Reversal of autoimmune disease in lupus-prone New Zealand black/New Zealand white mice by nonmyeloablative transplantation of purified allogeneic hematopoietic stem cells

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Patients with severe systemic lupus erythematosus (SLE) refractory to conventional treatment are candidates for autologous hematopoietic stem cell (HSC) transplantation if the intent is to reset the immunologic clock. These patients might be candidates for allotransplantation with (SLE)-resistant major histocompatibility complex (MHC) haplotype-matched HSC if partial or complete replacement of an autoimmune-prone system is the intent. Using lupus-prone New Zealand black × New Zealand white (NZBW) mice, we investigated the use of highly enriched, haploimmunocompromised, allogeneic HSC to prevent development of or to treat established autoimmune pathology. Young NZBW mice receiving purified allogeneic HSC transplants had improved survival, decreased proteinuria, circulating immune complexes, and autoantibodies to nuclear antigens than did untreated mice or mice given NZBW HSCs. NZBW mice with established lupus-like disease that received nonmyeloablative conditioning and transplants of (MHC) haplomismatched allogeneic HSCs also had greatly increased overall survival. Mice that received transplants exhibited stabilization or reversal of their lupus symptoms; stabilization or decreased proteinuria, and a lower frequency of elevated circulating immune complexes or autoantibodies than did control mice. Induction of durable mixed chimerism by transplantation of purified allogeneic HSCs after nonmyeloablative conditioning has the potential to reverse symptoms of established NZBW lupus. (Blood. 2007;110:1370-1378)

Introduction

Systemic lupus erythematosus (SLE) is a multifactorial, polygenic autoimmune disorder typified by the production of autoantibodies to nuclear antigens. Those afflicted with SLE often experience renal dysfunction and accelerated atherosclerosis, although almost all organ systems can be affected.1 Susceptibility to lupus is thought to be governed by a combination of genetic factors and environmental stimuli leading to a loss of self-tolerance.2,6 SLE is associated with several intrinsic defects of the immune system: autoreactive B and T cells, loss of suppressor function, impaired clearance of apoptotic bodies by phagocytes, and consumption of complement.7,9 SLE is most often controlled with aggressive immunosuppressive therapy, frequently as a course of intravenous cyclophosphamide and corticosteroids. These broad-spectrum treatments do not offer a cure for the disease but function to suppress the immune system and have significant side effects.10 Patients with severe lupus failing to respond to conventional therapies are considered for nearly ablative doses of cyclophosphamide alone or in combination with autologous hematopoietic cell transplant (HCT) in an effort to eliminate autoreactive lymphocytes and reset the immunologic clock.11-13 Transplantation is considered a salvage therapy. Patients with lupus have been immunocompromised for an extended period of time, and often have advanced end-organ dysfunction, and active or refractory disease at the time of transplant. In these patients, treatment-related mortality is significant, approaching 11%.14 To improve safety, less toxic nonmyeloablative conditioning regimens that use lower doses of chemoablation combined with polyclonal antibodies that eliminate lymphocytes are currently being investigated. These protocols target self-reactive lymphocytes with limited myeloablation. Initial trials for autologous nonmyeloablative HCT report a more tolerable treatment-related mortality of 4%.15 Although encouraging results have been seen in patients with long-term remissions of autoimmune diseases who have received allogeneic transplant for a coexisting disease, few patients have received allogeneic transplant primarily for autoimmune disease because of fears of fatal graft-versus-host disease (GVHD).16,17 Because mature T cells are the effectors of GVHD, their exclusion from the graft material should prevent its onset. Using a mouse model system of allogeneic hematopoietic stem cell transplantation, researchers demonstrated that engraftment of purified allogeneic hematopoietic stem cell (HSC) could effectively reconstitute a fully functional hematopoietic system without GVHD.18-23 Transplantation of SLE-resistant HSCs allows for partial or complete replacement of an autoimmune-prone system.24 Several reports have been published demonstrating that transplantation of hematopoietic cells can prevent or attenuate disease in spontaneous and induced animal models of autoimmune disease. These animal models have demonstrated that allogeneic hematopoietic cell transplantation is superior to syngeneic transplantation in controlling autoimmune disease in spontaneous models of autoimmune disease but does not confer an advantage in inducible models of autoimmune disease.19,25-28 Using the BXSB mouse model of spontaneous lupus-like autoimmune disease, experiments have
demonstrated prevention and treatment of lupus-like symptoms in ablative and nonablative models of transplantation with allogeneic T cell-depleted or whole bone marrow.\textsuperscript{25,29,31} Furthermore, highly purified allogeneic HSCs have been shown to be effective in preventing the onset of diabetes in nonobese diabetic mice.\textsuperscript{19,20} These studies suggest the reestablishment of tolerance in humans with a genetic background susceptible to autoimmune disease and may be more effective using allogeneic hematopoietic cell transplantation. Furthermore, the use of purified allogeneic HSCs free of immune cells eliminates the risk of GVHD and may offer a cure for autoimmune disease.

