Control of systemic B cell-mediated autoimmune disease by nonmyeloablative conditioning and MHC-mismatched allogeneic bone marrow transplantation

Brief Report

Running title: Control of autoimmune disease by allogeneic BMT

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ABSTRACT

Systemic autoimmune diseases (AID) can be controlled with conventional therapies in the majority of patients. However, relapses are common, leading to progressive disability and premature mortality. Nonmyeloablative conditioning and allogeneic bone marrow transplantation (BMT) could be an effective treatment for severe AID, because of mild toxicity of the conditioning and 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 as these antibodies disappeared rapidly after BMT. Although this type of treatment was able to treat 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.
INTRODUCTION

Systemic autoimmune diseases (AID) are characterized by immune dysregulation in which T and/or B cells play a pivotal role\(^1\). Preclinical studies and case reports indicate that *myeloablative* chemo(radio)therapy plus allogeneic bone marrow transplantation (BMT) may be an effective treatment for systemic AID in humans\(^2-6\). Its application, however, is limited because of the risk of graft-versus-host disease (GVHD) and 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 immunological effects of allogeneic BMT after nonmyeloablation on experimental arthritis in mice, a systemic inflammatory B cell-mediated autoimmune disease. More specifically, we wished to study whether stable, long-term and multi-lineage donor chimerism could be induced safely in mice with established polyarthritis, and whether this treatment would result in the reduction of both disease activity and serum levels of pathogenic autoantibodies produced by host plasma cells.
MATERIAL AND METHODS

Animals

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

Induction and clinical assessment of arthritis

CIA was induced and evaluated as described\textsuperscript{12}. Mice with a maximal score of 12 were euthanized.

Bone marrow transplantation

BMT was performed when >50% of the mice had developed CIA. Mice were subjected to sublethal total body irradiation (TBI) of 6.0 Gy, and received a single injection of anti-CD40 ligand (CD40L) mAb (MR1, 0.5 mg i.p.) before BMT with $10^7$ total BM cells i.v. 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\textsuperscript{d} (donor), biotinylated anti-H-2D\textsuperscript{d} (host), anti-CD3-FITC, anti-CD4-APC, anti-CD8\textalpha-APC, anti-Gr-1-FITC, anti-B220-FITC, and PE-conjugated streptavidin (Pharmingen, Erembodegem, Belgium).
**Measurement of IgG2 and Igh-1a antibodies in serum**

Anti-type II collagen (CII) antibodies were measured by ELISA\(^\text{12}\). In the case of Igh-1a, the plates were incubated with biotinylated anti-Igh-1a (Pharmingen) and subsequently with HRP-conjugated streptavidin. Total IgG2a antibodies in serum were measured by sandwich ELISA using plates coated with polyclonal anti-mouse total IgG (Dako, Heverlee, The Netherlands).

**Measurement of serum amyloid P (SAP)**

Levels of 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 Ig (Dako).

**Statistical analysis**

Differences in disease severity, antibody levels and SAP levels were analyzed with a nonparametric Mann-Whitney test. P<0.05 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, since 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 Gy) and a single injection of an anti-CD40L mAb (MR1), which is thought to result in tolerance and/or deletion of CD4+ and CD8+ host/donor-reactive T cells within a few days post-BMT. The mice subsequently received allogeneic BMT from BALB/c donor mice. The induction of stable, long-term and multi-lineage donor chimerism (>95%) up to day 300 post-BMT was observed in mice treated with TBI and anti-CD40L, but not in mice treated with TBI alone (P<0.0001, Table 1A), pointing to the importance of anti-CD40L. No clinically overt GVHD (defined by skin abnormalities or weight loss) was observed, nor were significant histological 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 MHC-mismatched allogeneic BMT. Mice treated with allogeneic BMT, but not syngeneic BMT, showed elevated SAP levels after treatment (P=0.006, Table 1B), indicative of an alloresponse. These data show that complete donor chimerism can be
induced using nonmyeloablation prior to allogeneic BMT, but also point to the presence of a HVG/GVH response, although without clinical GVHD.

