Available online at www.sciencedirect.com
SCIENCE DIRECT
Cytokine & Growth Factor Reviews 17 (2006) 235–244
Cytokine & Growth Factor Reviews
www.elsevier.com/locate/cytogfr

G-CSF 在後天性免疫中的作用 Mini review

The role of G-CSF in adaptive immunity

Anke Franzke *

Department of Hematology, Hemostasis and Oncology, Hannover Medical School, Hannover, Germany

Abstract

Granulocyte colony-stimulating factor (G-CSF) is a pleiotropic cytokine playing a major role as regulator of hematopoiesis and innate immune responses. There is growing evidence that G-CSF also exerts profound immunoregulatory effects in adaptive immunity. G-CSF mediates anti-inflammatory reactions accompanied by TH2 cell differentiation and promotes tolerogeneic cell populations at both poles of APC/T cell interaction. These recent findings have highlighted the novel impact of G-CSF in transplantation tolerance and autoimmunity. G-CSF represents a powerful and promising cytokine to promote T cell tolerance in pathological conditions associated with a TH1/TH2 imbalance.

Keywords: G-CSF; T cell; Autoimmunity; Transplantation; Tolerance

1. Introduction

In the last decades soluble factors have been identified that stimulate the growth of hematopoietic stem and progenitor cells [1]. These factors have been globally defined as colony-stimulating factors (CSFs) due to their ability to support the proliferation and maturation of stem-cell colonies *in vitro*. Granulocyte colony-stimulating factor (G-CSF) was characterized as a cytokine inducing both proliferation of normal granulocyte colonies and maturation of leukemic cell lines [2–4]. The gene for human G-CSF is localized on chromosome 17 [5]. G-CSF is produced by bone marrow stromal cells, endothelial cells, macrophages, fibroblasts, and its production can be induced by inflammatory stimuli. G-CSF acts through the G-CSF receptor (G-CSFR), which is expressed on early myeloid progenitors, mature granulocytes, and monocytes/macrophages, as well as endothelial cells [6]. Recently, the G-CSFR have also been described on human T and B lymphocytes [7–9]. G-CSF is known to stimulate granulocyte production, maturation and effector function [2,4,10,11], costimulates early progenitors in synergy with several other cytokines [12] and may also induce proliferation of pre-B cells, a variety of leukemic blasts, and solid tumor cell lines [1]. Mice deficient in G-CSF or the G-CSFR exhibit marked neutropenia, demonstrating the importance of G-CSF in steady-state granulopoiesis [13,14]. G-CSF has a broad effect on innate immune responses, especially on the monocyte/macrophage system by expansion and enhancement of phagocytosis [15] and regulation of inflammatory cytokine/chemokine production [16].

The clinical application of recombinant G-CSF mainly includes the mobilization of bone marrow hematopoietic stem and progenitor cells into the peripheral blood [17] and acceleration of neutrophilic reconstitution following chemotherapy-induced myelosuppression [18]. G-CSF was previously characterized as a major extracellular regulator of hematopoiesis and the innate immune system. Evidence is now accumulating that G-CSF has also pleiotropic effects on adaptive immune responses. Results from experimental models, *in vitro* studies and clinical data indicate that G-CSF alters T cell function and also has modulating effects on dendritic cells. The following review will discuss recent advances in our understanding of the immunoregulatory role of G-CSF, especially as a novel mediator of T cell tolerance (Fig. 1).

* Tel.: +49 511 5323202; fax: +49 511 5328205.
E-mail addresses: franzke.anke@mh-hannover.de, afranzke@gmx.de.

1359-6101/$ – see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cytogfr.2006.05.002

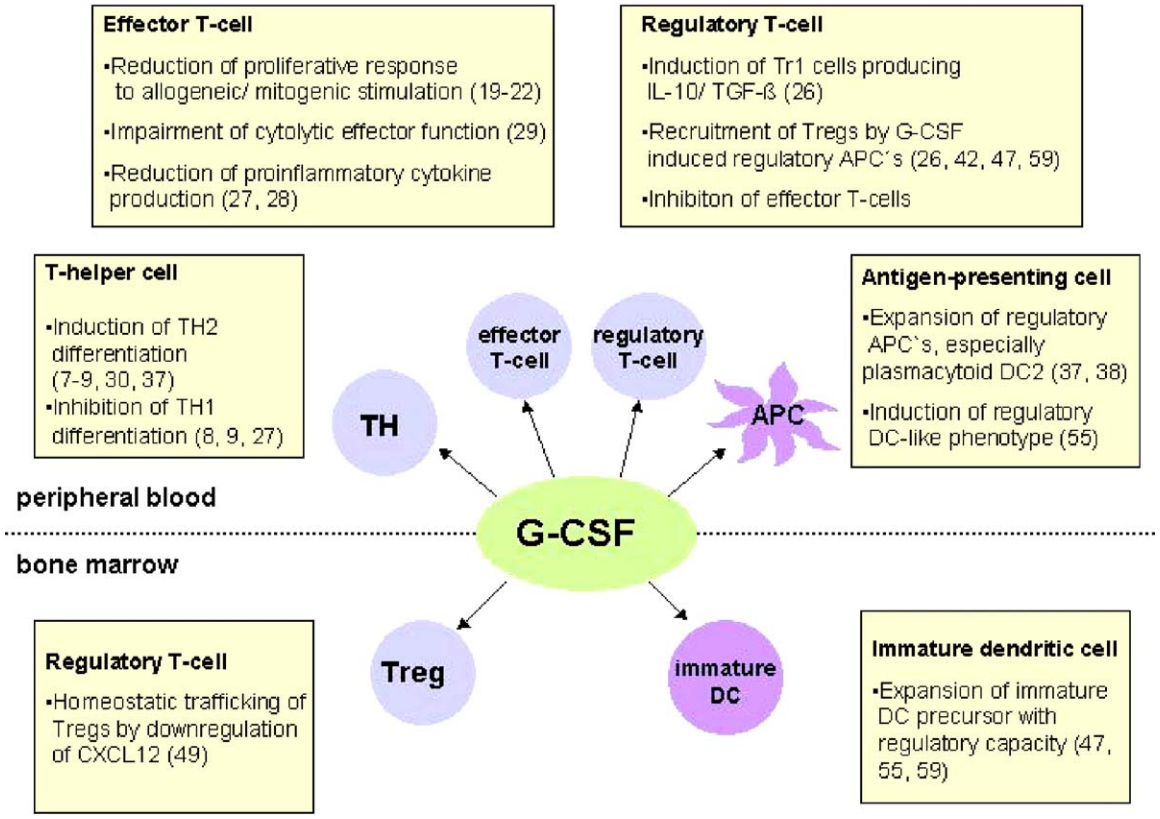

Fig. 1. G-CSF as mediator of peripheral T cell tolerance. G-CSF stimulation is leading to several key immunoregulatory effects: T cells upregulate GATA-3 expression and are biased directly [7–9] and indirectly [30,37] via other effector cells (i.e. monocyte, dendritic cell types) towards TH2 differentiation while inflammatory TH1 immune responses are inhibited [8,9,27]. G-CSF induces the generation of Tr1 cells producing IL-10 and TGF-β [26] that may limit effector T cells responses. G-CSF expands regulatory APC (i.e. DC2 [37,38], and immature precursor population [47,55,59]) that may recruit regulatory T cells contributing to tolerance induction [42,47,59]. Furthermore, homeostatic trafficking of Tregs from the bone marrow to the peripheral blood is promoted by G-CSF via downregulation of CXCL12 [49]. These mechanism seem to contribute to the tolerogeneic impact of G-CSF as documented in acute GVHD [30,31,37,40,42,94] and Th1-mediated autoimmune diseases, such as autoimmune type 1 diabetes [47,48], RA [63], lupus erythematosus [65], ileocolitis, [70–74], and EAE [75].

