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G-CSF: A Friend or Foe?

Ramya Sivakumar, Mark A Atkinson, Clayton E Mathews and Laurence Morel*

Department of Pathology, Immunology and Laboratory Medicine University of Florida, Gainesville, FL 32610, USA

***Corresponding author:** Dr. Laurence Morel, Department of Pathology, University of Florida, 1395 Center Drive, Gainesville, FL 32610-0275, Tel: (352) 273-5638; Fax: (352) 392-3053; E-mail: morel@ufl.edu

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Abstract

Granulocyte colony stimulating factor (G-CSF) is an essential cytokine frequently used in clinics to restore myelopoiesis and facilitate peripheral mobilization of hematopoietic stem cells. Endogenously secreted G-CSF acts as a pleiotropic growth factor and mediates its biological functions [granulopoiesis] by binding specifically to granulocyte colony stimulating factor receptor (G-CSF-R). The G-CSF-G-CSF-R pathway known for its pro-Th2 and anti- inflammatory properties has been successful in reversing the course of diseases such as type 1 diabetes (T1D) and myelin basic protein-induced experimental autoimmune encephalomyelitis (MOG-EAE). The promising benefits of G-CSF have helped in establishing it as a successful candidate for several clinical trials. Even though cytokine based immune intervention offers a highly feasible and attractive option for immune disease control, caution needs to exerted on its usage as the functions of most cytokines vary depending upon the disease where they are applied, the research model being tested, as well as the modality of treatment (e.g., dose, duration and route) and, G-CSF despite its beneficial anti- inflammatory properties has also exerted bifurcated roles depending upon the disorder and dosage in which it's applied.

This review aims to summarize the findings associated with the function and role of granulocyte colony-stimulating factor (G-CSF) and its dichotomous role in immune related therapies. Here we specifically focus on SLE and T1D, two autoimmune disorders in which G-CSF may exert physiological effects in opposite directions.

Keywords: G-CSF; G-CSFR; Systemic lupus erythematosus; Type 1 diabetes; Autoimmunity; Cytokines; Immune regulation

Introduction

Cytokine based therapies have long been considered as a potential means for treating several disorders of the immune system including cancer [1-5], Crohn's disease, multiple sclerosis (MS), rheumatoid arthritis (RA) [6-9], systemic lupus erythematosus (SLE), and type 1 diabetes (T1D) [10,11]. The advent of recombinant technology eased longstanding concerns regarding their production and hence, the availability of these small, short-term growth factors. As a result, cytokines now represent a highly feasible and attractive option for immune disease control. Indeed, translational and clinical investigators are actively pursuing the prospect for including these natural immune mediators as a part of a variety of therapeutic based approaches, including testing of combination cytokine-based strategies [12-15]. The goal of most such efforts, at least those involving autoimmunity, is to induce immunological tolerance through a restoration of appropriate immune regulatory mechanisms [10,16]. A complicating factor is that the functions of most cytokines vary depending upon the disease where they are applied, the research model being tested, as well as the modality of treatment (e.g., dose, duration and route). This review summarizes the findings associated with the function and role of one cytokine receiving much attention for its potential in immune related therapies, granulocyte colony-stimulating factor (G-CSF). Here we specifically focus on SLE and T1D, two autoimmune disorders in which G-CSF may exert physiological effects in opposite directions.

G-CSF and G-CSF Receptor Expression and Functions

G-CSF is a hematopoietic growth factor used widely in clinics to restore granulopoiesis during therapeutic or disease-induced myelosuppression [1-3]. G-CSF binds specifically to the granulocyte colony stimulating factor receptor (G-CSF-R; also designated CD114, encoded by the Csf3r gene) to promote the survival, proliferation, activation, and terminal differentiation of mature neutrophilic granulocytes from their precursors in the bone marrow (BM) [17,18].

