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The AJT Report

News and issues that affect organ and tissue transplantation

2017 American Transplant Congress Focuses on Hot Issues

Researchers presented work on allorecognition, mitochondrial targets, innate immune response, and other timely topics

This spring, McCormick Place in Chicago was packed with members of the transplantation community for the 2017 American Transplant Congress, held April 29 to May 3. The meeting is an important collaboration for the American Society of Transplant Surgeons (ASTS) and the American Society of Transplantation (AST).

Following are highlights from some of the key topics presented at this year's event.

New Research

Mitochondria. Dr. Li began with what he referred to as "the almighty mitochondria," drivers of tissue inflammation, and referenced a recently published review article concluding that mitochondrial targets could be exploited to modulate inflammation and cancer.[1]

He then drew the audience's attention to research presented by investigators at Duke University in Durham, N.C., that detailed how endothelial cells internalize extracellular mitochondria. The researchers concluded that internalized mitochondria influence the activation of the vascular endothelial cells, and this process can lead to alloreactive T cell activation as well as the promotion of graft rejection. They also hypothesized that tissue injury may result in the release of mitochondria, which can in turn activate endothelial cells. The mitochondrial release thus would serve as a danger signal that initiates allograft rejection. If this hypothesis proves true, this pathway could represent a link between innate and adaptive immunity during the initiation of the alloimmune response.

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The findings are intriguing and raise many questions, noted Dr. Li. "The cells do not randomly take extra mitochondria," he pointed out. "There must be a reason for endothelial cells to take in extra mitochondria."

Additionally, research out of Harvard Medical School in Boston presented at the conference indicates that mitochondrial DNA augments inflammation in aging. Specifically, aging increases the level of mitochondrial DNA, which in turn increases the likelihood that dendritic cells will be activated to orchestrate proinflammatory T cell responses. These T cell responses could then affect the survival of older grafts. The entire process might clarify the association between organ age and increased susceptibility to acute kidney injury.

Microbiota. Dr. Li then moved to the next hot topic, impactful microbiota. He explained that gut microbiota can now be classified in detail based upon 16S ribosomal RNA sequencing. "Remember, this is an ecosystem," said Dr. Li, "and they have to exist in a balanced, harmonious way." The ecosystem can vary depending on the circumstances of the individual, and new research indicates that the microbiome of the stool of pregnant mice differs substantially from that of nonpregnant mice, and from colitic mice.

Of interest to the transplantation community is the fact that *Bifidobacterium* in stool from pregnant mice can modulate cardiac allograft inflammation and fibrosis. In addition, researchers from the University of Chicago in Illinois reported to the audience that cutaneous commensal colonization is sufficient to accelerate skin graft rejection. They also suggested that changes in the local microbiota of a colonized organ can actually impact graft outcome. The work has led to the targeting of microbial constituents in specific anatomic compartments to possibly enhance graft survival.

Innate immune system. The renaissance of the innate immune system reigned as another hot topic at the Congress. "We are getting much better at studying the innate immune system," said Dr. Li, who described the work of Hehua (Lily) Dai, MD, a postdoctoral fellow, and colleagues at the University of Pittsburgh in Pennsylvania. The team found that cells of the innate immune system are capable of allorecognition. Moreover, these innate cells can exhibit adaptive features and can recognize antigens. Researchers in the same laboratory have also found that dendritic cells can crossdress with the major histocompatibility complex from donor grafts. These crossdressed dendritic cells are then able arrest antigen-specific effector cells in the graft. Dr. Li added, "We know this happens, but we don't know how it happens."

Additionally, medical student Tej Azad and colleagues at Stanford University in Palo Alto, Calif., have identified a three-gene M1 macrophage signature that can identify not only biopsies with acute rejection, but also subclinical injury. In particular, the M1 score was effective at diagnosing acute rejection in 403 allograft biopsies across four organs. The signature also correlated with all individual Banff diagnostic criteria used to quantify graft injury.

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When the investigators examined a longitudinal renal transplant cohort, the M1 score predicted subclinical injury 18 months before clinical manifestation. The researchers concluded that the M1 signature can be used as a prognostic marker of graft failure. They also suggested that M1-polarized macrophages may play an important role in solid organ transplant physiology. This conclusion was reinforced by the fact that, in an independent cross-sectional renal transplant cohort, the M1 signature was able to stratify patients at high risk of long-term graft failure as early as 15 days following biopsy. Transplant surgeons should thus keep these inflammatory macrophages in mind and be aware that research is underway to explore their association with acute rejection.

Regulatory T cells. Dr. Li then turned his focus to the Foxp3 promoter and regulatory T cells (Tregs). "We do see new check points for Foxp3 and Tregs," he noted, and described the work of the Hancock laboratory at the University of Pennsylvania in Philadelphia, which found that Hdac1 deletion decreases expression of the corepressor of RE1 silencing transcription factor (CoREST). Additional research from a different laboratory raised the possibility that Treg cells require p27kip1, the natural inhibitor of the cyclin-dependent kinase CDK2. This latter work suggests that there is a Treg-intrinsic role for p27kip1 in Treg stability and function. The research also highlights the CDK pathway as a potential new therapeutic target for the promotion of transplantation tolerance.