2. G-CSF as a regulator of T cell responses

There is growing evidence that G-CSF, in addition to its stimulatory effects on hematopoiesis, can exert immunomodulatory effects on T lymphocytes. However, it is still not clear how G-CSF is mediating these regulatory properties. Recently, G-CSF receptor could be detected in mitogen-activated T cells [7] as well as in unstimulated T cells at the mRNA level [8]. Whereas contribution of contaminating non-T cells cannot be excluded in these studies, we could demonstrate at the single-cell level that G-CSFR expression was detectable in CD4+ and CD8+ T cells after G-CSF exposure *in vivo* and *in vitro*. A kinetic study in more than 200 single T cells showed an induction of the G-CSFR in a time-dependent manner after direct stimulation of purified T cells with G-CSF [9]. Other investigators have observed an indirect effect of G-CSF on T cells via other effector cells, such as monocytes, dendritic cell types, and endogenous mediators. However, these results are not absolutely contradictionary as both mechanisms may simultaneously operate *in vivo*.

2.1. G-CSF affects T cell proliferation

In patients [19] and healthy stem cell donors [20] treated with G-CSF, the proliferative response of T cells to allogeneic and mitogenic stimulation is profoundly reduced. This oberservation was confirmed by Rutella et al. [21,22] showing that the inhibition of T cell proliferation after mitogenic challenge might result from a G1 arrest in cell cycling mediated by soluble factors. Others suggested that G-CSF administration suppresses T cell proliferation by indirect modulation of the monocyte function as depletion of monocytes partially reverted the proliferative capacity [20,23]. The downregulation of costimulatory molecules like CD86 and enhanced production of interleukin-10 might contribute to monocyte-mediated suppression of T cell proliferation [24]. The blockade of B7 with soluble CD28 fusion protein leads to subresponsiveness of T cells and thereby supports the theory that G-CSF might indirectly affect T cells through the inhibition of costimulatory signalling pathways [25]. Apparently, the upregulation of surface molecules associated with T cell activation, such as

CD69, CD25, CD71, and HLA-DR, is not affected after PHA stimulation following G-CSF exposure *in vivo* [21]. Additional functional analyses revealed that purified CD4+ T cells from G-CSF-mobilized stem cell donors were able to suppress allo-proliferative responses of autologous T cells in an antigen-non-specific and cell contact-independent manner [26].

2.2. G-CSF affects cytokine production of T cells

The impact of G-CSF on the cytokine secretion of T cells still remains controversial. Initial studies analyzed the *in vitro* effect of LPS stimulation on mononuclear cells of G-CSF-treated healthy volunteers and described an anti-inflammatory response with a reduction of pro-inflammatory cytokines such as TNF-α, IL-12, IL-1β, and IL-2 [27,28]. Further *in vitro* analyses demonstrated that G-CSF-mediated suppression of TNF-α production is regulated at the posttranscriptional level and might contribute to the impaired generation of cytolytic effector cells [29]. Additional murine and human studies investigated the effect of G-CSF on T helper type 1 (TH1) and type 2 (TH2) cell development. Several groups identified a shift towards a TH2 immune response with increased production of IL-10, IL-4 [8,30,31], accompanied by a decrease of interferon-γ [8,30] and IL-2 [30,28,31,32] upon alloantigeneic or mitogeneic stimulation *in vitro*. Interestingly, a spontaneous increase of IL-4 and decrease of IFN-γ secretion could be observed in peripheral stem cell donors after G-CSF application [9]. The molecular mechanism by which cytokine signalling and/or antigen stimulation via the T cell receptor (TCR) might control TH1 and TH2 effector cell differentiation is still poorly understood. Recently, we could identify the upregulation of the T cell-specific transcription factor GATA-3, which controls T helper cell differentiation, directs to TH2 commitment and stabilizes this phenotype [33,34] in peripheral stem cell donors after G-CSF application [9]. In addition, G-CSF may repress the opposing TH1 immune response by interfering with the IFN-γ transduction pathway through modulation of the ISGF3-γ subunit/p48 in CD4+ T cells of G-CSF-stimulated stem cell donors [9]. Whereas *in vitro* experiments could show the direct induction of GATA-3 activating a TH2 program in G-CSFR expressing T cells, the mode of action of G-CSF *in vivo* is still unknown. Some data argue for an indirect effect of G-CSF on the T cell phenotype via monocytes by downregulation of costimulatory molecules [30], increased production of IL-10 [24], and decreased production of IL-12 and TNF-α [20,31]. However, in peripheral blood and stem cell harvests from G-CSF-stimulated donors a selective mobilization of plasmacytoid dendritic cells (DCs) has been reported, which promote TH2-biased T cell responses [35] and regulatory T cell function [36]. It has been postulated that plasmacytoid DCs contribute indirectly to the skewed TH2 phenotype of donor T cells after G-CSF exposure [37,38]. But the inhibition of both TH1 and TH2 cytokine production in G-CSF-stimulated PBSC donors [39], especially after alloantigeneic and mitogeneic stimulation has also been reported [23,40]. In addition, recent murine and human studies applying G-CSF point to an increased production of immunoregulatory cytokines like TGF-β [41] and IL-10 [26,42] after allogeneic *in vitro* stimulation with potential impact on T cell-mediated diseases.

3. G-CSF as inducer of peripheral tolerance

Recent evidence supports an important role of G-CSF for the induction and maintenance of peripheral tolerance, but the mechanism by which G-CSF acts still remains controversial. There are two major possibilities for G-CSF as key player in T cell homeostasis: First, G-CSF may interfere by downregulating the differentiation, effector function and proliferation of potentially autoreactive T cells as described above. Second, G-CSF may be important for regulatory T cells (Treg) controlling autoreactive immune responses. It is well established that Treg cells play a pivotal role in the induction of antigen-specific tolerance by downregulating the activation and expansion of self-reactive T cells which seems to be a key mechanism for the control of T cell-mediated diseases [43]. Besides thymus-derived naturally occurring CD4+CD25+ Tregs, which have gained enormous interest [43], there are various types of *in vivo*- and *in vitro*-induced Tregs [44–46]. These regulatory cell types differ from naturally occurring Tregs in their dependency on cytokines, but not in their suppressive capacity or proliferative response upon antigenic stimulation. For example, T regulatory type 1 (Tr1) cells produce high levels of immunosuppressive IL-10 and can be generated by chronic activation in the presence of IL-10 both in the human and murine systems [44,45].

In particular, different regulatory T cell populations are induced after G-CSF stimulation *in vivo* and *in vitro*. In addition, G-CSF may modulate dendritic cell types and thereby lead to the expansion of potentially tolerogenic subsets at both poles of APC/T cell interaction. To date it is not yet clear whether these conflicting data are due to different experimental settings or result from the heterogeneity within the immunoregulatory cell populations.

