T CELL MEMORY IN TYPE 1 DIABETES BY PAOLO MONTI

State-of-art and future development/perspectives of the research area at international level (max 2000 characters):

T cells reactive to beta cell antigens (autoreactive T cells) play a fundamental role in the pathogenesis of type 1 diabetes (T1D). The strongest associations with disease thus far are with the presence of memory cell subsets. Three observations were key:
1) Autoreactive T cells are present in all individuals but develop a memory phenotype only in those who develop T1D
2) Long-lived memory subsets of autoreactive T cells remain for decades after the onset of the disease and can cause autoimmunity recurrence post islet or pancreas transplantation
3) Autoreactive T cells with a memory phenotype are less susceptible to regulation from immuno-suppressive drugs and regulatory T cells posing an additional set of therapeutic obstacles in the control of autoimmune responses.
Selective targeting of memory clones, with a specific focus of long-lived memory stem T cells is considered a therapeutic option to halt the chronic immune response that lead to beta cell destruction as well as autoimmunity recurrence in beta cell replacement strategies. The latest include also transplantation of beta cell derived from stem cells that recently reached the clinical testing. Strategies under evaluation for selective targeting of memory T cells include inhibition of metabolic pathways, blocking of homeostatic cytokines such as IL-7 and IL-15 and discovery of surface receptors for the development of monoclonals. On the other hand, the identification of pathways that regulates the development of long-lived memory T cells has gained attention in order to generate long-lived and fully suppressive regulatory T cells that can be adoptively trasferred to re-establish immune-tolerance in autoimmune and transplanted patients. In summary, to understand mechanisms of development of long-lived memory clones is a fundamental step to develop therapeutic strategies to control autoimmunity in type 1 diabetes, from the natural history of the disease to beta cell replacement therapies.

Actual lines of research (as is) of the Diabetes Research Institute (max 2000 characters):

Two project are currently active at the Diabetes Research Institute:
1) Generation of Treg with a stem cell memory phenotype and function for adoptive immunotherapy in patients with type 1 diabetes.
This project stems from previous findings that Treg can be expanded using homeostatic cytokines IL-7 and IL-15 to generate a progeny enriched in Treg with a stem cell memory (Treg-scm) phenotype and function. Treg-scm are more resistant to stress and apoptotic signals and survive longer in NSG mice overcoming a major barrier of Treg expanded with IL-2 whose life-span was shown to be short (few days/weeks) possibly impairing their therapeutic potential. A pilot study sponsored by EFSD allowed us to set up preliminary protocols of expansion using a combination of IL-2 and IL-7. A second pilot study sponsored by FID is currently ongoing to set up a protocol of expansion with IL-7 and IL-15 (without IL-2) which so far provided the best results in terms of superior performances of the final Treg product. A third study sponsored by JDRF has started in December 2021 with aiming at further optimizing the expansion protocol, characterize the final Treg product (RNAseq) and preliminary testing in preclinical models of disease.
2) Pharmacological GLUT1 inhibition to control the development of long-lived autoreactive memory T cell clones. This project aims at testing GLUT1 inhibitors to block activation of autoreactive and alloreactive T cells. The project was initially sponsored with a JDRF grant that was subsequently closed due to financial constraints imposed by the covid pandemic. Carla Di Dedda PhD student from my lab will move (Feb 2022 to Feb 2023) to the lab of Alberto Pugliese in Miami's DRI to study the dynamic of GLUT1 expression in patients undergoing simultaneous pancreas-kidney transplantation, several of whom developed well documented recurrence of autoimmunity to provide a rationale for the development of a pharmacological GLUT1 blockade strategy.

