blood

2007 109: 2643-2548
Prepublished online November 21, 2006;
doi:10.1182/blood-2006-07-035766

Development of a secondary autoimmune disorder after hematopoietic stem cell transplantation for autoimmune diseases: role of conditioning regimen used

Yvonne Loh, Yu Oyama, Laisvyde Statkute, Kathleen Quigley, Kimberly Yaung, Elizabeth Gonda, Walter Barr, Borko Jovanovic, Robert Craig, Dusan Stefoski, Bruce Cohen and Richard K. Burt

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.

Development of a secondary autoimmune disorder after hematopoietic stem cell transplantation for autoimmune diseases: role of conditioning regimen used

Yvonne Loh,[1] Yu Oyama,[1] Laisvyde Statkute,[1] Kathleen Quigley,[1] Kimberly Yaung,[1] Elizabeth Gonda,[1] Walter Barr,[2] Borko Jovanovic,[3] Robert Craig,[1,4] Dusan Stefoski,[5] Bruce Cohen,[6] and Richard K. Burt[1]

[1]Division of Immunotherapy, [2]Division of Rheumatology, [3]Department of Preventive Medicine, [4]Division of Gastroenterology, and [6]Department of Neurology, Northwestern University Feinberg Medical Center, Chicago, IL; [5]Department of Neurological Sciences, Rush University Medical Center, Chicago, IL

Patients undergoing autologous hematopoietic stem cell transplantation (auto-HSCT) for autoimmune disease may have an added propensity to develop a second autoimmune disorder, given the genetic predisposition to autoimmunity. Therefore, we undertook a retrospective analysis of all patients who have undergone auto-HSCT for an autoimmune disease in our institution to determine the occurrence of a second autoimmune disorder and possible risk factors. In all, 155 patients underwent auto-HSCT for various autoimmune diseases; of those patients, 6 manifested a distinct secondary autoimmune disease at a median of 8.5 months (range, 2-30 months) after auto-HSCT. There were 2 patients with systemic lupus erythematosus, conditioned with a regimen containing antithymocyte globulin (ATG), who developed factor VIII inhibitors with severe bleeding. There were 4 patients (2 with multiple sclerosis, one each with lupus and systemic sclerosis) who received an alemtuzumab-containing conditioning regimen who developed autoimmune cytopenias. Among the 155 patients, the frequency of secondary autoimmune complications was 16.0% with alemtuzumab (4/25), 1.9% for ATG (2/102), and 0% for conditioning regimens without lympho-depleting antibodies (0/28)—a difference that was found to be significantly higher with alemtuzumab exposure ($P = .011$). In contrast, sex, type of ATG used, and CD34-selection of peripheral blood stem cells were not found to be significantly associated with development of a secondary autoimmune disorder. (Blood. 2007;109:2643-2648)

Introduction

Autoimmune disorders have been reported to occur following both autologous and allogeneic hematopoietic stem cell transplantation (HSCT) for malignant and nonmalignant conditions, with autoimmune cytopenias being reported most frequently.[1-4] The underlying mechanism for the development of autoimmunity is postulated to involve impaired function of regulatory T cells allowing self-reactive T cells to act unchecked.[5] Delayed T-cell reconstitution following transplantation may thus predispose patients to the development of a secondary autoimmune disorder.

In addition to the immunologic milieu, a genetic predisposition to autoimmunity is well described, with epidemiologic studies demonstrating a higher rate of concordance among monozygotic compared with dizygotic twins, and association with certain HLA types.[6,7] Patients with autoimmune disorders who have undergone autologous HSCT (auto-HSCT), with the combination of genetic predisposition and the lympho-deficient state after undergoing transplantation, may thus be at risk of developing another autoimmune disorder.

We therefore undertook a retrospective analysis of all patients with autoimmune diseases who had undergone auto-HSCT in our institution to determine the occurrence of a secondary autoimmune disorder. We studied the characteristics of affected patients, and the treatment and outcome of the secondary autoimmune disease to assess the factors that may predispose to their development. We also discuss herein the possible contributory role played by the agents used in the conditioning regimen, with particular reference to alemtuzumab and antithymocyte globulin (ATG).

Patients and methods

We carried out a retrospective analysis of all patients who had undergone an auto-HSCT for an autoimmune disease in our institution. All patients underwent transplantation on protocols approved by the Institutional Review Board of Northwestern University, Chicago, IL. Patients met eligibility criteria for the respective disease-specific protocols, which have been previously described.[8-12] After informed consent in accordance with the Declaration of Helsinki, the patients received cyclophosphamide (2 g/m^2) and subcutaneous filgrastim for mobilization, and had peripheral blood stem cells (PBSCs) collected by apheresis upon neutrophil recovery. Depending on disease-specific protocols, the PBSC product was either CD34 selected with a cell separator (Isolex, Nexell, Irvine, CA; or Ceprate, Cellpro, Bothell, WA) or unmanipulated. The conditioning regimens consisted of various combinations of cyclophosphamide with either ATG (rabbit or equine) or alemtuzumab; or cyclophosphamide with either intravenous busulfan or total body irradiation (TBI), again depending on disease-specific protocols. PBSC infusion was on day 0, 36 hours after completion of cyclophosphamide. Subcutaneous filgrastim was administered from either day 0 or day +6 and continued until neutrophil recovery. Neutrophil engraftment was defined as the first day after transplantation when the absolute neutrophil count exceeded 0.5×10^9/L. Platelet engraftment was defined as the first of 3 days when platelet counts exceeded 20×10^9/L without transfusion. All blood products were irradiated and

Submitted July 19, 2006; accepted November 6, 2006. Prepublished online as *Blood* First Edition Paper, November 21, 2006; DOI 10.1182/blood-2006-07-035766.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

filtered and were cytomegalovirus safe. Packed red blood cells were transfused when hemoglobin declined below 80 g/L or where clinically indicated. Platelet transfusions were administered when platelet counts fell below 20×10^9/L or where clinically indicated.