3.1. G-CSF as inducer of regulatory T cells

G-CSF was reported to induce Tr1-like regulatory T cells in the peripheral blood of normal human recipients [26]. After TCR ligation *in vitro* these regulatory CD4+ T cells produce high amounts of IL-10 and to a lesser extent also TGF-β. They show an impaired proliferative capacity and mediate suppressive effects on antigen-driven proliferation of other T cells mainly via a cell contact-independent way. These effects seem to be consistent with observations *in vivo* where protection from graft-versus-host disease (GVHD)

following pretreatment of the donor with pegylated G-CSF is dependent on IL-10 production of regulatory donor T cells [42]. However, the precise phenotype of the protective IL-10 secreting T cell population remains unclear. Further evidence for the immunoregulatory function of G-CSF was provided by murine studies demonstrating that G-CSF treatment enhances a CD4+CD25+ T cell subset by producing high levels of membrane-bound and secreted TGF-β with regulatory capacity in adoptive transfer experiments [47]. In cyclophosphamid accelerated autoimmune diabetes G-CSF promoted the expansion of CD4+CD25+ Tregs in the spleen of NOD mice and critically controlled the early onset of disease [48]. Interestingly, a recent study postulated a major role for G-CSF by the induction of homeostatic trafficking of CD4+CD25+ Tregs from the human bone marrow to the periphery by downregulating CXCL12. Further evidence for the migration properties of G-CSF was provided by investigating chimeric non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice showing that CD4+CD25+ Tregs traffic to and are retained in the bone marrow as significant reservoir for Tregs through the regulation of CXCR4/CXCL12 signals [49].

3.2. G-CSF as regulator in dendritic cell types

Dendritic cells (DCs) as professional antigen-presenting cells (APCs) initiate primary T cell immune responses [50]. Different subsets of DCs have been described with distinct properties for the activation, clonal proliferation and functional differentiation of both naïve and memory T cells. According to their ability to induce naive T cell differentiation to TH1 and TH2 effector cells [51], two lineages have been described, DC1 (myeloid DCs) and DC2 (plasmocytoid DCs), respectively. Recently, DCs have also been identified as key regulators for the induction and maintenance of self-tolerance [44] being highly dependent on their maturation/activation stage. Especially immature DCs are able to induce regulatory T cells and may serve as stabilizing platform on which effector and Treg cell populations physically interact [52]. Furthermore direct DC/T cell interaction modulates the immunosuppressive environment by inhibiting the maturation and antigen-presenting function of DCs [53].

Arpinati et al. reported that G-CSF application in normal stem cell donors selectively increases the number of DC2 cells in the peripheral blood (approximately five-fold) and transplanted graft whereas DC1 counts did not change [37]. Functional characterization of the DC subsets after G-CSF application revealed that DC1 cells did not differ in their ability to stimulate allogeneic naive T cells, whereas DC2 cells behaved as poor stimulators but were not impaired in their capacity to induce a TH2 immune response [37,38]. The selective mobilization of DC2 cells to the peripheral blood and stem cell harvests from G-CSF-stimulated donors may result from an altered trafficking of DC subsets following the downregulation of CD62L and upregulation of CCR7 [54]. These alterations may also coordinate the migration of DCs into secondary lymphoid organs. Besides the selective increase of DC2 cell numbers in stem cell donors, *in vitro* studies demonstrated that G-CSF might induce a regulatory DC-like phenotype derived from monocytes [55]. This regulatory DC-like cell population shows an impaired ability to release IL12p70 and a poor allo-stimulatory capacity. Furthermore, co-culture of naïve CD4+ T cells with this DC-like population leads to IFN-α- and IL-10-dependent generation of regulatory T cells which secrete the immunosuppressive cytokines TGF-β and IL-10 [55]. These findings are well in line with another observation that human Tr1-like cells may be induced by IFN-α and IL-10 [56]. Interestingly, G-CSF treatment of NOD mice seems to prevent the spontaneous development of autoimmune diabetes by recruiting immature $CD11c^{lo}$ B220+ plasmacytoid DC subtype, a phenotype attributed to tolerogeneic pDCs, that was identified by the production of high levels of IFN-α [57], but low levels of IL-12p70. In addition this DC subset displayed features of immature DCs with a lower expression of the MHC II complex and the costimulatory molecule CD80. Specific tolerogeneic or immature pDC subsets have been shown to exert a major influence on regulatory T cell recruitment [57,58]. Even after adoptive transfer to untreated naïve NOD mice this immature tolerogeneic DC subset exhibits suppressive capacities by accumulating CD4+CD25+ regulatory T cells [47]. Interestingly, G-CSF can expand a novel granulocyte-monocyte precursor population that functions as regulatory APC and promotes tolerance in a murine transplant model through the induction of IL-10-producing antigen-specific regulatory T cells [59]. However, a better classification of dendritic cell types and their precursor population will facilitate the analysis of the complex role and pleiotropic effects G-CSF exerts on the functional differentiation in APCs.

4. G-CSF in autoimmune diseases

The accumulating evidence that G-CSF affects adaptive immune responses with skewing of T cells towards a TH2 phenotype and emergence of tolerogeneic APC with recruitment of regulatory T cells prompted investigations in autoimmune disease. However, in humoral autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) the analysis of exogenous and endogenous G-CSF lead to contradictionary results. In animal models of RA the blockage of endogenous G-CSF in collagen-induced arthritis markedly reduced disease manifestation and is as effective as TNF inhibition [60]. These results were well in line with studies in other animal models showing that the exogenous administration of G-CSF may induce a severe exacerbation of inflammatory joint disease [61,62]. The deleterious effects of G-CSF were explained by G-CSF-induced mobilization, recruitment and activation of

myeloid cells within inflamed tissues. However, in an adjuvant arthritis model of Lewis rats G-CSF application reduced the disease severity, which was associated with a decrease of IFN-gamma secretion [63]. Importantly, in patients suffering from RA the administration of G-CSF seemed to flare the ongoing disease in a dose-dependent manner [64]. An unexpected modulation of disease activity was observed in a murine model system for SLE. Whereas chronic treatment of MRL-lpr/lpr mice with low doses of G-CSF (10 μg/kg) increased glomerular deposition of immunoglobulins and accelerated lupus disease, high-dose treatment with G-CSF (200 μg/kg) prevented lupus nephritis by local down-modulation of FcγRIII expression within the glomeruli [65]. Interestingly, at lower dosages G-CSF seems to function as a key regulator of B cell homeostasis via the production of BlyS, a novel member of the TNF ligand superfamily important for B cell maturation and survival by G-CSF-stimulated neutrophils [66,67]. This novel finding might explain in part the exacerbation of humoral autoimmune diseases in animal models and susceptible humans [68]. Especially, in some patients with chronic neutropenia which is often associated with autoimmune diseases like RA and SLE flares of symptoms or development of leukocytoclastic vasculitis have been observed after G-CSF treatment [68,69]. The role of G-CSF has also been investigated in inflammatory bowel disease (IBD) including Crohn's disease and ulcerative colitis. Innate and adaptive immune responses have been identified as major components for the development of chronic mucosal inflammation and are currently investigated in different experimental model systems. Myeloid and epithelial cell mechanisms are studied in White New Zealand rabbits where immune complex colitis could be ameliorated dramatically by G-CSF [70]. The second component contributing to the intestinal inflammation of IBD is the disturbance in adaptive immunity, which, in most instances, comprises an imbalance between pro-inflammatory effector and regulatory T cell responses. Potentially beneficial effects of G-CSF have been demonstrated in a hapten-induced colitis model with TH1-associated mucosal damage [71]. Pretreatment with recombinant G-CSF (125 μg/kg twice per day) over 5 days before hapten challenge drastically attenuated the degree of colitis through selective downregulation of TH1-associated cytokines [71]. Interestingly, recombinant human G-CSF promoted healing of Crohn's disease-like intestinal lesions in patients with chronic granulomatous disease and glycogen storage disease Ib, which are both characterized by neutropenia and defective neutrophil functions. In this group of patients retrospective analysis demonstrate that G-CSF treatment can be an important protective factor for the development of IBD [72]. These preliminary clinical results together with promising data from experimental model systems have been recently translated to prospective studies with G-CSF in the treatment of Crohn's disease [73,74]. G-CSF was well tolerated in five patients with Crohn's disease treated for 12 weeks three times per week at a dosage of 300 μg. Neutrophil counts, IL-1 receptor antagonist and soluble TNF receptor p55 and p75 levels were found to be increased during G-CSF administration resulting in an amelioration of severe ileocolitis [73]. Another study enrolled 20 patients with Crohn's disease and could confirm the efficacy of G-CSF application by applying the Crohn's disease activity index demonstrating a statistically significant improvement at each assessment point [74].

