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RAPAMYCIN PREVENTS GVHD IN MOUSE MODEL THROUGH NOVEL MECHANISM: INCREASE IN REGULATORY T CELLS

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Rapamycin (RAPA) is an immunosuppressant that has been used for many years for prevention of rejection in solid organ transplantation. It also appears to have some impact on acute graft versus host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT). In previous experiments done in our lab, RAPA significantly improves survival in GVHD mouse model. Recently, it has been found that RAPA can increase numbers or enhance survival of regulatory T cells. In this experiment we further investigate the mechanisms behind RAPA in a murine GVHD model. In several experiments, B10. D2 bone marrow and splenocytes were injected into Balb/c mice. The mice had samples of peripheral blood and ear biopsies evaluated at two, four and six weeks post-transplantation. Regulatory T cells were analyzed based on intracellular FoxP3 expression via FACS or immunohistochemistry. At all three time points in the peripheral blood, the percent of regulatory T cells in total CD4+ cells was not significantly different. On days 14 and 28 there were significant differences seen in the ear biopsies, both in morphologic appearance and percent FoxP3 positive cells of mononuclear cells. On day 14 there was more inflammation in the control arm compared to the RAPA treated mice, however, there was a cellular infiltrate present in both. The percentage of FoxP3 positive cells was 13% in the treated arm as compared to 2.5% in the control arm ($p = 0.02$). On day 28, there was a decrease in cellularity in the control arm as compared to the RAPA arm, however in the RAPA arm there was a significant increase in percent of FoxP3 positive cells as compared to control arm: 9% vs 1.2% ($p = 0.04$). These results suggest that the mechanism through which RAPA prevents GVHD in this model is not only immunosuppression but also through the effects of regulatory T cells. It appears that the effect is mediated locally in the tissues, rather than through circulating regulatory T cells.

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INITIAL THERAPY FOR ACUTE GRAFT-VERSUS-HOST DISEASE WITH "LOW-DOSE" PREDNISONE (1 mg/kg/day) INSTEAD OF "STANDARD-DOSE" PREDNISONE (2 mg/kg/day) DOES NOT COMPROMISE MAJOR OUTCOMES AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

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The optimal glucocorticoid dose for initial treatment of acute graft-versus-host disease (GVHD) remains to be defined. We hypothesized that initial treatment with "low-dose" glucocortocoids (equivalent to prednisone, 1 mg/kg/day) would not compromise major transplantation outcomes compared to treatment with "standard-dose" glucocorticoids (equivalent to prednisone, 2 mg/kg/day). In a retrospective review, we identified 347 patients with acute GVHD who were treated initially with low-dose glucocorticoids and 386 who were treated at standard doses. All patients were at least 18 years of age and had transplants between 2000 and 2005 at a single institution. Fifty-eight (17%) of the 347 patients in the low-dose group subsequently received standard-dose treatment after inadequate response to low-dose treatment. The mean cumulative prednisone-equivalent doses across time in the low-dose group were reduced by approximately 48% compared to the standard-dose group. After adjusting for donor type (unrelated *vs* other), HLA-mismatch (any *vs* other), patient age (continuous), conditioning intensity (myeloablative *vs* nonmyeloablative), donor/recipient gender disparity, prophylactic use of ursodeoxycholic acid and tacrolimus, GVHD grade at onset of therapy and use of beclomethasone dipropionate (time-dependent covariate) in multivariate analysis, we found no statistically significant differences in outcomes in the low-dose group compared to the standard-dose group: overall mortality (hazard ratio [HR], 0.99; 95% confidence interval [CI], 0.8–1.3), relapse (HR, 1.14; 95% CI, 0.8–1.6), non-relapse mortality (HR, 0.92; 95% CI, 0.7–1.3). Although the proportion of patients receiving secondary therapy (including an increase from low dose to standard dose) was similar in the two groups, the onset of secondary therapy occurred sooner in the low-dose group (HR, 1.94; 95% CI, 1.3–2.8). Endpoints of prednisone-related morbidity were not assessed in this study. Results were similar in subgroups of patients with GVHD characterized by rash involving ≤50% body surface area and stool volumes ≤1.0 L with or without anorexia, nausea and vomiting (n = 425) and in those with more severe GVHD (n = 308). We conclude that, despite a nearly 50% reduction in the cumulative glucocorticoid dose across time, initial glucocorticoid treatment at a prednisone-equivalent dose of 1 mg/kg/day was not associated with inferior outcomes when compared to initial treatment with standard-dose glucocorticoids.

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OFF-THE-SHELF TUMOR IMMUNOTHERAPY WITH GENETICALLY ENHANCED ALLOGENEIC T-CELL PRECURSORS

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T cell deficiencies can occur in many physiological and pathophysiological settings such as aging, malignant diseases, and cytostatic therapy. We recently reported that co-transplantation of in vitro generated T cell precursors significantly enhances T cell reconstitution after allogeneic HSCT resulting in increased graft-versus-tumor activity without graft-versus-host disease. The aim of our present study was to evaluate if allogeneic T cell precursors can safely be transferred across MHC barriers in the absence of allogeneic HSCs to improve anti-tumor activity in immunosuppressed recipients. We found that adoptively transferred allogeneic (C57BL/6) precursor cells in irradiated hosts (BALB/c) develop into fully functional allogeneic T cells characterized by a host-MHC restricted and host-tolerant T cell receptor. We show that adoptively transferred allogeneic T cell precursors significantly improve survival of BALB/c mice after irradiation (675 cGy) and enhance anti-tumor activity against A20 lymphoma and renal cell carcinoma in syngeneic HSCT recipients. Furthermore, we demonstrate the feasibility of genetic engineering of antigen-specific T cell precursors, by transducing them to express a chimeric antigen receptor (CAR) targeting hCD19. Immunotherapy with CAR-expressing T cell precursors resulted in the in vivo generation of high numbers of appropriately selected T cells expressing the CAR, which persisted for at least two months after transfer and mediated significantly enhanced anti-tumor activity (compared with CAR-negative T cell precursors) against a CAR-sensitive tumor, without any undesirable auto/alloreactivity. We conclude that T cell precursors from any donor can be used universally for adoptive immunotherapy in any immunosuppressed individual irrespective of MHC disparities. The use of allogeneic precursors cells instead of autologous cells eliminates the risk of contamination with residual malignant patient cells and allows the generation and storage of virtually unlimited quantities of precursor cells for 'off-the-shelf' immunotherapy. This procedure is not only labor and cost-effective, but it facilitates the application of gene transfer technology, to generate antigen-specific or otherwise enhanced designer cells. Adoptive transfer of allogeneic and genetically enhanced T cell precursors therefore represents a promising novel strategy for targeted 'off-the-shelf' immunotherapy in immunosuppressed patients.

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DECREASED RISK OF ACUTE GVHD FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH THE 5,10-METHYLENETETRAHYDROFOLATE REDUCTASE 677TT GENOTYPE

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