

Brief report

Control of systemic B cell–mediated autoimmune disease by nonmyeloablative conditioning and major histocompatibility complex–mismatched allogeneic bone marrow transplantation

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Systemic autoimmune disease (AID) can be controlled with conventional therapies in most patients. However, relapses are common, leading to progressive disability and premature death. Nonmyeloablative conditioning and allogeneic bone marrow transplantation (BMT) could be an effective treatment for severe AID, because of mild toxicity of the conditioning and the potential benefits of donor chimerism. We examined the effects of

this treatment in experimental autoimmune arthritis. Our results demonstrate the induction of complete donor chimerism and significant suppression of disease activity. No clinical graft-versus-host disease (GVHD) was observed. The beneficial effects were most likely caused by the elimination of plasma cells producing pathogenic autoantibodies, because these antibodies disappeared rapidly after BMT. Although this type of treatment

was effective in organ-specific T-cell-mediated AID, the present study provides convincing evidence that nonmyeloablative conditioning and allogeneic BMT can effectively treat severe B-cell–mediated AID with a systemic inflammatory component. (Blood. 2005;105:2991-2994)

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Introduction

Systemic autoimmune disease (AID) is characterized by immune dysregulation in which T or B cells play a pivotal role.¹ Preclinical studies and case reports indicate that *myeloablative* chemoradiotherapy plus allogeneic bone marrow transplantation (BMT) may be an effective treatment for systemic AID in humans.²⁻⁶ The application of this treatment, however, is limited because of the risk for graft-versus-host disease (GVHD) and the toxicity of myeloablative therapy. Recently, preclinical protocols have been developed based on *nonmyeloablative* conditioning to achieve allogeneic donor chimerism without clinical GVHD.⁷⁻⁹ Preliminary results indicate that this treatment modality may be effective in treating human AID.^{1,10} The present study aimed to investigate the clinical and immunologic effects of allogeneic BMT after nonmyeloablation on experimental arthritis in mice, a systemic inflammatory B-cell–mediated autoimmune disease. More specifically, we wanted to study whether stable, long-term, and multilineage donor chimerism could be induced safely in mice with established polyarthritis and whether this treatment would result in the reduction of disease activity and serum levels of pathogenic autoantibodies produced by host plasma cells.

Study design

Animals

Male DBA/1 and BALB/c mice (8-12 weeks of age) were obtained, housed, and fed as described earlier.^{11,12}

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Induction and clinical assessment of arthritis

Collagen-induced arthritis (CIA) was induced and evaluated as described by Morgan et al.¹² Mice with maximum scores of 12 were humanely killed.

Bone marrow transplantation

BMT was performed when more than 50% of the mice developed CIA. Mice were subjected to sublethal total body irradiation (TBI) of 6.0 Gy, and each received a single injection of anti-CD40 ligand (CD40L) monoclonal antibody (mAb) (MR1, 0.5 mg intraperitoneally) before BMT with 1.0×10^7 total bone marrow (BM) cells intravenously collected from femurs and tibiae of donor mice.

Flow cytometric analysis for donor chimerism

The level of allogeneic donor chimerism was evaluated by flow cytometric analysis after staining of mononuclear cells with the following mAbs: biotinylated anti-H-2D^d (donor), biotinylated anti-H-2D^q (host), anti-CD3-fluorescein isothiocyanate (FITC), anti-CD4-allophycocyanin (APC), anti-CD8 α -APC, anti-Gr-1-FITC, anti-B220-FITC, and phycoerythrin (PE)-conjugated streptavidin (PharMingen, Erembodegem, Belgium).

Measurement of IgG2a and Igh-1a antibodies in serum

Anti-type II collagen (CII) antibodies were measured by enzyme-linked immunosorbent assay (ELISA).¹² In the case of Igh-1a, the plates were incubated with biotinylated anti-Igh-1a (PharMingen) and subsequently with horseradish peroxidase (HRP)-conjugated streptavidin. Total IgG2a

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antibodies in serum were measured by sandwich ELISA using plates coated with polyclonal anti-mouse total immunoglobulin G (IgG) (DAKO, Heverlee, The Netherlands).

