

**PATIENTS UNDERGOING TOTAL BODY IRRADIATION AND CHEMOTHERAPY FOR MYELOABLATIVE HEMATOPOIETIC STEM CELL TRANSPLANTATION DEMONSTRATE ENDOTOXEMIA AND DEFICIENCIES OF ENDOGENOUS PROTEINS THAT INHIBIT ENDOTOXIN-INDUCED TNF PRODUCTION, CORRELATING WITH POST-TRANSPLANT TOXICITIES.**

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Radiation-based murine hematopoietic stem cell transplantation (HSCT) models suggest that acute toxicities, particularly acute graft-versus-host disease (aGVHD), are driven in part by endotoxemia-induced production of the pro-inflammatory cytokine tumor necrosis factor (TNF). We hypothesized that in human HSCT, myeloablative radio-chemotherapy might not only cause gut permeability resulting in endotoxemia, but also compromise host mechanisms that inhibit endotoxin signaling. In aggregate, these changes might promote aGVHD and other inflammation-related toxicities. In an observational cohort (N = 30), patient blood obtained prior to myeloablative conditioning (baseline, B) and at Days 0 (D0) to D28 post-HSCT, demonstrated frequent endotoxemia, often in the absence of Gram-negative bacteremia, by both *Limulus* amoebocyte lysate (LAL) assay and by a novel endotoxin activity assay (EAA; Spectral Diagnostics). Changes in monocyte surface expression of CD14 and Toll-like receptor-4 (TLR4) as well as activation of a TLR transcriptome in whole blood RNA, suggested early (D0) exposure to endotoxin. There was a  $\geq 10$ -fold decline by D7 in plasma concentrations of bactericidal/permeability-increasing protein (BPI; an endotoxin-neutralizing molecule derived from neutrophils) and CCL5/RANTES (that inhibits TLR4-mediated TNF and IL-6 production). Concomitant (D7) increases in plasma IL-6 and LPS-binding protein (LBP) occurred at the time of peak fever incidence, indicating an acute phase response. Spontaneous in vitro TNF production by patient cells was inversely correlated with plasma CCL2/MCP-1, known to inhibit TLR4-mediated TNF. Patients who developed aGVHD had lower plasma BPI/LBP ratios (B) and RANTES concentrations (B and D7). This study demonstrates that radio-chemotherapy induces endotoxemia, often in the absence of bacteremia. The concurrent deficiency in BPI and RANTES likely favors TLR4-mediated TNF production potentially contributing to regimen-related toxicities, including aGVHD.