Granulocyte Colony-Stimulating Factor: A Novel Mediator of T Cell Tolerance

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In recent years, several investigators have unraveled a previously unrecognized role for G-CSF in the regulation of T cell and dendritic cell functions. The experimental evidence in favor of G-CSF-mediated immune regulation includes the ability to switch T cell cytokine secretion profile to Th2 responses and the promotion of regulatory T cell and tolerogenic dendritic cell differentiation. Interestingly, G-CSF is beneficial in animals for the prevention and/or treatment of immune-mediated diseases, e.g., graft-vs-host disease, multiple sclerosis, systemic lupus erythematosus, inflammatory bowel disease, and diabetes, suggesting a potential role in human autoimmune diseases. This review summarizes the growing body of evidence that supports a critical role for G-CSF as a novel mediator of T cell tolerance. The Journal of Immunology, 2005, 175: 7085–7091.

Granulocyte CSF was identified initially as a growth factor for neutrophils (1). Human G-CSF is encoded by a single gene located on chromosome 17q11–22 and is produced mainly by cells of monocyte/macrophage origin (1). The primary effects of G-CSF on cells of the hematopoietic system include stimulation of proliferation and differentiation of normal hematopoietic stem cells (HSCs), acceleration of neutrophil reconstitution following radiation- or chemotherapy-induced myelosuppression, activation of effector functions in mature neutrophils, and mobilization of bone marrow HSCs into the peripheral blood (2, 3).

A growing body of experimental evidence suggests that G-CSF might interact with the immune system by altering T cell reactivity and modifying APC function (1, 4). Expression of receptors for G-CSF (G-CSFR) on T cells remains controversial. It has been demonstrated that human CD4+ and CD8+ T cells might express the G-CSFR at the mRNA level (5, 6). In addition, biotin-labeled G-CSF binds to human mitogen-activated but not resting CD3+ T cells and to T lymphoblastoid cell lines, suggesting expression of G-CSFR at the protein level (7). Interestingly, G-CSF might activate T cell immunomodulatory genes, e.g., GATA-3 and Stat5, and directly inhibit proinflammatory cytokine production by T cells at pharmacologic doses (5, 6).

Effects of G-CSF on cytokine production

Initial studies showed that G-CSF modifies ex vivo cytokine production by human white blood cells. Specifically, G-CSF attenuated IL-1β, IL-12, IFN-γ, IL-18, and TNF-α production by LPS-stimulated whole blood and/or monocytes (8, 9). Furthermore, G-CSF enhanced the LPS-induced release of IL-1 receptor antagonist (IL-1ra) and soluble p55 and p75 TNFRs (10). Similarly, pretreatment with G-CSF in healthy volunteers subsequently given Salmonella abortus equi endotoxin increased plasma levels of soluble TNFRs and IL-1β compared with the control subjects not pretreated with the cytokine, suggesting in vivo anti-inflammatory effects (11). G-CSF reduces TNF-α production by normal, alloantigen-activated PBMCs at posttranscriptional level (12). More recently, G-CSF has been shown to promote the elevation of serum hepatocyte growth factor, an angiogenic cytokine that might contribute to microvesSEL formation induced by G-CSF (13) and to the in vitro differentiation of tolerogenic dendritic cell (DC)-like cells (S. Rutella and R. M. Lemoli, submitted for publication). Finally, G-CSF transiently increases soluble human HLA Ags, e.g., HLA-G and HLA class I, that reportedly modulate alloimmune responses (13).

Effects of G-CSF on T cell functions

G-CSF administration to healthy subjects has been shown to inhibit T cell proliferation in response to mitogens (14–16) through the release of soluble inhibitory factors (17–19). Interestingly, the induction of CD28 responsive complex, a T cell costimulator that interferes with CD28 signal transduction (20), in vivo following G-CSF administration might interfere with the CD28 signal transduction. In this respect, differences in monocyte quantity in post-G compared with pre-G

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3 Abbreviations used in this paper: HSC, hematopoietic stem cell; IL-1ra, IL-1 receptor antagonist; DC, dendritic cell; GVL, graft-vs-leukemia; Treg, regulatory T; Tr1, type 1 Treg; GVHD, graft-vs-host disease; RA, rheumatoid arthritis; MS, multiple sclerosis; TID, type 1 autoimmune diabetes.
PBMCs accounted for higher levels of immunomodulatory IL-10 in post-G PBMCs (21, 22). Additionally, monocytes in G-CSF-mobilized peripheral blood stem cell products might inhibit T cell function by inducing CD4+ T cell apoptosis through Fas-Fas ligand interaction (23), and cells of monocytic origin might interfere with the expression of CD3ζ chain in T cells, further contributing to T cell dysfunction (24, 25).

