Bone Marrow Transplantation (2006) 38, 453–460
© 2006 Nature Publishing Group All rights reserved 0268-3369/06 $30.00
www.nature.com/bmt

ORIGINAL ARTICLE

Effect of hematopoietic growth factors on severity of experimental autoimmune encephalomyelitis

L Verda[1], K Luo[1], D-A Kim[1], D Bronesky[1], AP Kohm[2], SD Miller[2], L Statkute[1], Y Oyama[1] and RK Burt[1]

[1]*Division of Immunotherapy, Feinberg School of Medicine, Northwestern University Medical Center, Chicago, IL, USA and*
[2]*Department of Microbiology-Immunology, Northwestern University, Chicago, IL, USA*

We have investigated the influence of different hematopoietic growth factors, including granulocyte colony-stimulating factor (G-CSF), stem cell factor (SCF), Flt-3 ligand (Flt-3L) and thrombopoietin (TPO), on the course of relapsing experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis. Disease course and central nervous system histology were evaluated in all groups. When given after immunization but before either disease onset or during remission, Flt-3L, SCF and G-CSF exacerbated disease severity whereas TPO had no effect compared to non-cytokine-treated controls. When compared to controls, TPO did not exacerbate disease. We conclude that autoimmune disease severity may be affected by hematopoietic growth factors currently being employed in hematopoietic stem cell transplantation of patients with autoimmune disease. The mechanism of their effects remains unknown: it may be related to both T helper (Th) 1/Th2 skewing and/or homing of inflammatory cells to the disease-affected organ.
Bone Marrow Transplantation (2006) **38,** 453–460. doi:10.1038/sj.bmt.1705473
Keywords: G-CSF; Flt-3 ligand; thrombopoietin; stem cell factor; EAE; multiple sclerosis

Introduction

Relapsing experimental autoimmune encephalomyelitis (R-EAE) is an animal model of multiple sclerosis (MS). Following an initial onset of neurologic deficits, symptoms resolve and then subsequently relapse in analogy to relapsing-remitting multiple sclerosis. Immunologic mechanisms involved in mediating remission and relapse are not well defined. EAE is an organ-specific, T-cell-induced autoimmune disease of the central nervous system (CNS), characterized by lymphocytic and monocytic infiltration, astrocytic hypertrophy and demyelination. CNS-infiltrating lymphocytes in EAE are T helper (Th) 1 cells that secrete primarily interleukin-2 (IL-2), interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) and promote inflammation and cellular immunity.[1,2] Perturbations in cytokine/chemokine expression patterns such as changes in lymphocyte Th1 and Th2 subsets, T regulatory cells and/or dendritic cell (DC)-1 and DC-2 alterations may contribute to the dynamic changes observed in the clinical course of EAE, and by inference, MS.

High-dose immunosuppression with hematopoietic stem cell transplantation (HSCT), first evaluated in EAE, has become an increasingly used form of therapy for MS. Animal studies in EAE suggested that syngeneic HSCT performed early in the course of the disease could improve or ameliorate disease severity.[3–5] However, HSCT applied in the late chronic phase of EAE does not influence disease severity owing to the irreversible pathologic changes initiated during early inflammatory demyelination.[3] Similar to chronic EAE, HSCT in the late progressive phase of MS did not improve neurologic deficits.[6] Studies of HSCT in the relapsing-remitting phase of MS, analogous to early inflammatory EAE, are now ongoing.[7]

Hematopoietic growth factors that are being used to mobilize stem cells and to minimize post transplant cytopenias in patients with MS have immune-modulating properties. Although granulocyte colony-stimulating factor (G-CSF)-based mobilization regimen remains the 'gold standard' of current clinical practice, several agents including thrombopoietin (TPO), stem cell factor (SCF), Flt-3 ligand (Flt-3L), etc. have shown significant promise in optimizing mobilization procedure as reviewed by Filshie.[8] G-CSF administration during mobilization has been associated with disease exacerbation in patients with MS and rheumatoid arthritis.[9,10] These reports heighten concern that G-CSF might adversely influence disease status in patients with MS or other autoimmune diseases undergoing HSC mobilization or HSCT. TPO, the primary regulator of megakaryocytopoiesis, also mediates biologic effects on hematopoietic cells more primitive than those committed to the megakaryocyte.[11] Both hematopoietic cytokines, SCF and TPO, play pivotal roles in stem cell renewal and expansion.[12,13] It has been reported that TPO could

Correspondence: Dr RK Burt, Division of Immunotherapy, Feinberg School of Medicine, Northwestern University Medical Center, 750 North Lake Shore Drive, ABA Bldg 649, Chicago, IL 60611, USA.
E-mail: rburt@northwestern.edu
Received 28 April 2006; revised 12 July 2006; accepted 13 July 2006

mobilize the CD34+ cell subset into peripheral blood.[14] We, therefore, evaluated and compared the effect of various HSC mobilizing growth factors including G-CSF, TPO, Flt-3L and SCF in R-EAE.

Materials and methods

Mice
Female Swiss Jackson Laboratory/Jackson (SJL/J) mice, 5–6 week old, were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and maintained under barrier conditions with easy access to water and food *ad libitum*. All animal experiments were approved by the Institutional Animal Care and Use Committee of Northwestern University.

Induction and clinical evaluation of PLP139–151-induced EAE
Proteolipid peptide sequence 139–151 (PLP139–151) (HSLGKWLGHPDKF) was obtained from Genemed Synthesis (South San Francisco, CA, USA) and reconstituted in phosphate-buffered saline (PBS). Six to 7-week-old mice were immunized subcutaneously (s.c.) with 100 μl of a complete Freunds adjuvant emulsion containing 200 μg of mycobacterium tuberculosis H37Ra (Difco Laboratories, Detroit, MI, USA) and 50 μg of PLP139–151 distributed over three sites on the flank.