The immunoregulatory properties and therapeutic potential of G-CSF have also been investigated in experimental autoimmune encephalomyelitis (EAE), a murine model for the human demyelinating disease multiple sclerosis [75]. EAE is mediated by the activation of autoaggressive TH1-differentiated T cells which trigger an inflammatory reaction [76]. Immunoprevention of EAE has been hitherto achieved either by inhibition of TNF-α [77] or by inducing regulatory TH2/TH3 cells that release anti-inflammatory cytokines [78]. Interestingly, a short treatment with G-CSF at the onset of clinical signs provides durable protection of SJL/J mice from EAE [75]. G-CSF reduces the T cell infiltration and autoimmune inflammation within the CNS, an effect based on immunoregulatory events that take place in the periphery. G-CSF application induces an immune deviation toward TH2, a reduction of TNF-α production, and an imbalance in the chemokine production ratio of MIP-1α/MCP-1. *In vivo* and *in vitro* studies have identified MIP-1α and MCP-1 as prototypical pro-TH1 and -TH2 chemokines, respectively [79,80]. However, the molecular and cellular mechanisms leading to this imbalanced chemokine profile remain unclear. These results in EAE corroborate recent microarray studies on multiple sclerosis lesions revealing high gene expression levels of G-CSF in the acute phase, thus suggesting an endogenous role for G-CSF in the control of autoreactive processes [81]. Altogether these data demonstrate that G-CSF targets mechanisms critical for the pathogenesis of EAE and constitute a promising rationale for the clinical evaluation of G-CSF in human autoimmune diseases, such as multiple sclerosis.

Recent experimental data provide emerging evidence that G-CSF treatment prevents another T cell-mediated disease, autoimmune type 1 diabetes, in the NOD mouse [47,48]. G-CSF treatment was administered to NOD mice over five consecutive days every 4 weeks and provided significant protection against the development of spontaneous diabetes. This protection correlated with marked recruitment of $CD11c^{lo}$ $B220^{+}$ plasmacytoid DCs (pDCs), an immature tolerogenic DC subset that in turn recruits CD4+CD25+ regulatory T cells. This immature pDC subset is characterized by reduced costimulatory signal expression and higher IFN-α but lower IL-12p70 release capacity than DCs in control mice. G-CSF recipients further displayed accumulation of functional CD4+CD25+ regulatory T cells that produced high levels of TGF-β1 and exhibited suppressive properties also after transfer into secondary NOD/SCID recipients. The ability of G-CSF to promote key tolerogenic

interactions between DCs and regulatory T cells was further demonstrated by enhanced recruitment of TGF-β1-expressing CD4+CD25+ cells after adoptive transfer of DCs isolated from G-CSF-treated mice into naive NOD recipients [42]. An interesting aspect is the possible role of infectious events that trigger endogenous production of G-CSF, which might protect from autoimmune diabetes in the early phase. Thus, adaptive immunity might be controlled by innate immunity.

There is compelling evidence that infections may influence diabetes frequency in human populations [82]. In addition, vaccination with mycobacteria products such as complete Freund adjuvant [83] can prevent the onset of type 1 diabetes in NOD mice. Recent reports suggest that mycobacterial components such as hsp65 [84], might also induce endogenous response factors to infections, such as G-CSF. Mycobacterial infection of IFN-$\gamma^{-/-}$ mice is characterized by enhanced extramedullar hematopoiesis and high levels of G-CSF in the serum of these mice. These observations have disclosed a critical balance between IFN-γ and G-CSF [85]. In addition, it has been shown that G-CSF is able to antagonize the progression of autoimmune diabetes induced by cyclophosphamide [48]. G-CSF accelerates the recovery of the T cell compartment, which might be reconstituted by the mobilization of lymphoid progenitors [86]. It can selectively prevent the loss of CD4+CD25+ regulatory T cells and abrogate the IFN-γ-burst triggered in immune cells by cyclophosphamide. These results suggest that G-CSF may be evaluated for the treatment of human type 1 diabetes.

5. G-CSF in stem cell transplantation

Recombinant human G-CSF is widely utilized to mobilize hematopoietic stem cells from their bone marrow niche to the peripheral blood. In the last decade G-CSF-stimulated peripheral blood has largely replaced bone marrow as a stem cell source for the treatment of malignant and non-malignant diseases [87]. Some of the advantages of peripheral stem cell transplantation (PBSCT) compared with conventional bone marrow transplantation (BMT) include easier harvesting of stem cells, improved yield of progenitor cells, and more rapid and robust engraftment with reduced transplant-related mortality [17]. In clinical studies, despite the presence of more than 10-fold higher number of mature T cells in the G-CSF-mobilized graft compared to the unstimulated bone marrow harvest [39], a reduced incidence and severity of acute graft-versus-host disease (GVHD) has been reported [31,88]. Similarly in murine transplant models T cells from G-CSF-treated donors show a reduced capacity to induce GVHD [30]. The mechanism by which G-CSF ameliorates GVHD still remains controversial. G-CSF has been shown to induce a TH2 polarization of donor T cells [22] and this has been suggested to be a major protective mechanism. The administration of other cytokines like IL-11 driving T cell responses toward TH2 differentiation also protects from severe GVHD supporting the relevance of this finding [89]. Several other reports have emphasised the role of cytokines as mediators in GVHD with compelling evidence for the protective role of type-2 cytokines [30,90,91] and deleterious effects of inflammatory TH1 immune responses [91]. G-CSF may exert its beneficial effects through the upregulation of GATA-3 expression [9] directing and stabilizing the TH2 phenotype [33] while opposing TH1 differentiation [34].