Human NK cells are defined by the presence of the invariant Vα24 Vβ11 TCR and are induced to proliferate in response to stimulation by α-galactosylceramide (26). NK cells from donors treated with G-CSF display an impaired ability to expand in vitro in response to α-galactosylceramide (26). Post-G CD14+ monocytes might also induce the expression of inhibitory NKRks on T cells that manifest potent cytolytic activity against leukemic cell lines, solid cancer cell lines, and patient’s leukemic cells (27) (Fig. 1). These findings suggest that expanded NKRk+ T cells from G-CSF-mobilized donors might be exploited for allogeneic T cell therapy to induce the graft-vs-leukemia (GVL) effect (27).

Importantly, G-CSF can mobilize bone marrow CD4− CD25− FoxP3− regulatory T (Treg) cells as well as natural suppressor CD4− CD8− TCRα−β+ T cells (28, 29). Specifically, G-CSF reduces the expression of CXCL12/SDF-1α, a CXCR4 ligand, in the bone marrow, thus favoring CD4+ CD25+ Treg cell trafficking (Fig. 1). Finally, the G-CSF-induced decrease in T cell mitogen responses and lymphokine-activated killer cell-mediated cytotoxicity have been correlated with expression of individual HLA alleles (30).

**Induction of Treg cells by G-CSF**

Recently, considerable effort has been devoted to the study of Treg cells and their role in human autoimmune diseases (31, 32). Among CD4+ T cells with inherent Treg activity, interest has been focused on naturally occurring CD4+CD25+ Treg cells and on adaptive Treg cells, including IL-10-secreting CD4+ type 1 Treg (Tr1) cells (33, 34).

We have recently shown a previously unrecognized role for G-CSF in the generation of human Tr1 cells (35). Freshly isolated CD4+ T cells challenged with alloantigens in vitro in the presence of post-G serum were hyporesponsive in terms of proliferation and were polarized to a Tr1-like functional profile (35). Post-G CD4+ T cells suppressed bystander T cells through a cell contact-independent but IL-10/TGF-β-dependent mechanism (35). Importantly, G-CSF per se was incapable of favoring Treg cell differentiation when added to an allogeneic MLR, and in vivo administration of the cytokine was an absolute requirement for T cell polarization (35).

Further evidence in favor of a role for G-CSF in the promotion of Treg cell differentiation stems from studies in mice demonstrating that treatment with pegylated G-CSF augments the generation of IL-10-producing Treg cells and promotes transplantation tolerance (36). Pegylated G-CSF-mediated protection from graft-vs-host disease (GVHD) was crucially dependent on IL-10 production by T cells.

**Effects of G-CSF on DC number and function**

DCs are highly specialized APCs with a unique capacity to fully activate and induce clonal expansion of naive and memory T cells. Different subsets of human DCs have been described, namely DC1 and DC2, according to the ability to induce naive T cell differentiation to Th1 and Th2 effector cells, respectively. Recently, DCs have also been implicated in the maintenance of tolerance in central lymphoid organs and in the periphery (31, 33). Studies in healthy controls have shown that G-CSF can mobilize DC2 that, in turn, induce allogeneic naive T cells to produce IL-4 and IL-10 (37, 38). Of interest, G-CSF treatment up-regulates CCR7 expression on blood DCs, thus affecting DC migratory and homing ability (39).

Recently, we reported that G-CSF might affect the differentiation pathway of human monocyte-derived DCs. DCs generated from post-G monocytes in the presence of autologous, post-G serum were phenotypically mature, e.g., expressed high levels of CD80, CD86, CD83, and MHC class II molecules, but released low levels of IL-12p70 (40), in accord with data on the IL-12 production ability of DCs recovering after myeloablative HSC transplantation and G-CSF treatment (41). Naive CD4+ T cells activated in vitro with post-G DCs were hyporesponsive in terms of proliferation and acquired an IL-10+TGF-β+ IL-2negIL-4negIL-5– cytokine secretion profile, which is consistent with Tr1 cells. Furthermore, those T cells were hyporesponsive to secondary stimulation with same-donor post-G DCs but not with third-party donor DCs, indicating Ag-specific hyporesponsiveness or anergy (40). Post-G DC-primed T cells mediated vigorous suppression of bystander T
cells through the release of IL-10 and TGF-β. Following the observation that IL-10 and IFN-α are involved in the differentiation of human Treg cells (42), serum levels of IL-10 and IFN-α were measured before and after G-CSF administration. Both cytokines were significantly elevated in post-G serum (40), and post-G DCs generated in the presence of blocking mAbs to IL-10 and IFN-α were unable to induce naïve T cells to a Th1-like profile.

