evaluated by microscopic examination. Eosinophilia was defined as a relative eosinophil count greater than 4% on at least one day within the first 100 days after allo-SCT. Eosinophilia was observed in 135 patients (57%). The cumulative incidence of grades II to IV acute GVHD was found to be significantly higher in patients without eosinophilia than in those with eosinophilia (68% vs. 43%; $P < 0.001$). In 15 of 58 (26%) patients with eosinophilia and 41 of 70 (59%) patients without eosinophilia, acute GVHD was resistant to standard doses of prednisolone and required salvage therapy ($P = 0.022$). The cumulative incidence of chronic GVHD was significantly higher in patients without eosinophilia than in those with eosinophilia (73% vs. 56%; $P < 0.011$). The cumulative incidence of relapse in patients with hematologic malignancies was similar between patients with and without eosinophilia (33% vs. 27%; $P = 0.438$). On the other hand, the cumulative probability of non-relapse mortality was 10% in patients with eosinophilia, which was significantly lower than that in patients without eosinophilia (31%; $P < 0.001$), and the estimated overall survival at 3 years was 67% in patients with eosinophilia, which was significantly higher than that in patients without eosinophilia (51%; $P = 0.003$). Multivariate analysis identified age above 40 years, high-risk disease, grade II to IV acute GVHD, sex disparity between patient and donor, and the absence of eosinophilia as significant factors for reduced overall survival. These data lead us to conclude that eosinophilia after allo-SCT may serve as a favorable prognostic marker. However, further prospective studies including detailed cytokine profiling are essential for an understanding of the pathophysiological mechanisms involved in posttransplant eosinophilia.

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A CRITICAL CONTRIBUTION OF DONOR -173G/C POLYMORPHISM OF MACROPHAGE MIGRATION INHIBITORY FACTOR GENE TO THE DEVELOPMENT OF CHRONIC GRAFT VERSUS HOST DISEASE

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Chronic graft versus host disease (cGVHD) severely impairs the clinical outcome and life quality after allogeneic stem cell transplantation (allo-SCT). Its pathophysiology is not fully understood, and treatment often fails. Macrophage migration inhibitory factor (MIF) is produced by various cell types including T cells, macrophages, epithelial cells and shows a broad range of proinflammatory properties. A G to C transition at position -173 of the MIF gene has been associated with the development of various inflammatory diseases. Here, we assessed the contribution of the minor C allele to cGVHD development.

Donor-recipient pairs of 405 patients receiving allo-SCT (matched unrelated donor (URD): n=225; matched related donor (MRD): n=180) at four independent centers and surviving >100 days after SCT were genotyped. Stem cells were derived from bone marrow (n=163) or from peripheral blood (n=242) and T cell depletion (TCD) was performed in 30.7% of cases. Mean follow up was 888±46 days. The C allele (GC or CC genotype) was present in 29.2% of recipients and 26.7% of donors. No association was seen between acute GVHD and the G/C polymorphism of either the donor or the recipient. Overall, 46.8% of all patients developed cGVHD: 43.1% of patients with a GG genotype donor and 55.4% of patients when the donor carried a C allele (p=0.04). The incidence of cGVHD was not altered by the patient's MIF status (GG: 45.3% vs. GC/CC: 49.1%; p=0.6).

In patients receiving URD SCT, the presence of a donor C allele resulted in an increase in cGVHD development from 47.2 to 63.9% (p=0.03). This effect was not seen in MRD SCT (p=0.58). Since T cells are a major source of MIF, we then tested the effect of TCD on the role of donor MIF in cGVHD development after URD SCT. cGVHD incidence was significantly increased in patients receiving non-TCD allo-SCT from GC or CC unrelated donors compared to recipients of GG donor cells (71.1 vs. 52.9%; p=0.045). When TCD was performed, cGVHD occurence was not affected, independent whether the donor carried the C allele or not (52.2 vs. 40.0%; p=0.2). The presence of a donor C allele further led to a significant increase in treatment related mortality (TRM) from 6.4 to 28.6% when no TCD was performed (p=0.002), whereas TRM after TCD in both groups ranged between 31.0 and 32.7%.

