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Effect of macrophage migration inhibitory factor (MIF) on acute graft-versus-host disease in a murine model of allogeneic stem cell transplantation

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Abstract

Macrophage migration inhibitory factor (MIF) may play an important role in the pathogenesis of acute graft-versus-host disease (aGVHD) after allogeneic hematopoietic stem cell transplantation (HSCT). We examined whether MIF has an influence on the development of aGVHD and survival using BALB/c-based MIF knock-out (MIF KO) mice. Although MIF expression was observed in lymphocytes that had infiltrated the liver during aGVHD in both wild-type (WT) and MIF KO mice that received bone marrow cells (BM) and spleen cells (SP) from C57BL/6N mice, no significant difference was found in severity of aGVHD or survival rate between the two groups of mice. However, MIF level had decreased at 1 week after HSCT when MIF KO mice were used as the recipients. In the experiment using MIF KO mice as the donors, the recipient mice transplanted with BM and SP from MIF KO mice had significantly lower aGVHD scores on days 14, 21, and 35 than those in the recipient mice transplanted with BM and SP from WT-BALB/c mice. Histopathological findings supported these observations, showing that the bile ducts and lobules in the liver were destroyed by infiltrating MIF-expressing lymphocytes in the recipients of BM and SP from WT-BALB/c mice, while the bile ducts were not destroyed even by infiltrating MIF-deficient lymphocytes in the recipients of BM and SP from MIF KO mice. Therefore, these findings suggest that MIF has an effect on the development of aGVHD in a murine model of allogeneic stem cell transplantation.

Keywords: Macrophage migration inhibitory factor (MIF); Acute graft-versus-host disease; Experimental hematopoietic stem cell transplantation; MIF knock-out (MIF KO) mice

1. Introduction

Graft-versus-host disease (GVHD) is a major complication after allogeneic hematopoietic stem cell transplantation (HSCT) [1]. The activation and expansion of donor T cells lead to the secretion of pro-inflammatory cytokines and the recruitment of additional inflammatory effector cells to several target organs such as the gut, liver, and skin and induce further damage to the affected tissues [2–4].

Macrophage migration inhibitory factor (MIF) was originally discovered as a lymphokine and was reported to prevent the random migration of macrophages and to recruit them to inflamed sites [5,6]. Recent studies have revealed that MIF is ubiquitously expressed in various types of cells, especially in T cells and macrophages, and it has been re-evaluated as a pluripotent cytokine involved in a broad-spectrum immune system [7,8] and also as a pro-inflammatory cytokine and pituitary-derived hormone that potentiates endotoxemia [9]. Subsequent work showed that T cells and macrophages secrete MIF in response to glucocorticoids as well as upon activation by various pro-inflammatory stimuli [10]. Moreover, it has been suggested that tumor necrosis factor alpha

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(TNF-α) and interferon gamma (IFN-γ) up-regulate MIF production in macrophages and, conversely, MIF induces TNF-α production, forming a pro-inflammatory loop within the cytokine network [10]. Furthermore, anti-MIF antibodies inhibit T cell proliferation and IL-2 production *in vitro* and suppress antigen-driven T cell activation and antibody production *in vivo* [11]. MIF plays an important role in the pathogenesis of inflammatory diseases. The serum level and the local expression level of MIF are significantly increased in inflammatory diseases such as rheumatoid arthritis [12], acute pulmonary diseases [13], glomerulonephritis [14], focal glomerular sclerosis [15], systemic sclerosis [16], chronic colitis and experimental colitis [17,18]. Recent studies have shown that local MIF expression is up-regulated in the allo-immune reaction during renal transplantation and bone marrow transplantation (BMT) and that MIF co-localizes with infiltrating macrophages and T cells [19–21]. It has also been shown that BMT causes a systemic increase in MIF [21]. These observations suggest that MIF also plays an important role in the pathogenesis of aGVHD after allogeneic HSCT. On the other hand, some studies have shown that MIF is not required for acute renal [22] or for acute and chronic cardiac allograft rejection in a murine model [23].

2. Objective

The aim of this study was to examine the association with MIF and acute GVHD in a murine model of allo HSCT. Since there was no study in a BMT murine model, we examined whether MIF has an influence on the development of aGVHD after allogeneic HSCT using MIF KO mice.

3. Material and methods

3.1. Mice

Eight- to 16-week-old female C57BL/6 (H-2^b) and BALB/c (H-2^d) wild-type (WT, MIF+/+) mice were purchased from CLEA Japan, Inc. (Shizuoka, Japan). In this study, by targeted disruption of the MIF gene, a mouse strain (bred onto a BALB/c WT background) deficient in MIF was established [24]. MIF KO mice develop normally in size and behavior and are fertile. No abnormalities of internal organs have been noted. The mice were maintained in standard wire cages and allowed free access to food and water. The age range of mice used as BMT donors and recipients was between 8 and 16 weeks. This study adhered to the Declaration of Helsinki and was approved by the Animal Experiment Ethics Committee of the Graduate School of Medicine of Hokkaido University.

