages and microglia (126-128). Further, GM-CSF secretion by T-cells lead to microglia activation in EAE (132). GM-CSF can also maintain EAE by sustaining autoimmune TH17 cells Indirectly through secretion of IL-6 by monocyte derived DCs (166).
GM-CSF administration ameliorates acquired pulmonary alveolar proteinosis (PAP) (139).	Macrophages	GM-CSF appears to be critical in the development of alveolar macrophages which are necessary for clearance of surfactant phospholipids and proteins (27, 80).
GM-CSF deficiency negatively affects the development of glomerulonephritis in mice (67).	Macrophages	This occurs possibly due to reduced macrophage activation as a consequence of the inability of T-cells to produce GM-CSF (67).
Maintains/sustains experimental autoimmune myocarditis (102).	Dendritic cells	Suggested mechanisms include mobilization of inflammatory monocyte derived DCs that infiltrate cardiac tissue (170) and indirect activation of autoantigen specific TH17 cells through increased secretion of IL-6 and IL-17 by GM-CSF stimulated dendritic cells (102).
Low-dose GM-CSF administration ameliorates experimental autoimmune thyroiditis (EAT) (7); experimental autoimmune myasthenia gravis (EAMG) (8) and type 1 diabetes (T1D) (9).	Dendritic cells, Indirectly on regulatory T-cells (Tregs)	GM-CSF differentiates precursor cells into tolerogenic DCs that can expand both natural and adaptive Tregs (174, 175). These Tregs suppress autoimmune Teffector responses through secretion of IL-10 (171).
GM-CSF administration has a protective effect against DSS induced colitis (10).	Dendritic cells, other myeloid cells	GM-CSF differentiates bone marrow cells into myeloid lineage CD11b+ cells and CD11b+CD11c+ DCs that contribute to the protective effect by inducing tissue repair (10).
Administration of GM-CSF ameliorates neutropenia in Felty's syndrome patients (but exacerbates concomitant RA) (69).	Neutrophils, other cells	GM-CSF ameliorates neutropenia through increased neutrophil survival, growth and differentiation but possibly worsens concomitant RA through the induction of IL-6 secretion by RA synovial cells (69).
GM-CSF treatment exacerbates autoantibody production in experimental epidermolysis bullosa acquisita (EBA) (63).	Neutrophils	It has been proposed that this could be due to neutrophil involvement in augmenting T cell– dependent production of antibodies (63).
Adoptive transfer of GM-CSF expanded Tregs suppressed diabetes in mouse models (142).	Tregs	It has been suggested that GM-CSF can directly expand Tregs which express the GM-Rα (142).
Administration of GM-CSF suppresses EAMG (146).	B cells	B-cells have been reported to express functional GM-CSF receptor (145). GM-CSF has been found to induce $CD1d^{high}CD5^{+}$ B-cells that suppress EAMG through secretion of IL-10 (146).