Antimicrobial prophylaxis was with oral ciprofloxacin, valacyclovir or acyclovir, fluconazole or voriconazole, and trimethroprim-sulfamethoxazole or nebulized pentamidine. During the period of neutropenia, patients were given intravenous cefepime or ceftazidime prophylactically. Herpes virus prophylaxis was continued for 12 months after transplantation, whereas antifungal and *Pneumocystis jiroveci* prophylaxis was continued for at least 6 months, or longer if the patient remained on immunosuppressive therapy. The patients were followed up at 6 and 12 months after undergoing transplantation, then yearly thereafter.

For purpose of analysis, patients were grouped by conditioning regimen into 3 categories, namely ATG-containing, alemtuzumab-containing, and without lympho-depleting antibodies (ie, cyclophosphamide-TBI or busulfan-cyclophosphamide). Fisher exact test was used to compute the statistical significance of observed differences in the frequency of secondary autoimmune disorders occurring among the 3 groups. Comparisons were also made pair-wise. In addition, the impact of sex, type of ATG used, and CD34 selection of the PBSC product were evaluated, and Fisher exact test was used to determine statistical significance. All *P* values were 2-sided and the statistical software employed was R version 2.2.0 (R Foundation for Statistical Computing; http://www.r-project.org). The same software was used to compute exact confidence intervals and bounds for odds ratios in 2-by-2 tables. An approximation of the odds ratio was obtained in cases where n was 0 by the addition of 0.25 to each cell in the 2-by-2 table, where the odds ratio would otherwise be expressed as "infinity."

Results

During the period from August 1996 to March 2006, 155 patients with various autoimmune disorders underwent an auto-HSCT. The autoimmune diseases represented were systemic lupus erythematosus (SLE; n = 60, 38.7%), systemic sclerosis (SSc; n = 13, 8.4%), multiple sclerosis (MS; n = 43, 27.7%), rheumatoid arthritis (n = 6, 3.9%), Crohn disease (n = 19, 12.3%), and others (n = 14, 9.0%). There were 6 patients (3 with SLE, 2 with MS, and 1 with SSc) who developed an autoimmune disorder distinct from their underlying autoimmune disease at a median of 8.5 months (range, 2-30 months) after auto-HSCT. The occurrence of a secondary autoimmune disorder and the possible contributory factors are summarized in Table 1.

The frequency of a secondary autoimmune disorder was 4 of 25 among patients receiving alemtuzumab, 2 of 102 among those receiving ATG, and none of 28 patients who received neither of these lympho-depleting antibodies. When a 3-way comparison is made, the differences were found to be statistically significant ($P = .011$). Pair-wise comparison between ATG and alemtuzumab also revealed a significantly increased occurrence of a secondary autoimmune disorder with alemtuzumab ($P = .014$; odds ratio 9.3; 95% confidence interval [CI] 1.2 to 108.8). In addition, pair-wise comparison between alemtuzumab and regimens without lympho-depleting antibodies revealed a significantly increased frequency of this complication with alemtuzumab ($P = .043$; estimated odds ratio 22.6; 95% CI 0.8 to infinity). On the other hand, a comparison between ATG and regimens without lympho-depleting antibodies was not statistically significant ($P > .999$; estimated odds ratio 2.5; 95% CI 0.1 to infinity). No significant difference was found between equine ATG (2/51) and rabbit ATG (0/51) ($P = .50$; estimated odds ratio 9.4; 95% CI 0.2 to infinity). CD34 selection of the PBSC product also did not appear to impact the development of a secondary autoimmune disorder. Two of 87 patients who received CD34-selected PBSCs developed a secondary autoimmune disorder, compared with 4 of 68 patients who received an unmanipulated graft ($P = .41$; odds ratio 0.4; 95% CI 0.03 to 2.7). In addition, sex did not have a statistically significant association with this complication (Table 1).

Table 1. Occurrence of secondary autoimmune disorders and possible risk factors

Possible risk factors	Affected by secondary autoimmune disorder, no.	Unaffected by secondary autoimmune disorder, no.	*P*
Conditioning regimen			
Cy/ATG	2	100	
Cy/alemtuzumab	4	21	
Cy/TBI or Bu/Cy	0	28	.011
Sex			
Female	6	106	
Male	0	43	.19
Primary disease			
Systemic lupus erythematosus	3	57	
Multiple sclerosis	2	41	
Systemic sclerosis	1	12	
Crohn disease	0	19	
Rheumatoid arthritis	0	6	
Others	0	14	ND
Type of ATG			
Rabbit	0	51	
Equine	2	49	.50
PBSC product			
CD34 selected	2	85	
Unmanipulated	4	64	.41

Pair-wise comparisons were as follows: Cy/alemtuzumab vs Cy/ATG, $P = .014$; Cy/alemtuzumab vs Cy/TBI or Bu/Cy, $P = .043$; and Cy/ATG vs Cy/TBI or Bu/Cy, $P > .999$.