The New Zealand black × New Zealand white F1 (NZBW) mouse model of lupus is considered the most similar to human SLE. These mice develop serologic abnormalities, including high mouse model of lupus is considered the most similar to human SLE. These mice develop serologic abnormalities, including high

>Materials and methods

**Mouse strains and conditioning**

Eight-week-old NZBW (H-2\textsuperscript{d}/z) female mice were purchased from Jackson Laboratories (Bar Harbor, ME). Mice were maintained and aged in the Stem Cells animal facility (Palo Alto, CA) following Institutional Animal Care and Use Committee-approved protocols and procedures. Recipient mice used in the myeloablative studies received transplants at 73 and 79 days of age. NZBW donor mice for the syngeneic HSCs and WBM were age-matched to the recipients. Donor mice for the allogeneic HSCs, female DBA\textsubscript{2} × C57/B16 (DBF1, H-2\textsuperscript{b}), were purchased from Charles River Laboratories (Hollister, CA). These are H2 haploidentical and minor histocompatibility loci mismatched to the NZBW hosts. DBF1 donor mice were 10 weeks of age at the time of bone marrow collection. Three separate cohorts of mice received transplants; in total, 26 mice received 25 to \(40 \times 10^3\) allogeneic HSCs, 33 mice received 1.5 to \(7.5 \times 10^3\) syngeneic HSCs and 16 mice received \(0.8 \times 10^3\) syngeneic WBM cells. A greater number of allogeneic HSCs than syngeneic HSCs were transplanted to both prevent the development of GVHD and may offer a cure for autoimmune disease.

To collect peritoneal exudate cells, the animal was humanely killed and the peritoneal cavity was washed with Hanks balanced salt solution. For personal use only.
Detection of lupus symptoms

Proteinuria was monitored using a Chemstrip (Roche Pharmaceutical, Indianapolis, IN). Urine was collected from each animal into a sterile tube. A volume of 20 to 30 μL was applied to a Chemstrip. Urine protein was determined according to the manufacturer’s instructions. Proteinuria was defined as urine protein 1 g/L (100 mg/dL) or more in concurrence with published studies.44 During the development of lupus-like disease in NZBW mice, the levels of urine protein fluctuates to some extent during disease progression and stabilizes with severe disease. We report peak levels of proteinuria.

Circulating immune complexes and autoantibodies were tested by enzyme-linked immunosorbent assay using test kits (Alpha Diagnostics, San Antonio, TX). Serum was collected monthly from all mice during the studies. The last time point before death was used for serology. Serology was not performed for mice not surviving 8 weeks after receiving the transplant. The donor strain, DBF, was used to establish baseline levels of autoantibodies. Circulating immune complexes test kit, anti-dsDNA test kit, antinuclear antigen test kit, and antihistone test kits were performed according to manufacturers’ instructions.

Statistical analysis

Statistical significance was evaluated using a 2-sample t test. For survival curves, statistical significance was assessed using Kaplan-Meier survival analysis with differences between groups analyzed by the log-rank test (GraphPad Prism 4.0, GraphPad Software, San Diego, CA). Results were considered statistically significant with a P less than .05.

Results

Transplantation in young New Zealand black × New Zealand white mice and establishment of donor chimerism

The initial experiments addressed the following 2 questions. First, is autologous transplantation or unrelated MHC haplomismatched transplantation superior when myeloablative conditioning is used? We compared the onset and severity of lupus-like disease in female NZBW mice receiving transplants of either purified unrelated MHC haplomismatched allogeneic HSCs or syngeneic (pseudoautologous) HSCs. Second, does an autologous transplantation with a graft that includes mature lymphoid cells fail to prevent the development of the lupus-like syndrome? We compared transplantation of purified syngeneic HSCs with syngeneic WBM in delaying or preventing disease onset. Hematopoietic stem cells were isolated with the phenotype c-Kit+ Sca-1+ lineage-foxn. Three cohorts of mice were lethally irradiated and received transplants of allogeneic HSCs, syngeneic HSCs, or syngeneic WBM. A fourth group was studied as unmanipulated age-matched controls. All mice receiving haplomismatched allogeneic HSCs had high stable donor cell contribution as measured in the peripheral blood and hematopoietic organs by MHC differences in the host and donor strains (Figure 1A,B). A significant number of host T cells remained in circulation after transplantation of purified allogeneic HSCs, as previously described.39,21 These host T cells probably survived radiation, perhaps as a result of the relative resistance gained by antigen activation.45 As noted in the peripheral blood, the frequency of host T cells present in the hematopoietic tissues was higher than in the B cell and myeloid compartments. However, the highest frequency of host cells was observed in the B cell population in the peritoneal cavity, which was composed almost exclusively of CD5+ B1 cells.

Effect of transplantation on the clinical course of lupus-like disease

Mice were monitored for approximately 350 days after transplantation for the onset of lupus-like symptoms: weight loss, proteinuria (urine protein more than or equal to 1 g/L [100 mg/dL]), and death. Serum was collected before death and screened for CIC and autoantibodies to dsDNA, nuclear antigens, and histones. Transplantation of either syngeneic HSCs or WBM accelerated mortality in these mice, resulting in a rate of death exceeding age-matched controls (P ≤ .001) (Figure 2). The median survival of mice receiving transplants of syngeneic HSCs or WBM was 215 and 219 days after transplantation, respectively, with no mice living until the conclusion of the study. There was no significant difference in survival between mice receiving transplants of syngeneic HSCs or WBM (P = .637). The age-matched control
mice lived to a median age of 272 days with 2 of 15 mice alive although moribund at the conclusion of the study. The occurrence and severity of proteinuria, CIC, and autoantibodies measured in the groups receiving syngeneic transplants was comparable with the age-matched controls. Many of the mice in these 3 groups developed severe proteinuria, with urine protein 5 g/L (500 mg/dL) or higher (Figure 3). In contrast, the mice receiving transplants of allogeneic HSCs had improved survival ($P = .024$). 14 of 26 survived to the conclusion of the study with a lower incidence of proteinuria, CIC, and autoantibodies ($P \leq .001$) than the age-matched controls. Only 4 of the mice receiving transplants of allogeneic HSCs developed proteinuria; only one of these progressed to severe proteinuria (urine protein more than or equal to 5 g/L [500 mg/dL]). In addition, mice receiving transplants of allogeneic HSCs had greatly reduced occurrences of CIC or serum autoantibodies (Figure 4). Of those mice receiving allogeneic HSCs that developed positive titers of CIC or autoantibodies, the serum levels were generally lower than the levels measured in the groups receiving syngeneic transplants or age-matched controls. As a result of the earlier deaths in the groups receiving syngeneic cells, the mice receiving allogeneic HSCs were, on average, 100 days older than the mice receiving syngeneic transplants when the serum was collected for analysis. There was no correlation between frequency of host T cells and survival or disease progression in the mice receiving allotransplants.