Mice were evaluated daily for clinical signs of diseases on a scale of 0–5 as follows: 0 = no abnormality; 1 = limp tail; 2 = limp tail and hind limb weakness (waddling gait); 3 = hind limb paralysis; 4 = hind limb paralysis and forelimb weakness and 5 = moribund. Daily clinical score evaluation was blinded. The data are reported as the mean daily clinical score.

Growth factors
Human G-CSF (hG-CSF), murine SCF (mSCF) and human TPO (hTPO), kindly provided by Kirin Pharmaceutical company (Takasaki, Japan), were prepared in 1% bovine serum albumin, and stored at −20°C. Human Flt-3L (hFlt-3L) was purchased from Immunex (Seattle, Washington, DC, USA).

Treatment
By definition, day 0 was the day of PLP139–151 injection. Mice were divided into several groups (nine mice per group). Control mice received no further therapy. Another control group (PBS group) was given PBS every day s.c. from day 1 to 10. G-CSF group received an injection of hG-CSF at a dose of 5 μg/mouse/day on days 1–10. TPO group was given 3 μg/mouse/day hTPO for 10 consecutive days starting on day 1. Flt-3L and SCF groups were given hFlt-3L at a dose of 10 μg/mouse/day and mSCF at a dose of 0.625 μg/mouse/day on days 1–10. Another set of experiments was designed to administer cytokines later in the disease course: mice were primed with PLP139–151 on day 0; on day 30, mice were divided into groups (nine mice per group) according to their mean and median clinical score and similar disease course, and cytokines at doses as described previously were given from day 30 to 40. Finally, we performed experiments comparing hG-CSF given at a dose of 5 μg/mouse/day and mouse G-CSF (mG-CSF) at a dose of 0.075 μg/mouse/day and an effect of Solu-Medrol if added to hG-CSF. G-CSF + Solu-Medrol group received Solu-Medrol (Pharmacia and Upjohn; Kalamazoo, MI, USA) at a dose of 50 μl/mouse/day in addition to G-CSF.

Collection of blood and spleen cells
Blood was drawn by cardiac puncture. Spleens were removed surgically. Cells were gently teased from the spleens with 25-gauge needles into Hanks balanced salt solution without calcium and magnesium. Clumps were dispersed by repeated aspiration with 1-ml syringe without a needle. Cells were washed once in Roswell Park Memorial Institute medium 1640 solution supplemented with 5% fetal calf serum (Bio-Technologies Inc., Parkerford, PA, USA). Red blood cells were lysed using an ammonium chloride lysing solution (Pharmingen, San Diego, CA, USA).

Complete blood count counting
Blood samples collected from five mice of each group were anti-coagulated in 100 U of heparin solution and transferred in K2EDTA-coated 500 μl Microtainer (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). The samples were counted using a CELL-DYN 1600 (Harrell Medical, Lake Oswego, OR, USA) calibrated for animal testing. Mice selected for blood sampling had clinical scores that represented the mean for their group.

Cytokine analysis was performed using LiquiChip kits
Splenocytes and lymph node (LN) lymphocytes (from nine mice per group) were washed and resuspended in Dulbecco's modified Eagle's medium/10% fetal bovine serum and plated at 5×10^5/well in 96-well flat-bottom microtiter plates with or without 50 mmol/l PLP139–151. After 72 h incubation at 37°C in 7.5% CO_2, supernatants were harvested. Final experiment cytokine concentrations were analyzed using LiquiChip mouse cytokine kits for IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12(p70), granulocyte–macrophage colony-stimulating factor (GM-CSF), IFN-γ and TNF-α (Qiagen Inc., Valencia, CA, USA), according to the manufacturer's instructions. Measurements were repeated in triplicate.

Histology
Mice that received cytokines from day 30 to 40 were killed at day 72 and spinal cords were collected for histologic examination (nine mice in each group). Ten to 12 1-μm thick, Epon-embedded sections stained with toluidine blue were examined from each spinal cord and analyzed blindly for degree of inflammation, demyelination and glial scarring as follows: ±, mild inflammation without demyelination; +, inflammation with focal demyelination; + +, inflammation with multiple foci of demyelination and + + +, marked inflammation with bilateral converging areas of demyelination. Glial scarring was characterized according to the majority of sections involved as

follows: absent, mild, focal glial bands of margin of the cord; moderate, glial bands covering more than 50% of the cord's margin or severe, glial scarring covering the entire thickness of the anterior column.

Statistical analysis

Comparison of the percentage of animals showing clinical disease and/or relapses between any two groups of mice was analyzed by χ^2 using Fisher's exact test.

Results

Effect of hematopoietic growth factors on clinical course of EAE

EAE was actively induced in SJL/J mice by immunization on day 0 with PLP139–151. Groups of mice were administered cytokines, including hG-CSF and mG-CSF, hFlt-3L, mSCF or hTPO for 10 consecutive days starting either on day 1 after immunization but before disease onset or day 30 during remission from first relapse.

We analyzed white blood cell (WBC) count in five mice for each group collected on day 14 or 44. Significantly increased WBC counts were observed in all cytokine-treated groups compared to the control group ($14.5 \pm 8.3 \times 10^9$/l vs $5.1 \pm 1.2 \times 10^9$/l; $P<0.01$). There were no statistically significant differences in WBC count between hG-CSF-, mG-CSF-, hTPO- and mSCF-treated groups (14.3–24.7×10^9/l; $P<0.05$), except in the Flt-3L-treated group, where leukocytosis was less pronounced ($9.7 \pm 4.5 \times 10^9$/l).