Besides direct effects of G-CSF on donor T cell populations [7–9], there are additional data suggesting that G-CSF may reduce GVHD through effects on dendritic cells [37,38], monocytes [23,24,27], natural killer cells [31], and NKT cells [92]. Most importantly, murine and human studies have described the selective increase of plasmacytoid DC2 cell numbers after G-CSF application in the stem cell donor and graft [37,38] which seem to contribute to the observed TH2 shift in T cell immune responses. There is growing evidence that G-CSF may also promote the emergence of tolerogenic immature DC subsets [47,55,59] that dampen allogeneic and mitogenic responses by recruiting regulatory T cell populations [26,42,47,59]. A novel murine granulocyte-monocyte precursor population has been described after G-CSF application that promotes transplant tolerance by generation of MHC class II-restricted IL-10 secreting, antigen-specific regulatory T cells [59]. So far, these observations are limited to *in vitro* studies [26,55] and experimental model systems [42,47,54,59]. However, adoptive transfer of these immature APCs potently inhibiting GVHD represents a promising therapeutic strategy as graft-versus-leukemia (GVL) effects were not affected. This observation is crucial as the eradication of malignant cells by immunocompetent cells of the donor represents a major therapeutic principle in stem cell transplantation. A murine allogeneic transplant model investigated whether G-CSF-mobilized PBSC maintained their GVL effect [93]. In comparison to non-mobilized donors the transplant survival was dramatically increased and all surviving recipients were leukemia-free at day 70 after transplantation. Transplantations from G-CSF-mobilized perforin-deficient mice could demonstrate a crucial role for perforin in mediating the GVL effect, as 90% of the recipients died from leukemia. While G-CSF treatment of the donor led to reduced systemic inflammation in the recipient with reduced systemic level of LPS and TNF-α, perforin-dependent donor CTL activity was preserved and responsible for separating GVL from GVHD in G-CSF-mobilized allogeneic PBSCT [93].

To date it is not yet clear whether G-CSF induced tolerogeneic APCs are essential for the generation of regulatory T cells promoting transplantation tolerance or if other effector cells/mediators contribute to the generation of regulatory T cell populations. An alternative possibility represents the homeostatic trafficking of Tregs from the bone marrow as important reservoir for regulatory T cells

through G-CSF regulated expression of CXCL12 [49]. However, studies in murine models show that CD4+CD25+ regulatory T cells are important for the induction of peripheral tolerance [94] and may potently inhibit GVHD [95–97] while preserving the GVL effect [98]. A recent murine study could show that donor pretreatment with G-CSF protects from GVHD in a dose-dependent fashion due to the generation of IL-10-producing Tregs [42]. These data collaborate with the observation in the human system where CD4+ T cells exposed to G-CSF *in vivo* acquire the properties of IL-10-producing Treg cells following TCR ligation *in vitro* [26]. However, the presence of additional effector molecules besides IL-10 must be assumed as T cells from G-CSF-treated IL-$10^{-/-}$ donors have residual regulatory function *in vivo* [42]. Gene expression profiling of CD4+ T cells isolated from G-CSF-stimulated donors revealed a downregulation of costimulatory molecules that may have contributed to the reduced alloreactivity observed in PBSCT [9]. In addition, a homing receptor of naïve T cells to the peripheral lymphoid tissues, LFA-1α, was drastically downregulated probably abrogating donor T cell activation to host antigens and thereby leading to an amelioration of alloreactive immune responses [9]. Interestingly, results from a murine model showed that transient a block of homing receptors reduced the alloreactivity of donor T cells and prevented acute GVHD [99].

G-CSF is often also administered to the recipients following transplantation to accelerate the hematopoietic reconstitution, which is currently a matter of debate. Whereas a recent meta-analysis studying more than 1000 patients revealed no effect of G-CSF on the incidence and severity of GVHD [100], an increased risk of GVHD with reduction of overall survival has been reported by the EBMT [101]. Furthermore, considerable concerns for routinely applied G-CSF after transplantation are based on studies in HLA-mismatched stem cell transplantation which show a delayed immune reconstitution following G-CSF administration [102]. Although the underlying mechanism remains unclear, the effects of G-CSF likely differ in transplant recipients from those observed in stem cell donors treated with G-CSF.

6. Conclusion

Despite the fact that our understanding of the role of G-CSF in adaptive immune responses is rapidly increasing, numerous questions remain. In particular, the mechanism of tolerance induction requires further clarification. To date, it is still not clear whether G-CSF induces regulatory T cells from naïve T cells in the periphery, selectively expanding predisposed populations capable of exerting regulatory functions (i.e. by promoting the emergence of tolerogeneic APCs) or whether, alternatively, G-CSF facilitates homeostatic trafficking of Tregs from the bone marrow. The generation of potentially tolerogeneic subsets at both poles of the APC/T cell interaction make G-CSF an interesting candidate for specific immune modulation in transplantation and autoimmune diseases associated with TH1/TH2 imbalance. In addition, there is growing evidence that G-CSF is an essential mediator in innate immune responses linking adaptive and innate immunity, which is of particular interest in infectious diseases and autoimmunity.

Acknowledgement

The author is very thankful to Arnold Ganser for critical discussion and review of the manuscript.

References

[1] Metcalf D. The molecular control of cell division, differentiation, commitment and maturation in haematopoietic cells. Nature 1989;339:27–30.
[2] Tsuchiya M, Asano S, Kaziro Y, Nagata S. Isolation and characterization of the cDNA for murine granulocyte colony-stimulating factor. Proc Natl Acad Sci USA 1986;83:7633–7.
[3] Nicola NA, Begley CG, Metcalf D. Identification of the human analogue of a regulator that induces differentiation in murine leukemia cells. Nature 1985;314:625–8.
[4] Welte K, Bonilla MA, Gillio AP, Boone TC, Potter GK, Gabrilove JL, et al. Recombinant human granulocyte colony-stimulating factor. Effects on hematopoiesis in normal and cyclophosphamide-treated primates. J Exp Med 1987;165:941–8.
[5] Simmers RN, Webber LM, Shannon MF, Garson OM, Wong G, Vadas MA, et al. Localization of the G-CSF gene on chromosome 17 proximal to the breakpoint in the t(15;17) in acute promyelocytic leukemia. Blood 1987;70:330–2.
[6] Demetri GD, Griffin JD. Granulocyte colony-stimulating factor and its receptor. Blood 1991;78(11):2791–808.
[7] Morikawa K, Morikawa S, Nakamura M, Miyawaki T. Characterization of granulocyte colony-stimulating factor receptor expressed on human lymphocytes. Br J Haematol 2002;118:296–304.
[8] Sloand E, Kim S, Maciejewski JP, van Rhee F, Chauduri A, Barrett J, et al. Pharmacologic doses of granulocyte colony-stimulating factor affect cytokine production by lymphocytes in vitro and in vivo. Blood 2000;95:2269–74.
[9] Franzke A, Piao W, Lauber J, Gatzlaff P, Könecke C, Hansen W, et al. G-CSF as immune regulator in T cells expressing the G-CSF receptor: implications for transplantation and autoimmune diseases. Blood 2003;102:734–9.
[10] Xu S, Hogland M, Venge P. The effect of granulocyte colony-stimulating factor (G-CSF) on the degranulation of secondary granule proteins from human neutrophils in vivo may be indirect. Br J Haematol 1996;93:558–68.
[11] Yong KL. Granulocyte colony-stimulating factor (G-CSF) increases neutrophil migration across vascular endothelium independent of an effect on adhesion: comparison with granulocyte-macrophage colony-stimulating factor (GM-CSF). Br J Haematol 1996;94:40–7.
[12] Jacobsen SE, Ruscetti FW, Dubois CM, Wine J, Keller JR. Induction of colony-stimulating factor receptor expression on hematopoietic progenitor cells: proposed mechanism for growth factor synergism. Blood 1992;80:678–87.
[13] Lieschke GJ, Grail D, Hodgson G, Metcalf D, Stanley E, Cheers C, et al. Mice lacking granulocyte colony-stimulating factor have chronic neutropenia, granulocyte and macrophage progenitor cell

