Granulocytic invasion of the central nervous system after hematopoietic stem cell transplantation for systemic lupus erythematosus

We report on the likely mechanism of an exacerbation of neurological symptoms developed during immune reconstitution after autologous non-myeloablative hematopoietic stem cell transplantation in a 33-year-old man with systemic lupus erythematosus-associated recurrent transverse myelitis. Cerebrospinal fluid examination revealed prominent neutrophilic pleocytosis and no evidence of infection or of reactivation of lupus. Following a course of corticosteroid treatment the exacerbation resolved completely and the patient's neurological function continued to improve, resulting in net gain above pre-treatment for over 1 year follow-up without maintenance immunosuppression. Granulocytic invasion of the central nervous system represents a novel and possibly preventable cause of neurological complications during haematologic reconstitution.

Haematologica 2006; 91:(5)e63-e66

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease that can affect multiple organs and systems. Immunoregulatory abnormalities involving primarily B cell responses are implicated in the pathogenesis of lupus.[1] Autologous hematopoietic stem cell transplantation (HSCT) can induce prolonged remissions of disease activity in patients with severe autoimmune disease.[2] A phase II pilot study of immune depletion followed by autologous HSCT to treat severe refractory SLE began at the NIH in 2004. Neurological complications frequently occur after allogeneic HSCT but are uncommon in autologous transplantation,[3] and the cause of the neurological adverse event remains in many cases elusive. We report on one patient with SLE-associated recurrent transverse myelitis who developed an exacerbation of neurological symptoms following lymphoablative autologous HSCT, and we propose a mechanism explaining this complication and its possible prevention.

Design and Methods

The study (http://clinicalstudies.info.nih.gov/detail/A_2004-C-0095.html) was approved by the National Cancer Institute Institutional Review Board and by the Food and Drug Administration. The patient is a 33-year-old male with 5-year history of recurrent transverse myelitis who met the American College of Rheumatology revised criteria for classification of SLE (4) based on arthritis, vasculitic skin rash, antinuclear (ANA) and anti-double stranded DNA (dsDNA) antibody positivity. He had previously failed treatments with hydroxychloroquine, mycophenolate mofetil, intravenous immunoglobulin and monthly pulse cyclophosphamide. Multiple attacks of SLE-associated transverse myelitis (as defined by the Transverse Myelitis Consortium Working Group[5] had resulted in severe spinal cord damage and paraparesis. His Expanded Disability Status Score [EDSS; a measure of neurological disability ranging from 0 = normal to 10 = death[6]] was 7.5 at baseline. In addition to the EDSS, we used the Scripps Neurological Rating Scale[7] to quantify neurological impairment during the study. The primary aim of HSCT was to prevent further worsening of neurologic disability (e.g. tetraplegia) and potentially fatal complications from extension of the pathologic process to upper segments of the spinal cord. Other than active transverse myelitis, the patient's SLE was not active enough to significantly impact the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). Since the SLEDAI does not cover transverse myelitis, the index was recorded but not used as an outcome measure (.

At the time of transplantation the patient was receiving symptomatic treatment but no maintenance glucorticosteroids. Hematopoietic stem cells (HSC) were mobilized with a regimen of methylprednisolone 1000 mg IV (day 1), rituximab 375 mg/m2 IV (days 1 and 4), cyclophosphamide 2000 mg/m^2 IV (day 2) and G-CSF (filgrastim) 10 μg/kg/day subcutaneously (started on day 6 for 7 days). HSC were collected by leukapheresis and CD34+ cells were enriched with the Isolex 300i, 2.5 system and cryopreserved. Conditioning regimen consisted of rituximab 750 mg/m^2 IV (day -7), fludarabine 30 mg/m^2 IV (day -6 to -3) cyclophosphamide 1200 mg/m^2 IV (days -6 to -3), and mesna 1200 mg/m^2 IV (days -6 to -3). Stem cells were infused at the dose of 4.59×10^6 cells/kg in January, 2005 (day 0). The patient received routine antimicrobial prophylaxis that included ceftazidime and caspofungin peritrasnsplantation and trimethoprim/ sulfamethoxazole and valacyclovir during six months post-transplant.