Marked diversity exists amongst the range of tissues and cells involved with the production and expression of G-CSF and its receptor. Apart from cells of monocyte/macrophage origin, which are considered to be its primary sources, endothelial cells, fibroblasts, and even cells of mesodermal origin have been included in the list of G-CSF producers [4]. Neutrophils and their precursors bear the maximum density of G-CSF-R, which is also expressed on myeloid progenitor cells, platelets, monocytes, and hematopoietic stem cells. Among lymphocytes, G-CSF-R expression is considered to be constitutive on B cells and inducible on T cells [19]. In addition, G-CSF-R expression has been identified in various non-hematopoietic tissues and cell lines such as human placenta and small cell lung cancer, among others [1,4]

The G-CSF receptor is a single polypeptide chain composed of three domains: an extracellular, a single transmembrane and a small intracellular. The extracellular portion of the receptor contains a conserved cytokine receptor homologous (CRH) domain, an Ig like domain and three fibronectin type III like domains. The Ig and CRH portions have been predicted to be associated with ligand binding whereas the three fibronectin domains likely confer stability and proper signaling for the receptor [20]. Despite lacking any intrinsic kinase activity, the intracellular portion of the receptor, composed of

conserved regions (Boxes 1, 2 and 3) and four tyrosine residues, serves as the signal transduction domain of the receptor [20].

Ligand binding induces homodimerization of the receptor and phosphorylation of its four intracellular tyrosine residues by non-receptor tyrosine kinases including Jak1, Jak2, Tyk2, Lyn, Syk and Hck [21]. As a result of the multiple cytosolic tyrosine kinases that undergo activation upon G-CSF stimulation, G-CSF-R signals through several pathways including JAK-STAT, RAS-RAF-MAP-ERK and PI3K-AKT. The specific function of each of these pathways relative to G-CSF-R activation is not well understood. However, several early studies have helped in determining that Jak1 along with Tyk2 are essential for receptor tyrosine phosphorylation and STAT activation following G-CSF stimulation. Jak1 plays a non-redundant role in G-CSF signaling and the JAK-STAT pathway associated STAT3 and SOCS3 play specific roles in G-CSF driven granulopoiesis. SOCS3 is an integral G-CSF/STAT3 target gene that controls the magnitude and duration of G-CSF-R signaling, thereby regulating the synthesis of bone marrow derived neutrophils. Therefore, STAT3 negatively regulates the terminal stages of bone marrow granulopoiesis and circulating neutrophil levels through SOCS3 dependent and independent mechanisms [22].

The SRC kinase LYN phosphorylates a variety of adaptor molecules, such as GAB2, SHC, CBL, as well as enzymes such as SHP-1, SHP-2, and SHIP-1. LYN plays a major role in G-CSF mediated cell proliferation, survival and metabolism, as well as in the induction G-CSF primed pro-inflammatory responses in neutrophils [23-25]. Phagocytic cells from Src-deficient ($Hck^{-/-}$, $Fgr^{-/-}$ or $Hck^{-/-}Fgr^{-/-}Lyn^{-/-}$ triple knock-out) mice are defective in superoxide production, degranulation, or migration [26-29]. Moreover, the SRC kinase associated AKT pathway apart, from promoting differentiation, also generates reactive oxygen species [23]. Thus different tyrosine kinases are activated following G-CSF stimulation depending upon the type of biological response summoned.

G-CSF and its receptor are essential for regulating granulopoiesis under both basal and emergent circumstances [22]. Deficiency or disruption of the cytokine or its receptor results in several complications in both mice and humans. Mice lacking G-CSF exhibit approximately 20% of normal circulating neutrophils and display a diminished ability to counter infection from Listeria monocytogenes, primarily due to a reduction in infection driven granulopoiesis [30]. Mice lacking G-CSF-R also have impaired production of neutrophils that exhibit an increased susceptibility to apoptosis [30,31]. In humans, several mutations in the intracellular region of G-CSF-R that affect myeloid maturation have been reported in congenital neutropenia and acute myeloid leukemia patients [32-36], thus shedding light on the prominent role of G-CSF-R mutations within myeloid disorders. As these mutations primarily truncate the C terminal cytoplasmic region of the receptor, understanding the role of each tyrosine and their associated pathway downstream of G-CSF-R can help in designing better and effective intervening strategies for these debilitating disorders.

Clinical Use of G-CSF

G-CSF has been used for almost two decades for the treatment of congenital and acquired neutropenia, as well as for reducing febrile neutropenia, either prior to or during intensive cytoreductive chemotherapy in cancer patients [37]. G-CSF was initially evaluated in phase I clinical trials for cancer patients to increase their neutrophil numbers. As it was well tolerated and potent in action [38-40], subsequent trials showed its efficacy as an adjunct to chemotherapeutic agents as well as a separate granulocytic agent. The capacity of G-CSF to thwart neuronal degeneration and to drive neurogenesis in acute ischemia has also led investigators to consider it as a promising drug for stroke, as well as degenerative and autoimmune diseases of the brain [41,42]. In addition, G-CSF has recently gained prominence for its role in the mobilization and transplantation of peripheral blood stem cells (PBSC), which represent a majority of allogenic stem cell transplants [43]. The beneficial effects of G-CSF have also been utilized in the treatment of autoimmune diseases, including MS and T1D in combination with anti-thymocyte globulin (ATG), where it has been shown to deliver long-term benefits through immune regulation [5,12,13,44], as it will be further discussed below.