**Effect of transplantation on the aberrant accumulation of B cells in the thymus**

B-cell abnormalities are one of the most recognized elements in the immunologic deregulation in NZBW mice. Although B cells are a normal component of the thymic microenvironment, aged NZBW have an aberrant increase of B cells in the thymus as well as other target organs. At death or at the conclusion of this study, we analyzed the thymi of transplanted and control mice for thymic B-cell accumulation. To establish a baseline, the thymi of young, female NWBW mice, 6 months of age or younger, were analyzed by flow cytometry for B cells. The frequency of B cells in the thymi of these mice ranged from 0.5% to 3.8%, with a mean of 1.6%. In comparison, the older age-matched control mice had levels of thymic B cells ranging from 4.7% to 70.4%, with a mean of 27.5% at the time of death. Of this group, 2 of 8 (25%) had a B-cell frequency of approximately 5%, near baseline levels. The mice receiving either syngeneic HSCs or WBM had a marked increase of thymic B cells, ranging from 21% to 68.8%, with a mean of 39.4%. In contrast, the mice receiving allogeneic HSCs had thymic B-cell numbers ranging from 1.3% to 36.8%, with a mean of 14.1%. Of the allotransplant group, 8 of 17 (47%) had approximately 5% or fewer thymic B cells, significantly lower frequency than the age-matched controls ($P = .042$) and mice receiving syngeneic cells ($P \leq .001$).

**Nonmyeloablative transplantation in 8-month-old New Zealand black × New Zealand white mice leads to reversal of lupus symptoms**

We also sought to determine whether nonmyeloablative transplantation of unrelated MHC haplomismatched allogeneic HSCs...
could halt progression or reverse autoimmune disease in older NZBW mice with established lupus-like disease. Because nonmyeloablative conditioning is also lymphoablative, we compared the progression of lupus-like disease in mice that received transplants with mice that received lymphoablative conditioning alone. To achieve lymphoablation and eliminate cells that posed a barrier to engraftment, the mice received a nonmyeloablative conditioning regimen with radiation, antithymocyte serum, and antiasialo GM1. The conditioned mice were randomized into 2 groups, one receiving haplomismatched allogeneic HSCs and the other receiving conditioning only. Because the conditioning regimen was truly nonmyeloablative, syngeneic stem cell rescue was unnecessary in the group receiving conditioning alone. A third group of mice was reserved as unmanipulated age-matched controls. At the time of conditioning, the mice were on average 240 days of age with established symptoms of lupus; 2 mice died from lupus-like disease before the day of treatment. The nonmyeloablative conditioning was well tolerated; no animals died in the 4 weeks after conditioning. The mice receiving the allogeneic HSCs developed mixed chimerism of 25% to 85% (Figure 5A).

**Attenuation of autoimmune disease by transplantation of allogeneic hematopoietic stem cells**

After the conditioning regimen, the mice were closely monitored for progression of lupus-like disease. The mice that received the conditioning treatment but not the stem cell transplant had a slight survival advantage more than the age-matched controls \( (P < .001) \), living to an average of 407 days of age versus an average of 350 days of age (Figure 5B). The longest surviving mouse from the group that received conditioning only lived to 518 days of age. In contrast, the mice receiving unrelated haplomismatched allogeneic HSCs had far greater overall survival compared with both groups \( (P \leq .001) \) with 60% still alive at 575 days of age, 335 days after transplantation. Proteinuria was monitored for approximately 330 days. All the age-matched controls developed proteinuria before death (Figure 6A). The frequency of mice with proteinuria increased after treatment in the group receiving conditioning only, from 47% having proteinuria \( (1 \text{ g/L} [100 \text{ mg/dL}] \) or more) before treatment to 70% with proteinuria at time of death (Figure 6B). The mice receiving allogeneic HSCs showed a reversal or stabilization of their lupus symptoms. The incidence of mice receiving transplants with proteinuria declined from 45% before transplantation to 30% after transplantation, a significant difference from both the mice receiving conditioning only \( (P = .015) \) and the age-matched controls \( (P < .001) \).

No correlation between the frequency of host B and T lymphocytes and disease progression or survival was observed.

