Xa-dependent coagulation assays. Thus, both observed that acylcarnitines had a hitherto functional effect on coagulation itself. Indeed, particularly, that acylcarnitines would have molecules for playing a causal role, the identifying acylcarnitines as candidate validating the accuracy and precision of the metabolomics were carried out as a means of studies, such is not a possibility; however, Deguchi et al take additional measures to address the correlation/causality issue. First, additional targeted and quantitative metabolomics were carried out as a means of validating the accuracy and precision of the antecedent and unbiased discovery-based untargeted approach. Second, having accomplished the discovery phase goal of identifying acylcarnitines as candidate molecules for playing a causal role, the investigators tested a prediction deduced from their new acylcarnitine hypothesis; in particular, that acylcarnitines would have a functional effect on coagulation itself. Indeed, using controlled assays of coagulation, it was observed that acylcarnitines had a hitherto unappreciated activity of inhibiting factor Xa-dependent coagulation assays. Thus, both verification of the untargeted analyte screen was carried out and the hypotheticoeductive process was explored, testing predictions from the hypothesis that acylcarnitines play a role in VTE (eg, that acylcarnitines would have activity in coagulation assays). Together, these data provide provocative evidence of a new pathway, which may yield insight into both the biology of coagulation and a potential target for therapeutic intervention. As a general property of omics-type explorations of natural phenomena, several essential next steps are required. The first is a mindful consideration that traditional statistics and metrics for significance (eg, P values with a .05 cutoff) are not meaningful in the context of many omics-based approaches. By traditional metrics, a type I error will be made in 1 of every 20 studies by chance alone (measuring a single variable). However, omics-type approaches measure hundreds of variables on each specimen, and for every 100 variables, 5 will be significant by chance alone. Statistics has evolved to test the deviation of P values from this predicted normal distribution, giving rise to more stringent metrics of significance (ie, q values); however, although a low q value indicates likely significance, a higher q value does not rule it out. Moreover, such statistical considerations assume that each variable is independent of each other variable, which is clearly not the case for metabolic pathways, and thus the nature of statistical predictions changes in omics-type studies. A second concern is what is meant by “verification.” A targeted analysis of the same samples on which the untargeted approach was carried out is a form of method verification and tests the accuracy and precision of the untargeted semiquantitative data. However, although this verification addresses whether the observed correlations actually occurred in the cohort studied, it does not verify that such correlations did not happen by chance alone. To evaluate this latter question, a new cohort, distinct from the group analyzed to generate the initial observation, must be analyzed. If the same correlation is observed in a separate cohort, then this will provide much confidence. The above cautions notwithstanding, the data put forth by Deguchi et al demonstrate a distinct biological basis for how acylcarnitines might play a causal role in VTE pathogenesis. These findings represent a new view of coagulation regulation, involving a class of compounds not previously appreciated to be intrinsic to this process. The previously ubiquitous and unnecessarily pejorative descriptor of “fishing expedition” has recently been replaced with the somewhat euphemistic label of “hypothesis-generating studies.” In this case, Deguchi et al have caught a very nice fish and generated a provocative and potentially seminal hypothesis.

Conflict-of-interest disclosure: J.C.Z. has a sponsored research agreement with Immucor Inc. that is unrelated to the current studies.

REFERENCES

DOI 10.1182/blood-2015-07-659532
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TRANSPLANTATION

Comment on Gartlan et al, page 1609

All pain, no gain: Tc17 phantoms in GVHD

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Elusive CD8\textsuperscript{+} T cells that transiently secrete interleukin (IL)-17 cause graft-versus-host disease (GVHD) but do not contribute to beneficial graft-versus-leukemia (GVL) responses, as reported by Gartlan et al in this issue of Blood.\textsuperscript{1} GVHD remains a lethal and morbid complication of allogeneic bone marrow transplantation, but GVHD is tightly linked to beneficial GVL effects, and removal of donor T cells that cause GVHD also diminish GVL, leading to greater relapse after bone marrow transplantation (BMT). This elegant paper from the laboratory of Geoffrey Hill has identified a rare “night fury” T-cell subset that causes much pain with no gain, a finding that may take us one large step closer to the long sought after goal of separating GVL and GVHD.