G-CSF in the Adaptive Immune System

G-CSF has received attention for its role as an immune regulator and modulator of adaptive immunity [41], with G-CSF treatment almost being synonymous with the induction of anti-inflammatory responses. G-CSF plays an immunosuppressive role on T cells, either directly or indirectly, as the expression of G-CSF-R on T cells is controversial [4,45]. Specifically, early studies were unable to detect G-CSF-R gene expression or binding to 125I-G-CSF by normal T cells, leading to the conclusion that T cells do not express G-CSF-R [4,46,47]. This view was later challenged when the expression of G-CSF-R was detected by single-cell RT-PCR on human $CD4^+$ and $CD8^+$ T cells after in vivo and in vitro exposure to G-CSF, suggesting that G-CSF-R expression is inducible on T cells exposed to its ligand [45]. In both mice and humans, G-CSF preferentially polarizes T cells from a Th1 to Th2 phenotype by altering cytokine production [45,48]. G-CSF also decreases T cell proliferation to both mitogens and alloantigens and reduces their cytotoxic activity [49].

Pretreatment with G-CSF protected mice against T cell-mediated bacterial superantigen shock by causing a systemic suppression of IL-2 production [50] and against a lethal dose of lipopolysaccharide (LPS) by reducing serum TNF-α levels [51]. T cells obtained from G-CSF-treated mice also secreted reduced levels of IL-2 and IFN-γ and elevated levels of IL-4 in response to an in vitro challenge with LPS [49], confirming the suppressive role of G-CSF on inflammatory cytokines. In experimental models of acute graft vs. host disease (aGVHD), G-CSF pre-treatment of donor mice reduced the severity of aGVHD and improved the survival of recipient mice[49]. A similar result was observed when PBSCs mobilized with G-CSF were transplanted in place of conventional BM stem cells. Although PBSCs produced a log more T cells than conventional BM stem cells, their mobilization using G-CSF helped with early engraftment and reconstitution of myeloid cells [52]. The selective mobilization of type 2 dendritic cells (DCs) and induced polarization of donor T cells towards the Th2 phenotype (with IL-4 and IL-10 secretion) have been attributed with attenuated allogenic responses in aGVHD [49,53].

G-CSF has also been instrumental in altering the inflammatory response in human studies. G-CSF treatment of healthy human volunteers modified the ex-vivo response from their whole blood cells to stimulants such as lipoteichoic acid, phytohemagglutinin or LPS, by reducing their capacity to secrete pro-inflammatory cytokines (TNF-α, GM-CSF, IFN-γ and IL-12) and by increasing the production of soluble TNF receptors and IL-1 receptor antagonist (IL-1ra) [54,55]. In vitro treatment with G-CSF also down regulated the responses of

peripheral blood mononuclear cells (PBMC) to allogenic Daudi cells by inhibiting their secretion of TNF-α [56]. In accordance with the anti-inflammatory role of G-CSF, a spontaneous increase of IL-4 and reduction of IFN-γ were observed in PBSCs after G-CSF administration [45]. Moreover, $CD4^+$ T cells obtained from healthy human volunteers primed in vivo with G-CSF secreted elevated levels of IL-10 and TGF-β in response to in vitro alloantigen stimulation and acquired the properties of T regulatory cells (Tr1), which suppressed allogenic T cell responses [57]. Finally, G-CSF has been used to mobilize human BM regulatory T cells (Tregs) by reducing the expression of CXCL12, thus favoring egress over retention within the BM niche [58] .