Cy indicates cyclophosphamide; ATG, antithymocyte globulin; TBI, total body irradiation; Bu, intravenous busulfan; PBSC, peripheral blood stem cell; and ND, not done.

The secondary autoimmune conditions manifested were acquired factor VIII inhibitors (n = 2), autoimmune thrombocytopenia (n = 2), autoimmune hemolytic anemia (AIHA; n = 1), and autoimmune neutropenia/AIHA (n = 1). Their presentation, management, and outcomes are summarized in Table 2.

Secondary acquired factor VIII inhibitor

Two patients developed a secondary acquired factor VIII inhibitor. The first was a 36-year-old female with refractory SLE with multisystem involvement including antiphospholipid syndrome (APS) with recurrent venous thromboembolism. She underwent auto-HSCT after conditioning with cyclophosphamide and equine ATG uneventfully, achieving both clinical and serologic remission from both SLE and APS, permitting discontinuation of anticoagulation and immunosuppression by 7 months. At 30 months after auto-HSCT, she developed severe postoperative bleeding following umbilical hernia repair and insertion of a peritoneal dialysis catheter, developing large intra-abdominal and retroperitoneal hematomas. Investigations revealed a partial thromboplastin time (PTT) exceeding 150 seconds, a factor VIII activity of less than 1%, and a factor VIII inhibitor at a level of 16.4 Bethesda units. Her lupus anticoagulant and anticardiolipin antibodies remained negative. The patient was treated with activated factor VII, prednisone, and rituximab and achieved remission from acquired hemophilia.

The second patient was a 28-year-old female with severe SLE, who was refractory to numerous immunosuppressants. She underwent auto-HSCT after conditioning with cyclophosphamide and

Table 2. Summary of patients who developed a secondary autoimmune disorder

	Primary disease	Regimen	CD34-selected PBSCs	Current status of primary disease	Secondary autoimmune disorder (time of onset)	Treatment of secondary disorder	Outcome of secondary disorder
1	SLE	Cy/ATG	Yes	Remission	FVIII inhibitor (30 mo)	FVIIa, RTX, pred	Resolved
2	SLE	Cy/ATG	Yes	Persistent	FVIII inhibitor (9 mo)	FVIIa, MP, RTX, Cy, MMF	Resolved
3	MS	Cy/alemtuzumab	No	Remission	ITP (8 mo)	IVIG, MP, RTX, pred	Resolved
4	SSc	Cy/alemtuzumab	No	Remission	AIHA (5 mo)	IVIG, MP, MMF, pred, RTX, Cy	Controlled
5	SLE	Cy/alemtuzumab	No	Remission	Autoimmune neutropenia/AIHA (2 mo)	IVIG, MP, RTX, Cy, MMF, pred, tacrolimus, splenectomy	Controlled
6	MS	Cy/alemtuzumab	No	Remission	ITP (14 mo)	IVIG, MP, RTX, MMF	Resolved

PBSCs indicates peripheral blood stem cells; HSCT, hematopoietic stem cell transplantation; SLE, systemic lupus erythematosus; MS, multiple sclerosis; SSc, systemic sclerosis; Cy, cyclophosphamide; ATG, antithymocyte globulin; FVIII, factor VIII; ITP, immune thrombocytopenia purpura; AIHA, autoimmune hemolytic anemia; FVIIa, recombinant activated factor VII; RTX, rituximab; pred, prednisone; MP, methylprednisolone; MMF, mycophenolate mofetil; and IVIG, intravenous immunoglobulin.

equine ATG; SLE disease activity remained persistent after the transplantation, albeit reduced in severity. At 9 months after auto-HSCT, she suffered a spontaneous abortion complicated by severe bleeding after evacuation of the uterus. She also developed multiple spontaneous bruises and hematomas on her upper and lower extremities. Her PTT exceeded 150 seconds, factor VIII activity was below 1%, and the factor VIII inhibitor was 1600 Bethesda units. She received activated factor VII which arrested the bleeding, and was also treated with rituximab, intravenous cyclophosphamide, mycophenolate mofetil (MMF), and prednisone. The factor VIII inhibitor cleared within several months but low-grade lupus activity has persisted.

Secondary autoimmune cytopenias

Four patients developed secondary autoimmune cytopenias. The first was a 46-year-old female with relapsing-remitting MS failing interferon therapy. She underwent auto-HSCT after cyclophosphamide and alemtuzumab (20 mg). She achieved platelet engraftment by day +8 and remained well until 8 months after transplantation when she presented with diffuse petechiae. Her platelet counts fell to 2×10^9/L with mild anemia (hemoglobin 100 g/L) and leukopenia. A bone marrow aspiration and biopsy revealed normal cellularity and maturation in all cell lineages. The direct antiglobulin test (DAT) was positive with biochemical evidence of hemolysis. She was treated with intravenous immunoglobulin (IVIG) and methylprednisolone, eventually requiring rituximab and cyclophosphamide to achieve remission. Immunosuppressants have been discontinued since, and she is in remission from both ITP and MS.

