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PREDICTION OF ACUTE GVHD FOLLOWING NONMYELOABLATIVE CONDITIONING BY MEASUREMENT OF TUMOR NECROSIS FACTOR-RECEPTOR I (TNFR1) AT BASELINE AND AT DAY 7 AFTER TRANSPLANTATION

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Acute graft-versus-host disease (GVHD) has remained a significant cause of nonrelapse mortality after allogeneic hematopoietic cell transplantation (HCT) with nonmyeloablative conditioning. The role of tumor necrosis factor-alpha (TNF-α) in the biology of acute GVHD following nonmyeloablative conditioning has not been studied thus far. Here, we measured TNF receptor 1 (TNFR1) as a surrogate marker for TNF-α in 106 patients before the start of the conditioning regimen (baseline) and 7 days after allogeneic HCT following nonmyeloablative conditioning. The nonmyeloablative regimen consisted of 2 Gy total body irradiation (TBI) alone (n = 15), 2 Gy TBI plus fludarabine 90 mg/m^2 (n = 73), or 4 Gy TBI plus fludarabine 90 mg/m^2 (n = 18). TNFR1 levels increased significantly from baseline to day 7 after nonmyeloablative HCT ($P < 0.0001$). Patients conditioned with 4 Gy TBI had higher TNFR1 day 7/baseline ratio than those conditioned with 2 Gy TBI (median 1.65 versus 1.25; $P = 0.01$). Patients with grade II-IV acute GVHD had higher relative (defined as TNFR1 level divided by baseline TNFR1 level) TNFR1 levels than those without grade II-IV acute GVHD on day 7 (NS), and on day 35 after HCT (P = 0.04), but not on day 63 after HCT. In a multivariate Cox model, high TNFR1 day7/baseline ratio was associated with grade II-IV (HR = 2.2, $P = 0.01$) and grade III-IV (HR = 2.9, $P = 0.007$) acute GVHD, but had no impact on overall survival (P = 0.8). In summary, our data suggest that nonmyeloablative conditioning induce the generation of TNF-α, and that the magnitude of TNF-α generation depends on the conditioning intensity (2 Gy versus 4 Gy TBI). Further, assessment of TNFR1 levels before and on day 7 after nonmyeloablative HCT provided useful information on subsequent risk of experiencing acute GVHD.

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CD103 DEFICIENCY SEPARATES GRAFT-VERSUS-HOST DISEASE AND GRAFT-VERSUS-LEUKEMIA EFFECTS MEDIATED BY DONOR CD8 T CELLS

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We have previously shown that CD103, an integrin conferring specificity for the epithelial ligand E-cadherin, is required for donor CD8 mediated intestinal GVHD. Owing to the lack of E-cadherin expression on hematopoietic cells, we hypothesized that CD103 deficiency would attenuate GVHD, while sparing GVL effects.

Methods: To examine the role of CD103 in CD8 mediated GVHD, lethally irradiated A/J hosts (H-2^a) were reconstituted by 1.0×10^7 BALB/c (H-2^d), bone marrow cells (BMCs) and 1.0×10^7 alloantigen-primed CD8 T cells from either WT or CD103-/- mice. To test whether CD103 deficiency compromises GVL effects mediated by donor T cells, 2.0×10^7 splenocytes from CD103-/- or WT Balb/c (H-2^d) mice were co transferred into BALB.scid (H-2^d) recipients, together with 1.0×10^7 splenocytes from a Eμ-TCL1 mouse (H-2^b) with end-stage chronic lymphocytic leukemia (CLL). A control group received CLL cells alone. T cells from the Eμ-TCL1 splenocytes were depleted to prevent alloreactivity against BALB.scid recipients.

