

and effective safety switches that can be deployed should severe GVHD be observed.^{9,10}

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

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● ● ● TRANSPLANTATION

Comment on Foley et al, page 2665

Natural revenge over cytomegalovirus

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In this issue of *Blood*, Foley et al show that, in allogeneic hematopoietic transplant recipients, donor-derived natural killer (NK) cells respond to cytomegalovirus (CMV) by acquiring features that are reminiscent of the specificity and memory of adaptive (T cell) immune responses.¹

Unfortunately, after allogeneic hematopoietic transplantation CMV disease is still, even in the era of the pre-emptive ganciclovir therapy, a major cause of death. Susceptibility to CMV reactivation is linked to immune incompetence as induced by conditioning regimens, immune suppressive measures to prevent/control graft-versus-host disease (GVHD), and the time needed for immune system maturation.

CMV has evolved escape mechanisms to evade both adaptive (CD8⁺ T cells) and innate (NK cells) immune responses. Virus-encoded genes down-regulate class I human leukocyte antigen (HLA) expression, which impairs antigen presentation and diminishes T-cell recognition but renders infected cells susceptible to NK-cell lysis (reviewed in Foley et al¹).

NK cells are well recognized for their ability to provide a first line of defense against viral pathogens. Their function is regulated by a balance between activating and inhibiting receptors. Clonally distributed inhibitory killer cell immunoglobulin-like receptors (KIRs)

recognize allotypic determinants shared by certain groups of HLA class I alleles. On interaction with self-HLA molecules, NK cells, which express inhibitory KIRs for self, are “licensed/educated” to become fully functional, exert “missing self-recognition,” and therefore, kill target cells that bear down-regulated HLA class I molecules, such as CMV-infected cells.² To escape NK-cell control, CMV encodes viral glycoproteins that mimic class I HLA (eg, UL-18) and interfere with expression of ligands for the activating NK receptors NKG2D and DNAM-1 (reviewed in Foley et al¹).

Foley and colleagues show a specific NK-cell subset expands and persists over time in response to CMV reactivation, suggesting a long-lasting specific memory had developed. As NK cells are the earliest immune cells to recover after transplantation, this observation is significant because it suggests they may contribute to controlling viral reactivation early after transplantation. Specifically, the authors show that NK cells expressing the NKG2C

activating receptor expand, display a mature CD56^{dim}/CD57⁺/NKG2A⁻ phenotype, produce IFN- γ , persist over time, and express KIR for self (donor)-HLA class I. NKG2C⁺ NK cells that expanded after CMV reactivation responded more robustly (ie, they produced more IFN- γ) when they expressed KIR that was specific for self-HLA class I molecules. The authors concluded that NKG2C⁺ NK cells were “licensed” through self-HLA recognition and proposed CMV-favored NK-cell education. The authors suggest NKG2C was probably directly involved in the response to human CMV.

Thus, their observations may be biologically very relevant as they are providing a model of virally induced NK-cell education in the setting of allogeneic hematopoietic transplantation. These data are in line with observations of a relationship between CMV and NKG2C expression. Normal immunocompetent CMV-seropositive individuals have an increased proportion of NK cells expressing NKG2C; this percentage remains high for years after CMV infection.³ Moreover, after solid organ transplantation, CMV reactivation is associated with expansion of NKG2C⁺ NK cells.⁴ Further investigation is needed to demonstrate whether the NK-cell NKG2C activating receptor specifically recognizes CMV and, if so, the exact nature of the putative CMV ligand. Most importantly, it remains to be seen whether, and to what extent, NK cells contribute to attenuate CMV reactivation in allogeneic hematopoietic transplant recipients.

Intriguingly, experimental evidence in mice showed that transfer of NK cells recognizing the CMV m157 protein from CMV-exposed mice protected naive mice from CMV challenge.⁵ Thus, NK cells are now known to display specificity and memory, that is, properties that are conventionally attributed only to T cells. Very interestingly, in murine models and in clinical trials, NK cells have been clearly demonstrated not to cause GVHD, even when specifically alloreactive across major MHC barriers.^{6,7} Thus, unlike T cells that carry the risk of causing lethal GVHD, NK cells are safe as a form of cellular immunotherapy in the allogeneic hematopoietic transplant setting. Furthermore, alloreactive NK cells are strong mediators of graft-versus-leukemia effects in T cell-depleted haploidentical hematopoietic transplantation for acute myeloid leukemia.^{6,7} In this regard, it is worth noting that after unmanipulated

HLA-matched sibling or unrelated hematopoietic transplantation, CMV reactivation was associated with a lower risk of leukemia relapse.⁸ The effect might have been because of CMV-induced NK-cell maturation and consequent elimination of residual leukemic cells.