The next patient, a 45-year-old woman, had diffuse cutaneous SSc and mild nephritis; baseline complete blood counts were normal. She underwent auto-HSCT after conditioning with cyclophosphamide and alemtuzumab (80 mg). At 5 months after undergoing transplantation, she presented with symptomatic anemia (hemoglobin 55 g/L). Investigations supported a diagnosis of autoimmune hemolytic anemia (AIHA) with positive DAT and both warm and cold auto-antibodies were present. She was treated with methylprednisolone, IVIG, oral prednisone, rituximab, and MMF with improvement in hemoglobin, but efforts to taper prednisone led to recrudescent hemolysis. She was then given intravenous cyclophosphamide in order to facilitate steroid taper.

The next patient was a 45-year-old female with severe SLE with refractory cerebritis. Prior to undergoing transplantation she had normal blood counts and a negative DAT. She underwent auto-HSCT after cyclophosphamide and alemtuzumab (60 mg), and had successful neutrophil engraftment on day +10. At 2 months after auto-HSCT, however, her white blood cell counts declined without evidence of an infectious etiology. She developed neutropenic sepsis, and for the next 7 weeks her white blood cell counts remained between 0.1×10^9/L and 0.4×10^9/L, with no discernible response to filgrastim. She also developed AIHA with warm and cold auto-antibodies. Serologies for lupus were negative and she had no clinical manifestations of active SLE. Bone marrow biopsy performed on 3 separate occasions repeatedly showed virtually complete absence of myeloid precursors, confirmed on myeloperoxidase staining of the core biopsy. Antineutrophil antibodies were detected in the serum. She received intravenous methylprednisolone, IVIG, ATG, cyclophosphamide, rituximab, prednisone, MMF, and tacrolimus. At 7 weeks after onset of pure white cell aplasia,[13] she recovered, but persistent hemolysis eventually necessitated a splenectomy. She currently remains on MMF and tacrolimus with normal neutrophil, hemoglobin, and platelet counts.

The last patient was a 51-year-old woman with refractory MS who underwent auto-HSCT after conditioning with cyclophosphamide and alemtuzumab (20 mg). She achieved platelet engraftment on day +8 and had normal complete blood counts within a month after the transplantation. She achieved remission from MS, and was well until 14 months after auto-HSCT when she presented with spontaneous bruising on her limbs and a platelet count of 8×10^9/L. She required treatment with methylprednisolone, IVIG, rituximab, and MMF, and has achieved remission from ITP.

Discussion

This study describes for the first time the development of a secondary autoimmune complication among patients undergoing auto-HSCT for an autoimmune condition, and possible contributory factors involved. The limitation of this study is its retrospective and nonrandomized nature, relatively small numbers, and its involvement of patients with different autoimmune diseases recruited under several different disease-specific protocols. It does, however, represent the largest single-center experience of auto-HSCT for autoimmune diseases, with the benefit of standardization of procedures, evaluation, and management. The timing of follow-up varies, as the regimens have evolved with time. Our first patient to receive an alemtuzumab-based regimen was in 2003, whereas our experience with ATG- or TBI-based regimens dates from 1996. It is noteworthy therefore that, despite a shorter duration of follow-up, we have seen a higher frequency of secondary autoimmunity among the alemtuzumab group, an observation that prompted this retrospective analysis. Among the factors analyzed, only alemtuzumab exposure had a statistically significant association with this complication.

The patients described in our series developed second autoimmune disorders distinct from their primary disease, and which are not usually associated with the underlying disorder. The onset of the complication was at a median of 8.5 months after undergoing transplantation, occurring as late as 30 months in the case of the first patient with SLE who developed acquired hemophilia. While autoimmune cytopenias are common manifestations of SLE, the fifth patient described never had significant cytopenia prior to auto-HSCT. In addition, with the exception of the second case, the patients developed the disorders despite achieving remission from the primary autoimmune disease. The occurrence of more than one autoimmune disorder in susceptible individuals has been well described.[14] This results from a genetic predisposition to autoimmune disease, which is supported by epidemiologic and genetic evidence.[6,7] We believe, however, that the secondary autoimmune disorders in our patients occurred because of a combination of factors: an underlying propensity to autoimmunity, the immune dysregulation/reset resulting from the auto-HSCT, and the conditioning regimen drugs used.

Autoimmune diseases developing after both autologous and allogeneic HSCT for hematologic malignancies have been reported to occur at a frequency of between 2% and 5%.[15-17] The spectrum of autoimmune disorders includes autoimmune cytopenias most frequently, but has also included autoimmune thyroiditis,[18] myasthenia gravis,[19] rheumatoid arthritis,[20] and a lupuslike illness.[2] Acquired hemophilia due to factor VII antibodies after HSCT has been described in 8 patients,[21] but acquired hemophilia due to factor VIII inhibitors has been reported only once to our knowledge, having occurred in a patient with MS after auto-HSCT during which he received conditioning with ATG.[22] The authors of the latter report concluded the coexistence of 3 factors (underlying autoimmunity, auto-HSCT, and use of interferon-beta) predisposed the patient to developing this autoimmune complication.