Measurement of serum amyloid P

Levels of serum amyloid P (SAP) were measured by sandwich ELISA using plates coated with polyclonal sheep anti-mouse SAP (Calbiochem, San Diego, CA). After incubation with serum, the plates were treated with polyclonal rabbit anti-mouse SAP (Calbiochem). Finally, the plates were incubated with HRP-conjugated polyclonal swine anti-rabbit immunoglobulin (DAKO).

Statistical analysis

Differences in disease severity, antibody levels, and SAP levels were analyzed using the nonparametric Mann-Whitney *U* test. *P* values than .05 was considered statistically significant.

Results and discussion

The aim of this study was to investigate the efficacy of allogeneic BMT in treating systemic AID after nonmyeloablative conditioning. First, we studied whether allogeneic BMT after myeloablation using lethal TBI is effective in treating arthritis. These experiments indicated that allogeneic BMT was more effective than syngeneic BMT (data not shown). However, given that both GVHD and the toxicity associated with myeloablation preclude application in a clinical setting, these results prompted us to investigate the effects of allogeneic BMT after nonmyeloablation. To establish whether nonmyeloablative conditioning allows the development of complete donor chimerism without GVHD, we treated healthy DBA/1 mice with sublethal TBI (6.0 Gy) and a single injection of an anti-CD40L mAb (MR1), which is thought to result in tolerance or deletion of CD4⁺ and CD8⁺ host/donor-reactive T cells within a few days after BMT.^{9,13,14} The mice subsequently received allogeneic bone marrow transplants from BALB/c donor mice. The induction of stable, long-term, and multilineage donor chimerism (greater than 95%) until day 300 after BMT was observed in mice treated with TBI and anti-MR1, but not in mice treated with TBI alone (*P* < .001; Table 1), pointing to the importance of anti-MR1. No clinically overt GVHD (defined by skin

Table 1. Percentage of donor cells after nonmyeloablative conditioning using anti-CD40L mAb (MR1) before allogeneic BMT in healthy DBA/1 mice

Time after allogeneic BMT, d	No. donor cells, mean ± SEM %	
	TBI alone	TBI/anti-CD40L
42	27.8 ± 10.6	83.0 ± 1.3
50	29.2 ± 13.4	84.8 ± 6.6
57	29.2 ± 8.0	90.0 ± 3.9
64	20.1 ± 19.8	83.7 ± 4.9
77	3.4 ± 1.5	78.3 ± 11.0
106	0.4 ± 0.1	93.9 ± 1.7
169*	NA	97.0
279*	NA	93.3
295*	NA	97.0

Percentages of total donor cells were analyzed by fluorescence-activated cell sorting (FACS) in peripheral blood (*n* = 2 mice per group; **n* = 1). Level of allogeneic donor chimerism was significantly higher in mice treated with TBI plus anti-CD40L compared with mice treated with TBI alone (*P* < .0001). NA indicates not available.

Table 2. Elevated SAP levels after allogeneic BMT, but not after syngeneic BMT, and conditioning alone at day 10 after treatment

Groups of mice	Mean SAP levels ± SEM	<i>P</i>
Positive control	88.9 ± 2.5	ND
Negative control	24.7 ± 9.8	ND
Conditioning alone	39.4 ± 5.6	NS
Syngeneic BMT	23.6 ± 1.6	NS
Allogeneic BMT	76.3 ± 9.7	.006

Arbitrary levels of SAP are expressed as mean ± SEM (*n* = 6 mice per group). NS indicates not significant. SAP levels were determined using 2 reference sera created from naive mice (negative control) or mice injected with CFA (positive control). *P* values of treatment groups are calculated compared with negative control. ND indicates not determined.

abnormalities or weight loss) was observed, nor were significant histologic abnormalities of liver, gut, and skin detected (data not shown). However, we could not exclude the presence of a subclinical host-versus-graft (HVG)/graft-versus-host (GVH) response. To address this point, we determined the levels of SAP, an acute-phase protein, after fully major histocompatibility complex (MHC)-mismatched allogeneic BMT. Mice treated with allogeneic BMT, but not those treated with syngeneic BMT, showed elevated SAP levels after treatment (*P* = .006; Table 2), indicative of an alloresponse. These data show that complete donor chimerism can be induced using nonmyeloablation before allogeneic BMT, but they also point to the presence of an HVG/GVH response, though without clinical GVHD.