Interestingly, DC precursors directly isolated from the peripheral blood of G-CSF-treated cancer patients are poor activators of allogeneic T cell proliferation, suggesting that preformed post-G DC precursors might share some functional features with in vitro differentiated post-G DCs (40).

**G-CSF-expanded myeloid precursors as tolerogenic APCs**

Treatment with G-CSF can expand a murine GM precursor population with regulatory activity (43). G-CSF-expanded, CD40− GM cells displayed features of APCs, produced high amounts of IL-10, and suppressed the development of GVHD when cotransplanted in allogeneic recipient animals (43). Furthermore, G-CSF-expanded GM cells favored the in vitro differentiation of Treg cells specific for host Ags.

Interestingly, immature GM-CSF-dependent mouse DCs might produce bioactive IL-2 upon encountering bacterial Ags (44). In this respect, tolerance might be crucially dependent on IL-2 production by non-T cell sources (45). In light of this emerging evidence, it is tempting to speculate that cytokine-expanded GM precursors might contribute to induction and/or maintenance of T cell tolerance through the release of IL-2 (Fig. 2). Additionally, tumor-derived GM-CSF might mobilize CD34+ HSCs, which nonspecifically suppress T cell responses, including antitumor reactivity (46). It remains to be ascertained whether G-CSF-mobilized CD34+ HSCs possess the ability to down-modulate T cell responses as well.

**G-CSF in HSC transplantation**

**Mouse models.** By using a hybrid resistance system in which NK cells mediate vigorous rejection, continuous pretreatment of F1 hybrid mice with G-CSF translated into engraftment of bone marrow parental cells and appearance of splenic colonies of donor origin (47). Also, transgenic mice expressing human G-CSF are capable of accepting xenotransplanted rat bone marrow cells (48).

Injection of G-CSF in mice has been shown to down-regulate T cell proliferation to mitogens and alloantigens and to decrease type 1 cytokine production by T cells (49). Release of IL-4 by G-CSF-treated T cells was significantly elevated, which is consistent with a shift to a Th2 cytokine pattern. Finally, G-CSF pretreatment of donor mice altered the ability of donor cells to mediate acute GVHD and determined an improvement of overall survival in recipient mice (49). The GVL effect of G-CSF-treated T cells was unchanged, being mediated by a perforin-dependent pathway that was unaffected by G-CSF treatment (50). Zeng et al. (51) described concordant results and showed an increase in the frequency of CD4−CD8− NK1.1+ T cells responsible for the secretion of large amounts of IL-4 in G-CSF-treated donor mice.

Importantly, the protective effects of G-CSF against GVHD might result from G-CSF administration to transplant donors, whereas the effects of G-CSF administration to allogeneic HSC recipients might be negligible. In this respect, recipients of G-CSF-mobilized splenocytes display a significant reduction in TNF-α and LPS production and in the GVHD score, translating into improved survival (52). Conversely, G-CSF treatment of the recipient animals alone without pretreatment of the donor had no impact on acute GVHD-related survival or in vivo TNF-α production. This elegant study clearly indicates that G-CSF effects on the donor rather than on the recipient might be responsible for the amelioration of experimental GVHD.

**Studies in humans.** G-CSF-mobilized HSC harvests contain ~1 log more T cells compared with conventional bone marrow harvests (53). Theoretically, a different graft composition might affect immune reconstitution, GVHD, and GVL. Surprisingly, incidence and severity of acute GVHD were similar with peripheral blood HSC transplantation to those reported for bone marrow transplantation tentatively attributed to G-CSF-driven expansion of immature myeloid precursor cells and/or functional alteration of T cells after in vivo priming with G-CSF (53).