We then sought to assess the therapeutic effects of nonmyeloablative conditioning and allogeneic BMT in mice with established polyarthritis. Both allogeneic and syngeneic BMT, but not conditioning alone, were able to arrest disease progression (P=0.007 and P=0.0002 respectively, compared to untreated mice, Figure 1A). Of note, a temporary exacerbation of arthritis was consistently observed within two weeks after allogeneic BMT, followed by a steady and prolonged suppression of disease activity similar to syngeneic BMT (Figure 1A). In accordance with the results shown in Table 1B, 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 (>95%) could be induced in both healthy and arthritic mice in all cell lineages analyzed (Table 1C), indicating that the presence of a systemic inflammatory autoimmune disease does not hamper the induction of allogeneic donor chimerism.

Since CII-specific serum antibodies (produced by plasma cells) are crucial and sufficient for the induction of CIA, we studied whether their presence and/or origin could explain the efficacy of allogeneic BMT. Therefore, sera were taken at 2 and 6 weeks post-BMT and tested for the presence of CII-specific IgG2a autoantibodies. Although differences between groups were already detectable at 2 weeks post-BMT, only allogeneic BMT was able to significantly suppress the production of CII-specific IgG2a antibodies, most evident at 6 weeks post-BMT (P<0.002, Figure 1B). This could not be explained by lower levels of total IgG2a in serum of allogeneic chimeras,
since no differences could be detected after syngeneic and allogeneic BMT (data not shown).

To exclude the possibility that the residual CII-specific antibodies result 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 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-life\textsuperscript{15}, and are predominantly produced by plasma cells, our data can be best explained by the disappearance of plasma cells producing CII-specific antibodies after allogeneic BMT. As allogeneic BMT is clearly correlated with a superior reduction of pathogenic antibody responses, it is tempting to speculate that a graft-versus-plasma cell and/or a cytokine 'storm' related to allografting is responsible for the disappearance of anti-CII producing plasma cells\textsuperscript{16,17}.

Altogether, our data indicate that allogeneic BMT is highly effective in suppressing both 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 and/or treat (spontaneous) T-cell mediated autoimmune disease\textsuperscript{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.
REFERENCES


FIGURES AND TABLES

**Table 1A.** The percentage of donor cells after nonmyeloablative conditioning employing anti-CD40L mAb (MR1) prior to allogeneic BMT in healthy DBA/1 mice.

<table>
<thead>
<tr>
<th>Days after allogeneic BMT</th>
<th>TBI alone</th>
<th>TBI / anti-CD40L</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>27.8 ± 10.6</td>
<td>83.0 ± 1.3</td>
</tr>
<tr>
<td>50</td>
<td>29.2 ± 13.4</td>
<td>84.8 ± 6.6</td>
</tr>
<tr>
<td>57</td>
<td>29.2 ± 8.0</td>
<td>90.0 ± 3.9</td>
</tr>
<tr>
<td>64</td>
<td>20.1 ± 19.8</td>
<td>83.7 ± 4.9</td>
</tr>
<tr>
<td>77</td>
<td>3.4 ± 1.5</td>
<td>78.3 ± 11.0</td>
</tr>
<tr>
<td>106</td>
<td>0.4 ± 0.1</td>
<td>93.9 ± 1.7</td>
</tr>
<tr>
<td>169*</td>
<td>N.A.</td>
<td>97.0</td>
</tr>
<tr>
<td>279*</td>
<td>N.A.</td>
<td>93.3</td>
</tr>
<tr>
<td>295*</td>
<td>N.A.</td>
<td>97.0</td>
</tr>
</tbody>
</table>

The percentages of total donor cells were analyzed by FACS in peripheral blood (n=2 mice per group, *n=1). Level of allogeneic donor chimerism was significantly higher in TBI plus anti-CD40L versus TBI alone (P<0.0001). N.A. = not available. Values are expressed as mean ± SEM.
Table 1B. Elevated SAP levels after allogeneic BMT, but not after syngeneic BMT and conditioning alone at day 10 after treatment.

<table>
<thead>
<tr>
<th>Groups of mice</th>
<th>SAP levels</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>88.9±2.5</td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td>24.7±9.8</td>
<td></td>
</tr>
<tr>
<td>Conditioning alone</td>
<td>39.4±5.6</td>
<td>N.S.</td>
</tr>
<tr>
<td>Syngeneic BMT</td>
<td>23.6±1.6</td>
<td>N.S.</td>
</tr>
<tr>
<td>Allogeneic BMT</td>
<td>76.3±9.7</td>
<td>0.006</td>
</tr>
</tbody>
</table>

The arbitrary levels of SAP are expressed as mean ± SEM (n=6 mice per group). N.S. = not significant. SAP levels were determined using two reference sera created from naïve mice (negative control) or mice injected with CFA (positive control). P-values of treatment groups are calculated compared to negative control.
Table 1C. The induction of complete donor chimerism is similar in arthritic versus healthy mice.