Acquired hemophilia due to factor VIII inhibitors is a rare condition with a reported incidence of only one person per million per year.[23] It is usually idiopathic, although the association with autoimmune disorders such as SLE and rheumatoid arthritis has been described.[24] In one of the largest series of acquired hemophilia published to date, with 215 patients, only 4% of the cohort had coexistent SLE.[25] The occurrence of this rare disorder with an infrequent association with SLE among 2 of our patients is thus notable. While acquired hemophilia is associated with pregnancy, it is usually mild in this subgroup of patients, with a median inhibitor titer of 20 Bethesda units, and spontaneous remissions often occur.[26,27] In contrast, our patient described in the second case had an inhibitor titer of 1600 Bethesda units and required multiple immunosuppressive agents to achieve control. This presentation, although perhaps exacerbated by pregnancy, is not typical of a case of postpartum acquired hemophilia.

The development of autoimmunity following autologous and allogeneic HSCT is postulated to be due to the immunologic dysregulation resulting from the transplant.[28] While high-dose immunosuppression and auto-HSCT have been employed in a variety of autoimmune disorders with varied success, the same process of immune ablation may alter the regulatory mechanisms within the immunological system keeping further autoimmunity in check. The role of regulatory T cells in maintaining self-tolerance has been demonstrated by the development of autoimmunity in thymectomized animals,[29] with restoration of self-tolerance through reconstitution with $CD4^+CD25^+$ splenocytes.[30] The role of these $CD25^+$ regulatory T cells in maintaining tolerance is now well established.[31] These T-cell homeostatic mechanisms are also altered during lymphopenia, with T-cell clones proliferating in response to self-antigens in the lymphopenic host.[32] The rate of immune reconstitution following transplantation of the various lymphocyte subsets differs, with B-cell recovery preceding T cell, and CD8 subsets occurring earlier than CD4.[33] These altered balances affect the immune rheostat and may set the stage for the emergence of autoimmune cytopenias and immune-mediated factor VIII deficiency. An analysis of T-cell subsets among our patients would serve to clarify the role regulatory T cells may play in the pathogenesis of secondary autoimmune disorders after transplantation.

In auto-HSCT for autoimmune diseases, the regimens chosen are selected specifically for lympho-depletion, with the aim of eliminating auto-reactive T-cell clones in the patient. Drugs like ATG and alemtuzumab are thus used for their lymphotoxicity. ATG induces a rapid and profound lymphopenia attributed to complement- and antibody-dependent cytolysis and macrophage phagocytosis[34] and apoptosis of T cells.[35] The effect of ATG on immune function has also been shown to persist for up to 12 months.[36,37] The net result is a lasting quantitative and qualitative defect in T-cell function. There have been several reports of various autoimmune disorders precipitated by antithymocyte or antilymphocyte globulin including thyroid disease,[38] Guillain-Barre syndrome,[39] immune hemolysis,[40] and alveolitis.[41] There was a case of a patient who developed asymptomatic factor XII inhibitors after allogeneic HSCT with an ATG-containing regimen for acute myeloid leukemia.[42] To our knowledge, the only other report of acquired hemophilia secondary to factor VIII inhibitors after ATG involved a patient with MS who underwent auto-HSCT after busulfan and ATG conditioning.[22]

Alemtuzumab is a monoclonal antibody directed against CD52 which is expressed on virtually all T and B lymphocytes (although preferentially on T lymphocytes)[43] and monocytes.[44] It has the ability to induce a rapid and profound lymphopenia following a single administered dose.[45] This lymphopenia persists beyond 6 months and the effect on the $CD4^+$ and $CD8^+$ subsets, compared with ATG, appears to be even more protracted, with some studies demonstrating decreased T-cell subsets beyond 18 months.[46]

The lymphopenia effected by alemtuzumab, with decreased $CD4^+CD25^+$ regulatory T cells, may predispose to the development of autoimmunity. In addition, CD52 antigens are also expressed on monocyte-derived dendritic cells and thus antigen presentation in the host is affected following alemtuzumab exposure.[47,48] Immature peripheral dendritic cells present antigen to T cells in a tolerogenic manner by silencing activated T cells, activating and causing expansion of regulatory T cells, and causing the differentiation of naive $CD4^+$ cells to regulatory T cells.[49,50] The effect of alemtuzumab in depleting these dendritic cells may therefore predispose to the development of autoimmunity. Furthermore, alemtuzumab has been shown to deplete natural killer cells; studies suggest these cells may play a role in both exacerbating and protecting against autoimmune mechanisms.[51] It can be seen, therefore, that alemtuzumab induces a wide range of perturbations in the various components of the adaptive and innate immune system, from which recovery and reconstitution occur within different time frames and to different extents. This immune dysregulation may set the stage for the development of autoimmune disorders.

There have been several reports of autoimmune disorders occurring after therapy with alemtuzumab. In a report of patients with MS given the drug, 32% developed Grave disease. The authors concluded that while the drug suppressed MS disease

activity, it permitted the development of autoimmunity, perhaps due to delayed $CD4^+$ T-cell recovery.[52] There have been 2 reports of fatal refractory ITP after treatment of chronic lymphocytic leukemia with alemtuzumab.[53,54] In 3 cases, ITP (one case fatal) occurred during a clinical trial of alemtuzumab for MS.[55] As with the published reports, we have found the autoimmune cytopenias developing after alemtuzumab and auto-HSCT to be generally severe and refractory to steroid therapy alone. All our patients required multiple agents to treat the cytopenias, and demonstrated a response to rituximab, which appears to be an effective agent in refractory cases.[56,57]