In addition to standard clinical and radiological assessments and laboratory tests, we obtained anti-nuclear and anti-dsDNA antibody titers from cerebrospinal fluid (CSF). To estimate the intrathecal synthesis of SLE-associated autoantibodies the autoantibody activity index was calculated according to the formula [CSF Ab / serum Ab] / [Albumin (CSF) / Albumin (serum)], as described.[8]

Results

The patient tolerated HSC mobilization, conditioning and autologous HSC infusion well and showed initial neurological improvement (Figure 1A). G-CSF (filgrastim) 5 μg/kg/day subcutaneously was started on day 1 and given daily for 8 days until absolute neutrophil counts (ANC) reached 500 cells/μl. A rapid increase of ANC ensued (peak of 7,768 cells/μL on day 11). On day 16 post-HSCT the patient developed fever of 39oC, malaise and increased weakness of his lower extremities progressing over 12 hours to flaccid paraplegia and reduced sensation with a T4 sensory level (EDSS 8.0). MRI of the spine showed new edema and swelling of the mid-thoracic spinal cord without frank pathological enhancement against a background of preexisting multiple cervical and thoracic cord lesions and thoracic cord myelomalacia (Fig. 1B). MRI of the brain showed no changes compared to previous exams. An expanded time-course of events is shown in Figure 1C. A lumbar puncture performed at onset of exacerbation revealed clear, colorless CSF with opening pressure of 270 mm H2O. CSF analysis showed pleocytosis with 89 WBC (86 polymorphonuclear granulocytes, 3 lymphocytes). Pertinent serum and CSF findings are presented in Table 1. Interestingly, ANA and anti-ds DNA antibodies that were positive both in serum and CSF before HSCT had become negative at the time of exacerbation and remained persistently negative thereafter. Immunofixation electrophoresis of CSF showed no abnormal immunoglobulins. Extensive microbiological testing of the CSF was negative, including sterile bacterial, fungal and mycobacteria cultures, anti-coccidioides antibody, Cryptococcus neoformans antigen PCR for EBV, CMV, HSV, VZV and Borrelia burgdorferi. Repeat blood cultures before and after the neurological onset were negative. Asymptomatic CMV antigenemia was detected on a routine screening on day 17 and treated with IV ganciclovir. The patient was treated for the neu-