deficiency, and impaired neutrophil mobilization. Blood 1994;84: 1737–46.
[14] Liu F, Wu H, Wesselschmidt R, Kornaga T, Link D. Impaired production and increased apoptosis of neutrophils in granulocyte colony-stimulating factor receptor-deficient mice. Immunity 1996;5: 491–501.
[15] Bober LA, Grace MJ, Pugliese-Sivo C, Rojas-Triana A, Sullivan LM, Narula SK. The effects of colony-stimulating factors on human monocyte cell function. Int J Immunopharmacol 1995;17(5):385–92.
[16] Xu S, Hoglund M, Hakansson L, Venge P. Granulocyte colony-stimulating factor (G-CSF) induces the production of cytokines in vivo. Br J Haematol 2000;108:848–53.
[17] Bensinger WI, Martin PJ, Storer B, Clift R, Forman SJ, Negrin R, et al. Transplantation of bone marrow as compared with peripheral blood cells from HLA-identical relatives in patients with hematologic cancers. N Engl J Med 2001;344(3):175–81.
[18] Moore MA. The clinical use of colony-stimulating factors. Annu Rev Immunol 1991;9:159–91.
[19] Reyes E, Garcia-Castro I, Esquivel F, Hornedo J, Cortes-Funes H, Solovera J, et al. G-CSF transiently suppresses mitogen-stimulated T cell proliferative response. Br J Cancer 1999;80:229–35.
[20] Mielcarek M, Martin PJ, Torok-Storb B. Suppression of alloantigen-induced T cell proliferation by CD14+ cells derived from granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cells. Blood 1997;89:1629–34.
[21] Rutella S, Rumi C, Lucia MB, Sica S, Cauda R, Leone G. Serum of healthy donors receiving recombinant human granulocyte colony-stimulating factor induces T cell unresponsiveness. Exp Hematol 1998;26:1024–33.
[22] Rutella S, Rumi C, Pierelli L, Morosetti R, Sica S, Bonanno G, et al. Granulocyte colony-stimulating factor pertubates lymphocyte mitochondria function and inhibits cell cycle progression. Exp Hematol 2000;28:612–25.
[23] Nawa Y, Teshima T, Sunami K, Hiramatsu Y, Maeda Y, Yano T, et al. G-CSF reduces IFN-gamma and IL-4 production by T cells after allogeneic stimulation by indirectly modulating monocyte function. Bone Marrow Transplant 2000;25:1035–40.
[24] Mielcarek M, Graf L, Johnson G, Torok-Storb B. Production of interleukin-10 by granulocyte colony-stimulating factor-mobilized blood products: a mechanism for monocyte-mediated suppression of T cell proliferation. Blood 1998;92:215–22.
[25] Hans JP, Koenen M, Joosten I. Blockade of CD86 and CD40 induces alloantigen-specific immunoregulatory T cells that remain anergic even after reversal of hyporesponsiveness. Blood 2000;95:3153–61.
[26] Rutella S, Pierelli L, Bonnano G, Sica S, Ameglio F, Capoluongo E, et al. Role for granulocyte colony-stimulating factor in the generation of human T regulatory type 1 cells. Blood 2002;100:2562–71.
[27] Boneberg EM, Hareng L, Gantner F, Wendel A, Hartung T. Human monocytes express functional receptors for granulocyte colony-stimulating factor that mediate suppression of monokines and interferon-gamma. Blood 2000;95:270–6.
[28] Hartung T, Docke WD, Gantner F, Krieger G, Sauer A, Stevens P, et al. Effect of granulocyte colony-stimulating factor treatment on ex vivo blood cytokine response in human volunteers. Blood 1995;85: 2482–9.
[29] Kitabayashi A, Hirokawa M, Hatano Y, Lee M, Kuroki J, Niitsu H, et al. Granulocyte colony-stimulating factor downregulates allogeneic immune responses by posttranscriptional inhibition of tumor necrosis factor-alpha production. Blood 1995;86:2220–7.
[30] Pan L, Delmonte J, Jalonen CK, Ferrara JL. Pretreatment of donor mice with granulocyte colony-stimulating factor polarizes donor T lymphocytes toward type-2 cytokine production and reduces severity of experimental graft-versus-host disease. Blood 1995;86:4422–9.
[31] Zeng D, Dejbakhsh-Jones S, Strober S. Granulocyte colony-stimulating factor reduces the capacity of blood mononuclear cells to induce graft-versus-host disease: impact on blood progenitor cell transplantation. Blood 1997;90:453–63.
[32] Pan L, Bressler S, Cooke KR, Krenger W, Karandikar M, Ferrara JL. Long-term engraftment, graft-vs.-host disease, and immunologic reconstitution after experimental transplantation of allogeneic peripheral blood cells from G-CSF-treated donors. Biol Blood Marrow Transplant 1996;2:126–33.
[33] Zheng W, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell 1997;89:587–96.
[34] Ouyang W, Ranganath SH, Weindel K, Bhattacharya D, Murphy TL, Sha WC, et al. Inhibition of Th1 development mediated by GATA-3 through an IL-4-independent mechanism. Immunity 1998;9:745–55.
[35] Shortman K, Liu YJ. Mouse and human dendritic cell subtypes. Nat Rev Immunol 2002;2:151–61.
[36] Wakkach A, Fournier N, Brun V, Breittmayer JP, Cottrez F, Groux H. Characterization of dendritic cells that induce tolerance and T regulatory 1 cell differentiation in vivo. Immunity 2003;18:605–17.
[37] Arpinati M, Green CL, Heimfeld S, Heuser JE, Anasetti C. Granulocyte colony-stimulating factor mobilizes T helper 2-inducing dendritic cells. Blood 2000;95:2484–90.
[38] Klangsinsirikul P, Russell NH. Peripheral blood stem cell harvests from G-CSF-stimulated donors contain a skewed Th2 CD4 phenotype and a predominance of type 2 dendritic cells. Exp Hematol 2002;30:495–501.
[39] Tayebi H, Kuttler F, Saas P, Lienard A, Petracca B, Lapierre V, et al. Effect of granulocyte colony-stimulating factor on phenotypical and functional properties of immune cells. Exp Hematol 2001;29: 458–70.
[40] MacDonald KP, Rowe V, Flippich C, Thomas R, Clouston AD, Welply JK, et al. Donor pretreatment with progenipoetin-1 is superior to granulocyte colony-stimulating factor in preventing graft-versus-host disease after allogeneic stem cell transplantation. Blood 2003;101:2033–42.
[41] Hirayama Y, Sakamaki S, Matsunaga T, Kuroda H, Kusakabe T, Akiyama T, et al. Granulocyte colony-stimulating factor enhances the expression of transforming growth factor-beta mRNA in CD4+ peripheral blood lymphocytes in the donor for allogeneic peripheral blood stem cell transplantation. Am J Hematol 2002;69:138–40.
[42] Morris ES, Kelli P, MacDonald A, Rowe V, Johnsosn DH, Banovic T, et al. Donor treatment with pegylated G-CSF augments the generation of IL-10-producing regulatory T cells and promotes transplantation tolerance. Blood 2004;103:3573–81.
[43] Sakaguchi S. Regulatory T cells: key controllers of immunologic self tolerance. Cell 2000;101:455–8.
[44] Wakkach A, Fournier N, Brun V, Breittmayer JP, Cottrez F, Groux H. Characterization of dendritic cells that induce tolerance and T regulatory 1 cell differentiation in vivo. Immunity 2003;18(5): 605–17.
[45] Dieckmann D, Bruett CH, Ploettner H, Lutz MB, Schuler G. Human CD4+CD25+ regulatory, contact-dependent T cells induce interleukin-10-producing, contact-independent type 1-like regulatory T cells [corrected]. J Exp Med 2002;196(2):247–53.
[46] Yamagiwa S, Gray JD, Hashimoto S, Horwitz DA. A role for TGF-beta in the generation and expansion of CD4+CD25+ regulatory T cells from human peripheral blood. J Immunol 2001;166(12): 7282–9.
[47] Kared H, Masson A, Adle-Biasette H, Bach JF, Chatenoud L, Zavala F. Treatment with granulocyte colony-stimulating factor prevents diabetes in NOD mice by recruiting plasmacytoid dendritic cells and functional CD4+CD25+ regulatory T cells. Diabetes 2005;54:78–84.
[48] Hadaya K, Kared H, Masson A, Chatenoud L, Zavala F. G-CSF treatment prevents cyclophosphamide acceleration of autoimmune diabetes in the NOD mouse. J Autoimmun 2005;24(2):125–34.
[49] Zou L, Barnett B, Safah H, LaRussa VF, Evdemon-Hogan M, Mottram P, et al. Bone marrow is a reservoir for CD4+CD25+ regulatory T cells that traffic through CXCL12/CXCR4 signals. Cancer Res 2004;64:8451–5.