3.2. BMT

We lethally irradiated BALB/c and MIF KO recipient mice with 8.0 Gy total body irradiation (TBI) and C57BL/6N recipient mice with 10.0 Gy TBI using an X-ray source and injected the mice with donor cells via the tail vein within 24 h after irradiation. All mice received 1×10^7 BM cells with or without 2×10^6 (BALB/c and MIF KO recipient mice) and 1×10^7 (C57BL/6N recipient mice) splenic T cells from the respective allogeneic or syngeneic donors on day 0. Mice were kept on antibiotic water (25 μg/ml neomycin and 0.3 U/ml polymyxin B) for the first 28 days. The survival and appearance of mice were monitored daily and body weight was measured daily until 28 days after HSCT and 2 times per week after day 28. Donor cell engraftment was determined by flow cytometric analysis of the percentages of H-$2K^{b+}$ and H-$2K^{d+}$ cells with the relevant monoclonal antibodies (BD Biosciences, San Jose, CA) in the spleen cells at 14 days after transplantation.

3.3. Assessment of GVHD

The degree of clinical GVHD was assessed weekly by a scoring system described by Cooke et al. [25] that sums changes in five clinical parameters: weight loss, posture, activity, fur texture, and skin integrity (maximum index = 10).

3.4. Assay for mice serum MIF by enzyme-linked immunosorbent assay (ELISA)

Serum MIF concentrations were measured by ELISA specific for MIF as described previously [18]. An anti-rat MIF IgG polyclonal antibody dissolved in 50 μl of phosphate-buffered saline (PBS) was added to each well of a 96-well microtiter plate and left for 30 min at room temperature. After the plate had been washed 3 times with distilled water, all wells were filled with PBS containing 0.5% bovine serum albumin for blocking and left for 20 min at room temperature. After removal of the blocking solution, serum samples were added in duplicate to individual wells and incubated for 1 h at room temperature. Serum was obtained by orbital vein puncture using a heparinized syringe. Absorbance was measured at 492 nm by an ELISA plate reader (Model 3550; Biorad, Hercules, CA).

3.5. Flow cytometric analysis

Cells were stained with the following mAbs to murine markers: FITC-conjugated anti-rat $IgG_{2b,\kappa}$, anti-H-$2K^b$, anti-H-$2K^d$ and anti-CD3 mAbs and PE-conjugated anti-CD4, anti-CD8 and anti-NK1.1 (all from BD Biosciences, San Jose, CA). Fluorescent staining was analyzed by a FACS Calibur (Becton Dickinson, San Jose, CA) using Cell Quest (BD Biosciences, San Jose, CA).

3.6. Immunohistochemistry

Immunohistochemical analysis was performed using a Vectastain ABC kit according to the manufacturer's protocol. Paraffin-embedded tissues were cut into 4-μm-thick sections. The sections were pretreated with 3% H_2O_2 for 10 min at 4 °C, then treated with 10% normal goat serum for 30 min at room temperature, followed by overnight incubation with the anti-MIF antibody at 4 °C. MIF-positive staining was visualized with diaminobenzidine as a chromogen.

3.7. Histologic examination

The tissues of the liver were longitudinally opened, fixed with 10% neutral buffered formalin, and embedded in paraffin. After the thin tissue sections had been deparaffinized on glass slides, samples were stained with hematoxylin and eosin.

3.8. Statistical analysis

Survival curves were plotted using Kaplan–Meier estimates. The Mann–Whitney *U*-test or Student's *t*-test was used for statistical analysis of *in vitro* data and clinical scores, and the log rank test was used to analyze survival data. A value of $p<0.05$ was considered statistically significant.

4. Results

4.1. Populations of spleen cells in WT-BALB/c and MIF KO mice

We examined the proportions of T cells ($CD3^+$, $CD4^+$, $CD8^+$) and NK cells ($NK1.1^+$) in spleen cells from WT-BALB/c and MIF KO mice by flow cytometry. We found that all of these cell proportions in spleen cells were not significantly different between WT-BALB/c mice and MIF KO mice (mean ± standard error, 59.19 ± 1.53% $CD3^+$, 34.89 ± 0.88% $CD4^+$, 19.66 ± 0.72% $CD8^+$ and 1.64 ± 0.14% $NK1.1^+$ cells in WT-BALB/c mice versus 48.60 ± 3.83% $CD3^+$, 36.75 ± 1.29% $CD4^+$, 15.28 ± 0.39% $CD8^+$ and 1.12 ± 0.12% $NK1.1^+$ cells in MIF KO mice, $p=0.99$, 1.00, 1.00 and 1.00, respectively) (Fig. 1).