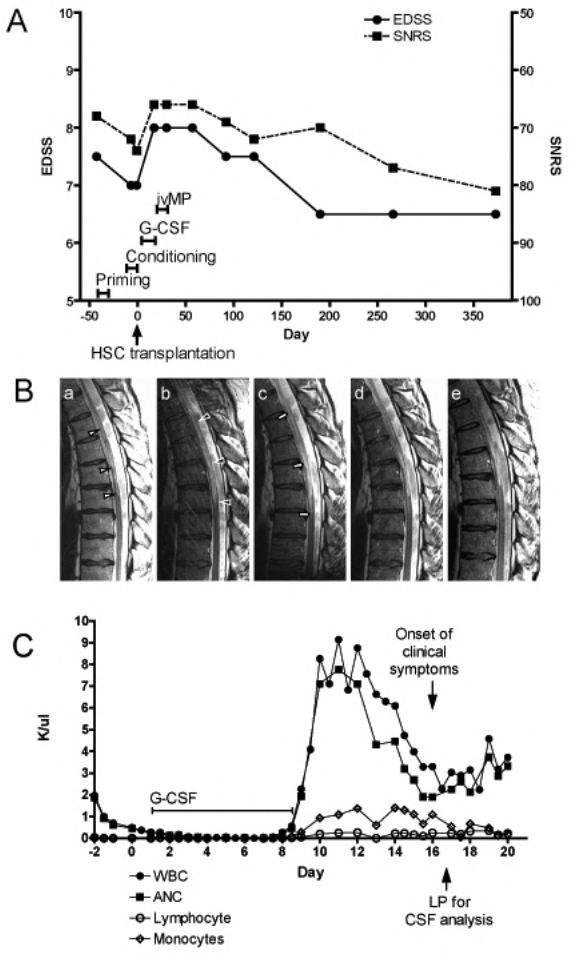

Figure 1. Peritransplantation clinical course and diagnostic findings. A.Quantitative measures of neurological impairment on a time course of days after stem cell infusion (X axis). The Expanded Disability Status Score (circles) is shown on the left Y axis and the Scripps Neurological Rating Scale on the right Y axis. The EDSS ranges from 0 (normal healthy person) to 10 (death) and the SNRS ranges from 100 (normal) to -10 (most severe disability). Brackets indicate time of priming, conditioning, G-CSF treatment per protocol, and IvMP the administration of methylprednisolone IV to treat the neurological complication. B. Serial MRIs of the thoracic spinal cord document development and resolution of cord edema. Preexisting myelomalacia (white arrowheads, a) is present throughout the upper thoracic cord from prior episodes of transverse myelitis on baseline MRI performed ~ 2 months prior to acute exacerbation. On the day of acute exacerbation, the thoracic cord is swollen, and hyperintense cord edema extends inferiorly into the lower thoracic cord (black arrowheads, b). MRI performed approximately 10 hrs later shows further progression inferiorly, despite high dose steroids (white arrows, c). Follow-up MRI four days later (d) shows reduction in cord edema and 11 days later (e) shows resolution of cord edema and swelling to that seen on the baseline MRI. C. Peripheral blood cell counts post-transplantation, demonstrating the rapid increase of neutrophils following treatment with G-CSF. The time points of neurological worsening and of lumbar puncture (LP) for CSF analysis are indicated by the arrows.

rological exacerbation with methylprednisolone 1 g IV for 5 days and discharged home with oral prednisone taper (discontinued at 3 months post-HSCT) and no additional immunosuppressive medications. Monthly follow-up visits showed return to his baseline condition at 3-month follow-up and subsequent 1.0 EDSS point improvement over pre-transplantation at 6-month follow-up, which persisted at 12 months (Figure 1A). Thoracic cord abnormalities seen on MRI also recovered to the pre transplant baseline (Figure 1 B).

Discussion

In lupus patients, recovery of neutrophil counts following G-CSF treatment for neutropenia or to hasten hematopoietic recovery after HSCT is typically uneventful.[9,10] Flares of preexisting lupus-associated neuropsychiatric symptoms (seizures and psychosis), however, have been described in the absence of clinical or serological signs of lupus activity in 2 out of 9 SLE patients who received G-CSF treatment for neutropenia.[11] In that report, Euler and colleagues speculated that increased granulocyte counts may have mediated intracerebral alterations with a mechanism similar to leukocytoclastic skin vasculitis.[11] Neurologic exacerbations were also reported in patients with multiple sclerosis (MS) following G-CSF treatment to mobilize hematopoietic stem cells before transplantation.[12] The authors of that study hypothesized that the flares were mediated by neutrophil infiltration in regions of demyelination. In addition, after HSCT, transient non-focal worsening of MS symptoms attributable to fever-related fatigue and impairment of nerve conduction was reported in conjunction with the engraftment syndrome.[13-15] Taken together, those observations suggested that pre-existing chronic CNS disease may predispose to neurological complications during hematological recovery, yet the mechanism remained matter of conjecture.

Here, we described an exacerbation of neurological symptoms during immune recovery in a patient with severe CNS lupus who had undergone autologous non-myeloablative HSCT. The intensive clinical, radiological and laboratory monitoring provided by the trial protocol allowed us greater insight than previous studies into the mechanism underlying the complication. We considered four possible options to explain the neurological event: (a) new flare of lupus involving the central nervous system (CNS); (b) CNS infection; (c) fever-related functional neurological impairment; and (d) G-CSF induced neutrophil expansion and infiltration of the CNS. In our patient there were no clinical or laboratory signs in serum or CSF of reactivation of lupus post-transplantation. The patient never had CSF neutrophilic pleocytosis at two previous analyses (one during a flare of SLE-TM before entering the study) nor during clinical remission at 3-, 6- and 9-month follow-up post-HSCT. Extensive evaluation for an infectious agent in the CSF was nonrevealing. The patient developed CMV antigenemia near the time of the exacerbation but CMV was not detectable in the CSF and there were no signs of CMV encephalitis. Although fever preceded the onset of exacerbation, clinical and MRI evidence excluded a hyperthermia-induced neurological decline.

Based on the rapid leukocyte recovery preceding the exacerbation, the unusual granulocytosis in the CSF, the involvement of previously affected spinal cord segments, the presence of CSF indices of blood-brain barrier disruption and myelin breakdown in the sustained absence of SLE autoantibodies in blood and CSF, and the response to methylprednisolone treatment, we concluded that granulocytic invasion of the CNS, targeting sites of pre-existing injury, best explained the pathogenesis of the exacerbation. Possible mechanisms of granulocyte-mediated damage to the CNS include leukocytoclastic vasculitis[11]and direct or excitotoxic neuronal damage.[16] Pre-existing tissue injury could target neutrophils inappropriately to the CNS during rapid hematopoietic reconstitution resulting

Table 1. CSF and relevant serum findings from pre-HSCT baseline to 9-month post-HSCT follow-up.