[50] Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998;392:245–52.
[51] Rissoan MC, Soumelis V, Kadowaki N, Grouard G, Briere F, de Waal Malefyt R, et al. Reciprocal control of T helper cells and dendritic cell differentiation. Science 1999;283:1183–6.
[52] Vlad G, Cortesini R, Suciu-Foca N. Licence to heal: bidirectional interaction of antigen-specific regulatory T cells and tolerogenic APC. J Immunol 2005;174:5907–14.
[53] Cederbom L, Hall H, Ivars F. CD4+CD25+ regulatory T cells downregulate co-stimulatory molecules on antigen-presenting cells. Eur J Immunol 2000;30:1538–43.
[54] Vuckovic S, Kim M, Khalil D, Turtle CJ, Crosbie GV, Williams N, et al. Granulocyte colony-stimulating factor increases CD123hi blood dendritic cells with altered CD62L and CCR7 expression. Blood 2003;101:2314–7.
[55] Rutella S, Bonanno R, Pierelli L, Mariotti A, Capoluongo E, Contemi AM, et al. Granulocyte colony-stimulating factor promotes the generation of regulatory DC through induction of IL-10 and IFN-α. Eur J Immunol 2004;34:1291–302.
[56] Levings MK, Sangregorio F, Galbiati S, Squadrone S, de Waal Malefyt R, Roncarolo MG. IFN-α and IL-10 induce the differentiation of human type 1 T-regulatory cells. J Immunol 2001;166: 5530–9.
[57] Nakano H, Yanagity M, Gunn MD. CD11c(+)B220(+)Gr-1(+) cells in mouse lymph nodes and spleen display characteristics of plasmacytoid cells. J Exp Med 2001;194:1171–8.
[58] Moser M. Dendritic cells in immunity and tolerance: do they display opposite functions? Immunity 2003;19:5–8.
[59] MacDonald KP, Rowe V, Clouston AD, Welply JK, Kuns RD, Ferrara JLM, et al. Cytokine expanded myeloid precursors function as regulatory antigen-presenting cells and promote tolerance through IL-10-producing regulatory T cells. J Immunol 2005;174:1841–50.
[60] Lawlor KF, Campbell IK, Metcalf K, O'Donnell A, van Nieuwenhuijze A, Roberts AW, et al. Critical role for G-CSF in inflammatory arthritis. Proc Natl Acad USA 2004;101:11398–403.
[61] Campbell IK, Rich MJ, Bischof RJ, Hamilton JA. The colony-stimulating factors and collagen-induced arthritis: exacerbation of disease by M-CSF and G-CSF and requirements for endogenous M-CSF. J Leukocyte Biol 2000;68:144–50.
[62] Miyahata H, Horokebuchi T, Saikawa I, Arita C, Takagishi K, Sugioka Y. The effects of recombinant human granulocyte colony-stimulating factor on passive collagen-induced arthritis transferred with anti-type II collagen antibody. Clin J Immunol Immunpathol 1993;69:69–76.
[63] Brendolan A, Higuchi M, Sibley R, Strober S. Treatment of adjuvant arthritis with granulocyte-colony stimulating factor and peptide derived from heat shock protein 65. Cell Immunol 2003;221:6–14.
[64] Snowden JA, Biggs JC, Milliken ST, Fuller A, Staniforth D, Passuello F, et al. A randomised, blinded, placebo-controlled, dose escalation study of the tolerability and efficacy of filgastrim for hematopoietic cell mobilization in patients with severe active rheumatoid arthritis. Bone Marrow Transplant 1998;22:1035–41.
[65] Zavala F, Masson A, Hadaya K, Ezine S, Schneider E, Babin O, et al. Granulocyte colony-stimulating factor treatment of lupus autoimmune disease in MRL-lpr/lpr mice. J Immunol 1999;163:5125–32.
[66] Scapini P, Nardelli B, Nadali G, Calzetti F, Pizzolo G, Montecucco C, et al. G-CSF stimulated neutrophils are a prominent source of functional BLyS. J Exp Med 2003;197:297–302.
[67] Scapini P, Carletto A, Nardelli B, Calzetti F, Roschke V, Merigo F, et al. Proinflammatory mediators elicit secretion of the intracellular B lymphocyte stimulator pool (BLyS) that is stored in activated neutrophils: implications for inflammatory diseases. Blood 2005;105: 830–7.
[68] Euler HH, Harten P, Zeuner RA, Schwab M. Recombinant human granulocyte colony-stimulating factor in patients with systemic lupus erythematosus associated neutropenia and refractory infections. J Rheumatol 1997;24:2153–7.
[69] Starkebaum G. Chronic neutropenia associated with autoimmune diseases. Sem Hematol 2002;39:121–7.
[70] Hommes DW, Meenan J, Dijkhuizen S, Ten Kate FJ, Tytgat GN, van Deventer SJ. Efficacy of recombinant granulocyte colony-stimulating factor in experimental colitis. Clin Exp Immunol 1996;106:529–33.
[71] Egi H, Hayamizu K, Yoshimitsu M, Shimamoto F, Oishi K, Ohmori I, et al. Regulation of T helper type-1 immunity in hapten-induced colitis by host treatment with granulocyte colony-stimulating factor. Cytokine 2003;23:23–30.
[72] Melis D, Parenti G, Della Casa R, Sibilio M, Berri Canani R, Terin G, et al. Crohn's like ileo-colitis in patients affected by glycogen storage disease Ib: two years' follow-up of patients with a wide spectrum of gastrointestinal signs. Acta Paediatr 2003;92:1415–21.
[73] Dejaco C, Lichtenberger C, Miehsler W, Oberhuber G, Herbst F, Vogelsang H, et al. An open-label pilot study of granulocyte colony-stimulating factor for the treatment of severe endoscopic postoperative recurrence in Crohn's disease. Digestion 2003;68: 63–70.
[74] Korzenik JR, Dieckgraefe BK. An open-labeled study of granulocyte colony-stimulating factor in the treatment of active Crohn's disease. Aliment Pharmacol Ther 2005;68:63–70.
[75] Zavala F, Abad S, Ezine S, Taupin V, Masson A, Bach JF. G-CSF therapy of ongoing experimental allergic encephalomyelitis via chemokine- and cytokine-based immune deviation. J Immunol 2002;168:2011–9.
[76] Antel JP, Owens T. Immune regulation and CNS autoimmune disease. J Neuroimmunol 1999;100:181–9.
[77] Korner H, Riminton DS, Strickland DH, Lemckert FA, Pollard JD, Sedgwick JD. Critical points of tumor necrosis factor action in central nervous system autoimmune inflammation defined by gene targeting. J Exp Med 1997;186:1585–90.
[78] Khoury SJ, Hancock WW, Weiner HL. Oral tolerance to myelin basic protein and natural recovery from experimental autoimmune encephalomyelitis are associated with downregulation of inflammatory cytokines and differential upregulation of transforming growth factor beta, interleukin 4, and prostaglandin E expression in the brain. J Exp Med 1992;176:1355–64.
[79] Kennedy KJ, Karpus WJ. Role of chemokines in the regulation of Th1/Th2 and autoimmune encephalomyelitis. J Clin Immunol 1999;19:273–9.
[80] Karpus WJ, Lukacs NW, Kennedy KJ, Smith WS, Hurst SD, Barrett TA. Differential CC chemokine-induced enhancement of T helper cell cytokine production. J Immunol 1997;158:4129–36.
[81] Lock C, Hermans G, Pedotti R, Brendolan A, Schadt E, Garten H, et al. Gene-microarray analysis of multiple sclerosis yields new targets validated in autoimmune encephalomyelitis. Nat Med 2002;8:500–8.
[82] Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. N Engl J Med 2002;347:911–20.
[83] Sadelain MW, Qin HY, Lauzon J, Singh B. Prevention of type 1 diabetes in NOD mice by adjuvant immunotherapy. Diabetes 1990;39:583–9.
[84] Elias D, Reshef T, Birk OS, van der Zee R, Walker MD, Cohen IR. Vaccination against autoimmune mouse diabetes with a T cell epitope of the human 65-kDa heat shock protein. Proc Natl Acad Sci USA 1991;88:3088–91.
[85] Murray PJ, Young RA, Daley GQ. Hematopoietic remodeling in interferon-gamma-deficient mice infected with mycobacteria. Blood 1998;91:2914–24.
[86] Imamura R, Miyamaoto T, Yashimoto G, Kamozyki K, Ishikawa F, Henzan H, et al. Mobilization of human lymphoid progenitors after treatment with granulocyte colony-stimulating factor. J Immunol 2005;175(4):2647–54.
[87] Gratwohl A, Baldomero H, Horisberger B, Schmid C, Passweg J, Urbano-Ispizua A. Current trends in hematopoietic stem cell transplantation in Europe. Blood 2002;100:2374–86.

