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Pathophysiological Aspects of Transplantation Related Complications Following Busulphan-Cyclophosphamide Conditioning Regimen in Mouse Model

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Abstract

Hematopoietic stem cell transplantation (HSCT) is a curative treatment for several malignant and non-malignant disorders. However, transplantation related morbidity and mortality limit its use. The complications of the HSCT procedure can be caused by several factors including toxicity of the conditioning regimen and allogeneicity. Despite the fact that 50% of the transplanted patients are conditioned with chemotherapy, the majority of transplantation models are based on radiation. In the present thesis, we utilized a HSCT mouse model following conditioning using busulfan-cyclophosphamide (Bu-Cy) to explore mechanisms and factors that might affect graft versus host disease (GVHD) and/or treatment related toxicity, thus altering transplantation outcome.

Study I: Was designed to investigate early cell dynamics during the development of GVHD after allogeneic HSCT. We found an early expansion and activation of dendritic cells (DCs) that peaked by day +3 post HSCT. The T cell expansion started later and reached its peak by day +5 post HSCT. The majority of these cells were donor CD8⁺ cells. The inflammatory cytokines (IL-2, INF- γ and TNF- α) also reached maximum levels by day +5. The results showed the important role of donor DCs in GVHD.

Study II: We studied the early histopathological changes in several organs at different time points from conditioning, as well as during the development of GVHD, until day +21. The study showed that the liver and spleen were the most affected organs; however, no morphological effects were detected in the pancreas, heart, lungs or kidneys after the conditioning regimen. Histopathological changes such as vasculitis, inflammation and apoptotic cell forms in the liver, spleen, pancreas, lungs and heart were observed during GVHD development, however, only hypocellular spleen and extramedullar hematopoiesis were detected in syngeneically transplanted animals. No morphological changes were observed in the kidneys in either HSCT setting. These results may help in understanding mechanisms underlying the development of GVHD.

Study III: We investigated the toxicity of Bu-Cy conditioning regimen on the arteries. We found that the conditioning regimen enhanced acetylcholine relaxation in the mesenteric arteries through the increased expression of endothelial nitric oxide. In contrast, the sensitivity of the aorta to the acetylcholine was similar between the Bu-Cy treated group and the controls. However, the aortas from the treated animals had a higher sensitivity to noradrenalin. The Bu-Cy treated animals had lower blood pressure, lower hematocrit and more endothelial damage compared to the controls. These results might help in developing prophylactic treatment for cardiovascular complications.

Study IV: We studied the effect of omega-3 on the Bu-Cy conditioning regimen and on the allogeneic HSCT outcome focusing on GVHD. We used corn oil and standard food as controls. The mice that were fed omega-3 food had the lowest survival rate and showed early signs of GVHD. Omega-3 enhanced the effect of the conditioning regimen by increasing its myeloablative properties, decreasing the expression of CD4⁺CD25⁺FoxP3⁺ T cells and reducing their function. Less GVHD and a higher rejection rate were observed in the corn oil group. The higher death rates in the omega-3 fed group might be explained by a greater myeloablative effect and increased severity of acute GVHD.

Taken together, these studies increase our knowledge of GVHD and conditioning related toxicity. This may improve treatment strategies and hence the clinical outcome of HSCT.

Key words: Busulfan, cyclophosphamide, conditioning regimen, HSCT, vascular, omega-3, GVHD, mouse model, treatment related toxicity.

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