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TRANSPLANTATION

Comment on Thangavelu et al, page 1090

New hope offered to reduce GVHD

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In this issue of *Blood*, Thangavelu et al have developed a novel pharmacological approach to inhibit graft-versus-host disease (GVHD) by activating retinoid X receptor (RXR) in donor T cells.[1]

The success of allogeneic hematopoietic cell transplantation (allo-HCT) has been hampered by GVHD, which is initiated by host antigen-presenting cells (APCs). GVHD is characterized by the induction of donor effector T cells, which recognize host antigens, and the suppression of functional regulatory T cells (Tregs).[2] Donor effector T-cell expansion is a prerequisite for GVHD induction. Alloreactive effector T cells produce high levels of proinflammatory cytokines (eg, interferon-γ [IFN-γ] and tumor necrosis factor-α) and cytolytic molecules (eg, Fas ligand, perforin, and granzyme B).

Alloreactive effector T cells mediate tissue injury during GVHD and selective inhibition of alloreactive effector T cells by targeting the epigenetic regulator Ezh2 can arrest ongoing GVHD.[3]

$CD4^+$ Tregs are also an important cell population that is needed to control GVHD.[4] FoxP3 expression levels are important for Treg development and function.[5] Natural Tregs (nTregs) develop within the thymus. However, hematopoietic cell transplantation (HCT) grafts may contain insufficient numbers of nTregs to suppress alloreactive T-cell responses, and the use of nTregs in HCT would require ex vivo expansion. In contrast, induced Tregs (iTregs) arise from activated $CD4^+$ T cells in the periphery, but they are often unable to maintain their suppressive activity because of unstable FoxP3 expression. Furthermore, GVHD inflammation and immunosuppressive calcineurin inhibitors (CNIs; used for GVHD prevention) are known to impair Treg expansion and function.[6,7] As such, novel and clinically relevant approaches that could reduce alloreactive effector T-cell expansion and induce stable iTreg generation would be ideal for GVHD prevention.

RXRs are master regulators that control cell growth, differentiation, and survival.[8] Studies have suggested that RXRα signaling in T cells suppresses differentiation of IFN-γ–producing T helper 1 (Th1) cells in mice.[9] Du et al demonstrate that mice with a mutation of Rxra (I273N) have a dramatic decrease in ligand-inducible transactions. Homozygous RXRα I273N mutant mice had severe alopecia, exacerbated Th1 responses, and decreased Tregs' suppressive functions.[9] Interestingly, when naive $CD4^+$ T cells were stimulated under Th1–skewing or mixed Th1/Th2 conditions, RXRα I273N cells produced significantly higher amounts of IFN-γ than their wild-type counterparts, indicating a direct impact of RXRα signaling on inhibiting Th1 cell differentiation.[9] Thus, under physiological conditions, RXRα signaling represses $CD4^+$ T-cell differentiation into Th1 cells while promoting Treg function.

Thangavelu et al demonstrate that administration of the RXR homodimer–selective agonist IRX4204 decreases the generation of Th1 cells and promotes Treg generation, leading to inhibition of GVHD while preserving antileukemia activity. IRX4204 treatment reduced donor T-cell proliferation and Th1 differentiation, decreased intestine injury, and reduced expression of genes critical for regulating proinflammatory pathways (eg, Sema7a, Stat1, Irf1). Notably, IRX4204 activation of RXR signaling leads to enhanced Treg generation and maintenance. The investigators found that IRX4204 treatment in vivo under GVH conditions increased the conversion of donor $FoxP3^-$ T cells into peripheral $FoxP3^+$ Tregs in mice and stabilized them by sustaining FoxP3 expression. The direct effect on Tregs was also confirmed using in vitro cultures of murine and human T cells. IRX4204 failed to prevent acute GVHD in recipients given $CD25^-$ T cells derived from scurfy donor mice that have a deletion of FoxP3. Two important conclusions can be drawn from these findings. IRX4204-mediated repression of GVHD requires the presence of functional Tregs, despite its suppressive effects on Th1 responses against host tissues. Additionally, in contrast to the CNI FK506 (aka tacrolimus)–mediated suppression of Tregs, IRX4204 treatment provides a beneficial effect on Treg generation and maintenance.