The frequency of mice with elevated levels of CIC or autoantibodies was also lower in the mice receiving allogeneic HSCs than in the mice receiving the conditioning regimen alone (Figure 6C). One cohort of mice was analyzed for autoantibodies just before conditioning or transplantation and again before death or at 508 days of age (Figure 7A). Of the mice receiving allogeneic HSCs, 13 of 18 mice (72%) had a positive titer of antihistone autoantibodies before transplantation. After transplantation, 7 of 18 (39%) mice had positive titers, a decrease in frequency of 33%. In contrast, in the mice given the conditioning regimen only, 10 of 12 mice (83%) had positive antihistone titers before treatment; after conditioning no change was detected with 10 of 12 mice (83%) having positive titers. The difference between the groups receiving transplants and receiving conditioning after treatment was significant \( (P = .008) \). Differences in CIC or dsDNA titers were not significantly different before and after treatment in either group.

Four of the animals that received transplants and one that received conditioning were killed during the study because they developed abscesses. These mice are included as dead on the survival graph. Most of the abscesses occurred late in the study, more than 427 days of age, after most of the conditioned animals and age-matched controls had died from lupus-related indications. At necropsy, we noted that 5 of the 11 moribund or dead mice from the group receiving only the conditioning regimen had developed thymic lymphomas.\(^{45,49}\) The mice analyzed had lymphomas of T-cell origin. We did not observe any thymic lymphomas in the mice that received allotransplants.

**Progression of lupus as related to disease course**

Stabilization of disease symptoms and long-term survival was associated with the severity of disease at the time of transplantation. Eight-month-old NZBW mice with severe proteinuria at the time of allogeneic HSC transplantation, 5 g/L (500 mg/dL) or more urine protein, had poor overall survival of 20%. In comparison, mice from the same cohort with mild to moderate lupus symptoms at the time of stem cell transplantation, 1 g/L (100 mg/dL) or less urine protein, had superior overall survival with 64% alive and healthy at 575 days of age. Eight-month-old NZBW mice with mild to moderate lupus symptoms at the time of transplantation had a stabilization or reversal of their disease and 79% did not have measurable proteinuria after transplantation (Figure 7B). In this group, the frequency of mice with proteinuria decreased from 37% before transplantation to 21% after transplantation. In contrast, the frequency of mice with proteinuria in the conditioning-only group increased from 32% with proteinuria before treatment to 61% with proteinuria before death. The difference between the mice that received transplants and those that received conditioning after

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**Figure 4. Serology of New Zealand black × New Zealand white mice after transplantation.** Serum was screened by enzyme-linked immunosorbent assay for titers of circulating immune complexes (CIC), and autoantibodies to dsDNA, nuclear antigen, and histone. Serum was collected before death or at the conclusion of the study. The levels of CIC and all autoantibodies analyzed was significantly lower in the mice that received allogeneic hematopoietic stem cells (red) than in mice that received syngeneic hematopoietic stem cells (blue) or whole bone marrow (green) and the age-matched control mice (gray) \( (P < .001) \). Horizontal lines represent mean of each group. Serum from donor strain mice, DBF, was used to establish baseline levels of autoantibodies. Data were combined from 3 experiments.

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**Figure 5. Comparison of healthy and lupus-like disease mice.** The nonmyeloablative conditioning was well tolerated; no animals died in the 4 weeks after conditioning. The mice receiving the conditioning regimen only lived to 518 days of age. In comparison, the mice receiving unrelated haplomismatched allogeneic HSCs had far greater overall survival compared with both groups \( (P \leq .001) \) with 60% still alive at 575 days of age, 335 days after transplantation. Proteinuria was monitored for approximately 330 days. All the age-matched controls developed proteinuria before death (Figure 6A). The frequency of mice with proteinuria increased after treatment in the group receiving conditioning only, from 47% having proteinuria \( (1 \text{ g/L} [100 \text{ mg/dL}] \) or more) before treatment to 70% with proteinuria at time of death (Figure 6B). The mice receiving allogeneic HSCs showed a reversal or stabilization of their lupus symptoms. The incidence of mice receiving transplants with proteinuria declined from 45% before transplantation to 30% after transplantation, a significant difference from both the mice receiving conditioning only \( (P = .015) \) and the age-matched controls \( (P < .001) \).