Regulatory B cells. The basic science review ended with a quick summary of new findings related to B cells and transplantation. While B cells are best known for their role in humoral immunity, they also express cytokines that can influence transplantation outcomes. In addition, there are distinct B cell subsets that can be differentiated by expression of T cell immunoglobulin and mucin domain (TIM)-1 and TIM-4. New research indicates that TIM-1$^+$ and TIM-4$^+$ B cells transferred from alloimmunized mice have opposite effects on islet allograft survival. The results imply that targeting TIM-4$^+$ B cells and/or B cell interleukin (IL)-17 might help reduce rejection and leverage the potent activity of regulatory B cells.

The Innate Immune Response to Allogeneic Grafts

Fadi Lakkis, MD, professor of surgery, immunology and medicine at the University of Pittsburgh in Pennsylvania, presented his research on the role of donor signal regulatory protein α (SIRPα) polymorphism on allorecognition. He explained that SIRPα is expressed on myeloid cells as well as neurons. Although its expression was initially believed to be restricted to these cells, new research suggests that it is also expressed elsewhere throughout the body.

Dr. Lakkis noted that alloimmune responses can occur in the absence of obvious danger signals, microbial stimuli or any other signaling pathways triggered by damage-associated molecular patterns (DAMPS) and pathogen-associated molecular patterns (PAMPS). This observation prompted his laboratory to ask whether innate recognition mechanisms exist to distinguish between self and allogeneic. Dr. Lakkis's review of the literature suggested that, as he said, "There is, indeed, an allogeneic recognition mechanism in the innate immune response."

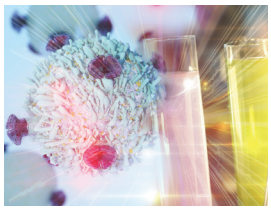
Although it was clear in the study that monocytes were recognizing nonself, the mechanism behind this phenomena was not obvious. In pursuing this question, the researchers observed that when they sourced donor organs from the very similar congenic nonobese diabetic (NOD) and the related nonobese diabetes-resistant (NOR) donor mice, the organs elicited markedly distinct responses in the same host. The response was independent of lymphoid cells and representative of the innate immune system. "We are dealing with a single Mendelian locus, which is really a bonus," said Dr. Lakkis.

When Dr. Lakkis and his colleagues looked more closely at the mice, they found that the allorecognition was likely due to variation in SIRPα. These findings raised the question of whether SIRPα is a marker of nonself. The team's mouse studies suggested that it is, because donors who carried SIRPα variants from the NOD and *Mus musculus castaneus* (CAST) strains not only displayed enhanced binding to the cognate ligand CD47, but also produced the strongest response. Moreover, when the investigators looked more closely at SIRPα, they found that disrupting SIRPα–CD47 interaction blocked the alloresponse.

Dr. Lakkis explained that SIRPα is highly polymorphic and the polymorphisms are primarily present in the CD47 binding domain of SIRPα. Thus, these SIRPα polymorphisms modulate binding to CD47, and the findings are consistent with the fact that the allorecognition response could be disrupted by treatment of the recipient with a recombinant CD47-Fc decoy protein.

Taken together, the results point to an innate allorecognition model that includes two signals, one that is stimulatory and one that is inhibitory. A mismatch in the SIRPα between the donor and the recipient results in the stimulation of dendritic cells. The findings thus delineate a mechanism of allorecognition that monocytes perform using CD47 to detect donor SIRPα polymorphism. Most important for transplantation surgeons, this allorecognition appears to be relevant to the rejection of cell and organ transplants.

Regulatory T Cells: Gene Expression and Gene Suppression



Wayne Hancock, MBBS, PhD, professor of pathology and laboratory medicine at the University of Pennsylvania in Philadelphia, described the highly conserved corepressor complex (CoREST) as a master regulator of Foxp3+ Treg cells. The CoREST complex includes histone deacetylase 1 (HDAC1) and HDAC2 and, using both in vitro and in vivo studies, the investigators identified contrasting effects of HDAC1 and HDAC2 deletion on regulatory T cell function. Specifically, HDAC1 deletion disrupts the CoREST complex, decreases induced Treg (iTreg) development, and impairs Treg function. Deletion of HDAC1 thus resulted in decreased Treg expression of CoREST. In contrast, deletion of HDAC2 increases CoREST function and enhances Treg function. So, despite their high structural homology and dimerization within the CoREST complex of regulatory T cells, HDAC1 and HDAC2 hold opposite roles.

Using this information, the investigators sought to develop CoREST inhibitors. They identified some that had dose-dependent effects and could be used as tools to pharmacologically target Tregs. In addition, they developed kinetically selective HDAC2i compounds that were able to promote regulatory T cell function. Their work thus brings new insights into the dynamic composition and functions of multimolecular complexes in Foxp3+ Treg cells and reveals some pharmacological options for influencing these cells. AJT

Reference

1. Mills EL, Kelly B, O'Neill LAJ. Mitochondria are the powerhouses of immunity. Nat Immunol 2017; 18: 488–498.

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