Several studies have investigated whether G-CSF modulates immune responses through monocytes, since these cells express G-CSF-R [46]. G-CSF-treated monocytes - inhibited-T cell receptor-mediated T cell proliferation, while the effect was abrogated by addition of neutralizing IL-10 antibodies [59]. As a parallel to experimental murine aGVHD studies using G-CSF [49], in vitro experiments were carried out using cells obtained from humans treated with G-CSF. G-CSF-primed monocytes reduced T cell alloreactivity in mixed leukocyte reactions and this suppression was identified as an indirect modulation of T cells by G-CSF through monocytes [60]. G-CSF has also been shown to mediate the conversion of monocyte-derived DCs into regulatory DCs using Tr1 cytokines such as IL-10 and IFN-α. These regulatory DCs functioned as poor allo-stimulators, express altered levels of co-stimulatory molecules and are impaired in their inability to secrete IL-12p70. In addition, these regulatory DCs converted naïve CD^{4+} T cells into TGF-β and IL-10 secreting Treg cells [61]. G-CSF treatment also expanded a murine granulocyte myeloid precursor population with GVHD suppressive functions [62], thus elucidating the interplay between various cell types involved in G-CSF mediated immunomodulation.

Immunoregulatory Role of G-CSF in Autoimmune Diseases

The immunoregulatory role of G-CSF in adaptive immunity has been tested in several autoimmune disease models. G-CSF treatment conferred protection in T cell-mediated autoimmune diseases such as MS and T1D. A 7-d long administration of a 200ug/kg/d dose of G-CSF starting at the onset of clinical symptoms conferred a substantial protection against myelin basic protein-induced experimental autoimmune encephalomyelitis (EAE, a model for MS) in SJL mice [5]. The significant reduction in demyelination was accompanied by a reduced recruitment of T cells to the central nervous system. The G-CSF treatment also induced an imbalance in chemokine production from macrophages and a deviation of T cell response to a Th2 phenotype. G-CSF treatment protected C57BL/6 mice from MOG-induced EAE in a similar fashion [6].

Similarly, a 200 ug/kg daily dose of G-CSF for 5 weeks protected NOD mice against cyclophosphamide (CY) accelerated T1D (Table 1). G-CSF reversed the disease by preventing the loss of $CD4^+CD25^+$ Treg cells and by accelerating the recovery of CY-depleted T cells. Furthermore, G-CSF negated the effects of IFNγ and the chemokine burst triggered by the CY treatment [44]. Monotherapy of NOD mice with G-CSF from 4 to16 weeks of age reversed the incidence of spontaneous T1D and protected the mice from destructive insulitis. Protection was attributed to the recruitment of immature CD11clo B220+ plasmacytoid DCs (pDC) and $CD4^+CD25^+$ Treg cells. TGF-β secreted by these Treg cells was instrumental in suppressing diabetes transfer through diabetogenic effector T cells in NOD-SCID recipients [12]. However, the modest difference in Treg cell numbers suggests that other regulatory mechanisms may also participate. The impact of myeloid derived suppressor cells (MDSC) was explored upon treatment with G-CSF in NOD mice. Treated mice exhibited an increase in both the Ly6G+ granulocytic MDSCs (gMDSC) and the Ly6C+ monocytic MDSC (mMDSC) in accord with a report demonstrating that adoptive transfer of MDSCs could reduce T1D incidence in NOD models [63]. The above finding suggests a novel role for G-CSF mediated MDSCs in regulating T1D. A combination therapy of G-CSF with ATG was also successful in reversing new-onset diabetes in NOD mice [13]. A recent clinical trial testing this combination therapy met the primary endpoint of preserved c-peptide at 12 months, suggesting that G-CSF, when combined with a T lymphocyte depleting therapy, can provide beneficial therapeutic effects in patients with T1D [64].

To summarize G-CSF reduces autoimmune inflammation in T1D and MS models by inducing tolerogenic DCs, Treg cells and MDSCs, inhibiting T cell activation and proliferation, as well as by inhibiting pro- inflammatory cytokines.

Pro-inflammatory Role of G-CSF in Autoimmune Diseases

Although the above studies argue in favor of anti-inflammatory clinical benefits of G-CSF, this cytokine plays a pro-inflammatory role in autoimmune diseases such as SLE and RA [7,65]. Neutralization of endogenous G-CSF significantly reduced the severity of collagen-induced arthritis in the mouse and was proven equally effective as treatment with a TNF inhibitor. The reduced disease severity was associated with a blunted mobilization of granulocytic cells from the BM to the inflamed joints and accompanied by lesser cellular infiltrate and cellular activation from the joints [8]. Similarly, G-CSF treatment induced flares in a dose-dependent manner in patients suffering from RA [9]. To the contrary, G-CSF treatment lowered disease severity in rats with adjuvant arthritis [66]. These contrasting results suggest that alternate mechanisms can be deployed by this cytokine for the same disease in different systems.

Dosage seems to play a role in the dual effects of G-CSF (Table 1). G-CSF administered in chronically low doses (10 ug/kg) accelerated lupus severity in MRL/lpr mice in spite of a polarized Th2 phenotype. In contrast, a high dose regimen of G-CSF (200 ug/kg) prevented nephritis, considered as the end stage of lupus disease in mice [67]. We have also reported a link between G-CSF and lupus pathogenesis using the bm12-induced chronic graft vs. host disease (cGVHD) model of T-cell mediated systemic autoimmunity [68]. Sle2c2 is a suppressor locus in the NZM2410 lupus-prone mouse. Congenic mapping narrowed down Csf3r as the primary candidate gene for Sle2c2, and the NZM2410 allele of Csf3r (Csf3rN as opposed to the wild type B6 allele Csf3rS) carries a polymorphism in exon 10 (rs13477964) that results in a S378N change in its extracellular domain [68]. Mixed BM chimeras and functional assays identified non-B, non-T BM derived cells as the primary cells mediating this suppression, suggesting that Csf3rN-expressing myeloid cells are responsible for Sle2c2 suppression [69]. Induction of cGVHD was blunted in mice carrying the Sle2c2 locus. We hypothesized that Sle2c2 suppression is mediated by defective G-CSFR signaling, predicting that G-CSF therapy would exacerbate autoimmune responses. In support of this hypothesis, treatment of Sle2c2 congenic mice with G-CSF broke their resistance to bm12-cGVHD in a dose-dependent manner [68]. In addition, a few clinical

reports have associated G-CSF treatment of neutropenic SLE patients with flares [70,71], suggesting that a partial inhibition of the G-CSF pathway could prevent flares in SLE patients. Overall, these studies indicate that G-CSF plays a dichotomous role, by acting as a friend promoting tolerance in T1D and EAE, but as a foe in SLE and RA by inducing pro-inflammatory responses. Since G-CSF dosage seems to play an important role in mediating these opposite functions, G-CSF-R signaling strength and kinetics could contribute towards to the dichotomy of G-CSF treatments. In addition, studies focusing on the responses of G-CSF-R expressing cell subsets to G-CSF could provide important clues regarding the mechanisms employed by G-CSF in these disorders.

Agent	Dose	Subjects	Outcome	References
T1D				
Neupogen	200 ug/kg/d for 5 wks	Male NOD mice	Protected from CY-accelerated T1D by expanding Treg cells and abrogating CY-mediated cytokine and chemokine burst	[44]
Neupogen	200 ug/kg/d for 5 d	Female NOD mice	Recruits immature pDCs and functional Treg cells and suppresses diabetes transfer in NOD-SCID recipients	[12]
Neupogen + ATG	6 ug/d for 8 wks	Female NOD mice	Combination therapy reversed new onset diabetes and improved glucose control overtime with attenuation of pancreatic inflammation and increased CD4/CD8 ratio and splenic Treg cell numbers	[13]
Neulasta	1 mg/kg single dose	Female NOD mice	Protected against T1D by inducing CD8α(-) DCs to recruit Treg cells	[98]
rh-G-CSF	600 ug/d for 5 d	T1D patients	Improved lower limb pain and ulcers	[99]
Neulasta + ATG	6 mg every 2 wks for 6 treatments	T1D patients	Combination therapy preserved β cell function in T1D established patients	[64]
SLE				
Neupogen	10 ug/kg for 5 d for 6 weeks	MRL-lpr/lpr mice	Accelerated disease	[67]
Neupogen	200 ug/kg for 5 d for 6 wks or 200 ug/kg for 5 d at 13 wks of age	MRL-lpr/lpr mice	Reduced disease	[67]
Neulasta	12 ug 3 times per wk	B6.Sle2c2 mice	Restored induced autoimmunity in lupus resistant mice	[68]
rh-G-CSF	12 cycles of 48 U /d for 6 d	SLE patients	Reduced neutropenia but induces flares in 3 of 9 patients	[100]
rh-G-CSF	1ng/ml- in-vitro culture	Neutrophils from SLE patients and healthy controls	Neutrophils from SLE patients displayed resistance to apoptosis- inhibiting effects of G-CSF ; neutropenic patients displayed highest resistance	[101]
Neupogen	variable	Neutropenic SLE patient	Induced lupus severe flares in 2/18 patients and mild flares in 4/18 patients	[71]
rh-G-CSF	NA	Neutropenic SLE patient	Cutaneous flare	[70]

Table 1: G-CSF treatment studies in T1D and SLE.