We then sought to assess the therapeutic effects of nonmyeloablative conditioning and allogeneic BMT in mice with established polyarthritis. Allogeneic and syngeneic BMT, but not conditioning alone, were able to arrest disease progression (*P* = .007 and *P* = .001, respectively, compared with untreated mice; Figure 1A). Of note, a temporary exacerbation of arthritis was consistently observed within 2 weeks after allogeneic BMT, followed by a steady and prolonged suppression of disease activity similar to that of syngeneic BMT (Figure 1A). In accordance with the results shown in Table 2, only allogeneic BMT recipients had elevated SAP levels during the exacerbation (data not shown), again pointing to the presence of a subclinical HVG/GVH response after allogeneic BMT.

Complete and stable donor chimerism (greater than 95%) could be induced in healthy and arthritic mice in all cell lineages analyzed (Table 3), indicating that the presence of a systemic inflammatory autoimmune disease does not hamper the induction of allogeneic donor chimerism.

Given that CII-specific serum antibodies which are produced by plasma cells are crucial and sufficient for the induction of CIA, we studied whether their presence or origin could explain the efficacy of allogeneic BMT. Therefore, sera were taken at 2 and 6 weeks after BMT and were tested for the presence of CII-specific IgG2a autoantibodies. Although differences between groups were already detectable 2 weeks after BMT, only allogeneic BMT was able to significantly suppress the production of CII-specific IgG2a antibodies, which was most evident at 6 weeks after BMT (*P* < .002; Figure 1B). This difference could not be explained by lower levels of total IgG2a in the sera of allogeneic chimeras, because no differences could be detected after syngeneic and allogeneic BMT (data not shown).

To exclude the possibility that the residual CII-specific antibodies resulted from a de novo autoimmune response by allogeneic donor cells against CII, we analyzed the presence of CII-specific Igh-1a, an IgG2a allotype found in BALB/c mice but not in DBA/1