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The frequency of host T cells after transplantation did not influence survival or disease progression, suggesting down-regulation of the host T-cell response occurs. The autoantibodies detected in some of the mice that received allotransplants may have been associated with the surviving host B1 cells. The large populations of host-derived peritoneal B1 cells observed are probably the result of radioreistance, poor de novo generation of B1 cells from adult stem cells, and the ability of B1 cells to maintain their numbers by self-replenishment.51-55 Peritoneal B1 cells have been linked to the production of autoantibodies in nonautoimmune and NZBW mice.46,54-57 Aberrant B-cell infiltration of target organs, including the thymus, kidneys, and lungs, is a hallmark of aged NZBW mice. Defective homing of B1 cells resulting from the overexpression of chemokines by dendritic cells in these tissues is one factor that has been linked to this event in treatment was significant (P < .001). All age-matched control mice progressed to severe proteinuria.

Discussion

NZBW mice develop a complex, spontaneous autoimmune disease involving the misregulation of many aspects of the immune system that is very similar to SLE.50 The lupus-like disease in these mice is 100% lethal with few female mice surviving more than 390 days.33,34 Our studies show that transplantation of purified, unrelated, haploidentical but minor histocompatibility locus mismatched allogeneic HSC can prevent the occurrence of the symptoms of autoimmune disease, successfully blocking the strong genetic predisposition for development of the lupus-like disorder in this strain of mice and improving overall survival. Of the young myeloablated mice receiving allogeneic HSC transplants, only one developed severe proteinuria. The mice receiving allogeneic HSC also had a significantly reduced tendency toward the generation of autoantibodies. T cells surviving lethal doses of irradiation have been shown to be able to mediate disease pathogenesis in autoimmune mice.79 The frequency of host T cells after transplantation did not influence survival or disease progression, suggesting down-regulation of the host T-cell response occurs. The autoantibodies detected in some of the mice that received allotransplants may have been associated with the surviving host B1 cells. The large populations of host-derived peritoneal B1 cells observed are probably the result of radioreistance, poor de novo generation of B1 cells from adult stem cells, and the ability of B1 cells to maintain their numbers by self-replenishment.51-55 Peritoneal B1 cells have been linked to the production of autoantibodies in nonautoimmune and NZBW mice.46,54-57 Aberrant B-cell infiltration of target organs, including the thymus, kidneys, and lungs, is a hallmark of aged NZBW mice. Defective homing of B1 cells resulting from the overexpression of chemokines by dendritic cells in these tissues is one factor that has been linked to this event in...
NZBW mice. We observed that NZBW mice that received allogeneic HSCs had a decreased occurrence of B-cell infiltration of the thymus.

These studies demonstrate that young NZBW mice that received transplants of unrelated haplomismatched allogeneic HSCs had a significant decrease in autoantibody production and proteinuria and reduced accumulation of B cells in the thymus, indicating that complete or partial replacement of the immune system and down-regulation of the host T-cell response is effective in controlling several aspects of this complex disease. In contrast, NZBW mice treated by transplantation with purified syngeneic HSCs or WBM experienced acceleration in the rate of death. No difference was observed in the disease course whether the T and B cells were eliminated from the syngeneic graft before transplantation. Several published models of autoimmune disease (AID) have shown comparable or a slightly improved survival over control animals after receiving syngeneic HSCs or bone marrow. The difference in our model may be attributed to treatment-related factors complicated by the exceptionally high dose of irradiation required to reach the minimum lethal dose in the NZBW. Allogeneic HSC transplantation may well offer a curative treatment for AID by regulation of the autoimmune response by immune modulation or induction and maintenance of tolerance.

However, patients being treated for AID by hematopoietic cell transplantation have already developed often severe symptoms of disease. To address this scenario, we treated NZBW mice having established symptoms of lupus-like disease with nonmyeloablative allogeneic HSC transplantation. Eight-month-old NZBW mice receiving unrelated, allogeneic haplotype-mismatched HSC transplants, with the establishment of mixed chimerism, successfully showed a halt or reversal of the progression of lupus-like disease in approximately 80% with mild to moderate disease and showed a significant increase in overall survival. We found the benefits of lymphoablative conditioning alone was transient, with 70% of the mice that received conditioning but not a HSC transplant demonstrating a continuance, worsening, or development of proteinuria. Mice with severe proteinuria at the time of treatment did not respond well to either therapy, indicating that transplantation would be more effective when attempted before major organ damage occurs. Although the number of mice with positive antihistone titers decreased after transplantation, a higher percentage of aged mice that received allogeneic HSCs had positive titers than those mice that received the transplant at a younger age. Similarly, aged BXSB mice transplanted with allogeneic WBM also maintained positive levels of autoantibodies after transplantation. These titers may be the result of surviving host autoreactive B, plasma, or B1 cells. Our data in this nonmyeloablative model demonstrate that the induction of mixed chimerism is sufficient to control the autoreactive mechanisms in these mice.

Currently, several trials in the United States and Europe are in progress for treatment of severe AID by autologous HCT. More than 1000 patients worldwide have been treated, with more than 30% maintaining sustained disease-free durable remission without the need of immunosuppressive drugs. The most current results for treatment of SLE are encouraging, with approximately 50% disease-free survival at 5 years. Additional trials investigating the use of high-dose cyclophosphamide without stem cell rescue to treat severe SLE have reported 40% of patients achieving long-lasting remission. Nonmyeloablative conditioning regimens are being investigated to reduce the risk of the transplantation procedure, to reduce treatment-related mortality. However, it is clear that not all patients respond to intense lymphoablation or autologous transplantation. Depending on their genetic predisposition and the nature of the environmental trigger initiating the disease, some patients may benefit from allogeneic HSC transplantation. Often transplantation is used clinically as a salvage regimen when patients have failed or become refractory to other therapies. Our results demonstrate the value of transplantation before the disease progresses significantly.

In addition, the complications and risks of stem cell mobilization for autologous transplantation are higher in patients with autoimmune disease. Mobilization is associated with flare of AID and bacteremia caused by severe cytopenias, leading to increased morbidity and mortality. In addition, with autologous HCT, there is a risk of relapse from reinfection of autoreactive lymphocytes. Although many patients with malignancies have been treated by allogeneic HCT, autologous HCT has been favored over allogeneic HCT for treatment of AID because of the increased risks of morbidity and mortality from GVHD. In the treatment of malignancy, GVHD is tolerated as a result of the related graft-versus-leukemia effect that has been shown to be important in maintaining remission. A graft-versus-autoimmunity effect has been postulated; however, no conclusive data have yet demonstrated this effect. Allogeneic transplantation is associated with acute organ toxicity, increase in treatment-related mortality, delayed reconstitution of the immune system, persistence of autoreactive lymphocytes, and an increase in the rate of opportunistic infection. However, these effects are mostly attributable to acute and chronic GVHD from the T cells in the graft. Our data in the NZBW mouse model indicate that nonmyeloablative transplantation of purified unrelated haplomismatched allogeneic HSC with the establishment of mixed chimerism can alleviate immune symptoms.

These data with the NZBW mouse indicate that active autoimmunity can be halted and controlled by the transplantation of purified allogeneic HSCs with nonmyeloablative conditioning. The purification of HSCs eliminates T-cell contamination, avoiding...
GVHD, and immunologically naive donor immune cells develop within the host environment. The use of haplo-type-matched family members without AID or unrelated donors should place these therapies in the reach of most patients if a conditioning regimen that eliminates all or most host T cells and natural killer (NK) cells is available. The ability of purified allogeneic HSC transplantation with the establishment of mixed chimerism to reverse established symptoms of lupus makes this approach a reasonable strategy to test in humans not only in SLE, but in other autoimmune diseases as well.

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Authorship

Contribution: S.S.B. designed and performed research, and collected and analyzed data; D.G. collected data; I.L.W. reviewed data and edited the manuscript; J.L.C. designed research, analyzed and interpreted data, and drafted the manuscript; and all authors checked the final version of the manuscript.

Conflict-of-interest disclosure: S.S.B., D.G., and J.L.C. are employed by and have stock options in Celleneral Therapeutics; I.L.W. owns significant Amgen stock and is a cofounder and director of Cellerant Therapeutics, and StemCells.

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