Thus, an exciting potential clinical application of the data presented by Foley et al might be the use of donor NK-cell immunotherapies after transplantation to help control not only leukemia relapse, but also CMV reactivation.

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

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filipodia formation, in agreement with previous findings from Kuehbacher and colleagues where they showed that inhibition of miR-27b significantly reduces endothelial cell sprouting in an in vitro setting.⁵ Urbich and colleagues showed that miR-27a/b promotes angiogenesis by targeting endogenous angiogenesis inhibitor SEMA6A and controlling endothelial sprouting.⁶ Now Biyashev et al demonstrate that Dll4 and Spry2 are targets of miR-27b and therefore the effectors of miR-27b action on the angiogenic switch. Dll4 and Spry2 have been previously implicated in vascular guidance and branching of the tubular structures and further supports this study.^{7,8} miR-27b acts posttranscriptionally by silencing Dll4 and Spry2 expression that contributes to vessel sprouting and venous specification. This report also shows that miR-27b controls arterial-venous specification via silencing of Dll4, thus impairing Notch and Ephrin B2 (EfnB2) signaling. Spry2 silencing further enhances Flt4, another determinant of venous specification (see figure). Importantly, knockdown of Spry2 or Dll4 activity rescues the phenotype resulting from miR-27b silencing in zebrafish and mice.

The structural differences between arteries and veins were largely attributed to distinct blood flow dynamics and structural differences. But lately, distinct genetic footprints associated specifically with either arterial or venous endothelial cells have been identified.⁹ This reports by Biyashev et al adds to such previous studies and identifies miR-27b as a molecule that participates in differentiating between arterial and venous endothelial cells via controlled expression of EfnB2, EfnB4, Flt1 and Flt4. Biyashev and colleagues further show that pigment epithelial-derived factor (PEDF), an endogenous angiogenesis inhibitor, can down-regulate miR-27b in the activated endothelial cells, thus leading to impaired angiogenesis via up-regulation of Dll4 and Spry2 proteins and affecting venous specification.

Recently the miR-23b cluster, of which miR-27b is part, as been demonstrated to have proangiogenic properties and targets Spry2 and Semaphorin.¹⁰ While miR-23b regulates Spry2, whether this represents a direct targeting of by the miR-23b cluster remains unknown. Collectively, Biyashev and colleagues demonstrate the involvement of miR-27b in the control of Notch signaling and regulating

● ● ● VASCULAR BIOLOGY

Comment on Biyashev et al, page 2679

Angiogenesis is controlled by miR-27b associated with endothelial tip cells

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In this issue of *Blood*, Biyashev and colleagues report their findings about the critical role of miR-27b in controlling endothelial tip cell fate, branching and venous specification through down-regulation of the newly described miR-27b targets, Spry2 and Dll4.¹

Angiogenesis is a fundamental and dynamic process in vertebrates for generation of new blood vessels and capillaries from pre-existing blood vessels. This process initially involves proliferation, sprouting, and migration of endothelial cells. The newly generated blood vessel sprout is guided by migrating tip cells, in response to growth factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). Endothelial cells are extremely sensitive to signals emanating from the extracellular microenvironment and a substantial number of studies implicate endothelium-associated small noncoding RNAs, known as microRNAs (miRNAs), in fine-tuning this process.

The first evidence implicating miRNAs in the regulation of angiogenesis was provided by a study involving mice homozygous for a hypomorphic allele of DICER1, an endoribonu-

lease in the RNase III family that is essential for processing of functional miRNAs.² These hypomorphs lacked angiogenesis and died between embryonic days 12.5 and 14.5. This and other studies have clearly implicated a pivotal role for endothelial miRNAs in the regulation of angiogenesis. miRNAs that promote angiogenesis include miR-126, miR-130a, miR-210, and miR-292. The most studied is miR-126, an endothelial cell-specific miRNA that promotes angiogenesis in response to proangiogenic stimuli, and represses negative regulators of angiogenic signaling pathway.^{3,4}

In this report Biyashev and colleagues show that miR-27b has a crucial role in determining tip cell sprouting and is also involved in venous specification. They demonstrate that silencing of miR-27b in zebrafish and mouse tissue impairs vessel sprouting and



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Natural revenge over cytomegalovirus

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