Results: Upon adoptive transfer into A/J recipient mice, primed WT CD8 T cells elicited severe GVHD resulting in 75% GVHD mortality. In contrast, CD103-/- CD8 T cells elicited mild or undetectable GVHD (23% GVHD mortality). At autopsy compared to the CD103-/- recipients, WT CD8 recipients exhibited severe lesions in the intestinal and hepatic epithelium. These data demonstrate that CD103 expression is required for GVHD pathogenesis mediated by primed CD8 T cells in this model. To assess the role of CD103 in effector T cell mediated GVL effects, splenocytes from either WT or CD103-/- BALB/c mice were adoptively transferred in BALB.scid mice along with CLL cells from Eμ-TCL1. A control group of BALB.scid mice received CLL cells only. FACS analyses of peripheral blood revealed development of CLL in BALB-scid mice receiving only CLL cells as early as day +14 post cell transfer. In contrast no CLL was detected in peripheral blood of mice receiving WT or CD103-/- splenocytes at any time point. BALB.scid mice receiving CLL cells only, displayed poor grooming, decreased activity, dyspnea and massive heptosplenomegaly. Mean survival time of BALB.scid mice in CLL only group was 73 days. In contrast all the mice receiving WT or CD103-/- cells survived beyond day +110 with no evidence of CLL (p-value = 0.0001).

Conclusion: Our data demonstrate that CD103 deficiency attenuates GVHD while sparing GVL effects and thus hold great translational potential.

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HUMAN T CELL RAPAMYCIN RESISTANCE AND TH1/TC1 POLARIZATION AUGMENT XENOGENEIC GRAFT-VERSUS-HOST DISEASE

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Adoptive transfer of co-stimulated T cells represents an immunotherapeutic method currently under clinical investigation. We hypothesized that the ex vivo acquisition of rapamycin resistance and type I cytokine polarization during co-stimulation would augment the in vivo effect of adoptively transferred human T cells. In the presence of rapamycin, T cell co-stimulation and polarization with IL-12 or IFN-γ permitted human CD4+ and CD8 + T cell differentiation towards a Th1/Tc1 phenotype: by intracellular flow, median percentage expression of Foxp3, IFN-γ, and T-bet was 4%, 20%, and 72%, respectively. Phosphoflow cytometry revealed that such Th1/Tc1 cells expressed activated STAT1 and STAT4 in spite of mTOR blockade; STAT activation was abrogated by PI3 kinase inhibition. Rapamycin-resistant human Th1/Tc1 cells (Th1/Tc1.R cells): (1) had increased expression of the autophagy-related gene LC3BII by gene array and protein analysis; (2) preferentially expressed anti-apoptotic bcl-2 family members (reduced Bax, Bak; increased phospho-Bad); (3) maintained mitochondrial membrane potentials; and (4) had reduced apoptosis relative to control Th1/Tc1 cells not generated in rapamycin (p = 0.04). The anti-apoptotic phenotype of Th1/Tc1.R cells was abrogated by co-incubation with the autophagy inhibitor, 3-methyl adenine. The in vivo effect of the Th1/Tc1.R cells was evaluated using two xenogeneic GVHD (x-GVHD) models. First, in an LPS-induced x-GVHD model, Th1/Tc1.R cells resulted in lethality in 75% recipients; soluble TNF-α receptor therapy with etanercept reduced the frequency of lethality to 15%. Second, using a non-LPS natural history model of x-GVHD, recipients of Th1/Tc1.R cells (relative to recipients of control Th1/Tc1 cells) had increased human T cell engraftment (day 30 post-BMT, p = 0.001), increased human T cell cytokine levels, increased human T cell expression of the cytotoxic degranulation molecule CD107 (p = 0.05), and increased human T cell infiltration of skin, gut, and liver. In this model, lethality due to x-GVHD was also increased in Th1/Tc1.R cell recipients (lethality increased from 20% to 70%, p = 0.04). We conclude that rapamycin therefore does not impair human T cell capacity for type I differentiation. Rather, by promoting autophagy rapamycin permits stable expression of T-bet and generates an anti-apoptotic Th1/Tc1 effector phenotype, thereby yielding increased x-GVHD.

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B- CELL DEPLETION AS THERAPY FOR STEROID REFRACTORY CHRONIC GVHD IS MOST EFFECTIVE FOR SCLEROSIS OF THE SKIN

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