Tim-3 Serum Levels Are Associated With Severe GVHD

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Graft-versus-host disease (GVHD) is a frequent complication in patients receiving allogeneic hematopoietic cell transplantation (HCT). In acute GVHD, the donor immune cells attack and destroy the cells of the transplant recipient in various organs, including the gut, skin and liver. Mortality of patients with acute GVHD is correlated with the severity and location of gastrointestinal involvement. Acute GVHD of the upper gut manifests as anorexia, nausea, vomiting and diarrhea, which can be controlled by low doses of steroids, while acute GVHD of the mid-gut leads to more severe gastrointestinal problems that require higher doses of steroids, requiring increased immunosuppression and leading to non-relapse-related mortality. The ability to identify and treat patients with severe GVHD before clinical onset could potentially reduce patient side effects and deaths. Several clinical studies have found candidate biomarkers in patient plasma that modulate the immune response or indicate tissue damage in acute GVHD. To expand upon these efforts, Drs. John Hansen, George McDonald and colleagues in the Clinical Research Division utilized proteomic analysis of plasma to identify novel proteins associated with GVHD.

The researchers analyzed proteins and protein fragments in patients' serum by mass spectrometry in collaboration with the laboratory of Dr. Samir Hanash (Public Health Sciences Division). Several proteins were detected at high levels only in patients who later developed severe GVHD compared to serum of patients without GVHD. A soluble fragment of the protein T cell Ig and mucin domain 3 (Tim-3) was one of the proteins identified. Importantly, increased levels of Tim-3 were detected before clinical onset of severe mid-gut GVHD compared to upper-gut GVHD. A microbead assay for detecting Tim-3 in individual serum samples was developed by the FHCRC Cytokine Laboratory Shared Resource, led by Rick Lawler. Using a larger 185-patient cohort, Hansen *et al*. detected significantly higher levels of soluble Tim-3 in the plasma of patients with the more severe mid-gut GVHD compared to those with upper-gut GVHD ($P=0.005$), those without GVHD ($P=0.002$) and normal controls ($P<0.0001$, see figure). In a 64-patient cohort with serum samples three weeks after tranplant, Tim-3 was the strongest univariate risk factor examined to predict mid-gut GVHD development. The sample size was too small to perform multivariate analysis with other GVHD risk factors.

The Tim-3 receptor regulates the immune response by suppressing the activation of T lymphocytes. Tim-3 is expressed on the cell surface of activated effector T cells and mediates its suppressive function by binding the ligand galectin-9. Tim-3 is also expressed on activated B cells, dendritic cells, and natural killer (NK) cells. The researchers detected a soluble form of Tim-3 in circulation that contained the extracellular domain of the receptor, but lacked the transmembrane and intracellular portions of the protein. In addition, cell-surface expression of Tim-3 was significantly increased in CD8+ T lymphocytes from patients with more severe GVHD (P=0.01) as determined by flow cytometry.

While the mechanism for generating soluble Tim-3 is unknown, previous studies have shown that defective Tim-3 signaling exacerbates autoimmune reactivity. In a mouse model of GVHD, blocking the interaction of Tim-3 with its ligand galectin-9 resulted in a more severe GVHD reaction (Oikawa et al., 2006). Results from a different mouse model using soluble a Tim-3 fusion protein further suggest that soluble Tim-3 can inhibit the negative regulatory activity of Tim-3 on immune cells and inflammation, resulting in more severe GVHD (Veenstra et al., 2012). Conversely, reducing soluble Tim-3 levels in plasma could serve as a treatment to reduce severity of GVHD reactions by allowing normal function of Tim-3 in regulating the immune response.

"One key concept here is that measuring Tim-3 before a patient becomes sick with GVHD is a little like having a crystal ball -- to predict the future before it happens," according to Dr. McDonald. The goal is to develop a panel of biomarkers to treat patients for GVHD even before the disease appears. Current and future studies will examine how well serum levels of Tim-3 perform as a predictor of the onset of more severe GVHD, in comparison or in combination with twenty-two other putative biomarkers of GVHD previously reported in the literature. Dr. McDonald surmises that "because Tim-3 plays a role in immune regulation, the correlation of blood Tim-3 levels with more severe GVHD might lead to deeper insight into the cellular mechanisms of GVHD and possibly to novel therapies."

Hansen JA, Hanash SM, Tabellini L, Baik C, Lawler RL, Grogan BM, Storer BE, Chin A, Johnson M, Wong CH, Zhang Q, Martin PJ, McDonald GB. 2013. A Novel Soluble Form of Tim-3 Associated with Severe Graft-versus-Host Disease. *Biology of Blood and Marrow Transplantation*. Epub ahead of print, doi: 10.1016/j.bbmt.2013.06.011.

Also see: Oikawa T, Kamimura Y, Akiba H, Yagita H, Okumura K, Takahashi H, Zeniya M, Tajiri H, Azuma M. 2006. Preferential involvement of Tim-3 in the regulation of hepatic CD8+ T cells in murine acute graft-versus-host disease. *Journal of Immunology* 177, 4281-4387.

Veenstra RG, Taylor PA, Zhou Q, Panoskaltsis-Mortari A, Hirashima M, Flynn R, Liu D, Anderson AC, Strom TB, Kuchroo VK, Blazar BR. 2012. Contrasting acute graft-versus-host disease effects of Tim-3/galectin-9 pathway blockade dependent upon the presence of donor regulatory T cells. *Blood* 120:682-690.

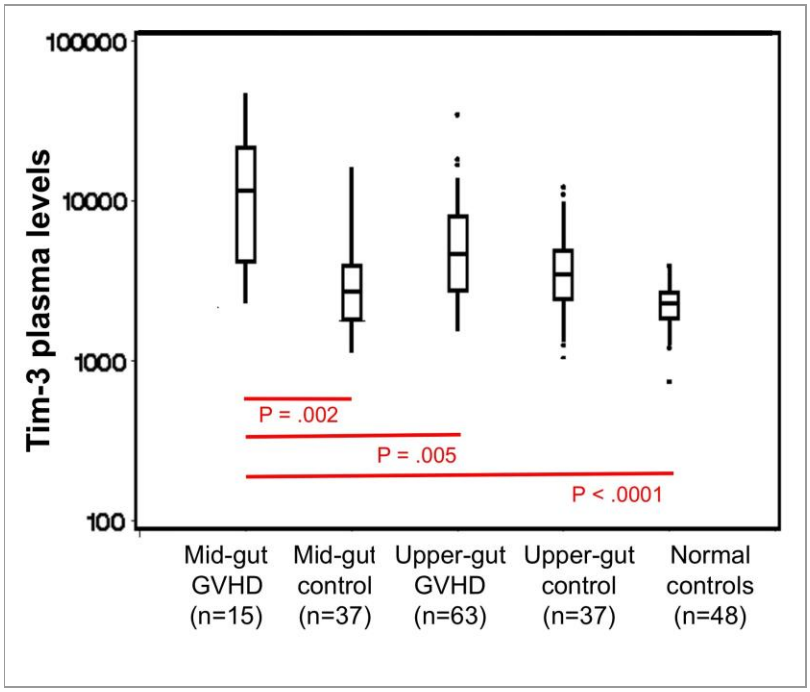

Image modified from the manuscript

Levels of Tim-3 are significantly higher in the bloodstream of patients about to develop severe GVHD than in transplant patients who will never develop GVHD. Box plots represent serum levels detected by a Tim-3 microbead assay showing 25th, 50th, and 75th percentiles; whiskers indicate 5th and 95th percentiles.