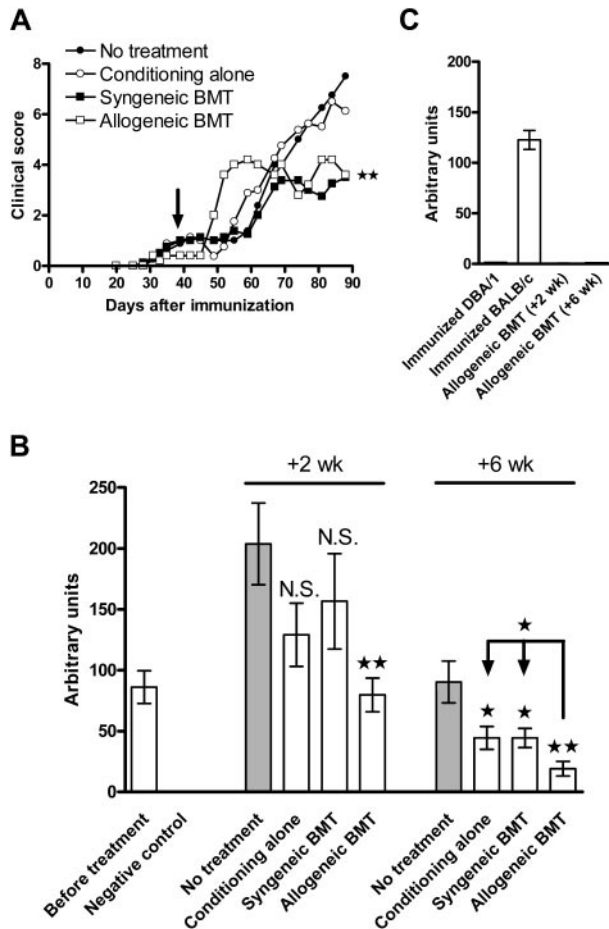


Figure 1. Allogeneic BMT can effectively treat CIA, a severe B cell-mediated autoimmune disease. (A) Allogeneic and syngeneic BMT have a suppressive effect on arthritis after nonmyeloablative conditioning (***P* = .007 and ***P* = .001, respectively). Clinical data of arthritic DBA/1 mice (n = 8 mice per group) treated with sublethal TBI of 6.0 Gy (day 37) plus a single injection of anti-CD40L mAb (0.5 mg intraperitoneally, day 38) and subsequently injected with 1.0×10^7 total BM cells intravenously from syngeneic DBA/1 mice or fully major histocompatibility complex (MHC)-mismatched allogeneic BALB/c mice are shown (day 38; arrow indicates start of treatment). No statistical differences were observed at the time of treatment. Results from 1 of 2 experiments are shown. (B) Allogeneic BMT results in a marked decrease of pathogenic anti-type II collagen autoantibodies. Sera were taken at 2 and 6 weeks after BMT (days 52 and 81, respectively) and were tested by ELISA for the presence of anti-type II collagen antibodies (data are shown for the IgG2a isotype). Allogeneic BMT was more effective in suppressing the production of anti-type II collagen antibodies (***P* < .002) than syngeneic BMT or conditioning alone (**P* < .05) compared with untreated animals. Allogeneic BMT compared with syngeneic BMT and conditioning alone (**P* = .02) at 6 weeks after BMT. (C) Anti-type II collagen antibodies after allogeneic BMT are of recipient origin. None of the allogeneic BMT recipients developed anti-type II collagen antibodies of the Igh-1a allotype, demonstrating that the antibodies present after allogeneic BMT are of recipient origin. As positive and negative controls for the presence of Igh-1a, we immunized normal BALB/c and DBA/1 mice, respectively, with CII. Immunized BALB/c mice were able to produce CII-specific antibodies to the same extent as immunized DBA/1 mice without the clinical symptoms of arthritis (data not shown).

Table 3. Induction of complete donor chimerism is similar in arthritic and healthy mice

	Mean donor cells \pm SEM, %			
	PB	SP	LN	BM
CD4⁺ T cells				
Healthy	99.6 \pm 0.1	99.1 \pm 0.2	98.9 \pm 0.5	99.7 \pm 0.1
Arthritic	99.3 \pm 0.3	99.2 \pm 0.2	99.1 \pm 0.2	99.2 \pm 0.4
CD8⁺ T cells				
Healthy	98.3 \pm 1.2	99.0 \pm 0.4	98.1 \pm 0.7	99.4 \pm 0.3
Arthritic	98.8 \pm 0.6	98.8 \pm 0.3	97.7 \pm 0.7	99.3 \pm 0.3
B cells				
Healthy	99.6 \pm 0.1	99.7 \pm 0.1	99.2 \pm 0.2	99.4 \pm 0.2
Arthritic	99.7 \pm 0.1	99.7 \pm 0.1	99.3 \pm 0.2	99.8 \pm 0.1
Granulocytes				
Healthy	97.4 \pm 1.3	98.2 \pm 0.4	NA	92.8 \pm 1.4
Arthritic	98.4 \pm 0.3	98.4 \pm 0.4	NA	93.7 \pm 1.2

Percentages of donor cells were analyzed using FACS in different cell lineages (ie, CD3⁺ T cells, B220⁺ B cells, and Gr1⁺ granulocytes) in the following lymphoid compartments: peripheral blood (PB), spleen (SP), lymph node (LN), and bone marrow (BM). Values are expressed as mean \pm SEM (n = 8 mice per group). NA indicates not available because of low numbers of Gr-1⁺ cells in lymph nodes.

mice. None of the allogeneic BMT recipients had developed CII-specific Igh-1a antibodies (Figure 1C), demonstrating that the antibodies present after allogeneic BMT are of recipient DBA/1 origin. Because serum antibodies in mice have a short half-life¹⁵ and are produced predominantly by plasma cells, our data can be best explained by the disappearance of plasma cells producing CII-specific antibodies after allogeneic BMT. Allogeneic BMT is clearly correlated with a superior reduction of pathogenic antibody responses, so it is tempting to speculate that a graft-versus-plasma cell or a cytokine “storm” related to allografting is responsible for the disappearance of anti-CII-producing plasma cells.^{16,17} Together, our data indicate that allogeneic BMT is highly effective in suppressing clinical disease and autoantibody production by host plasma cells without eliciting a de novo autoimmune response after BMT.

Although nonmyeloablative conditioning and allogeneic BMT has been successfully used in several animal models to prevent or treat (spontaneous) T-cell-mediated autoimmune disease,^{1,18,19} our study provides convincing evidence that nonmyeloablative conditioning followed by allogeneic BMT can effectively treat severe B-cell-mediated autoimmune disease with a systemic inflammatory component.

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