Herrmann et al compared the PD-1_{ex} domain-containing CiTE to a conventional CD33 × CD3 BiTE as well as to a single-chain triplebody (CD33 × CD3 × PD-L1) in which the PD-1_{ex} module was replaced by a high-affinity anti-PD-L1 scFv. All 3 molecules induced T-cell activation in a CD33-restricted manner, provided efficient in vitro killing of $CD33^+$ AML cells at very low concentrations (picomolar range), and cleared a human $CD33^+$ cell line in a murine xenograft model. The CiTE significantly increased (1) T-cell secretion of interferon-γ (IFN-γ) and granzyme B, (2) killing of $CD33^+PD\text{-}L1^+$ dual expressing targets, and (3) in vitro T-cell proliferation compared with the traditional CD33 × CD3 BiTE (see figure panel D). Importantly, the CiTE exhibited less binding to $CD33^-/PD\text{-}L1^+$ targets compared with the single-chain triplebody. This decreased binding of the CiTE to CD33-negative targets may have reduced the body weight loss (potentially immune-related adverse event) associated with the single-chain triplebody in a murine xenograft model.

The CD33 target antigen appears during commitment of hematopoietic stem cells to the myelomonocytic lineage and is expressed on ~90% of AML myeloblasts. It is also expressed on monocytes, myeloid dendritic cells, and less so, on macrophages and granulocytes.[2] The ligands for PD-1 and the PD-1_{ex} domain included in the new CiTE are PD-L1 and PD-L2. Both ligands are constitutively expressed on B cells, dendritic cells, and macrophages, and proinflammatory signals are known to induce much higher levels of both PD-L1 and PD-L2.[8] Taken together, these expression patterns suggest that the new CiTE may target normal myeloid cell populations in addition to AML blasts. This targeting may exacerbate cytokine release syndrome, the major dose-limiting toxicity observed to date with bispecific antibody therapy. Future preclinical studies examining the new CiTE molecule in humanized NSG mice reconstituted with human T cells and carrying established primary human AML tumors are warranted.

Early phase clinical trials combining T-cell recruiting antibody constructs with PD-1/PD-L1 antibodies are ongoing. The increased specificity afforded by the new PD-1_{ex} domain-containing CiTE described by Herrmann et al might be an important step forward if immune-related adverse events are limiting in these initial trials.

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PLATELETS AND THROMBOPOIESIS