	Pre-HSCT Baseline (day -40)	*Exacerbation (day 17)*	*Follow-up (day 30)*	*Follow-up (day 92)*	*Follow-up (day 190)*	*Follow-up (day 266)*	*Follow-up (day 373)*
CSF cells/mm[3]	2 WBC	86 PMN, ↑ 3 lymphs, 11 other cells	1 WBC	1 WBC	1 WBC	2 WBC	3 WBC
CSF protein (mg/dL)	34	175 ↑	23	29	26	30	30
CSF glucose (mg/dL)	56	94	85	76	71	80	82
Albumin ratio[2] (n.v. 3.2-9.0)	4.54	27.9 ↑	3.13	4.16	3.5	3.69	3.93
IgG index (n.v. 0.26-0.62)	0.61	0.73 ↑	0.90 ?	0.89 ↑	0.61	0.55	0.55
Myelin basic protein[3] (n.v. 0-1.4 ng/mL)	0.2	8.4 ↑	0.2	0.1	<0.1	<0.1	0.3
ANA[4], CSF	0.25	Negative	Negative	Negative	Negative	Negative	Negative
ANA, serum (n.v. 0-0.9 units)	1.7 ↑	NegativeNegative	Negative	Negative	Negative	Negative	Negative
ANA activity index	32.4↑	-	-	-	-	-	-
Anti-dsDNA, CSF	7.0	Negative	Negative	Negative	Negative	Negative	Negative
Anti-dsDNA, serum	47.0	Negative	Negative	Negative	Negative	Negative	Negative
Anti-dsDNA activity index[2]	32.8 ↑	-	-	-	-	-	-

[1]Includes monocytes; does not include RBC; [2]An index of blood-brain barrier damage; [3]A marker of myelin injury; [4]Abbreviations: ANA, anti-nuclear antibodies; Anti-dsDNA, anti-double stranded DNA; ↑ indicates abnormally elevated value; n.v. = normal values; [5]For explanation of antibody (ANA or anti-dsDNA) activity indices, see Methods and Ref. 6.

in inflammatory damage.[17] It is possible that the conditioning regimen used in this study, which is lympho- but not myeloablative, might also contribute to an increased susceptibility to adverse events from neutrophilic recovery and G-CSF administration.

Corticosteroid treatment has been used to treat MS flares during mobilization with G-CSF[12] and to treat engraftment syndrome.[13] Glucocorticoids can reduce neutrophil extravasation and migration into tissues through modulation of the expression of selectins and integrins.[18,19] Interestingly, a second patient with severe SLE-associated myelopathy treated in our protocol, who unlike the case reported here remained on oral prednisone during post-HSCT immune recovery, did not develop neurologic complications. Both patients have now completed 15-month follow up post-HSCT, are free of all immunosuppressive treatment and no signs of disease exacerbation have been reported. Although further work is required to define the preventive efficacy and best scheme of administration, we suggest considering corticosteroid prophylaxis during immune reconstitution in patients with recent inflammatory or vasculitic CNS damage who undergo autologous HSCT.

Paolo A Muraro, Nikolay P Nikolov, John A Butman, Andrea Abati, Juan Gea-Banacloche, Ronald Gress, Peter Lipsky, Gabor Illei, Steven Pavletic

From the National Institute of Neurological Disorders and Stroke (Dr. Muraro), the National Institute of Arthritis and Musculoskeletal and Skin Diseases (Drs. Nikolov and Lipsky), the Diagnostic Radiology Department (Dr. Butman), the National Cancer Institute (Drs. Abati, Gea-Banacloche, Gress and Pavletic), and the National Institute of Dental and Craniofacial Research (Dr. Illei), National Institutes of Health, Bethesda, MD, USA.

Acknowledgments: This research was supported by the Intramural Research Program of the NIH, NCI, NIAMS and NINDS. We thank C. Yarboro, M. Krumlauf, K. Castro, N. Frenkel-Arons and R. Babb for excellent nursing and research support. We also thank Drs. S. Leitman and E. Read for the stem cell processing expertise, Drs. U. Oh and R. Martin for helpful advice. We have no conflict of interest to report.

