Acute Graft-Versus-Host Disease Disrupts Fibroblastic Reticular Cell Expression of Tissue-Restricted Antigens and Impairs Peripheral Regulation of Autoaggressive T Cells

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Acute graft-versus-host disease (GVHD) is a known risk factor for the later development of chronic GVHD. Although thymic injury during acute GVHD leads to the de novo generation of self-reactive T cells, the “two-hit” hypothesis proposes that the additional loss of peripheral mechanisms is required for autoimmunity. Non-hematopoietic lymph node stromal cells (LNSC) provide a platform for peripheral tolerance by expressing and directly presenting tissue-restricted antigens (TRAs) to T cells. We hypothesized that alloreactive T cells could directly kill LNSC in the GVHD recipient, thus disrupting a critical mechanism for preventing injury by auto-aggressive donor T cells. To test this hypothesis, we used a clinically relevant model of MHC-matched, B6 female → B6 male bone marrow transplantation (BMT) where GVHD is induced by the co-transfer of anti-HY TCR-transgenic MannaTα CD8+ T cells. We found that fibroblastic reticular cells (FRC), a LNSC subset identified as CD45-CD31-gp38+→Ccl19-Cxcl12-Ccl22, were reduced ~15-fold in mice with GVHD whereas other LNSC, including lymphatic or blood endothelial cells were less affected. Consistent with injury to FRC, transcription of both Il-7 and Ccl19 genes was significantly reduced in LN stroma of mice with GVHD. Furthermore, the repertoire of putative TRAs expressed by FRC was also substantially reduced.

To test the hypothesis that acute GVHD interferes with FRC capacity to express TRAs and induce peripheral tolerance, we exploited the iFABP+Tova model where truncated ovalbumin (tOVA) antigen is expressed by intestinal epithelial cells and ectopically by FRC: in the steady state, direct presentation of tOVA by FRC induces clonal elimination of antigen-specific T cells. Transfer of HY-specific T cells into iFABP+Tova male BMT recipient reduced intranalonal tOVA expression by 15-fold compared to no GVHD controls. To test whether acute GVHD-mediated FRC damage impairs tolerance induction, 10^6 OT-1 T cells (a surrogate for autoreactive T cells) were adoptively transferred to GVHD- or GVHD- iFABP+Tova recipients at 6 weeks following BMT. Transferred OT-1 T cells were efficiently tolerated and clonally deleted in GVHD- iFABP+Tova recipients. However, in GVHD+ iFABP+Tova, OT-1 T cells were not eliminated, and a large number of IFNγ+ effector cells expanded in LN and intestinal epithelium, leading to severe weight loss and intestinal injury. Taken together, our data demonstrate that acute GVHD reduces TRA display by FRC and induces loss of a critical peripheral tolerance mechanism that prevents expansion and pathogenicity of autoreactive T cells. These findings provide a potential mechanism to explain the link between acute GVHD and the succeeding autoimmune disorder in chronic GVHD.

Gut GVHD is critical for determining the outcome of allo- genic hematopoietic cell transplantation (HCT). Damage Paneth cells and loss of defenses but increase of anti- bacterical lectin regenerating islet-derived IIIγ (RegIIIγ) can lead to dysregulation of intestinal microbiota and gut GVHD pathogenesis. RegIIIγ can be produced by Paneth cells and intestinal epithelial cells, and its secretion is augmented by IL-22, and the functional effect of IL-22 is regulated by IL-22 antagonists. IL-22-binding protein (IL-22–BP). However, the circumstances leading to RegIIIγ dysregulation and dysbiosis remain largely unknown.

Here we show that, in a MHC-mismatched model of C57BL/6 donor to BALB/c recipient, one injection of depleting anti-CD4 effectively prevents acute gut GVHD in recipients given wild-type (WT) transplants but has no effect in recipients given IFN-γ–/- transplants. Most of the latter recipients died with diarrhea and severe colitis by two weeks after HCT. Interestingly, PD-L1–/- recipients given IFN-γ–/- transplants showed spontaneous recovery of the disease. The disease induction was dependent on alloreactive donor CD8+ T cells and mismatched host MHC I. As compared to recipients given WT transplants, recipients given IFN-γ–/- transplants showed expansion of gut tissue infiltrating CD8+ Tc17 cells that produced both IL-17 and IL-22, and the Tc17 cells were required for the disease induction, as administration of anti-IL-22 but not anti-IL-17 effectively prevented the disease induction. Furthermore, we found that host tissue expression of PD-L1 augmented expansion of Tc17 and reduced the DC subsets that produce IL-22BP. In addition, recipients given IFN-γ–/- transplants showed no damage of Paneth cells but their intestinal tissues had markedly reduced mRNA and protein levels of RegIIIγ. Gut GVHD in recipients given IFN-γ–/- transplants resulted from dysbiosis, because those recipients had significant reduction of anaerobic bacteria Barnesella in feces and significant increase of E. Coli and Lactobacillus. Co-housing recipients given IFN-γ–/-transplants together with recipients given WT transplants significantly reduced tissue RegIIIγ, changed microbiome profile, and reduced the disease severity. Treatment with antibiotics of Ampicillin, Neomycin, Metronidazole,