Comment on Morales-Ortíz et al, page 2495

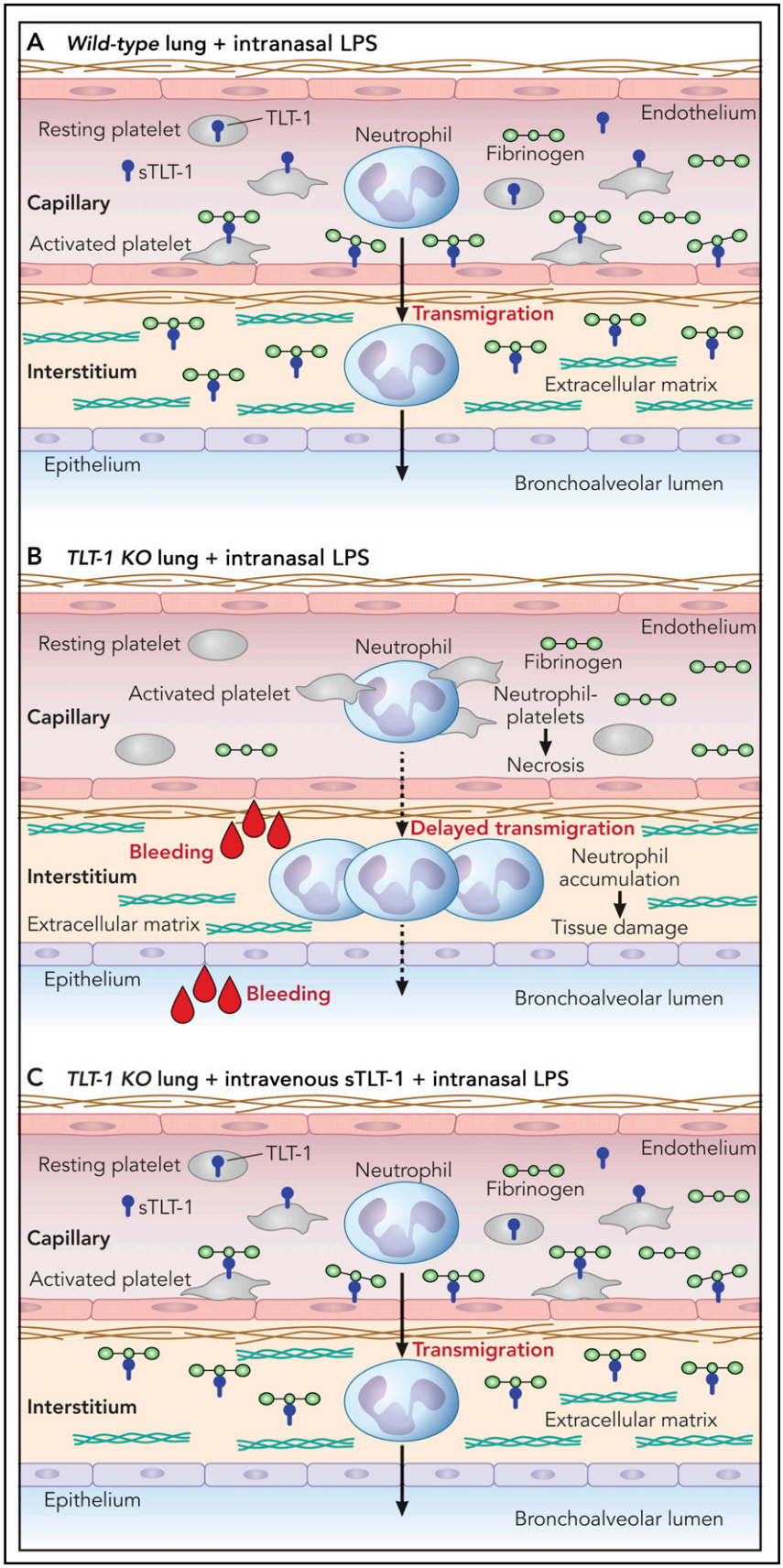
TLT-1: please release me, let me go

Yotis A. Senis | University of Birmingham

In this issue of *Blood*, Morales-Ortíz et al establish triggering receptor expressed in myeloid cells (TREM)-like transcript-1 (TLT-1) as an independent risk factor for acute respiratory distress syndrome (ARDS), and they show in a murine model of acute lung injury (ALI) that TLT-1 mediates fibrinogen deposition in the lungs and facilitates platelet-neutrophil release during transmigration.[1]

TLT-1 is a type-1 transmembrane protein that is highly and exclusively expressed in megakaryocytes and platelets.[2-4] Structurally, it consists of a single immunoglobulin-variable domain in its extracellular region, a transmembrane domain, and a cytoplasmic tail containing 2 highly conserved tyrosine residues, 1 of which is found within an immunoreceptor-tyrosine-based inhibitory motif (ITIM) consensus sequence (I/V/LxYxxL/V, where x represents any amino acid), and the other is found within an ITIM-like sequence.[5] Phosphorylation of these tyrosine residues provides a docking site for the Src homology-2 (SH-2) domain-containing tyrosine phosphatases Shp1 and Shp2 in activated platelets.[3,4]

TLT-1 is stored in α-granules and possibly in another platelet compartment, and it is rapidly upregulated to the surface of activated platelets.[4,6] A soluble form of TLT-1 (sTLT-1) also exists, plasma levels of which are significantly elevated in sepsis.[7] The majority of sTLT-1 is shed from the surface of activated platelets,



Schematic representation of lung response to intranasal lipopolysaccharide (LPS) treatment in (A) wild-type and (B) TLT-1 knockout (KO) mice. (C) Intravenous treatment with sTLT-1 rescues the LPS response in TLT-1 KO mice. Professional illustration by Patrick Lane, ScEYEnce Studios.

and the remainder is an alternatively splicing isoform stored and released from α-granules. Washington et al[7] previously showed that TLT-1 binds fibrinogen, which they further investigate and which features prominently in the Morales-Ortíz et al study. However, despite the high levels of TLT-1 on the surface of activated platelets, which binds fibrinogen, mice that lack TLT-1 exhibit only a minor bleeding diathesis, which raises this question: What is the physiological function of this highly abundant platelet fibrinogen receptor? The answer from Morales-Ortíz et al is that it regulates neutrophil transmigration in inflammatory conditions.

The hypothesis underpinning the study by Morales-Ortíz et al is that platelet TLT-1 regulates the progression of ALI/ARDS. The authors show retrospectively that ARDS patients with plasma levels of sTLT-1 >1200 pg/mL have a 91% increased risk of mortality compared with patients with <1200 pg/mL. Patients with <80 000 platelets/μL or coagulation failure had significantly lower survival rates than those with >80 000 platelets/μL and intact coagulation. These parameters did not, however, maintain significance when controlled for other covariates, including age, ventilator volume, creatinine, and Acute Physiology and Chronic Health Evaluation III scores, whereas sTLT-1 levels did, providing a better independent predictor of mortality than either platelet count or coagulation failure. These findings suggest that platelet-derived sTLT-1 plays a role in the underlying mechanism of disease progression, which the authors explored through the use of a murine model of lipopolysaccharide (LPS)-induced ALI (see figure). TLT-1–deficient mice treated intranasally with LPS showed increase alveolar bleeding, delayed neutrophil transmigration, and accumulation in the lung interstitial tissue, all of which are associated with reduced fibrinogen deposition and increased pulmonary tissue damage compared with control mice expressing TLT-1. Intriguingly, the absence of TLT-1 on platelets resulted in significantly more platelet-neutrophil conjugates and correlated with increased neutrophil necrosis. The interpretation is that TLT-1 somehow releases platelets from neutrophils when they transmigrate into the interstitium through a mechanism that seems to involve platelets sticking to fibrinogen in a TLT-1–dependent manner, details of which remain ambiguous.