In recent years, prophylaxis with G-CSF has been associated with higher incidence of acute GVHD in patients with hematological malignancies receiving allogeneic HSCs from HLA-identical siblings (54). Similarly, a retrospective analysis by the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation in >2000 patients with acute leukemia has shown that patients treated with G-CSF after transplant had a greater incidence of acute and chronic GVHD and an increase in transplant-related mortality (55). A recent meta-analysis of 1198 patients, including 9 prospective randomized trials, 8 retrospective cohort studies, and 1 case control study, showed that G-CSF exerted no effect on GVHD or transplant-related mortality compared with patients not treated with the cytokine (56). Collectively, clinical studies suggest that G-CSF might be of limited value as prophylactic treatment in recipients of allogeneic HSCs (55, 57, 58). However, it must be emphasized that the effects of G-CSF in donors treated to mobilize HSCs are likely to differ from those observed in HSC transplant recipients. Therefore, powered randomized trials of sufficient patient size and homogeneity to address the

![FIGURE 2. Preclinical models of G-CSF-induced inhibition of autoimmune and allogeneic T cell responses. The beneficial (green arrow) and detrimental (red arrow) effects of G-CSF in animal models of immune-mediated diseases are summarized. G-CSF might modulate T cell alloreactivity and affect incidence and severity of acute and chronic GVHD by expanding myeloid precursors (see also Fig. 1) and altering allograft composition. See main text for further details.](http://www.jimmunol.org/)
impact of G-CSF on GVHD incidence and severity are warranted (59) (Fig. 2).

The in vitro alloresponse of donor cells 5 days after G-CSF administration might be predictive of the occurrence of acute GVHD in transplanted patients (60), and T cells from donors with a significant G-CSF-induced suppression of in vitro alloreactivity less frequently induce GVHD in the recipients (60).

The risk of chronic GVHD might be moderately increased following allogeneic peripheral blood HSC compared with bone marrow transplantation due to a different graft composition (61, 62). The production of TGF-β after allogeneic HSC transplantation might provide an additional explanation for the opposing effects of G-CSF administration to donors on acute vs chronic GVHD (63). In this respect, G-CSF administration to donor mice might induce TGF-β- and IL-10-dependent protection from acute GVHD but contribute to TGF-β-dependent exacerbation of chronic GVHD (63). Neutralization of TGF-β in transplanted mice protects from chronic GVHD in gastrointestinal tract and skin but not from liver abnormalities. Moreover, G-CSF-expanded myeloid cells might be a major source of TGF-β, thus dictating the induction of chronic GVHD in this model of G-CSF-driven disease.

A further word of caution against the administration of G-CSF after HSC transplantation stems from studies in HLA-mismatched HSC transplantation (64). In this cohort of patients, posttransplantation G-CSF impaired functional immune recovery and mediated delayed reconstitution of Aspergillus-specific and Candida-specific immunity and reduced production of IL-12 by APCs (64).

G-CSF in inflammatory bowel disease

G-CSF has been successfully used in experimental colitis in White New Zealand rabbits (65). Animals were pretreated either 24 h before or at colitis induction either with 50 or 200 μg/kg rG-CSF. Cytokine administration at either dose translated into increased tissue myeloperoxidase levels, despite a histologically similar mucosal polymorphonuclear cell infiltrate in the G-CSF-treated compared with the control colitis group. Moreover, dialysis fluid levels of leukotriene B4 and thromboxane B2 were significantly lower in treated animals (65). Similar results were obtained in the 2,4,6-trinitrobenzene sulfonic acid colitis in rats, a model of Th1 disease. G-CSF at 250 μg/kg/day remarkably attenuated both the loss of body weight and colonic wall thickening due to progressive transmural inflammation (66). This effect was associated with a significant inhibition of IFN-γ and IL-12p35 transcription (66). Promising preclinical data have been translated recently into the treatment of human inflammatory bowel disease. G-CSF proved to be efficacious in severe endoscopic postoperative recurrence of Crohn’s disease (67). Five patients were treated with 300 μg of recombinant human G-CSF three times per week for 12 wk. G-CSF was safe and well tolerated, and a significant increase in neutrophil counts, IL-1ra, and soluble TNFR p55 and p75 was shown in the plasma of G-CSF-treated patients. In a recent open-labeled study, G-CSF demonstrated safety and efficacy for the treatment of active Crohn’s disease (68). Patients received 300 μg of G-CSF for 12 consecutive weeks, achieving a statistically significant decrease in disease activity. Although a randomized controlled placebo trial has not been performed yet, these studies suggest that rG-CSF might be a promising therapeutic approach for Crohn’s disease.