Interestingly, alemtuzumab has also been employed in the treatment of refractory autoimmune cytopenias with responses seen in two-thirds of patients, although a third subsequently relapse.[58,59] The drug also has demonstrated activity in treating MS, but the occurrence of autoimmune thyroid disease and severe delayed ITP has dampened enthusiasm toward its use in MS. As with our 2 patients with MS who developed ITP at 8 and 14 months after transplantation, respectively, the development of ITP among the patients who received alemtuzumab for MS was relatively delayed and manifested up to one year after treatment. It is therefore essential to continue monitoring the blood cell counts in these patients beyond a year. Alemtuzumab thus appears to be a somewhat double-edged sword in autoimmunity: its effective long-term lympho-depletion removes auto-reactive T-cell clones, whereas the loss of T-cell homeostasis may favor subsequent development of another autoimmune condition.

Alemtuzumab has been used increasingly in solid organ and hematopoietic stem cell transplantation, and in treating lymphoid malignancies; nevertheless, the reports of autoimmune complications have been relatively few. In fact, in a long-term study of immune reconstitution in patients with chronic lymphocytic leukemia who had been treated with alemtuzumab, there was no reported increase in autoimmunity.[60] In contrast, the occurrence of autoimmune complications among patients with underlying autoimmune disorders receiving alemtuzumab appears to be increased.

Among the 155 patients with autoimmune diseases who underwent transplantation at our institution thus far, the frequency of secondary autoimmune complications was 16.0% with alemtuzumab (4/25), 1.9% for ATG (2/102), and 0% for conditioning regimens without lympho-depleting antibodies (0/28), a difference that was found to be significantly higher with alemtuzumab exposure. In the interest of safety, therefore, we have ceased to use alemtuzumab in our conditioning regimens for auto-HSCT.

Growing evidence supports the potential of auto-HSCT in controlling severe autoimmune diseases, but a second autoimmune disorder may complicate the procedure. The underlying susceptibility of an "autoimmune-prone" patient, coupled with the immune dysregulation resulting from auto-HSCT and lympho-depleting agents used in the conditioning regimen, may set the stage for immune-mediated cytopenias and factor VIII deficiency, especially with long-term lympho-depleting agents such as alemtuzumab. With the increasing employment of auto-HSCT in the treatment of autoimmune diseases, physicians need to be alert to the occurrence of this complication. The increased likelihood of a secondary autoimmune disorder with alemtuzumab recommends against using it in autologous HSCT regimens for autoimmune diseases.

Authorship

Contribution: Y.L. collected and analyzed data, reviewed literature, and wrote and revised the paper; R.B. and Y.O. designed and formulated research, and revised the paper; B.J. performed statistical analysis; Y.O., L.S., K.Q., K.Y., E.G., R.C., D.S., B.C., W.B., Y.L., and R.B. performed the study and collected data.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Richard Burt, Division of Immunotherapy, Northwestern University Feinberg School of Medicine, Chicago, IL 60611; e-mail: rburt@northwestern.edu.

References

1. Horn B, Viele M, Mentzer W, Mogck N, DeSantes K, Cowan M. Autoimmune hemolytic anemia in patients with SCID after T cell-depleted BM and PBSC transplantation. Bone Marrow Transplant. 1999;24:1009-1013.
2. Hartert A, Willenbacher W, Gunzelmann S, et al. Successful treatment of thrombocytopenia and hemolytic anemia with IvIG in a patient with lupus-like syndrome after mismatched related PBSCT. Bone Marrow Transplant. 2001;27:337-340.
3. O'Brien TA, Eastlund T, Peters C, et al. Autoimmune haemolytic anaemia complicating haematopoietic cell transplantation in paediatric patients: high incidence and significant mortality in unrelated donor transplants for non-malignant diseases. Br J Haematol. 2004;127:67-75.
4. Raj A, Bertolone S, Cheerva A. Successful treatment of refractory autoimmune hemolytic anemia with monthly rituximab following nonmyeloablative stem cell transplantation for sickle cell disease. J Pediatr Hematol Oncol. 2004;26:312-314.
5. Marleau AM, Sarvetnick N. T cell homeostasis in tolerance and immunity. J Leukoc Biol. 2005;78:575-584.
6. Poser CM. The multiple sclerosis trait and the development of multiple sclerosis: genetic vulnerability and environmental effect. Clin Neurol Neurosurg. 2006;108:227-233.
7. Svendsen AJ, Holm NV, Kyvik K, Petersen PH, Junker P. Relative importance of genetic effects in rheumatoid arthritis: historical cohort study of Danish nationwide twin population. BMJ. 2002;324:264-266.
8. Burt RK, Traynor A, Statkute L, et al. Nonmyeloablative hematopoietic stem cell transplantation for systemic lupus erythematosus. JAMA. 2006;295:527-535
9. Burt RK, Cohen BA, Russell E, 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.
10. Burt RK, Georganas C, Schroeder J, et al. Autologous hematopoietic stem cell transplantation in refractory rheumatoid arthritis: sustained response in two of four patients. Arthritis Rheum. 1999;42:2281-2285.
11. Oyama Y, Craig RM, Traynor AE, et al. Autologous hematopoietic stem cell transplantation in patients with refractory Crohn's disease. Gastroenterology. 2005;128:552-563.
12. Burt RK, Oyama Y, Traynor A, et al. Hematopoietic stem cell transplantation for systemic sclerosis with rapid improvement in skin scores: is neoangiogenesis occurring? Bone Marrow Transplant. 2003;32(suppl 1):S65–67.
13. Levitt LJ, Ries CA, Greenberg PL. Pure white-cell aplasia: antibody-mediated autoimmune inhibition of granulopoiesis. N Engl J Med. 1983;308:1141-1146.
14. Dittmar M, Kahaly GJ. Polyglandular autoimmune syndromes: immunogenetics and long-term follow-up. J Clin Endocrinol Metab. 2003;88:2983-2992.
15. Hequet O, Salles G, Ketterer N, et al. Autoimmune thrombocytopenic purpura after autologous stem cell transplantation. Bone Marrow Transplant. 2003;32:89-95.
16. O'Brien TA, Eastlund T, Peters C, et al. Autoimmune haemolytic anaemia complicating haematopoietic cell transplantation in paediatric patients: high incidence and significant mortality in unrelated donor transplants for non-malignant diseases. Br J Haematol. 2004;127:67-75.
17. Lambertenghi-Deliliers GL, Annaloro C, Della Volpe A, Oriani A, Pozzoli E, Soligo D. Multiple autoimmune events after autologous bone marrow transplantation. Bone Marrow Transplant. 1997;19:745-747.
18. Karthaus M, Gabrysiak T, Brabant G, et al. Immune thyroiditis after transplantation of allogeneic $CD34^+$ selected peripheral blood cells. Bone Marrow Transplant. 1997;20:697-699.
19. Smith CI, Aarli JA, Biberfeld P, et al. Myasthenia gravis after bone-marrow transplantation: evidence for a donor origin. N Engl J Med. 1983;309:1565-1568.
20. Imamura R, Inoue H, Kato K, et al. Development of rheumatoid arthritis following autologous peripheral blood stem cell transplantation. Bone Marrow Transplant. 2002;30:527-529.
21. Toor AA, Slungaard A, Hedner U, Weisdorf DJ,