No statistically significant difference in disease severity was observed between the two control groups: PBS-injected and naive mice (Figure 1a). Flt-3L and G-CSF administered daily for 10 days after immunization of PLP139–151 increased peak of disease severity (Figure 1a). Flt-3L resulted in the worst mean clinical scores throughout whole period of follow-up ($P<0.01$) (Figure 1a). Differences in mean score between the G-CSF-treated group and controls were found to be significant only on day 13 and 14 post immunization ($P<0.05$). The later course of disease in both groups was similar (Figure 1a). Mean peak clinical disease scores were no different between TPO-treated and control groups (PBS-treated as well as no treatment group) (Figure 1a).

When cytokines were administered after peak of disease but before relapse (from day 30 to 40, that is, during remission), subsequent disease severity was significantly worse for Flt-3L- (from day 51 post immunization to the end of follow-up day 72) ($P<0.05$), G-CSF- (from day 56) ($P=0.05$) and SCF- (from day 50) ($P<0.05$) treated mice (Figure 1b). There was no significant difference in disease severity between TPO and control groups (Figure 1b). The effect of G-CSF in EAE was independent of the origin of cytokine (mouse or human). As shown in Figure 1c, both mG-CSF and hG-CSF when administered daily from day 30 to 40 caused significantly worse mean clinical scores compared to control. When administered during disease remission, the disease-exacerbating effect of cytokines such as Flt-3L, SCF, and G-CSF did not become manifest until

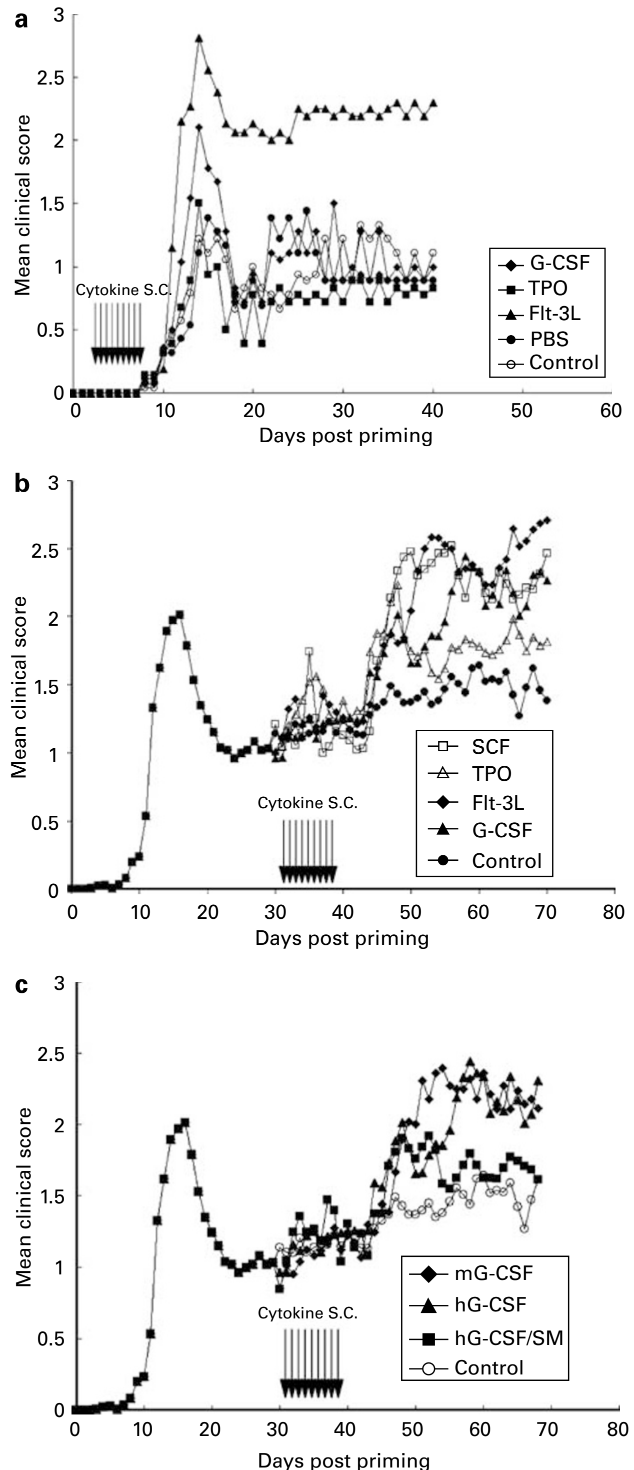

Figure 1 EAE disease progression after induction with PLP139–151 at day 0. Data are presented as the mean clinical score of total number of animals per group. (**a**) G-CSF, TPO or Flt-3L were administered s.c. from day 1 for 10 consecutive days. Total number of animals per group out to day 14 was 18; beyond day 14, the number of animals per group was nine. (**b**) SCF, TPO, Flt-3L or G-CSF were administered s.c. from day 30 for 10 consecutive days. Total number of animals per group out to day 44 was 14; beyond day 44, the number of animals per group was nine. (**c**) mG-CSF, hG-CSF or combination of hG-CSF and Solu-Medrol (SM) were administered s.c. from day 30 for 10 consecutive days. Number of mice per group was nine.

1–2 weeks later (Figures 1b–c). Treatment with corticosteroids (Solu-Medrol) during the time of G-CSF administration prevented G-CSF-related disease flare (Figure 1c).

