Our results have identified the microbiota as a potent therapeutic target that can be recruited to significantly reduce GVHD. Approaches to potentially translate these findings include investigating the safety of introduction of Blautia to allo BMT recipients, or alternatively developing nutritional strategies to support endogenous Blautia during the transplantation process.

2

CD4\(^+\) Invariant Natural Killer T Cells Protect from Acute Graft-Versus-Host Disease Lethality through a Dramatic Expansion of Donor-Derived CD4\(^+\)FoxP3\(^+\) Regulatory T Cells

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CD4\(^+\) FoxP3\(^+\) regulatory T cells are a rare but potent immunomodulatory subset of lymphocytes in humans (TCR\(^V\) \(\alpha 24-j3x18\)) and mice (TCR\(^V\) \(\alpha x14-j3x18\)). It has been shown previously that host iNKT cells require interaction with CD4\(^+\)FoxP3\(^+\) regulatory T cells to protect from acute GVHD after conditioning with total lymphoid irradiation and anti-thymocyte globulin (Pillai et al., Blood 2009). In this study, we investigated the role of highly purified adoptively transferred donor-derived CD4\(^+\) iNKT cells in the setting of myeloablative conditioning. Balb/c (H-2Kb) recipient mice were irradiated with 8 Gy and transplanted with T cell-depleted bone marrow together with 5x10\(^4\) CD4\(^+\)CD8\(^-\) T lymphocytes (Tcon) from C57Bl/6 (H-2Kb) donor mice. Mice co-injected with as low as 5x10\(^4\) highly purified (>99%) CD4\(^+\) iNKT cells showed significantly reduced proliferation and activation of alloreactive Tcon towards BCL1 cells measured by bioluminescence imaging (\(p<0.0001\)). With \(\alpha\)-GalCer and IL-2 in vitro expanded CD4\(^+\) iNKT cells had the same protective effect from lethal acute GVHD compared to freshly isolated CD4\(^+\) iNKT cells (\(p<0.0001\)). We conclude that low numbers of highly purified CD4\(^+\) iNKT cells protect from lethal acute GVHD in mice through a dramatic expansion of donor-derived CD4\(^+\)FoxP3\(^+\) regulatory T cells with retained GVL effect. Despite the fact that iNKT cells are a rare cell population, the feasibility of in vitro expansion with retained functionality of CD4\(^+\) iNKT cells provide the basis for clinical translation. EM and RN contributed equally to this study.

3

ATG16L1 Prevents Lethal T-Cell Alloreactivity Mediated By Dendritic Cells

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The role of autophagy in GVHD is unknown. We used a MHC-disparate allo-HSCT model (B10.BR into B6) and compared wild-type (WT) vs ATG16L1\(^\text{TM}\) (hypomorphic (HM) for expression of the ATG16L1 protein) recipients. HM recipients developed significantly increased GVHD mortality (Figure A, \(P < 0.001\)) and morbidity. This was associated with significantly increased levels of inflammatory cytokines, including TNF-alpha and IL-12 (\(P < 0.05\)). CFSE analysis demonstrated significantly increased proliferation and activation of alloreactive donor T cells in HM recipients. Alloreactive donor T cells in HM recipients expressed higher levels of LPAM-1,
which is involved in T cell trafficking to the intestines. This correlated with significantly increased numbers of infiltrating donor T cells in the intra-epithelial compartment of the small intestines (P < 0.05). HM recipients had greater loss of intestinal integrity as demonstrated by increased intestinal permeability after oral FITC-dextran gavage (P < 0.01) and increased bacterial colony growth from spleen and blood (P < 0.05). To further assess the increased alloreactivity of donor T cells in HM recipients, we analyzed recipient-derived dendritic cells (DC) in HM vs WT recipients and noted a) increased numbers, b) increased expression of activation/maturation molecules (CD40, CD80 and CD86; Figure B) by flow cytometry and transcriptome microarray and c) increased alloactivation of donor T cells when tested in a mixed lymphocyte reaction. In addition, we demonstrated that in vitro culture of DCs with an autophagy-inducing drug (resveratrol) resulted in decreased expression of activation/maturation molecules (P < 0.001).

To determine if ATG16L1 deficiency in recipient DCs is sufficient, we performed experiments with CD11c-Cre/Atg16l1fl/fl recipients (in which the ATG16L1 deficiency is restricted to CD11c+ DC in the recipient) and found increased GVHD. Finally, to assess whether ATG16L1 contributes to GVHD through its role in autophagy, we treated allo-HSCT recipients with an autophagy inhibitor (LysoS5; 10 mg/kg i.p., days 3 to 30) and found significantly increased GVHD.

In conclusion, these preclinical studies demonstrate that loss of ATG16L1 expression in allo-HSCT recipients results in increased activation/maturation of DC leading to increased alloactivation of donor T cells and worse GVHD. Based on these observations in mice we analyzed a cohort of 122 allo-HSCT patients, who had received an allograft from an HLA-identical sibling and found that the presence of ATG16L1 SNPs either in donor or recipient resulted in increased mortality, although only donor ATG16L1 SNP reached statistical significance in this small cohort (P = 0.026).

Taken together, our results in mouse and man suggest that ATG16L1 and autophagy are involved in the pathophysiology of GVHD and raise the possibility that strategies to induce autophagy could ameliorate GVHD.

CIBMTR BEST ABSTRACT AWARDS FOR CLINICAL RESEARCH

Retrospective Study of 240 Patients with Severe Combined Immunodeficiency Transplanted from 2000-2009: A Report from the Primary Immune Deficiency Treatment Consortium of North America

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