Collectively, our data demonstrate a distinct role for donor T cell derived MIF in the development of cGVHD, which may allow MIF as a new immunotherapeutic target in future.

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TREATMENT OF EXPERIMENTAL ACUTE GRAFT-VERSUS-HOST DISEASE USING EXTRACORPOREAL PHOTOTHERAPY: A NOVEL MURINE MODEL

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Extracorporeal phototherapy (ECP) is an emerging therapy for clinical graft-versus-host disease (GVHD) that exposes a patient's peripheral white blood cells (WBCs) to photo-activatable 8-methoxypsoralen (8-MOP) and UVA light before re-infusing them. We have developed a novel murine model that closely mirrors ECP treatment of clinical GVHD to investigate its mechanism of action. C3H.SW mice were conditioned with 11Gy total body irradiation and injected with 5.0×10^6 bone marrow and 0.5×10^6 purified T cells from either syngeneic (C3H.SW) or allogeneic (B6-Ly5.2) donors. In order to model ECP as a treatment strategy rather than as prevention, animals were not treated until GVHD was clinically evident 7 days after BMT and then received 4 weekly infusions with 30.0×10^6 8-MOP+UVA treated splenocytes from similarly transplanted mice. Control mice were infused with untreated splenocytes, and did not show any differences from mice treated with L-15 (data not shown). Infusion of 8-MOP+UVA-treated splenocytes into allo-BMT recipients resulted in significantly better day +56 survival (74% vs 42%, p=0.0007), reduced GVHD clinical scores (2.9 vs 5.9, p<0.004) and GVHD histopathology in liver (7.3 vs 13.4), gut (11.4 vs 18.4) and skin (0.6 vs 1.2; all organs p<0.03) compared to mice infused with untreated splenocytes. ECP treated allo-BMT recipients also had significantly better immune reconstitution (p=0.01) on day +56, with both total thymocytes and distribution of thymocyte compartments indistinguishable from syngeneic controls. In vitro studies showed that >98% of cells were Annexin$^+$ within 24h of ECP treatment and experiments using Ly5 congenic donors demonstrated that ECP treated cells are undetectable in the thymus and spleen following injection. Because apoptotic cells are known to induce tolerance in several systems, we hypothesized that ECP increased the number of T regulatory cells (Treg). The number of CD4$^+$CD25$^+$Foxp3$^+$ Treg in ECP-treated allo-BMT recipients was significantly increased compared to mice infused with untreated splenocytes in the thymus (2.5 vs 1.0×10^4, p<0.02) and the spleen (1.8 vs 1.1×10^5, p=0.008). We conclude that 4 weekly infusions of ECP treated cells beginning after GVHD induction significantly decreases acute GVHD by all clinical, pathological and cellular parameters and is associated with increased numbers of Tregs.

	SYN +/- ECP	ALLO+ No ECP Spl	ALLO+ ECP Spl	p-value*
Number	15	19	34	
Day +56 Analysis:				
Survival	100%	42%	74%	0.0007
GVHD Clinical Score	1.1±0.4	5.9±0.3	2.9±0.4	0.004
Pathology				
Liver	2.1±0.9	13.4±5.5	7.3±2.5	0.0001
Intestine	7.4±2.8	18.4±5.7	11.4±3.1	0.0003
Skin	0	1.2±0.7	0.6±0.6	0.03
Thymic reconstitution				
Thymocytes (x10^6)	12.4±9.9	2.2±2.1	7.7±7.2	0.01
Thymic Treg (x10^4)	5.1±2.6	1.0±0.6	2.5±1.4	0.02
Spleen Treg (x10^5)	6.2±2.9	1.1±0.4	1.8±0.1	0.008

* 95% confidence level, Allo+ECP Spl vs Allo+No ECP Spl