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ANTI-GVHD EFFECT OF ANTI-THYMOCYTE GLOBULIN IS MEDIATED BY ANTIBODIES BINDING TO T CELLS AND REGULATORY NK CELLS, AND LESS SO OR NOT AT ALL BY ANTIBODIES BINDING TO OTHER MONONUCLEAR CELL SUBSETS

Introduction: Polyclonal rabbit-anti-human T cell globulin (ATG) may decrease the likelihood of graft-vs-host disease (GVHD) without increasing the likelihood of relapse. ATG is polyclonal and the anti-GVHD effect may be mediated through killing/inhibition of one or several lymphocyte subsets (eg, T cells) or their subsets (eg, naive T cells). To understand the mechanism of action of ATG on GVHD, we determined levels of which ATG fraction (capable of binding to which cell subset) are associated with subsequent development of GVHD.

Patients & Methods: We studied 121 patients whose myeloablative conditioning included 4.5 mg/kg ATG (Thymoglobulin). Using flow cytometry, levels of the following ATG fractions in serum from day 7 were determined: capable of binding to naive or memory B cells, naive, central memory (CM) or effector memory (EM) CD4 and CD8 T cells, CM and EM CD4 T cells not expressing CD45RA (EMRA-); cytolytic, regulatory or CD16+CD56- NK cells, monocytes and dendritic cells (DCs). ATG levels in patients with vs without aGVHD or cGVHD were compared using Mann-Whitney test. For each fraction where levels were significantly different (p < 0.05), we determined if patients with high fraction level had a significantly lower likelihood of aGVHD or cGVHD using log-binomial regression models.

Results: In univariate analyses, significantly lower levels of ATG fractions on the following subsets were found in patients developing aGVHD: binding to naive CD4 and CD8 T cells, EM CD4 T cells, and regulatory NK cells, or cGVHD: binding to naive CD4 and CD8 T cells, CM and EM CD4 T cells, and regulatory NK cells. In multivariate analyses, high levels of the following ATG fractions were associated with a low likelihood of aGVHD: binding to naive CD4 T cells (RR = 0.33, p < .001), EM CD4 T cells (RR = 0.30, p < .001), naive CD8 T cells (RR = 0.33, p < .002) and regulatory NK cells (RR = 0.36, p < .001) or cGVHD: binding to naive CD4 T cells (RR = 0.59, p = 0.028), CM CD4 T cells (RR = 0.49, p = 0.009), EM CD4 T cells (RR = 0.51, p = 0.006), naive CD8 T cells (RR = 0.46, p < .005) and regulatory NK cells (RR = 0.55, p = 0.036).

Conclusion: For both aGVHD and cGVHD, the anti-GVHD effect with relapse-neutral effect of ATG appears to be mediated by antibodies to antigens expressed on naive T cells (both CD4 and CD8), EM CD4 T cells and regulatory NK cells. This is the first step towards identifying the antibodies within ATG important for the anti-GVHD effect without impacting relapse.

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DISSECTING THE MECHANISMS OF GVHD CONTROL: USING THE PRIMATE GVHD MODEL TO DETERMINE THE ROLE THAT SIROLIMUS PLAYS IN GVHD PREVENTION
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The Rhesus Macaque GVHD Model is uniquely able to support a mechanistic dissection of the contribution that individual immunomodulating agents make to GVHD prevention, in a clinically relevant primate system. In this study, we determined the primary mechanism by which sirolimus prevents GVHD in primates. Rhesus macaques underwent MHC-mismatched HSCT while receiving sirolimus monotherapy (sirolimus trough 3-15 ng/mL). Recipients exhibited modestly prolonged survival compared to untreated controls (MST 19 days versus 6.5 days in controls).

Sirolimus markedly inhibited T cell proliferation in transplanted animals. Thus, while CD4+ and CD8+ T cells expanded by as much as 300-fold and 2000-fold, respectively, in the absence of immunophrophylaxis, in sirolimus-treated animals, the accumulation of these cells was significantly blunted, with their expansion being only 4- and 3.6-fold, respectively compared to the post-TBI nadir. Consistent with this marked impact on T cell proliferation, sirolimus-treated recipients also better controlled the upregulation of the proliferation marker Ki-67 on CD4+ or CD8+ T cell subpopulations. Thus, while untreated recipients upregulated Ki-67 expression by as much as 10-fold after engraftment, (with > 80-98% of both CD4+ and CD8+ T cells expressing high levels of Ki-67 post-transplant versus 5-10% pre-transplant) sirolimus-treated recipients displayed blunted Ki-67 expression (with an average of only 20-30% of both CD4+ and CD8+ T cells expressing high levels of Ki-67 post-transplant).

While the impact of sirolimus on T cell proliferation was profound, its impact on the expression of CD127 (the IL-7Ra), a marker of T cell activation, was small. Thus, similar to the untreated control animals, who demonstrated a total loss of CD127 in the context of severe clinical GVHD, up to 60% of CD4+ T cells in sirolimus-treated recipients down-regulated CD127, consistent with breakthrough activation and functional maturation of these cells despite mTOR inhibition.

These results indicate that the predominant effect of sirolimus during primate GVHD prophylaxis is its striking ability to inhibit T cell proliferation. These results imply that therapies that are added to sirolimus to achieve multimodal GVHD prevention should be directed at inhibiting T cell activation and function rather than proliferation, in order to target non-redundant pathways of alloimmunone activation during GVHD control.