Effect of hematopoietic growth factors on EAE splenic and LN lymphocyte cytokine production profile

In vitro lymphocyte proliferative responses and cytokine (IL-2, IL-6, IL-10, IL-12, IFN-γ, GM-CSF and TNF-α) production was performed on LN (Figure 2) and splenic (SP) (Figure 3) lymphocytes to identify changes associated with administration of hematopoietic growth factors. Compared to control groups (PBS-treated and true controls), LN lymphocytes of the Flt-3L-treated group showed increased production of IL-2 (475.6$\pm$35.6 vs 116.4$\pm$23.8 vs 60.4$\pm$8.4), TNF-α (64.9$\pm$5.7 vs 4.3$\pm$0.6 vs 1.6$\pm$2.2), GM-CSF (82.7$\pm$27.3 vs 3.1$\pm$1.4 vs 0) and IFN-γ (92.2$\pm$2.7 vs 0 vs 0) when stimulated (Figure 2). Splenocytes from the Flt-3L-treated mice demonstrated slightly increased production of TNF-α compared to PBS and control groups (6.7$\pm$1.2 vs 4.4$\pm$0.8 vs 4.2$\pm$2.3) (Figure 3). Analyzing results of the TPO group, we noticed that stimulated LN and SP lymphocytes had increased production of IL-6 compared to controls (200.2$\pm$14.2 vs 0; 15.5$\pm$2.5 vs 0.3$\pm$0.2), which is twice as much amount as observed in the Flt-3L group (Figures 2 and 3).

Effect of hematopoietic growth factors on EAE CNS histology

Histologic examination of the spinal cords from mice of all groups, including controls, revealed focal demyelination or multiple foci of demyelination (+/++) accompanied by mild focal glial scarring. Demyelination was more severe (+++) in the Flt-3L-treated group compared to all other groups (Table 1). Differences in the level of inflammation were observed between Flt-3L-, SCF- and G-CSF-treated groups compared to controls (Figure 4). Although the most prominent lymphocytic/macrophagic infiltration was seen in the Flt-3L- and SCF-treated groups, the G-CSF group also exhibited a significantly greater degree of inflammation compared to controls. In contrast, the histological presentation of the disease did not differ between the TPO-treated and control groups (Figure 4 and Table 1).

Discussion

As G-CSF is being used worldwide to mobilize stem cells and to treat cytopenias in patients with MS and other autoimmune diseases,[15] we evaluated the effect of G-CSF as well as other hematopoietic cytokines such as TPO, SCF and Flt-3L on the course of EAE.

One mechanism associated with EAE initiation and relapse is induction of Th1-derived cytokines (IL-2, IFN-γ and TNF-α), whereas recovery is associated with polarization towards a Th2 cell response and production of anti-inflammatory cytokines such as IL-4 and IL-10.[2] Accumulating evidence indicates an immunosuppressive role for naturally occurring regulatory CD4+ cells which express the IL-2Rα chain (CD25) (Tregs) in autoimmune diseases.[16] It has been shown that adoptive transfer of CD4+CD25+ cells results in significant protection against EAE induction/progression.[17] The mechanism by which Tregs exert their suppressive capacity is still a subject of debate. It has been speculated that Tregs influence either

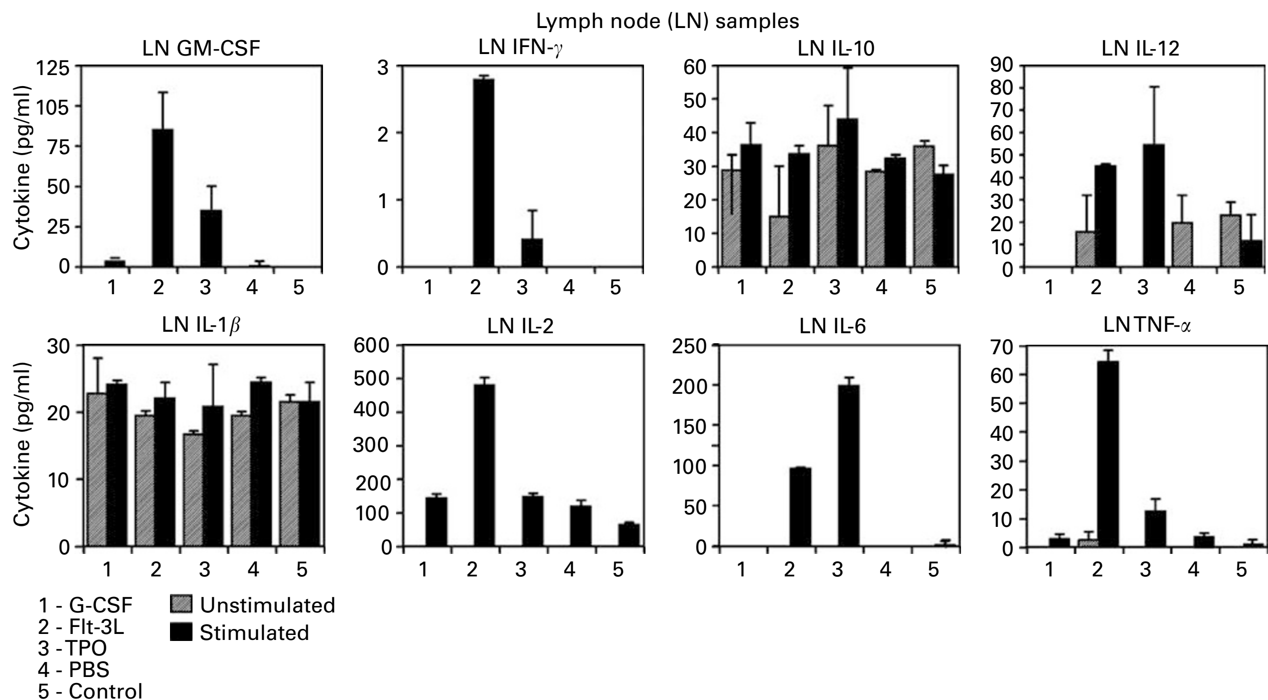

Figure 2 Effect of G-CSF, TPO and Flt-3L given s.c. for 10 consecutive days on cytokine production by LN lymphocytes during EAE course. LN was harvested at day 14 after immunization.