Infusion of sTLT-1 intravenously into TLT-1–deficient mice was taken up and detected on the surface of platelets, and it rescued the ALI response in these mice.

Collectively, findings from the Morales-Ortíz et al study not only establish TLT-1 as a new prognostic indicator of ARDS but also identify an important new player in the progression of the condition. As in all good science, additional questions are raised, the most important being how TLT-1 regulates platelet-neutrophil interactions and transmigration, whether a counter-receptor of TLT-1 exists on neutrophils and the downstream signaling pathways triggered in both platelets and neutrophils following engagement, and what sTLT-1-fibrinogen conjugates bind to on endothelial cells and in the vessel wall. This study lays the foundation for further clinical-scientific investigation into the functional roles of TLT-1 in health and disease.

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TRANSPLANTATION

Comment on Swimm et al, page 2506

Stool can soften GVHD

Mette D. Hazenberg | Amsterdam University Medical Centers

In this issue of *Blood*, Swimm et al report that the tryptophan metabolite indole that is produced by the intestinal microbiome limits graft-versus-host disease (GVHD). In mouse models of GVHD, they found that indoles act via type I interferons (IFNs) to limit chemotherapy- and radiotherapy-induced damage to the intestinal epithelium.[1]

The relationship between transplantation hematologists and their patients' gut microbiota has had its ups and downs but has never been as good as in the past years. Taking advantage of novel technologies that no longer depend on culturing to determine the composition of the gut microbiome, Jenq, Taur, and colleagues demonstrated that allogeneic hematopoietic stem cell transplantation (allo-HSCT) is associated with a loss of intestinal microbial diversity that precedes the development of GVHD and has a poor prognosis.[2,3] Subsequent studies showed that when certain bacterial families such as the Ruminococcaceae and the Lachnospiracaea were maintained, the risk to develop GVHD was significantly reduced. Indeed, gut epithelium protective properties have been ascribed to bacteria of the class Clostridiales (to which the Ruminococcaceae and the Lachnospiracaea belong). Via production of the short-chain fatty acid butyrate, these bacteria activate regulatory T cells. Moreover, butyrate protects intestinal epithelial cells against apoptosis and enhances epithelial cell junctions, thereby maintaining epithelial barrier function. In experimental GVHD, supplementation of butyrate mitigated GVHD.[4] Thus, although gut bacteria may still cause harm when they translocate an inflamed and damaged intestinal epithelium, the immune modulating and epithelial homeostasis promoting properties of the intestinal microbiome are increasingly acknowledged and embraced (see figure).

An important question to be answered is whether immunological and epithelial gut homeostasis is maintained by individual bacterial species or by the diversity of the community. Swimm et al may have added a piece to the puzzle, by demonstrating that indoles, either produced in the intestine by *Escherichia coli* or added as a compound (the indole derivative indole-3-carboxaldehyde [ICA]) mitigate GVHD in a dose-dependent manner. In a series of comprehensive experiments, they demonstrated that indoles induce type I IFN genes in epithelial cells, whereas in IFN-I receptor knockout mice that received sublethal irradiation therapy (without HSCT), the gut epithelium protective effect of indoles was abrogated. Indoles are metabolites from tryptophan, an essential amino acid that is metabolized by tryptophanase-expressing commensal bacteria. Swimm et al used *E coli* as an indole-producing commensal in their experiments (and a mutant that lacks the tryptophanase gene). *E coli* belongs to the family Enterobacteriaceae; other gut commensals that produce indoles are, for example, *Enterococcus faecalis* (Enterococcaceae) and *Clostridium sporogenes* (Clostridiaceae). The observation of Swim et al that bacteria-produced indoles and exogenously administered indole derivates mitigate GVHD suggests that the source of indoles and indole derivates is of less importance and that other indole-producing bacteria may have similar effects. In other words, as long as the microbiome as a community provides indoles to protect the gut epithelium against chemotherapy- or radiotherapy-induced damage, the exact composition of the microbiome may be of minor importance.

Indoles have been shown to enhance tight junctions between epithelial cells and enforce the epithelial barrier in the intestine.[5] In addition to this direct effect on the gut epithelium, Swimm et al report that indoles induced tolerance (not anergy) of splenic T cells. To what extent T-cell tolerization contributed to the GVHD mitigating effect of indoles remains unclear. Although tolerized splenic