Fingering a Natural Culprit During Antibody-Mediated Rejection

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The introduction of currently used immunosuppression regimens has decreased the incidence of T cell-mediated graft rejection.¹ Alternatively, the incidence of antibody-mediated rejection (AMR) is more frequently observed and is less sensitive to this immunosuppression. The production of donor-specific antibodies (DSA) is associated with acute graft injury and loss, as well as with chronic injury that promotes fibrogenesis leading to the late graft loss that continues to undermine long-term graft survival. The mechanisms by which DSA mediate acute and chronic graft injury have been difficult to dissect and remain largely unclear. Recognition of AMR as an important problem in clinical transplantation has motivated the development of animal models to test potential mechanisms underlying DSA-mediated allograft and xenograft injuries. However, differences in human versus rodent Fc receptors with regard to cellular distribution, affinity for binding antibody, and activation versus regulatory function have added additional layers of complexity that may limit contributions of animal models to clinically relevant insights to AMR.

Earlier studies from Hidalgo and colleagues² investigating expression of transcripts in kidney graft biopsies from patients with versus without detectable DSA indicated the appearance of unique endothelial-associated and NK cell-associated transcripts and the presence of CD16⁺, but not CD3⁺, cells in biopsies from patients with high DSA. These investigators then went on to document distinct patterns of transcript expression in biopsies from grafts with early/cell-mediated rejection versus late/AMR.³ Early cell-mediated rejection was associated with transcripts indicative of T cell activation within the allograft (eg, CD3D, TcR α chain, and CXCR6) whereas the late AMR episode transcript profiles were indicative of NK cell activation (eg, CX₃CR1, KLRF1, Mybl1, and Sh2D1B). These initial studies indicated the presence of NK cells in the microcirculation of kidney grafts during AMR. However, it has been more difficult to provide insights into

mechanisms of NK cell function that might mediate kidney graft injury during AMR. In vitro studies have demonstrated the role of the NK cell-expressed Fc receptor, CD16a/Fc γ RIIIa, in antibody-mediated activation of NK cells to produce cytokines and mediate antibody-dependent cell-mediated cytotoxicity, resulting in lysis of tumors and allogeneic bone marrow cells.⁴

In the current issue of *Transplantation*, Parkes⁵ have used a clever strategy to link CD16a signaling during NK cell activation within kidney grafts during AMR. First, activation of peripheral blood NK cells in vitro by crosslinking CD16a resulted in the upregulated expression of more than 270 transcripts when compared with transcript profiles from control, nonactivated NK cells. Then, the CD16a-activated NK cell transcript profile was compared with the profile obtained in kidney graft biopsies during AMR to identify 8 shared transcripts. Two of these shared transcripts are unique to NK cells, the chemokine XCL1 and CD160, a glycoprotein expressed on NK cells and T cells. One interesting facet of these studies is that the upregulation of the previous NK cell transcript profile they had reported during AMR was not observed during crosslinking of CD16a on the NK cells, suggesting that expression of these genes is more likely constitutively expressed by NK cells.

The key observation of these studies is the identification of 2 genes expressed during CD16a-mediated activation of NK cells that appear in biopsies during ongoing AMR. An obvious caveat is that such CD16a-mediated NK cell activation is certain to occur in an inflammatory environment within the graft that is not present in the in vitro study design. DSA binding to the graft microvasculature may induce the expression of endothelial adhesion molecules, chemokines, or other proinflammatory cytokines that could synergize with signals transduced by CD16a crosslinking to provoke additional functions of the NK cells during AMR. With this in mind, it might be worthwhile extending the current in vitro studies by testing the impact of integrin, chemokine receptor, and/or cytokine stimulation on the transcription profile following CD16a crosslinking on NK cells.

Although these studies provide strong evidence linking NK cell activation through CD16a crosslinking with AMR, the CD16a induced functions that mediate graft injury remain unclear. In mouse models, cotransfer of allograft-reactive monoclonal antibody and NK cells to immunodeficient recipients of heterotopically transplanted heart allografts has promoted the development of graft aortic vasculopathy.^{6,7} These studies have identified NK cell-derived IFN- γ as an important mediator of this vasculopathy, and studies from the Gill laboratory have indicated that both NK cell IFN- γ and cytolytic function mediated through either FasL or

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perforin/granzyme B are required for the cardiac allograft vasculopathy. NK cells have also been implicated in antibody-independent kidney allograft injury in wild type and Rag1^{-/-} mice as allograft injury was reduced in recipients treated with NK cell-depleting antibodies.⁸ In support of the clinical studies, a mouse model of kidney allograft AMR indicates the requirement for NK cells to reject the allografts and expression of the NK cell-associated transcripts in the allograft during AMR, including CX₃CR1, Mybl1, and Sh2D1B.⁹ Collectively, these animal models have provided strong evidence that NK cells can mediate antibody-dependent chronic injury/vasculopathy of heart allografts and AMR of kidney allografts. Furthermore, studies by the Chong laboratory have demonstrated that IgG1 antibody-mediated hyperacute rejection of xenogeneic rat hearts in α Gal-deficient mice is dependent on Fc γ receptor expression on NK cells.¹⁰

The results of the current report from Parkes⁵ are important in linking CD16-mediated NK activation with kidney graft injury during AMR. These studies as well as those in animal models are now building strong evidence that NK cells function to mediate graft injury during AMR. Different parameters including the specificity and isotype of the graft reactive antibodies, the microvasculature of the transplant, and the inflammatory environment are likely to impact NK cell function during AMR. It will be important to understand the synergy of these factors with NK cell function in mediating graft injury and to identify targets that can be neutralized to inhibit

or attenuate the intensity of ongoing AMR that is confounding the success of organ transplantation.

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