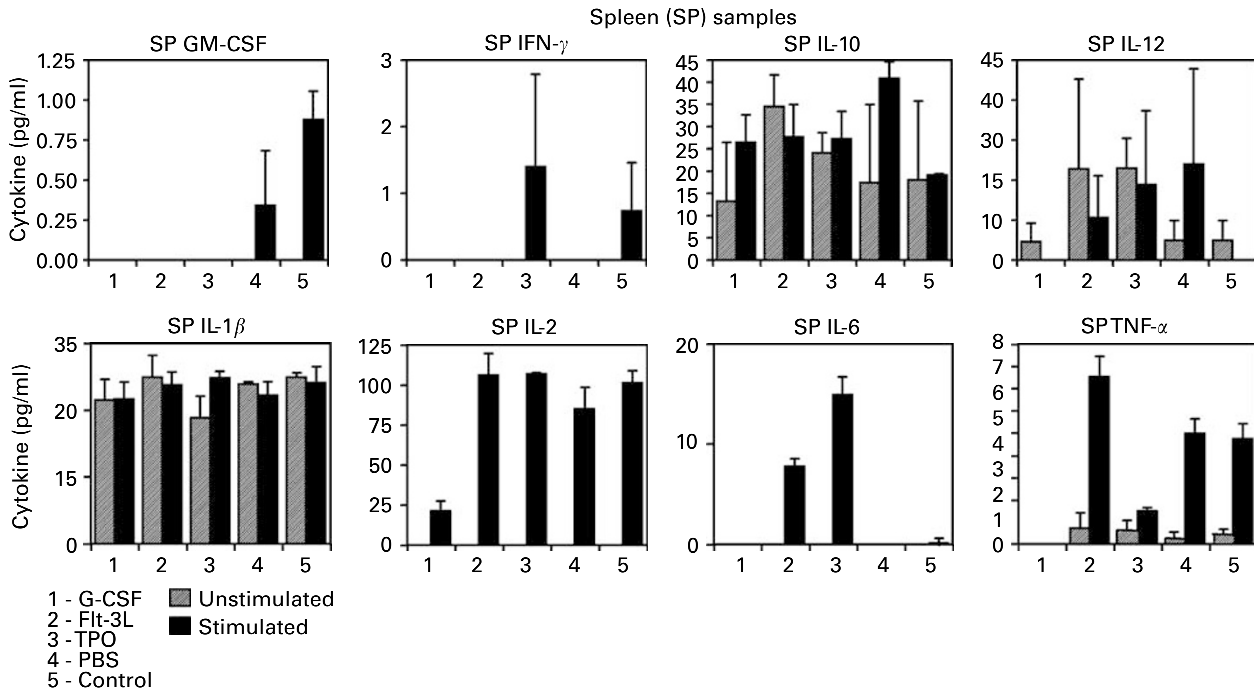

Figure 3 Effect of G-CSF, TPO and Flt-3L given s.c. for 10 consecutive days on cytokine production by splenocytes during EAE course. Spleens were harvested at day 14 after immunization.

Table 1 Comparison of histological examination of spinal cords from G-CSF-, Flt-3L-, TPO- or SCF-treated mice and controls

Group	*Inflammation*	*Demyelination*	*Glial scarring*
G-CSF	Modest	+ +	Mild
Flt-3L	Severe	+ + +	Mild to moderate
TPO	Mild	+ +	Mild
SCF	Modest to severe	+ +	Mild
Control	Mild	+ +	Mild

Abbreviations: Flt-3L = Flt-3 ligand; G-CSF = granulocyte colony-stimulating factor; SCF = stem cell factor; TPO = thrombopoietin.

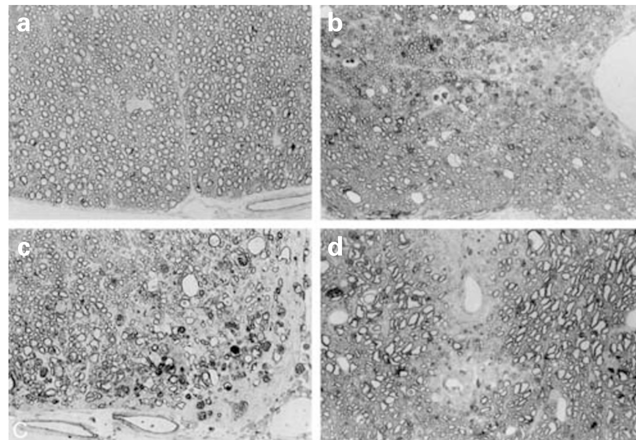

Figure 4 Histological examination of spinal cords during the course of EAE induced with PLP139–151. (**a**) Control (EAE unaffected mouse). (**b**) Spinal cord from Flt-3L-treated group showing moderate–severe inflammation with extensive demyelination. (**c**) Spinal cord from TPO-treated mouse demonstrating mild inflammation with moderate demyelination. (**d**) Spinal cord from G-CSF-treated mouse with numerous mononuclear cell infiltration (moderate–severe inflammation) and mild demyelination. Sections are 1-μm thick, Epon-embedded, stained with Toluidine blue ($\times$ 200).

the differentiation or effector function of Th1/Th2 cells[17] and regulate the production of suppressive cytokines, for example, IL-10 and TGF β.[18] However, the interplay and sequence of cytokines and cellular interactions in the pathology and immunoregulation of CNS autoimmune diseases such as R-EAE and MS remains incompletely understood.

Our results from Flt-3L- and TPO-treated mice are consistent with a role for Th1/Th2 polarization on disease activity. Flt-3L synergizes with other hematopoietic cytokines such as IL-3, IL-6 and SCF, resulting in proliferation of primitive progenitor cells.[19,20] Administration of Flt-3L leads to the expansion and mobilization of HSC.[21,22] Flt-3L is also a potent Th1-inducing cytokine and mobilizes both the CD11c^{+} and the CD11c^{-} DC subsets into the blood leading to skewing of Th1/Th2 balance.[23,24] Our data demonstrate that administration of Flt-3L in EAE mice exacerbates disease activity and tends to polarize towards a Th1 immune response. The capacity of Flt-3L to activate production of Th1-derived cytokines may make its use a relative contraindication in Th1-mediated autoimmune disease.

TPO, first identified as the major regulator of thrombocyte production, exerts significant influence on other hematopoietic lineages as well, including erythroid, granulocytic and lymphoid lineages.[11,25–28] Virtually all primitive HSC that display marrow repopulating activity express on their surface the receptor for TPO–c-Mpl.[26] Studies in

mice have shown that priming of donor bone marrow cells with TPO can accelerate the reconstitution of platelets and red cells and ameliorate post transplant thrombocytopenia.[27] When TPO was added to G-CSF and chemotherapy as a part of the mobilizing regimen in patients with breast cancer, enhanced mobilization of HSC was observed.[29] Murray *et al.*[14] demonstrated that TPO alone can efficiently mobilize primitive hematopoietic progenitor and stem cells with long-term repopulating ability into peripheral blood. Our data showed that administration of TPO in mice resulted in the same level of WBC increase as seen following G-CSF treatment and higher WBC count than post Flt-3L injections. We did not observe a more severe disease course in the TPO-treated group as compared to the control group and histological appearance of the disease in TPO-treated group did not differ from controls.

It is probable that growth factor-related effects on EAE disease severity cannot be completely explained by alteration of Th1- and Th2-derived cytokines from peripheral lymphocytes assayed. Ongoing pathologic events in EAE would be better represented by analyzing lymphocytes derived from the disease-involved organ, that is, the CNS.

Our knowledge about the role of cytokines in pathology and immunoregulation of EAE is still sparse with existing controversies about the effects of some cytokines such as TNF-α, IL-4, IL-10 and IFN-γ.[30] Although the role of IL-6, if any, in EAE is unknown, IFN-γ has been reported to reduce disease activity.[31–33] IFN-γ belongs to a group of proinflammatory cytokines produced by Th1 cells. EAE disease activity, therefore, appears to be determined by more factors than just Th1/Th2 skewing of SP or LN lymphocytes. Besides growth factor dose and timing of administration in terms of disease stage (onset, remission, relapse, etc.), factors influencing disease activity may include tissue infiltration by other cells such as neutrophils, macrophages, B cells and DCs, as well as chemokines, adhesion and homing molecules and cytokine expression within the target organ, that is, the CNS.

G-CSF is a hematopoietic colony-stimulating factor that plays a role in the activation of DCs as well as the survival and homing of neutrophils, both of which could be involved in EAE.[24,34–36] G-CSF, although regarded by some as a Th2-inducing cytokine, seems to have inconsistent effects in terms of Th1/Th2 skewing. It has been shown that G-CSF can exert immunomodulatory effects on T lymphocytes and affect the production of IFN-γ and IL-4, possibly owing to the preferential mobilization of Th2-inducing DCs.[24,36–38] Although IL-4 is an anti-inflammatory Th2 cytokine, IFN-γ is a proinflammatory cytokine. It has also been reported that G-CSF administration induces a 25-fold increase in TNF-α plasma level which is not consistent with G-CSF-induced Th2 modulation.[39]

We have consistently observed that G-CSF, given after immunization but either before disease onset or during remission, enhanced EAE disease severity. Two clinical reports have also documented disease flare when using G-CSF in patients with MS.[9,10] However, timing of G-CSF administration may impact disease severity.[40,41] Zavala *et al.*[40] used G-CSF in MBP- not PLP-immunized mice, at different times then used in our study. They reported that G-CSF treatment before immunization with MPB or during peak of disease (from days 10 or 20 post immunization) significantly reduced disease severity.[40]

Although peripheral lymphocyte Th1/Th2 cytokine skewing did not explain G-CSF disease exacerbation, the severity of inflammatory infiltration within the CNS did correlate with both G-CSF and Flt-3L and SCF-mediated disease exacerbation. Specifically, moderate to extensive infiltration of spinal cords by T lymphocytes and macrophages was observed in all the G-CSF-, SCF- and Flt-3L-treated groups. When compared to controls, TPO, which did not exacerbate EAE, did not affect inflammatory infiltration within CNS. Thus, one potential mechanism that correlates with or might be responsible for the exacerbation of EAE after injection of growth factors such as G-CSF, SCF and Flt-3L is increased infiltration and migration of inflammatory cells into regions of demyelinating plaques.

In our experiments, the combination of G-CSF with Solu-Medrol protected from G-CSF-mediated EAE exacerbation. These data are consistent with clinical findings where MS patients experiencing neurologic worsening during G-CSF treatment improved following treatment with methylprednisolone.[9] High-dose glucocorticosteroids applied in MS patients, seems to reduce cellular inflammation via diminished T-cell and macrophage/neutrophil infiltration in the CNS and possibly via reduction of TNF-α production.[42] The ability of corticosteroids to decrease the capacity of neutrophils and monocytes to migrate from the vascular space[43] might explain their disease ameliorating effect in G-CSF-treated mice.

In summary, administration of G-CSF, Flt-3L and SCF resulted in exacerbation of EAE disease severity. These observations parallel the results of clinical case reports showing flare of disease activity in patients with MS receiving G-CSF during stem cell mobilization. Concomitant treatment with glucocorticosteroid effectively abrogated the disease-enhancing effect of G-CSF in EAE and parallels clinical reports of corticosteroid prevention of G-CSF-mediated disease flare in MS patients undergoing hematopoietic stem cell mobilization. TPO treatment failed to affect disease activity. Further evaluation of TPO as an alternative mobilizing or post HSCT engraftment growth factor may be appropriate. New insight into physiology of CXCR4 in HSC has brought into development a novel promising mobilizing agent AMD3100, a bicyclam antagonist of chemokine receptor CXCR4.[44] Extensive research on this compound's potential efficacy and safety in different diseases and patient populations will also be of interest.

Acknowledgements

We thank Dr Stephen M Shinall for critical review of this manuscript.

References

1 Jee Y, Matsumoto Y. Two-step activation of T cells, clonal expansion and subsequent Th1 cytokine production, is

essential for the development of clinical autoimmune encephalomyelitis. *Eur J Immunol* 2001; **31**: 1800–1812.
2 Begolka WS, Vanderlugt SM, Rahbe SM, Miller SD. Differential expression of inflammatory cytokines parallels progression of central nervous system pathology in two clinically distinct models of multiple sclerosis. *J Immunol* 1998; **161**: 4437–4446.
3 Burt RK, Padilla J, Begolka WS, Dal Canto MC, Miller SD. Effect of disease stage on clinical outcome after syngeneic bone marrow transplantation for relapsing experimental autoimmune encephalomyelitis. *Blood* 1998; **91**: 2609–2616.
4 van Gelder M, van Bekkum DW. Effective treatment of relapsing experimental autoimmune encephalomyelitis with pseudoautologous bone marrow transplantation. *Bone Marrow Transplant* 1996; **18**: 1029–1034.
5 Karussis DM, Vourka-Karussis U, Lehmann D, Ovadia H, Mizrachi-Koll R, Ben-Nun A *et al*. Prevention and reversal of adoptively transferred, chronic relapsing experimental autoimmune encephalomyelitis with a single high dose cytoreductive treatment followed by syngeneic bone marrow transplantation. *J Clin Invest* 1993; **92**: 765–772.
6 Burt RK, Cohen BA, Russell E, Spero K, Joshi A, Oyama Y *et al*. Hematopoietic stem cell transplantation for progressive multiple sclerosis: failure of a total body irradiation-based conditioning regimen to prevent disease progression in patients with high disability scores. *Blood* 2003; **102**: 2373–2378.
7 Burt RK, Cohen B, Rose J, Petersen F, Oyama Y, Stefoski D *et al*. Non-myeloablative hematopoietic stem cell transplantation for multiple sclerosis. *Arch Neurol* 2005; **62**: 860–864.
8 Flishie RJA. Cytokines in haemopoietic progenitor mobilization for peripheral blood stem cell transplantation. *Curr Pharm Des* 2002; **8**: 379–394.
9 Openshaw H, Stuve O, Antel JP, Nash R, Lund BT, Weiner LP *et al*. Multiple sclerosis flares associated with recombinant granulocytes colony-stimulating factor. *Neurology* 2000; **54**: 2147–2150.
10 Burt RK, Fassas A, Snowden JA, van Laar JM, Kozak T, Wulffraat NM *et al*. Collection of hematopoietic stem cells from patients with autoimmune diseases. *Bone Marrow Transplant* 2001; **28**: 1–12.
11 Young JC, Bruno E, Luens KM, Wu S, Backer M, Murray LJ. Thrombopoietin stimulates megakaryocytopoiesis, myelopoiesis, and expansion of $CD34^+$ progenitor cells from single $CD34^+Thy\text{-}1^+Lyn^-$ primitive progenitor cells. *Blood* 1996; **88**: 1619–1631.
12 Kaushansky K. Thrombopoietin and the hematopoietic stem cell. *Ann NY Acad Sci* 2005; **1044**: 139–141.
13 Broudy VC. Stem cell factor and hematopoiesis. *Blood* 1997; **90**: 1345–1364.
14 Murray LJ, Luens KM, Estrada MF, Bruno E, Hoffman R, Cohen RL *et al*. Thrombopoietin mobilizes CD34+ cell subsets into peripheral blood and expands multilineage progenitors in bone marrow of cancer patients with normal hematopoiesis. *Exp Hematol* 1998; **26**: 207–216.
15 Burt RK, Slavin S, Burns WH, Marmont AM. Induction of tolerance in autoimmune diseases by hematopoietic stem cell transplantation: getting closer to a cure? *Blood* 2002; **99**: 768–784.
16 Bluestone JA, Tang Q. How do CD4+CD25+ regulatory T cells control autoimmunity? *Curr Opin Immunol* 2005; **17**: 638–642.
17 Kohm AP, Carpentier PA, Anger HA, Miller SD. Cutting edge: CD4+CD25+ regulatory cells suppress antigen-specific autoreactive immune responses and central nervous system inflammation during active experimental autoimmune encephalomyelitis. *J Immunol* 2002; **169**: 4712–4716.
18 Read S, Malmstrom V, Powrie F. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25+CD4+ regulatory cells that control intestinal inflammation. *J Exp Med* 2002; **192**: 295–302.
19 Lyman SD, James L, Vanden Bos T, de Vries P, Brasel K, Gliniak B *et al*. Molecular cloning of a ligand for the flt2/flk-2 tyrosine kinase receptor: a proliferative factor for primitive hematopoietic cells. *Cell* 1993; **75**: 1157–1167.
20 Hudak S, Hunte B, Culpepper J, Menon S, Hannum C, Thompson-Snipes L *et al*. FLT3/FLK2 ligand promotes the growth of murine stem cells and the expansion of colony-forming cells and spleen colony-forming units. *Blood* 1995; **85**: 2747–2755.
21 Brasel K, McKenna HJ, Morrissey PJ, Charrier K, Morris AE, Lee CC *et al*. Hematologic effects of flt3 ligand *in vivo* in mice. *Blood* 1996; **88**: 2004–2012.
22 Robinson S, Mosley RL, Parajuli P, Pisarev V, Sublet J, Ulrich A *et al*. Comparison of the hematopoietic activity of flt-3 ligand and granulocyte–macrophage colony-stimulating factor acting alone or in combination. *J Hematother Stem Cell Res* 2000; **9**: 711–720.
23 Maraskovsky E, Brasel K, Teepe M, Roux ER, Lyman SD, Shortman K *et al*. Dramatic increase in the numbers of functionally mature dendritic cells in Flt3 ligand-treated mice: multiple dendritic cell populations identified. *J Exp Med* 1996; **184**: 1953–1962.
24 Pulendran B, Banchereau J, Burkeholder S, Kraus E, Guinet E, Chalouni C *et al*. Flt3-ligand and granulocyte colony-stimulating factor mobilize distinct human dendritic cell subsets *in vivo*. *J Immunol* 2000; **165**: 566–572.
25 Wagemaker G, Neelis KJ, Hartong SC, Wognum AW, Thomas GR, Fielder PJ *et al*. The efficacy of recombinant thrombopoietin in murine and nonhuman primate models for radiation-induced myelosuppression and stem cell transplantation. *Stem Cells* 1998; **16**: 375–386.
26 Ninos JM, Jefferies LC, Cogle CR, Kerr WG. The thrombopoietin receptor, c-Mpl, is a selective surface marker for human hematopoietic stem cells. *J Translat Med* 2006; **4**: 9; doi:10.1186/1479-5876-4-9.
27 Ratajczak MZ, Ratajczak J, Machalinski B, Mick R, Gewirtz AM. *In vitro* and *in vivo* evidence that *ex vivo* cytokine priming of donor marrow cells may ameliorate posttransplant thrombocytopenia. *Blood* 1998; **91**: 353–359.
28 Bojko P, Pawloski D, Stellberg W, Schroder JK, Seeber S. Flt3 ligand and thrombopoietin serum levels during peripheral blood stem mobilization with chemotherapy and recombinant human glycosylated granulocyte colony-stimulating factor (rhu-G-CSF, lenograstim) and after high-dose chemotherapy. *Ann Hematol* 2002; **81**: 522–528.
29 Gajewski JL, Rondon G, Donato ML, Anderlini P, Korbling M, Ippoliti C *et al*. Use of thrombopoietin in combination with chemotherapy and granulocyte colony-stimulating factor for peripheral blood progenitor cell mobilization. *Biol Blood Marrow Transplant* 2002; **8**: 550–556.
30 Willenborg DO, Staykova MA. Cytokines in the pathogenesis and therapy of autoimmune encephalomyelitis and multiple sclerosis. In: Santamaria P (ed) *Cytokines and chemokines in autoimmune disease*. Eurekah.com and Kluwer Acad/Plenum Publishers: The Netherlands, 2003, pp 97–116.
31 Espejo C, Penkowa M, Saez-Torres I, Hidalgo J, Garcia A, Montalban X *et al*. Interferon-γ regulates oxidative stress during experimental autoimmune encephalomyelitis. *Exp Neurol* 2002; **177**: 21–31.
32 Billiau A, Heremans H, Vandekerckhove F, Dijkmans R, Sobis H, Meulepas E *et al*. Enhancement of experimental allergic encephalomyelitis in mice by antibodies against IFN-γ. *J Immunol* 1988; **140**: 1506–1510.