G-CSF in preclinical models of autoimmune diseases

Systemic lupus erythematosus and rheumatoid arthritis (RA). The anti-inflammatory properties of G-CSF and its capacity to switch T cell cytokine profiles toward Th2 and to promote tolerogenic DC and Treg cell differentiation prompted its therapeutic evaluation in experimental models of autoimmune diseases (Fig. 2).

G-CSF exerted contrasting effects in humoral and cellular autoimmune diseases. In humoral diseases such as systemic lupus erythematosus and RA, polarized T cell phenotypes and inflammation often display a complex relationship to the pathogenesis. Thus, despite an increase in the serum levels of IgG1 over IgG2a and IgG3 autoantibodies and reduced IFN-γ and TNF-α production, demonstrating an effective shift toward Th2 of the autoimmune response, a chronic treatment with low doses of G-CSF (10 μg/kg) accelerated lupus disease in MRL/lpr/lpr mice (69). This deleterious effect of the low-dose G-CSF regimen may relate to the ability of the growth factor to increase the production of IFN-α (40) and B lymphocyte stimulator (70), which play a central role in plasmocyte differentiation, B cell autoantibody production and lupus disease development (71–73).

On the contrary, nephritis, the end-stage of lupus disease of which inflammation is the hallmark, was prevented in mice given a high-dose G-CSF regimen (200 μg/kg), even when treatment was started in animals already suffering from a beginning proteinuria (69) (Fig. 2). An uncoupling between immune complex deposition and kidney damage was observed, which corresponded to a profoundly reduced expression of FcγRIII (CD16) within the glomeruli, a receptor mediating the inflammatory response of kidney mesangial cells to IgGs. Accordingly, inflammatory IL-12 serum response was limited, and mortality was delayed significantly.

In inflammatory arthritis, neutralization of endogenous G-CSF markedly reduced the progression of disease to the same extent as anti-TNF treatment (74). Contradictory results have been obtained with the administration of exogenous G-CSF being reported to prevent adjuvant-arthritis in rats (75) but to exacerbate murine collagen-induced arthritis (76) and a passive transfer model of collagen-induced arthritis in rats (77). The deleterious role of G-CSF (Fig. 2) was suggested to relate to mobilization, expansion, and trafficking to the periphery of myeloid cells, which contribute to inflammatory joint disease.

Collectively, the body of experimental results obtained so far in lupus and arthritis models, together with clinical reports of vasculitis and aggravation of neurolupus (78), as well as RA exacerbation in G-CSF recipients (79), raise a word of caution for the use of G-CSF in susceptible individuals. Studies further suggest that the dose should be chosen with caution, if G-CSF is needed to restore neutrophil counts in lupus patients (78).

Experimental autoimmune encephalomyelitis and diabetes.

Different from the results in humoral autoimmune disease models, the beneficial effects of G-CSF in T cell-dependent animal models of multiple sclerosis (MS) and type 1 autoimmune diabetes (T1D) proved to be of potential interest for translation into therapeutic strategies (Fig. 2).

A short 7-day treatment with G-CSF (200 μg/kg), initiated at the onset of clinical signs, conferred durable protection of SJL-J mice against myelin basic protein-induced experimental autoimmune encephalomyelitis (80). Protected mice displayed...
limited demyelination and reduced recruitment of T cells to the CNS as well as very discrete autoimmune inflammation and barely detectable cytokine and chemokine mRNA levels in the CNS. These effects were based on immunoregulatory events that took place in the periphery and included an imbalance in the chemokine (MIP-1α/MCP-1, e.g., CCL3/CCL2) production ratio by macrophages and autoreactive lymphocytes, which correlated with an immune deviation of the autoreactive response toward Th2. A dramatic reduction of systemic and lymphocyte TNF-α production was also observed.

The protective effect of G-CSF was confirmed in C57BL/6 mice immunized with the MOG peptide (81). The same authors showed that elevated G-CSF gene expression occurred selectively in brain lesions of patients at the acute phase of MS, suggesting an endogenous protective role of the growth factor. Recent results demonstrating the capacity of G-CSF to counteract acute neuronal degeneration and to drive neurogenesis in acute ischemia further encourage its evaluation as a promising drug for stroke, degenerative, and autoimmune diseases of the nervous system (82). The therapeutic window of efficacy, however, remains to be ascertained, given the complex evolution of the different phases of MS, of which no single experimental disease model is representative enough.