<table>
<thead>
<tr>
<th>% of donor cells</th>
<th>Mice</th>
<th>PB</th>
<th>SP</th>
<th>LN</th>
<th>BM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD4+ T cells</strong></td>
<td>Healthy</td>
<td>99.6±0.1</td>
<td>99.1±0.2</td>
<td>98.9±0.5</td>
<td>99.7±0.1</td>
</tr>
<tr>
<td></td>
<td>Arthritic</td>
<td>99.3±0.3</td>
<td>99.2±0.2</td>
<td>99.1±0.2</td>
<td>99.2±0.4</td>
</tr>
<tr>
<td><strong>CD8+ T cells</strong></td>
<td>Healthy</td>
<td>98.3±1.2</td>
<td>99.0±0.4</td>
<td>98.1±0.7</td>
<td>99.4±0.3</td>
</tr>
<tr>
<td></td>
<td>Arthritic</td>
<td>98.8±0.6</td>
<td>98.8±0.3</td>
<td>97.7±0.7</td>
<td>99.3±0.3</td>
</tr>
<tr>
<td><strong>B cells</strong></td>
<td>Healthy</td>
<td>99.6±0.1</td>
<td>99.7±0.1</td>
<td>99.2±0.2</td>
<td>99.4±0.2</td>
</tr>
<tr>
<td></td>
<td>Arthritic</td>
<td>99.7±0.1</td>
<td>99.7±0.1</td>
<td>99.3±0.2</td>
<td>99.8±0.1</td>
</tr>
<tr>
<td><strong>Granulocytes</strong></td>
<td>Healthy</td>
<td>97.4±1.3</td>
<td>98.2±0.4</td>
<td>N.A.</td>
<td>92.8±1.4</td>
</tr>
<tr>
<td></td>
<td>Arthritic</td>
<td>98.4±0.3</td>
<td>98.4±0.4</td>
<td>N.A.</td>
<td>93.7±1.2</td>
</tr>
</tbody>
</table>

The percentages of donor cells were analyzed by FACS in different cell lineages (i.e. CD3+ T cells, B220+ B cells and Gr1+ granulocytes) in the following lymphoid compartments: peripheral blood (PB), spleen (SP), lymph nodes (LN) and bone marrow (BM). Values are expressed as mean ± SEM (n=8 mice per group). N.A. = not available, because of low numbers of Gr-1+ cells in lymph nodes.
**Figure 1.** Allogeneic BMT can effectively treat CIA, a severe B cell-mediated autoimmune disease. **A,** Both allogeneic and syngeneic BMT have a suppressive effect on arthritis after nonmyeloablative conditioning (**P=0.007 and **P=0.0002, respectively). Clinical data of arthritic DBA/1 mice (n=8 mice per group) which were treated with sublethal TBI of 6.0 Gy (day 37) plus a single injection of anti-CD40L mAb (0.5 mg i.p., day 38) and subsequently injected with 1.0 x 10^7 total BM cells i.v. from either syngeneic DBA/1 mice or fully MHC-mismatched allogeneic BALB/c mice are shown (day 38, start of treatment is indicated by the arrow). No statistical differences were observed at the time of treatment. One out of two experiments is shown. **B,** Allogeneic BMT results in a marked decrease of pathogenic anti-type II collagen autoantibodies. Sera were taken at 2 and 6 weeks post-BMT (i.e. day 52 and 81, respectively) and 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<0.002) than mice receiving syngeneic BMT or conditioning alone (*P<0.05) as compared to untreated animals. Allogeneic BMT versus syngeneic BMT and conditioning alone (*P=0.02) at 6 weeks post-BMT. **C,** The 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 a positive and negative control for the presence of Igh-1a, respectively, we immunized normal BALB/c and DBA/1 mice with CII. Immunized BALB/c mice are able to produce CII-specific antibodies to the same extent as immunized DBA/1 mice, but lack the clinical symptoms of arthritis (data not shown).
Figure 1A

Figure 1B

Figure 1C
Control of systemic B cell-mediated autoimmune disease by nonmyeloablative conditioning and MHC-mismatched allogeneic bone marrow transplantation

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