Key words: systemic lupus erythematosus, hematopoietic stem cell transplantation, autoimmune disease, cerebrospinal fluid

Correspondence: Dr. Steven Pavletic,
Head, Graft-Versus-Host and Autoimmunity Unit
Experimental Transplantation and Immunology Branch National Cancer Institute, Bethesda, MD 20892
9000 Rockville Pike, 10/CRC 3-3330
Phone: 301-402-4899; Fax: 301-480-3444
E-mail: pavletis@mail.nih.gov

References

1. Lipsky PE. Systemic lupus erythematosus: an autoimmune disease of B cell hyperactivity. Nat Immunol. 2001 Sep;2(9):764-6.
2. Tyndall A, Saccardi R. Haematopoietic stem cell transplantation in the treatment of severe autoimmune disease: results from phase I/II studies, prospective randomized trials and future directions. Clin Exp Immunol. 2005 Jul;141(1):1-9.
3. Saiz A, Graus F. Neurological complications of hematopoietic cell transplantation. Semin Neurol. 2004;24(4):427-34.
4. Guidelines for referral and management of systemic lupus erythematosus in adults. American College of Rheumatology Ad Hoc Committee on Systemic Lupus Erythematosus Guidelines. Arthritis Rheum. 1999 Sep;42(9):1785-96.
5. Proposed diagnostic criteria and nosology of acute transverse myelitis. Neurology. 2002 Aug 27;59(4):499-505.
6. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale. Neurology. 1983;33:1444-52.
7. Sipe JC, Knobler RL, Braheny SL, Rice GP, Panitch HS, Oldstone MB. A neurologic rating scale (NRS) for use in multiple sclerosis. Neurology. 1984;34(10):1368-72.
8. Mevorach D, Raz E, Steiner I. Evidence for intrathecal synthesis of autoantibodies in systemic lupus erythematosus with

neurological involvement. Lupus. 1994 Apr;3(2):117-21.
9. Jayne D, Tyndall A. Autologous stem cell transplantation for systemic lupus erythematosus. Lupus. 2004;13(5):359-65.
10. Hellmich B, Schnabel A, Gross WL. Treatment of severe neutropenia due to Felty's syndrome or systemic lupus erythematosus with granulocyte colony-stimulating factor. Semin Arthritis Rheum. 1999;29(2):82-99.
11. Euler HH, Harten P, Zeuner RA, Schwab UM. Recombinant human granulocyte colony stimulating factor in patients with systemic lupus erythematosus associated neutropenia and refractory infections. J Rheumatol. 1997 Nov;24(11):2153-7.
12. Openshaw H, Stuve O, Antel JP, Nash R, Lund BT, Weiner LP, et al. Multiple sclerosis flares associated with recombinant granulocyte colony-stimulating factor. Neurology. 2000 Jun 13;54(11):2147-50.
13. Oyama Y, Cohen B, Traynor A, Brush M, Rodriguez J, Burt RK. Engraftment syndrome: a common cause for rash and fever following autologous hematopoietic stem cell transplantation for multiple sclerosis. Bone Marrow Transplant. 2002 Jan;29(1):81-5.
14. Lee CK, Gingrich RD, Hohl RJ, Ajram KA. Engraftment syndrome in autologous bone marrow and peripheral stem cell transplantation. Bone Marrow Transplant. 1995 Jul;16(1):175-82.
15. Nash RA, Bowen JD, McSweeney PA, Pavletic SZ, Maravilla KR, Park MS, et al. High-dose immunosuppressive therapy and autologous peripheral blood stem cell transplantation for severe multiple sclerosis. Blood. 2003 Oct 1;102(7):2364-72.
16. Dinkel K, Dhabhar FS, Sapolsky RM. Neurotoxic effects of polymorphonuclear granulocytes on hippocampal primary cultures. Proc Natl Acad Sci U S A. 2004 Jan 6;101(1):331-6.
17. Viswanathan K, Dhabhar FS. Stress-induced enhancement of leukocyte trafficking into sites of surgery or immune activation. Proc Natl Acad Sci U S A. 2005 Apr 19;102(16):5808-13.
18. 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(2):110-5.
19. Filep JG, Delalandre A, Payette Y, Foldes-Filep E. Glucocorticoid receptor regulates expression of L-selectin and CD11/CD18 on human neutrophils. Circulation. 1997 Jul 1;96(1):295-301.