[88] Schmitz N, Dreger P, Suttorp M, Rohwedder EB, Haferlach T, Loffler H, et al. Primary transplantation of allogeneic peripheral blood progenitor cells mobilized by filgrastim (granulocyte colony-stimulating factor). Blood 1995;85:1666–72.
[89] Hill GR, Cooke KR, Teshima T, Crawford JM, Keith JC, Brinson YS, et al. Interleukin-11 promotes T cell polarization and prevents acute graft-versus-host disease after allogeneic bone marrow transplantation. J Clin Invest 1998;102:115–23.
[90] Krenger W, Snyder KM, Byon JC, Falzarano G, Ferrara JL. Polarized type 2 alloreactive CD4+ and CD8+ donor T cells fail to induce experimental acute graft-versus-host disease. J Immunol 1995;155 (2):585–93.
[91] Rus V, Svetic A, Nguyen P, Gause WC, Via CS. Kinetics of Th1 and Th2 cytokine production during the early course of acute and chronic murine graft-versus-host disease. Regulatory role of donor CD8+ T cells. J Immunol 1995;155(5):2396–406.
[92] Morris ES, MacDonald KP, Rowe V, Banovic T, Kuns RD, Don AL, et al. NKT cell-dependent leukemia eradication following stem cell mobilization with potent G-CSF analogs. J Clin Invest 2005;115: 3093–103.
[93] Pan L, Teshima T, Hill GR, Bungard D, Brinson YS, Reddy VS, et al. Granulocyte colony-stimulating factor-mobilized allogeneic stem cell transplantation maintains graft-versus-leukemia effects through a perforin-dependent pathway while preventing graft-versus-host disease. Blood 1999;93:4071–8.
[94] Wood KJ, Sakaguchi S. Regulatory lymphocytes: regulatory T cells in transplantation tolerance. Nat Rev Immunol 2003;3:199–210.
[95] Taylor PA, Noelle RJ, Blazar BR. CD4(+)CD25(+) immune regulatory cells are required for induction of tolerance to alloantigen via costimulatory blockade. J Exp Med 2001;193:1311–8.
[96] Ermann J, Hoffmann P, Edinger M, Dutt S, Blankenberg FG, Higgins JP, et al. Only the CD62L+ subpopulation of CD4+CD25+ regulatory T cells protects from lethal acute GVHD. Blood 2005;105:2220–6.
[97] Hoffmann P, Ermann J, Edinger M, Fathman CG, Strober S. Donor-type CD4+CD25+ regulatory T cells suppress lethal acute graft-versus-host disease after allogeneic bone marrow transplantation. J Exp Med 2002;196:389–99.
[98] Edinger M, Hoffmann P, Erman J, Drago K, Fathman CG, Strober S, et al. CD4+CD25+ regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation. Nat Med 2003;9:1144–50.
[99] Li B, New JY, Yap EH, Lu J, Chan SH, Hu H. Blocking L-selectin and alpha4-integrin changes donor cell homing pattern and ameliorates murine acute graft versus host disease. Eur J Immunol 2001;31:617–24.
[100] Ho VT, Mirza NQ, Junco Ds D, Okamura T, Przepiorka D. The effect of hematopoietic growth factors on the risk of graft-versus-host disease after allogeneic hematopoietic stem cell transplantation: a meta-analysis. Bone Marrow Transplant 2003;32:771–5.
[101] Ringden O, Labopin M, Gorin NC, Le Blanc K, Rocha V, Gluckman E, et al. Treatment with granulocyte colony-stimulating factor after allogeneic bone marrow transplantation for acute leukemias increases the risk of graft-versus-host disease and death: a study from the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. J Clin Oncol 2004;22:416–23.
[102] Volpi I, Perruccio K, Tosti A, Capanni M, Ruggeri L, Posati S, et al. Postgrafting administration of granulocyte colony-stimulating factor impairs functional immune recovery in recipients of human leukocyte antigen haplotype-mismatched hematopoietic transplants. Blood 2001;97:2206–14.