Key NS. Acquired factor VII deficiency in hematopoietic stem cell transplant recipients. Bone Marrow Transplant. 2002;29:403-408.

22. Kaloyannidis P, Sakellari I, Fassas A, et al. Acquired hemophilia-A in a patient with multiple sclerosis treated with autologous hematopoietic stem cell transplantation and interferon beta-1a. Bone Marrow Transplant. 2004;34:187-188.
23. Delgado J, Jimenez-Yuste V, Hernandez-Navarro F, Villar A. Acquired haemophilia: review and meta-analysis focused on therapy and prognostic factors. Br J Haematol. 2003;121:21-35.
24. Trotta F, Bajocchi G, La Corte R, Moratelli S, Sun LY. Long-lasting remission and successful treatment of acquired factor VIII inhibitors using cyclophosphamide in a patient with systemic lupus erythematosus. Rheumatology (Oxford). 1999;38:1007-1009.
25. Green D, Lechner K. A survey of 215 non-hemophilic patients with inhibitors to Factor VIII. Thromb Haemost. 1981;45:200-203.
26. Hauser I, Schneider B, Lechner K. Post-partum factor VIII inhibitors: a review of the literature with special reference to the value of steroid and immunosuppressive treatment. Thromb Haemost. 1995;73:1-5.
27. Solymoss S. Postpartum acquired factor VIII inhibitors: results of a survey. Am J Hematol. 1998;59:1-4.
28. Sherer Y, Shoenfeld Y. Autoimmune diseases and autoimmunity post-bone marrow transplantation. Bone Marrow Transplant. 1998;22:873-881.
29. Sakaguchi S, Fukuma K, Kuribayashi K, Masuda T. Organ-specific autoimmune diseases induced in mice by elimination of T cell subset, I: evidence for the active participation of T cells in natural self-tolerance; deficit of a T cell subset as a possible cause of autoimmune disease. J Exp Med. 1985;161:72-87.
30. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25): breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol. 1995;155:1151-1164.
31. Zwar TD, Read S, van Driel IR, Gleeson PA. $CD4^+CD25^+$ regulatory T cells inhibit the antigen-dependent expansion of self-reactive T cells in vivo. J Immunol. 2006;176:1609-1617.
32. Krupica T Jr, Fry TJ, Mackall CL. Autoimmunity during lymphopenia: a two-hit model. Clin Immunol. Prepublished on June 9, 2006, as DOI 10.1016/j.clim.2006.04.569.
33. Peggs KS, Mackinnon S. Immune reconstitution following haematopoietic stem cell transplantation. Br J Haematol. 2004;124:407-420.
34. Bonnefoy-Berard N, Revillard JP. Mechanisms of immunosuppression induced by antithymocyte globulins and OKT3. J Heart Lung Transplant. 1996;15:435-442.
35. Michallet MC, Saltel F, Preville X, Flacher M, Revillard JP, Genestier L. Cathepsin-B-dependent apoptosis triggered by antithymocyte globulins: a novel mechanism of T-cell depletion. Blood. 2003;102:3719-3726.
36. Meijer E, Bloem AC, Dekker AW, Verdonck LF. Effect of antithymocyte globulin on quantitative immune recovery and graft-versus-host disease after partially T-cell-depleted bone marrow transplantation: a comparison between recipients of matched related and matched unrelated donor grafts. Transplantation. 2003;75:1910-1913.
37. Weimer R, Staak A, Susal C, et al. ATG induction therapy: long-term effects on Th1 but not on Th2 responses. Transpl Int. 2005;18:226-236.
38. Todd A, Todd J. Graves' disease following successful treatment of severe aplastic anaemia with antilymphocyte globulin. Clin Lab Haematol. 1999;21:69-70.
39. Kaya B, Davies CE, Oakervee HE, Silver NC, Gawler J, Cavenagh JD. Guillain Barre syndrome precipitated by the use of antilymphocyte globulin in the treatment of severe aplastic anaemia. J Clin Pathol. 2005;58:994-995.
40. Prchal JT, Huang ST, Court WS, Poon MC. Immune hemolytic anemia following administration of antithymocyte globulin. Am J Hematol. 1985;19:95-98.