The investigators' success in this preclinical study of IRX4204 opens new possibilities for exploring T-cell alloimmunity. For example, what is the mechanism by which IRX4204-activated RXR signaling reduces Th1 differentiation? The investigators correlate the inhibition of Th1 differentiation with decreases in CD98, Glut1, and carnitine palmitoyl-transferase 1 (CPT-1), which are known to be important for regulating T-cell metabolism. However, these molecules are found to be readily upregulated in alloantigen-activated T cells.[10] Will the inhibition of alloreactive T-cell expression of CD98, Glut1, and CPT-1 result in impaired T-cell proliferation? In addition, genetic inactivation of RXRα increases interleukin-12 production by dendritic cells,[9] which may augment effector T-cell responses. Will IRX4204 reduce APC activation and, thereby, modulate Th1 cell responses in vivo?

In summary, the investigators provide clear evidence that activating RXRs with IRX4204 inhibits GVHD via complex effects on alloreactive effector T-cell

expansion and Treg stability and function. Because IRX4204 is in clinical trials for cancer treatment, repurposing to attenuate acute GVHD in allo-HCT is warranted and feasible.

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

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LYMPHOID NEOPLASIA

Comment on Ollila et al, page 1120

CNS relapse in DLBCL: a calculable risk?

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In this issue of *Blood*, Ollila et al[1] address a challenging problem: Can the risk for central nervous system (CNS) relapse in patients with diffuse large B-cell lymphoma (DLBCL) be predicted better on a molecular basis? The authors found that most tumors with CNS recurrence are well defined molecularly and fall into 2 categories. The most frequent is the hc-MCD subtype, based on MYD88L265P and CD79B mutations, which molecularly resembles primary central nervous system lymphoma (PCNSL). Its association with some extranodal disease sites suggests that the molecular underpinning is the major factor driving the increased risk of CNS invasion. The second subgroup encompasses high-grade tumors characterized by double-hit biology or TP53 mutations, which frequently exhibit high-grade B-cell lymphoma signature on gene expression profiling.

To search for a molecular subtype that could be used to identify patients at high risk for CNS recurrence of DLBCL, the authors used a clinically validated 592-gene assay. DLBCL can be clustered into distinct molecular subgroups.[2,3] Further analyses have also revealed different molecular signatures for both extranodal DLBCL and PCNSL.[4,5] Currently, the decision on CNS prophylaxis relies on clinical risk stratification. Nevertheless, many patients have a CNS relapse despite being considered low risk for a CNS recurrence by these criteria at initial diagnosis. Thus, extending this risk stratification to include additional molecular information, as Ollila et al explored, is an interesting approach and of great clinical importance. Secondary involvement of the CNS is a dreaded complication of DLBCL and is associated with an extremely poor prognosis. In the MiNT study, the median time to secondary CNS involvement was 7.2 months; with a median survival of only 3.5 months after development of CNS involvement.[6] The so-called CNS International Prognostic Index (CNS-IPI) is a useful tool to roughly estimate the risk of CNS involvement during the course of disease.[7] Because kidney and adrenal gland involvement is significantly associated with CNS recurrence, the CNS-IPI includes these parameters in addition to the classic IPI, which is validated for systemic DLBCL. The CNS-IPI identifies 3 risk groups: low, intermediate, and high; the latter accounts for 12% of all patients analyzed in this cohort. Patients assigned to the high-risk group carry a 10.2% risk of subsequent CNS-involvement. Conversely, only about half of patients with secondary CNS involvement during the disease course, were considered CNS-IPI high risk at their initial systemic-lymphoma diagnosis. Gene expression analyses of 1418 patients of the GOYA trial showed that activated B-cell–like and unclassified cell-of-origin (COO) subtypes were associated with CNS relapse in DLBCL, irrespective of a high CNS-IPI score.[8] Currently, the addition of intravenous high-dose methotrexate (MTX) or intrathecal MTX to standard chemotherapy with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) is recommended for patients at a high risk for secondary CNS involvement. However, this approach's effectiveness has only been evaluated in a single retrospective trial.[9] The challenge we face is to develop a prognostic score that reliably predicts CNS recurrence and that better targets the use of CNS prophylaxis. To address this challenge, the authors recommend a validated 592-gene assay, used on formalin-fixed, paraffin-embedded material, to identify a subgroup at significantly higher risk for secondary CNS involvement. In my opinion, the authors describe a promising approach that succeeds via next-generation sequencing (NGS). The LymGen classifier recently published by Wright et al[10] divides DLBCL into different clusters. On the basis of the subgroups described therein, the authors ultimately constructed a simplified hierarchical classifier (hc) with 3 subtypes: