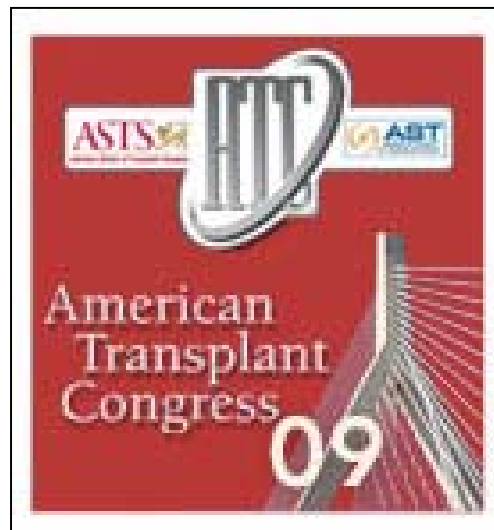


**Report of the American Transplant Congress,  
Boston, USA, June 2009 For the Novartis  
Transplantation Advisory Board**

By Lloyd D'Orsogna  
Leiden University Medical Centre  
Department of Immunohematology and  
Blood Transfusion  
[l.j.a.d\\_orsogna@lumc.nl](mailto:l.j.a.d_orsogna@lumc.nl)



The American Transplant Congress (ATC) is a joint meeting between the American Society of Transplant Surgeons (ASTS) and the American Society of Transplantation (AST), this years meeting was the 9<sup>th</sup> joint American transplant meeting. The ATC is the premier educational event in the field of basic and clinical organ transplantation. A broad range of topics were covered with a focus on controversial topics and new developments, as such the meeting provided an excellent educational environment.

At this meeting I presented my own research which focuses on the immunology of antigen presentation and allorecognition in the kidney transplantation setting.

- Summary of my own work presented at this meeting: *“New tools to monitor the impact of viral infection on the alloreactive T-cell repertoire”*

Accumulating evidence suggests that alloreactive memory T-cells may be generated as a result of viral infection, however, a suitable tool to define the individual HLA cross-reactivity of virus-specific memory T-cells is not available. We aimed to develop a novel system for the detection of heterologous immunity using single HLA antigen expressing cell lines (SALs) as stimulator. We generated a HLA-B8 restricted EBNA-3A specific CD8 memory T-cell clone and assayed for alloreactivity against a panel of SALs, using IFN $\gamma$  Elispot as readout. Generation of the T-cell clone was performed by single cell sorting, based on staining with viral peptide/MHC complex specific tetramer. Firstly we confirmed alloreactivity of the HLA B8 restricted EBV specific T-cell clone (EBNA-3A B8-FLR) against SAL expressing HLA B\*4402. Further screening against the entire panel of SALs also revealed cross-reactivity against SAL expressing HLA B\*5501. Therefore we have shown that SALs are a new and effective tool to detect heterologous cross-reactivity of viral specific CD8 memory T-cell clones,

against individual class I HLA molecules. My future work will focus on the in-vivo implications of viral specific T-cells for renal transplantation patients.

My work was completed within the department of Immunohematology and Blood Transfusion and in collaboration with the department of Hematology at the Leiden University Medical Centre, the Netherlands. Our work was presented as both a poster and a oral presentation. Our work was well received with much positive feedback and ideas for future experiments.

Aside from the presentation of my own work I also attended this meeting for the excellent educational opportunities offered. There were many cutting edge and new developments presented at this meeting. Below are summaries of five (5) presentations that are of special interest to the research area of (basic) transplantation immunology.

### **1. Potential of regulatory T-cells to control rejection: Kathryn Wood, England**

Prof Wood presented her data on the potential of Tregs to control rejection during concurrent symposium V: "Cutting edge topics in Transplantation". Her work may have major future therapeutic potential for the development of transplantation tolerance. CD127<sup>low</sup>, CD4+, CD25+ Tregs were isolated and expanded ex-vivo from a humanized mouse model. She found that these human Tregs generated ex-vivo can control the immune response to an allograft in-vivo. Co-transfer of these cells was able to facilitate specific unresponsiveness in transplantation, including the deletion of donor reactive T-cells. Drawbacks of this model include the fact that this therapy must be individualized (autologous) if it is to be transferred to human setting and that these regulatory T-cells are less effective at controlling memory T-cells.

### **2. Prevention of Acute Islet Graft Rejection by In Vitro Generated CD4+CD25+Foxp3+ Regulatory T Cells: Qing Yang, USA**

This group has shown that CD4+CD25+Foxp3+ Tregs with islet antigen specificity can be induced by autoantigen-pulsed splenic dendritic cells in the presence of high concentration of TGF- $\beta$ 1. Balb/c (donor) BMDCs were generated in GM-CSF and conditioned overnight in sirolimus and TGF- $\beta$ 1. The DCs were then co-cultured with C57BL/6 (recipient) CD4+CD25- T cells in the presence of IL-2, retinoic acid and TGF- $\beta$ 1 for 7 – 10 days. Resulting CD25+ T cells were harvest and co-adoptively transferred with B6 Thy1.2 cells to diabetic B6.RAG-/- mice bearing Balb/c islet grafts. BMDCs conditioned with sirolimus

and TGF- $\beta$ 1 were superior to untreated BMDCs in inducing CD4+CD25+Foxp3+ T cells in the presence of IL-2, RA and TGF- $\beta$ 1. The induced CD25+Foxp3+ T cells suppressed naïve B6 T cell proliferation (measured by 3H-thymidine uptake) and IFN- $\gamma$  production. Both suppression of proliferation and IFN- $\gamma$  production was donor specific, as no suppression was seen with third party APCs. Therefore, alloantigen-specific CD4+CD25+ cells with high levels of Foxp3 can be generated from naïve CD4+CD25- cells by donor dendritic cells in the presence of IL-2, retinoic acid, and TGF- $\beta$ 1.

### **3. New Insights into the Interaction between Regulatory B7-H3 Molecule and Costimulatory CD28-B7 Pathway in Determining the Outcome of the Alloimmune Responses In Vivo: □Takuya Ueno, Japan/USA.**

B7-H3 is a new member of the B7 family and its receptor TLT-2 was recently described. This group aimed to elucidate the functions of B7-H3 and its relationship to the CD28-B7 costimulatory pathway during alloimmune responses in-vivo. Treatment of B7-1/B7-2 DKO and CD28KO recipients resulted in accelerated cardiac allograft rejection. Accelerated rejection was associated with a significant increase in the frequency of IFN- $\gamma$  and granzyme B-producing alloreactive T cells. B7-H3 blockade also abrogated the graft-prolonging effects of CD28-B7 blockade by CTLA4Ig. Studies with B7-H3KO recipient of BALB/c heart confirmed above results when compared to WT recipients treated with CTLA4-Ig. Finally they observed a significant synergistic effect of an agonistic B7-H3-Ig when combined with CTLA4-Ig. These data confirmed the inhibitory function of B7-H3. Targeting this pathway combined with costimulatory blockade may help promote tolerance in transplant models.

### **4. STAT6 or T-bet Is Required for Acute Allograft Rejection: □Yu Yang, USA.**

Mice deficient in T-bet are severely impaired in their ability to produce IFN $\gamma$  and Th1 cells, yet reject allografts promptly. Further, mice deficient in Stat6 are defective in IL-4 production and Th2 development, yet also reject allografts rapidly. This group performed vascularized cardiac transplants in wild type, Stat6 Knockout (KO), T-bet KO or Stat6/T-bet double KO (DKO) BALB/c mice by using MHC mismatched C57BL/6 donors. Neither Stat6 nor T-bet single KO prolonged allograft survival. In contrast, Stat6/T-bet DKO mice had delayed graft rejection up to 30 days. This delayed transplant rejection was not due to impaired lymphocyte development, defective alloantigen presentation, altered effector migration, or lack of alloantibody production. These findings suggest that acute transplant rejection requires either Stat6 or T-bet, and their corresponding Th1 and Th2 cytokines.

### **5. Co-Transplantation of Syngeneic MSCs Delays Islet Allograft Rejection Generates a Local Immunoprivileged Site: □Andrea Augello, USA □□**

Mesenchymal stem cells (MSCs) are multipotent cells derived from bone marrow that have been shown to play a role in immunomodulation. This group aimed to evaluate the effect of a local or systemic administration of B6 MSCs on islet transplant rejection in a fully allogeneic model of islet transplantation (BALB/c→B6). A single dose of syngeneic MSCs in B6 recipients co-transplanted under the kidney capsule with allogeneic islets (from BALB/c donors) delayed islet rejection, reduced infiltration and induced long-term islet function in 30% of islet transplanted mice. Interestingly, systemic injection of the same dose of B6 MSCs was unable to delay islet rejection. B6 MSCs inhibited the autoimmune response, displayed multiple immune-regulatory abilities in vitro, showed a specific Th2/TGF- $\beta$  profile production, and delayed islet allograft rejection in-vivo when co-transplanted with islets into B6 syngeneic recipients, but failed to prevent rejection when transplanted systemically. MSCs are a promising therapeutic option for the induction of transplantation tolerance.

The next ATC will be held May 1-5 2010 at the San Diego Convention Centre, CA.