41. Zomas A, Marsh JC, Harrison NK, et al. Rapid progression of fibrosing alveolitis and thyrotoxicosis after antithymocyte globulin therapy for aplastic anemia. Ann Hematol. 1995;71:49-51.
42. Kawano C, Muroi K, Akioka T, Izumi T, Kodera Y, Ozawa K. Cytomegalovirus pneumonitis, activated prothrombin time prolongation and subacute thyroiditis after unrelated allogeneic bone marrow transplantation. Bone Marrow Transplant. 2000;26:1347-1349.
43. Ginaldi L, De Martinis M, Matutes E, et al. Levels of expression of CD52 in normal and leukemic B and T cells: correlation with in vivo therapeutic responses to Campath-1H. Leuk Res. 1998;22:185-191.
44. Hale G, Xia MQ, Tighe HP, Dyer MJ, Waldmann H. The CAMPATH-1 antigen (CDw52). Tissue Antigens. 1990;35:118-127.
45. DelleKarth G, Laczika K, Scholten C, et al. Clearance of PCR-detectable lymphoma cells from the peripheral blood, but not bone marrow after therapy with campath-1H. Am J Hematol. 1995;50:146-147.
46. Brett S, Baxter G, Cooper H, Johnston JM, Tite J, Rapson N. Repopulation of blood lymphocyte sub-populations in rheumatoid arthritis patients treated with the depleting humanized monoclonal antibody, CAMPATH-1H. Immunology. 1996;88:13-19.
47. Ratzinger G, Reagan JL, Heller G, Busam KJ, Young JW. Differential CD52 expression by distinct myeloid dendritic cell subsets: implications for alemtuzumab activity at the level of antigen presentation in allogeneic graft-host interactions in transplantation. Blood. 2003;101:1422-1429. Erratum in: Blood. 2005;105:3018.
48. Buggins AG, Mufti GJ, Salisbury J, et al. Peripheral blood but not tissue dendritic cells express CD52 and are depleted by treatment with alemtuzumab. Blood. 2002;100:1715-1720.
49. Rutella S, Lemoli RM. Regulatory T cells and tolerogenic dendritic cells: from basic biology to clinical applications. Immunol Lett. 2004;94:11-26.
50. Kubach J, Becker C, Schmitt E, et al. Dendritic cells: sentinels of immunity and tolerance. Int J Hematol. 2005;81:197-203.
51. Johansson S, Berg L, Hall H, Hoglund P. NK cells: elusive players in autoimmunity. Trends Immunol. 2005;26:613-618.
52. Coles AJ, Wing M, Smith S, et al. Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis. Lancet. 1999;354:1691-1695.
53. Otton SH, Turner DL, Frewin R, Davies SV, Johnson SA. Autoimmune thrombocytopenia after treatment with Campath 1H in a patient with chronic lymphocytic leukaemia. Br J Haematol. 1999;106:261-262.
54. Haider I, Cahill M. Fatal thrombocytopaenia temporally related to the administration of alemtuzumab (MabCampath) for refractory CLL despite early discontinuation of therapy. Hematology. 2004;9:409-411.
55. Food and Drug Administration. FDA alert for healthcare professionals: Alemtuzumab (marketed as Campath). http://www.fda.gov/cder/drug/InfoSheets/HCP/alemtuzumabHCP.pdf. Accessed June 30, 2006.
56. Raj K, Narayanan S, Augustson B, et al. Rituximab is effective in the management of refractory autoimmune cytopenias occurring after allogeneic stem cell transplantation. Bone Marrow Transplant. 2005;35:299-301.
57. Shanafelt TD, Madueme HL, Wolf RC, Tefferi A. Rituximab for immune cytopenia in adults: idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia, and Evans syndrome. Mayo Clin Proc. 2003;78:1340-1346.
58. Willis F, Marsh JC, Bevan DH, et al. The effect of treatment with Campath-1H in patients with autoimmune cytopenias. Br J Haematol. 2001;114:891-898.
59. Marsh JC, Gordon-Smith EC. CAMPATH-1H in the treatment of autoimmune cytopenias. Cytotherapy. 2001;3:189-195.
60. Lundin J, Porwit-MacDonald A, Rossmann ED, et al. Cellular immune reconstitution after subcutaneous alemtuzumab (anti-CD52 monoclonal antibody, CAMPATH-1H) treatment as first-line therapy for B-cell chronic lymphocytic leukaemia. Leukemia. 2004;18:484-490.