G-CSF also provided protection against experimental autoimmune diabetes in NOD mice. G-CSF (200 μg/kg) reversed the accelerating effects of cyclophosphamide, prevented the loss of CD4⁺CD25⁺ Treg cells and abrogated the robust cytokine—particularly IFN-γ—and chemokine burst triggered in immune cells by cyclophosphamide (83).

In the spontaneous diabetes model, treatment of NOD mice at 4 wk of age for 5 consecutive days, repeated every 4 wk thereafter until 16 wk of age, durably prevented disease onset and destructive insulitis (84). This protection correlated with marked recruitment of two major regulatory subsets, i.e., plasmacytoid DCs and CD4⁺CD25⁺ Treg cells. Thus, DCs in protected mice were enriched in CD11c⁺B220⁻Gr-1⁺PDCA-1⁺ cells, and produced higher IFN-α levels but nearly no IL-12p70 relative to DCs from excipient-treated mice. These plasmacytoid DCs exhibited a partially immature phenotype with reduced expression of MHC class II Ags and CD80 costimulatory molecule but normal levels of CD86 and CD40. Moreover, G-CSF recipients displayed an accumulation, particularly at the peripancreatic lymph nodes, of CD4⁺CD25⁺ Treg cells, which produced high levels of TGF-β1 and remained functional, actively suppressing diabetes transfer in secondary NOD-SCID recipients. Adoptive transfer experiments demonstrated the mutual interaction between the two regulatory subsets. Indeed, sorted DCs from mice given a single 5-day-long G-CSF treatment, relative to DCs from excipient-treated donors, were able upon adoptive transfer to secondary NOD recipients to trigger markedly enhanced accumulation of CD4⁺CD25⁺ Treg cells that expressed significantly higher levels of membrane TGF-β1. These experiments suggest that G-CSF most likely recruits CD4⁺CD25⁺ Treg cells by restoring the balance between immunogenic and tolerogenic DCs in the NOD mouse.

Future perspectives

Altogether, the results obtained in animal models argue favorably for therapeutic evaluation of G-CSF in human MS and T1D. However, optimal schedule and dose of G-CSF administration remain to be determined with appropriately conducted phase I/II clinical trials. Particularly, in T1D, there is a need to extend the window of efficacy of G-CSF to later stages of the disease (G-CSF was inactive if treatment was started at 9 wk of age in the NOD mouse), as patients are usually diagnosed at relatively advanced stages of the autoimmune destruction of pancreatic islets. Several combinations might be envisaged: 1) combining G-CSF, as a DC maturation inhibitor, with T cell activation inhibitors such as the anti-CD3 Ab (85–87); 2) combining G-CSF with other hemopoietic factors, such as Flt3-L, either separated or combined in a chimeric molecule (progenetin) (88); and 3) sorting a particular cellular subset induced by G-CSF, which may provide protection against autoimmune and allograft responses.

CD4⁺CD25⁺ Treg cells mobilized by G-CSF (28, 84) might represent a promising source of Treg cells for clinical application since they can be expanded up to 40,000-fold with artificial APCs and high-dose IL-2 (89). Additional cell subsets of interest include GM precursors, which reportedly protect against GVHD (43). However, a similar population of CD11c⁺CD11b⁺Gr-1⁺ granulocyte-monocyte precursors was able to prevent diabetes in NOD mice only if they were expressing the autoantigenic proinsulin as a transgene (90).

We have gathered experimental evidence that particular subsets of immature hemopoietic progenitor cells, mobilized by a combination of growth factors, including G-CSF, have the capacity to halt the onset of overt diabetes when G-CSF injection is no more able to prevent disease (H. Kared and F. Zavala, submitted for publication). Cell therapy with G-CSF-mobilized hemopoietic progenitor cells, devoid of other G-CSF-induced proinflammatory cell subsets, may show therapeutic promise in patients with autoimmune diseases (91). G-CSF-expanded CD34⁺ cells and/or myeloid precursors might further downmodulate T cell alloreactivity through the release of TGF-β and possibly other cytokines, e.g., IL-2 (45, 46, 63, 92).

The results described above also raise the hypothesis that endogenous response factors to infections such as G-CSF could be involved in protection against T1D onset provided by infectious events, particularly if they take place in early phases of diabetes development (93). In view of the large body of experimental evidences presented in this review, G-CSF should certainly be considered as one factor linking innate and adaptive immunity.

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References


