inside blood commentary

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TRANSPLANTATION

Comment on Suessmuth et al, page 3835

CMV after transplant: T-cell repertoire crooks

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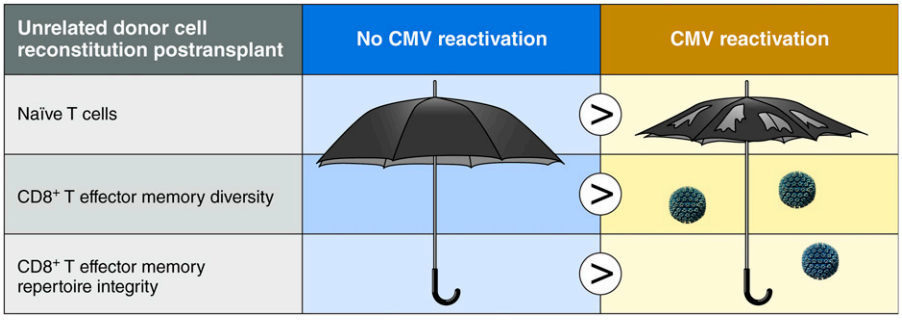
In this issue of *Blood*, Suessmuth et al demonstrate that cytomegalovirus (CMV) reactivation in unrelated donor transplant recipients associates with quantifiable defects in T-cell repertoire recovery.[1]

Reconstituting the donor immune system to normal functionality is a critical part of hematopoietic stem cell transplantation. Although transplant outcomes have steadily improved because of better management of common posttransplant immune complications such as CMV reactivation, we remain largely in the dark about how to measure and define a normal immune repertoire.

In the last 6 years, high-throughput nucleotide sequencing of T- and B-cell receptors has advanced from infancy to an early new articulation of the immune repertoire.[2] For T cells, the method involves the targeted polymerase chain-reaction amplification of the T-cell receptor Vβ variable region usually through the use of multiplexed primers that bind to V and J subunits. The end result is a detailed list of T-cell Vβ sequence clones and their relative frequency in any sample. At the same time, a new global view could provide us with a comprehensive window into T-cell population dynamics, especially as the statistics of measuring T-cell repertoires develop.

How CMV alters T-cell population dynamics is an area of intensive research in part because CMV is among the viruses with the strongest influence on T-cell immunity. By comparing people with and without CMV, studies have estimated that an enormous fraction of the T-cell repertoire is devoted to controlling the virus.[3] CMV infection is linked to the depletion of the naïve T-cell pools and other hallmark markers of early immune aging in thymectomized patients and the general population.[4,5] In the posttransplant setting, CMV reactivation strongly associates with abnormal immune reconstitution.[6,7]



Depiction of T-cell repertoire deficits at 1 year after allogeneic hematopoietic stem cell transplant in recipients who reactivate with CMV. Professional illustration by Patrick Lane, ScEYEnce Studios.

In the present study, Suessmuth et al study how CMV reactivation affects T-cell dynamics in 17 patients who had myeloablative unrelated donor allogeneic transplant for myelodysplastic syndrome, acute leukemia, or myelofibrosis. These patients had posttransplant immune suppression with a calcineurin inhibitor and methotrexate with (n = 10) and without abatacept (n = 7). Seven recipients reactivated CMV (>300 copies/mL) that resolved by 1 year after transplant. None of the patients had CMV organ involvement.

On first pass, those transplant recipients with CMV reactivation might appear to be doing smashingly well at 1 year after transplantation, with significantly more peripheral blood T cells than their nonreactivating counterparts or even healthy controls. However, closer inspection shows that the process of CMV reactivation props up a straw man of CD8 T-effector memory cells (Tem) so strong that it inverts the normal ratio of CD8 to CD4 T cells. These CD8 Tem cells show severe repertoire skewing favoring many more copies of fewer T-cell clones. The authors provide evidence that these expanded CD8 clones are involved in CMV immunity directly because T-cell receptor sequences of CMV-specific T cells sorted from recipients occur at a remarkably high frequency in the CD8 Tem fraction.