33 Duong TT, St Louis J, Gilbert JJ, Finkelman FD, Strejan GH. Effect of anti-interferon-γ and anti-interleukin-2 monoclonal antibody treatment on the development of an actively and passively induced experimental allergic encephalomyelitis in the SJL/J mouse. *J Neuroimmunol* 1992; **36**: 105–115.
34 Welte K, Gabrilove J, Bronchud MH, Platzer E, Morstyn G. Filgrastim (r-metHuG-CSF): the first 10 years. *Blood* 1996; **88**: 1907–1929.
35 Hock BD, Haring LF, Ebbett AM, Patton WN, McKenzie JL. Differential effects of G-CSF mobilization on dendritic cell subsets in normal allogeneic donors and patients undergoing autologous transplantation. *Bone Marrow Transplant* 2002; **30**: 733–740.
36 Arpinati M, Green CL, Heimfeld S, Heuser JE, Anasetti C. Granulocyte-colony stimulating factor mobilizes T helper 2-inducing dendritic cells. *Blood* 2000; **95**: 2484–2490.
37 Sloand EM, Kim S, Maciejewski JP, Van Rhee F, Chaudhuri 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**: 2269–2274.
38 Pan L, Delmonte Jr J, Jalonen CK, Ferrara JLM. 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**: 4422–4429.
39 Xu S, Hoglund 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**: 558–568.
40 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**: 2011–2019.
41 Lock C, Hermans G, Pedotti R, Brendolan A, Schadt E, Garren H *et al*. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. *Nat Med* 2002; **8**: 500–508.
42 Schmidt J, Metselaar JM, Gold R. Intravenous liposomal prednisolone downregulates in situ TNF-α production by T-cells in experimental autoimmune encephalomyelitis. *J Histochem Cytochem* 2003; **51**: 1241–1244.
43 Crockard AD, Boylan MT, Droogan AG, McMillan SA, Hawkins SA. Methylprednisolone-induced neutrophil leukocytosis down-modulation of neutrophil L-selectin and Mac-1 expression and induction of granulocyte-colony stimulating factor. *Int J Clin Lab Res* 1998; **28**: 110–115.
44 Larochelle A, Krouse A, Metzger M, Orlic D, Donahue RE, Fricker S *et al*. AMD3100 mobilizes hematopoietic stem cells with long-term repopulating capacity in nonhuman primates. *Blood* 2006; **107**: 3772–3778.