Strengths of the research area (as is) of the Diabetes Research Institute (max 2000 characters):

Project 1: Generation of Treg with a stem cell memory phenotype and function for adoptive immunotherapy in patients with type 1 diabetes. Two phase 1 clinical trials testing the adoptive transfer of polyclonal Treg in patients with T1D and patients with T1D undergoing to islet transplantation proved feasible and safe but also revealed important limits of the Treg product infused, including limited cell yield and short life-span. Using homeostatic cytokines to expand Treg we are in the position to generate a final Treg product with improved performances. This is attractive in the field since overcoming these limitations is considered a must to proceed to a phase 2 trial. In general terms the improvement of Treg performances is of interest also in other field of autoimmunity, transplantation and GVDH in which adoptive transfer of Treg is under evaluation as therapeutic approach. Another strength of this research area is the possibility to apply the expansion protocol with homeostatic cytokines also to novel Treg based therapeutics such as TCR transgenic and CAR-Treg. Preliminary agreement of collaboration have been set up with the groups of Dott Georgia Fousteri and Prof Chiara Bonini at the San Raffaele Institute.
Project 2: Pharmacological GLUT1 inhibition to control the development of long-lived autoreactive memory T cell clones. This project will benefit from a collection of samples of patients undergone to islet allo-transplantation which can be studied in term of GLUT1 dynamic and metabolic changes in autoreactive T cells using MHC multimers. In perspective, if successful a GLUT1 inhibition strategy as part of the induction therapy could be tested in a clinical trial, taking advantage of a unique and well established islet transplantation program. The need to find novel end effective therapies to control autoimmunity recurrence is still a strong need in the field.

Weaknesses of the research area (as is) of the Diabetes Research Institute (max 2000 characters):

In perspective, for both projects, a weakness in the lack of a structured and available facility to test both strategies in animal models, beside personal agreement between individual labs. This would include specialized personell familiar with animal models of diabetes and transplantation. Animal models of interest include humanized mice, models of GVHD, NOD mice and tranplanted mice. Another weakness for project 1 is the lack of a clinical programme testing adoptive transfer of Treg in type 1 diabetes or islet transplantation.

Short-medium term OSR/UniSR goals (0-18 months): milestones and deliverables (max 1000 characters):

For project 1 (Treg) the goal is to set up the best possible protocol for Treg expansion in terms of cell yield, stability, resistance to stress, suppressive capacity, and life-span once transplanted in animal models.
Milestone: Optimized expansion protocol with IL-7 and IL-15
Deliverables: A treg product enriched in memory stem T cells with improved performances in vitro and long-term persistence in vivo.
For project 2 (GLUT1) the goal is to provide data about the upregulation of GLUT1 in SPK patients experiencing autoimmunity recurrence and in vitro data that GLUT1 blockade result in exhaustion of activated T cells. As GLUT1 is broadly expressed on several cell types, preliminary data on the toxicity of drugs targeting GLUT1 on other cells and organs (beta-cells, CNS, Treg)
Milestone: Demonstration that autoreactive T cells upregulate GLUT1 post transplantation
Deliverable: GLUT1 blocking strategy effective in animal model of islet tranplantation.

Medium term OSR/UniSR goals (18-36 months): milestones and deliverables (max 1000 characters):

For project 1 medium term goals will be to adapt the Treg expansion protocol to clinical grade GMP systems (MACSprodigy) thanks to a collaborative effort with Anke Theil/ Ezio Bonifacio in Dreden Germany. The Treg product will be tested in comparison to the standard one (with IL-2) in animal models of GVHD and islet transplantation.
Milestone: effectiveness in animal models
Deliverable: GMP grade Treg product
For project 2, the pharmacological GLUT1 blockade strategy will be tested in a mouse model of islet transplantation. As GLUT1 is differentially expressed in mouse and human T cells, the development of a humanized mouse model is strongly required for this purpose.
Milestone: effectiveness/low toxicity of the approach in animal models
Deliverables: Protocol of a GLUT1 blocking strategy in combination with standard immunosuppressive drugs

Long term OSR/UniSR goals (36-60 months): milestones and deliverables (max 1000 characters):

The long term goals for both project is the clinical testing of these strategies in islet transplantation.
Deliverable: ethics commitee approvals

Investments of the Diabetes Research Institute (e.g. personnel, space, technology) to achieve the short-medium-long term goals (max 2000 characters):

-Available pre-clinical models of humanized, NOD and islet transplanted mice with a trained animal technician
-Lab and office space (already available)
-2 postdoc (1 already available) and 2 research fellows (2 already available) to follow two research projects. 1 technician for general lab management.