Cytolytic T cells (CTLs) that mediate GVL effects are predominantly CD8\textsuperscript{+}, and therefore, elimination of the entire CD8\textsuperscript{+} T-cell subset usually leads to greater relapse. Current GVHD prophylaxis efforts, such as calcineurin inhibitors or antithymocyte globulin, are nonspecific and target all T cells. It has not been clear which donor T-cell subpopulations could be eliminated without impairing GVL effects or damaging reconstitution of the patient’s immune system after BMT. The Hill group had previously reported that IL-17, an inflammatory cytokine that is important in autoimmune disease, also mediates GVHD in experimental models.\textsuperscript{2} Here they identified a weakly cytolytic but
highly inflammatory subset of CD8⁺ cells whose removal costs nothing in terms of GVL activity but virtually eliminates lethal GVHD. Because the functions of many T cells are not firmly fixed when they first encounter their targets, these functions can alter during rapid clonal expansion. The authors therefore tracked their quarry using an elegant fate-mapping technique and identified phantom CD8⁺ cells that secrete IL-17 for only a brief period of time but continue to secrete other cytokines such as interferon (IFN)-γ (see figure).

The IL-17 axis is complex and important, particularly as it relates to GVHD in the gastrointestinal tract, which is the target organ most resistant to therapy and most likely to give rise to transplant-related mortality. Subpopulations of T cells that produce IL-17 have already been identified: one is pathogenic and secretes IFN-γ and the other other is nonpathogenic and secretes IL-10.² Tc17 cells also produce IL-22, as the authors demonstrate, but IL-22 can protect intestinal stem cells and can regulate GVHD.³ The story is further complicated by the fact that excess IL-17 can decrease the production of IL-22, disturbing gastrointestinal barrier function, which can be reversed with endogenous IL-22.⁴ The IL-17/IL-22 axis also induces regenerative 3α, a Paneth cell protein that is a validated biomarker of gastrointestinal GVHD.⁵ Thus, IL-17 after BMT is key to the reconstitution of mucosal immunity and the restoration of normal barrier function after BMT, but in excess, it is primarily inflammatory, even for short periods, and can cause severe gastrointestinal damage.

It should be noted that it is not yet clear that these cells are present in clinical GVHD, and given their elusive nature and the morbidity of liver and gastrointestinal biopsies early after BMT in thrombocytopenic and neutropenic patients, it will likely be exceedingly difficult to demonstrate them. Nevertheless, strategies that may be able to eliminate them are already being tested in the clinic. The authors show that IL-6 is a key inducer of inflammatory Tc17 cells (as expected, because Tc17 cells are known to depend on IL-6), and the blockade of IL-6 after BMT prevents their proliferation and subsequent GVHD damage. Recently, an anti–IL-6 receptor monoclonal antibody, tocilizumab, was tested as GVHD prophylaxis in a small clinical trial of 48 patients. The overall incidence of grade 2 to 4 GVHD was 12% and that of grade 3 to 4 GVHD was only 4%. Relapse of leukemia was within the expected range: ~23% at 2 years. This study provides a strong rationale as to why the use of anti–IL-6 is unlikely to increase the risk of relapse even though it significantly reduces GVHD and engenders enthusiasm for larger definitive trials of this approach.

Conflict-of-interest disclosure: The author declares no competing financial interests.

REFERENCES

1. Gartlan KH, Markey KA, Varelias A, et al. Tc17 cells are a proinflammatory, plastic lineage of pathogenic CD8⁺ T cells that induce GVHD without antileukemic effects. Blood. 2015;126(13);1609-1620.

DOI 10.1182/blood-2015-08-661512
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