Although these detailed results advance reports of the same general phenomena, a strength of the study is that it provides a nascent tool to answer unresolved questions, such as: (1) what happens to the T-cell repertoire eeking out life around the swell of CMV-reactive CD8 Tem cells?; and (2) what is their T-cell repertoire integrity?

To begin to answer these questions, the authors use a novel approach to measure T-cell

repertoire integrity. They first established what diversity of T cells one might expect to find in any human subject by compiling the V and J subunit use of normal healthy controls as a sort of VJ use reference nomogram. They then compare the V and J use of T-cell clones in the CD8 Tem of recipients with CMV reactivation vs nonreactivation to the reference. They show that recipients with CMV reactivation have more pockets of V and J use with significantly fewer clones than expected. Thus, these patients appear to have more repertoire "holes" than expected, likely explained by CMV's crooked actions in shaping T-cell immunity (see figure).

Transplant recipients with CMV reactivation also show statistically fewer naïve CD8 T cells, fewer CD8 memory T cells, and fewer CD4-naïve CD31$^+$ recent thymic emigrants. These results suggest a potential defect in the repertoire needed to establish de novo adaptive immunity.

CMV-specific T cells are often dysfunctional in transplant patients with CMV reactivation.[8] In addition, not all transplant patients who are CMV carriers reactivate. This leads to more questions. Are the repertoire defects caused by the CMV virus itself or other underlying susceptibilities in the genetic and cell architecture of the immune system, and if so, what are these factors? How can we contextualize potentially beneficial aspects of CMV reactivation like reduced relapse for patients with acute myeloid leukemia[9]?

Although this study of relatively few patients is a landmark evaluation of T-cell repertoire health and CMV, there are some limitations. Many of the patients in the study had graft-versus-host disease and different immunosuppression doses. Both factors interact with CMV and affect T-cell repertoire. The authors looked at this and saw little effect on repertoire, but this needs further evaluation in more patients and transplant conditions.

The metric of repertoire "holes" is new, with some assumptions that have yet to be fully explored. We do not know if the reference VJ use "nomogram" employed uses the best human control comparator statistically. This study uses state-of-the-art sequencing depth, but this is still a very shallow level, many orders of magnitude below the T-cell population size in any human; therefore, although holes might exist at this sampling depth, does that really indicate a problem? What about T-cell subsets other than CD8 Tem, do they show the same patterns?

Despite these caveats, Suessmuth et al help advance how we interrogate the immune system after transplantation. Perhaps this takes us a step closer to using quantitative measurements of the adaptive immune system in clinical decision-making. As a result of studies like this, one day we may be answering a new type of question from our patients: "How's my T-rep doing, doc?"

Conflict-of-interest disclosure: E.M. is a founder of and holds stock options in GigaGen, Inc. ■

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Comment on Burnett et al, page 3878

Beyond the first glance: anthracyclines in AML

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In this issue of *Blood*, Burnett et al present the results of United Kingdom (UK) National Cancer Research Council Acute Myeloid Leukemia 17 (NCRI AML17), a randomized comparison of daunorubicin (90 vs 60 mg/m^2) in induction in 1206 patients with acute myeloid leukemia (AML), showing no benefit to the higher dosing.[1]

AML is a heterogeneous disorder with devastating consequences. It requires an aggressive treatment approach to control the disease and ultimately cure it. AML has been treated in a similar fashion for >40 years, with variations on the main theme. The mainstay of therapy has been an injected anthracycline (usually for 3 days) and continuously infused cytarabine for 7 days.[2] The addition of other medications to this duet or dose intensification of cytarabine has not significantly improved outcomes.[3-5]

More recent studies have looked at the dose intensification of the anthracycline component to improve complete remission (CR) rates and overall survival (OS). The benefit of this method was first suggested by the large randomized trial from the Eastern Cooperative Oncology Group (ECOG), where high-dose daunorubicin of 90 mg/m^2 for 3 days was superior to the then standard of 45 mg/m^2 for 3 days.[6] This improvement was noted in favorable and intermediate-risk disease. With further follow-up, the benefit has remained for these groups of patients and is now evident in unfavorable risk, including *FLT3-ITD*–positive disease.[7]

Other anthracyclines and dosing schedules of anthracyclines have recently been compared with daunorubicin. Most have demonstrated