Neutrophils in T1D and SLE

Neutrophils, being the primary target of G-CSF pathway, have recently gained prominence both in the innate and adaptive arms of the immune system [65,72,73]. Here we review the various roles played by neutrophils in T1D, SLE, and EAE, [65,74,75] as well as explain how G-CSF might act in opposite directions in these autoimmune diseases.

At disease onset, T1D patients present with low circulating numbers of neutrophils [75,76]. The mild neutropenia generally observed in the preclinical phase of the disease persists for a few years after disease onset but eventually resolves in the later phase of the disease. Neutrophils in patients with T1D are also considered to be hyporesponsive and exhibit lower levels of oxidative metabolism in comparison with those isolated from healthy individuals [77]. The reduced activity of neutrophils has been demonstrated to be a function of hyperglycemia as decreased degranulation has been exhibited in patients with diabetes lacking tight glycemia control, and can be modeled using glucose infusions [78]. The reduction of neutrophil numbers in these studies was not a singular phenomenon as basophils, eosinophils, monocytes, and total white blood cell counts were reduced at onset [80], suggesting that a stem-cell "Mobilopathy" may be present in T1D and be a root cause of the lower white blood cell counts [79,81]. Indeed, treatment of the NOD mouse model of spontaneous T1D with G-CSF from early in life resulted in increased immunoregulatory potential [44] increasing both Treg cell numbers as well as MDSCs [13]. The fact that G-CSF treatment increases neutrophil numbers while protecting NOD mice from T1D runs counter to the idea that neutrophils are pathogenic. The postulate that neutrophils are not necessary for T1D initiation in the NOD mouse

model was bolstered by our data demonstrating that long-term depletion of neutrophils using an anti-Ly6G antibody had no impact on diabetes development [82]. These data suggest that G-CSF therapy could promote enhanced mobilization of immature immunoregulatory cells and in doing so induce a regulatory environment that dampens the autoimmune assault on the beta cells.

In parallel to the hypo-responsiveness of neutrophils in T1D, evidence has been accumulating in support of a pathogenic role for neutrophils in lupus [65]. A positive correlation has been found between disease severity and neutrophil aggregation in lupus patients [83]. Lupus neutrophils are generally more responsive against any stimuli and exhibit a higher propensity to undergo spontaneous and enhanced NETosis when treated with lupus serum containing high amounts of nucleic acid immune complexes [84,85]. Increased number of apoptotic neutrophils [86]; impaired degradation of NETs [87,88] and enhanced complement activation [89]–leading to autoantibodies against NETs and subsequent activation of pDCS with IFN-α secretion have also been reported [90-92]. The number of low density granulocytes is expanded in the circulation of lupus patients and this alternate subset of neutrophils mediates pathogenic effects by enhancing vascular damage and inhibiting vascular repair [90].

A subset of neutrophils coined neutrophil B helpers (NBH) found in the marginal zone of mice and humans [93] promote B cell activation and antibody production through the secretion of BAFF [94]. It has not been formally established whether lupus is associated with an expanded NBH subset, but elevated BAFF levels have been consistently associated with systemic autoimmunity [94,95]. A causal variant within neutrophil cytosolic factor (NCF2) identified as a susceptibility gene in both childhood- and adult-onset SLE reduced the production of reactive oxygen species (ROS) and enhanced the susceptibility to lupus within patients [96]. This was similar to the association between loss of function polymorphisms in Ncf1 and RA [97-101]. On the other hand, ROS reduction due to Ncf1 deficiency delayed T1D in NOD mice [82], highlighting again the dichotomous function of genes regulating neutrophil activities.

Conclusion

As neutrophils, the primary cellular targets of G-CSF, function in a variety of ways in autoimmune disease, this cytokine cannot be presumed to play a universal anti-inflammatory role. We hypothesize that G-CSF activates two opposite ends of an activity based-spectrum, resulting in tolerance induction in settings of T1D versus one of disease amplification in SLE. Further studies of G-CSF/G-CSFR pathway are needed to test the hypothesis that its modulation in two opposing directions may have therapeutic effects in these two autoimmune diseases. In addition, more information is needed on the specific role that G-CSF plays in activating or modulating cells other than neutrophils in these disorders. Dissecting the role of G-CSF by narrowing down its cellular targets and potential side effects could help in designing better immunomodulatory therapies against disorders in the future.

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