2% GZMB$^+$ B cells. Nevertheless, using an expansion mixture containing IL-21, anti-BCR, CpG oligodeoxynucleotide, CD40L, and IL-2, we were able to obtain more than 90% GZMB$^+$ B cells after 3 d culture. GZMB$^+$ B cells obtained through this protocol suppressed the proliferation of autologous and allogenic CD4$^+$CD25$^-$ effector T cells. The suppressive effect of GZMB$^+$ B cells was partially GZMB dependent and totally contact dependent but was not associated with an increase in effector T cell apoptosis or uptake of GZMB by effector T cells. Interestingly, we showed that GZMB produced by B cells promoted GZMB$^+$ B cell proliferation in ERK1/2-dependent manner, facilitating GZMB$^+$ B cell expansion. However, GZMB$^+$ B cells tended to undergo apoptosis after prolonged stimulation, which may be considered a negative feedback mechanism to limit their uncontrolled expansion. Finally, we found that expanded GZMB$^+$ B cells exhibited a regulatory phenotype and were enriched in CD307bhi, CD258hiCD72hi, and CD21loPD-1hi B cell subpopulations.
Conclusions: Our study shed new insight into biology of GZMB$^+$ B cells and provided an efficient method to expand GZMB$^+$ B cells for potential cell therapy in transplantation and autoimmune diseases.

OP521 GAMMA-DELTA T CELL THERAPY FOR THE TREATMENT OF POST-TRANSPLANT CMV INFECTION: PROOF OF CONCEPT

Gabriel Marseres[1], Anais Cosentino[2], Maxime Courant[2], Victor Bigot[1], Hannah Kaminski[1,2], Vincent Pitard[1], Atika Zouine[3], Isabelle Garrigue[4], Pierre Merville[1,2], Julie Déchanet-Merville[1], Lionel Couzi[1,2]
[1]ImmunoConcEpT UMR 5164, Bordeaux University, CNRS, Bordeaux, France, [2]Bordeaux University Hospital, Department of Nephrology, Transplantation, Dialysis and Apheresis, Bordeaux, France, [3]Flow cytometry facility, TBM Core, Bordeaux University, CNRS UMS 3427, INSERM US 005, Bordeaux, France, [4]Laboratory of Virology, Bordeaux University Hospital, Bordeaux, France

Background: Human cytomegalovirus (CMV) infection in solid organ transplant recipients (SOTRs) is associated with increased risks of allograft loss, morbidity and mortality. Current gold standard treatment, based on the use of Valganclovir, fails to prevent late CMV reactivation or emergence of viral resistance in a significant percentage of SOTRs. Importantly, long-term CMV clearance relies on the establishment of an anti-CMV T-cell response. There is therefore a growing interest in developing anti-CMV cell therapy. Both $\alpha\beta$ and $\gamma\delta$ T cells are key effectors of the anti-CMV immune response. The goal of this study was to explore a potential $\gamma\delta$ T cells-based immunotherapy.
Methods: Healthy donors (both CMV-positive and CMV-negative) and kidney transplant recipients (KTRs) undergoing refractory CMV infection were enlisted in this preclinical study. $\gamma\delta$ T cells were sorted from peripheral blood, then amplified and activated *in vitro,* using a T cell receptor (TCR) agonist combined to different cytokines, notably IL-4 and IL-15. The reactivity of expanded $\gamma\delta$ T cells against CMV-infected target cells was then measured *in vitro*.
Results: $\gamma\delta$ T cells amplification from both CMV-positive and CMV-negative healthy donors, as well as KTRs, was reproducible and compatible with a human cell-immunotherapy. Amplified cells displayed an activated and differentiated phenotype, but low exhaustion, produced IFNγ in the presence of infected target fibroblasts, endothelial cells and macrophages, and were able to control viral dissemination *in vitro*. At the mechanistic level, anti-CMV reactivity was independent of the $\gamma\delta$TCR but involved the co-stimulatory receptor lymphocyte function-associated antigen 1 (LFA-1).
Conclusions: Altogether, these data provide a proof of concept for a future use of amplified $\gamma\delta$ T cells, in the prevention and curative treatment of CMV disease in SOTRs. These results pave the way for a future phase I clinical trial.

OP522 MESENCHYMAL STEM CELLS LOCAL THERAPY FOR INDUCTION OF IMMUNOSUPPRESSION IN LIVER TRANSPLANTATION: RESULTS OF PILOT STUDY.

Sergey Korotkov[1], Aliaksei Shcherba[1], Denis Efimov[1,2,3], Evgeniya Primakova[1], Ekaterina Petrovskaya[1], Ekaterina Nazarova[1], Ivan Shturich[1], Svetlana Krivenko[1], Oleg Rummo[1]
[1]Minsk Medical Center for Surgery, Transplantology and Hematology, Minsk, Belarus, [2]Minsk Scientific and Practical Center for Surgery, Transplantology and Hematology, Minsk, Belarus, [3]Minsk Scientific and Practical Center for Surgery, Transplantology and Hematology, transplantation, Minsk, Belarus

Aims: The aim of the study was to evaluate the efficiency of the adipose tissue mesenchymal stem cells (MSC) local therapy (LT) for induction of immunosuppressive therapy (IIT) in patients after liver transplantation (KT) in the early postoperative period.
Methods: This is a report of pilot, prospective, single center, open label, randomized study of the superiority MSC induction of immunosuppression over standard IIT in regard of immunological dysfunction development and liver transplant function improvement. Inclusion criteria: adult liver transplant recipients who received liver transplant from DBD. Exclusion criteria were living-donor LTx, recipient portal vein thrombosis. MSCs introduction was performed in 10 patients through the portal vein during the reperfusion in total dose of 20 million cells. Maintenance therapy includes calcineurin inhibitor, mycophenolic acid, steroids. The protocol liver transplant biopsies were performed on the 7th day.
Results: Results of our research showed that the frequency of graft dysfunction which were associated with rejection, was developed in 2 patients. The severity of ACR was mild (RAI 5). Median GFR at the beginning and end of protocol was 27 $\pm$ 6.2 ml/min. Median trough level of tacrolimus was 2.1 $\pm$ 1.5 ng/ml with range of 0–5.3 ng/ml. There were no severe adverse effects of MSC therapy. All patients were switched to 3 component IS composed of tacrolimus (5–8 ng/ml), MMF (1 g/day) and steroids (medrol 16 mg tapering after 28 POD). Patients with decreased GFR were managed by the minimizing of Tac and substitution of overall IS by prolonged steroids, increased MMF and addition of Everolimus.
Conclusions: Local infusion through the portal vein of allogeneic adipose tissue MSC for induction of immunosuppressive therapy in liver transplantation is effective and safely.

OP523 CREATION OF BIOENGINEERED VASCULARIZED SPLEEN MATRIX AS AN ENDOCRINE CELL SUPPORT

Louis Maistriaux[1,2,3], Matias Ramirez[2], Lies Fievé[1], Chloe Heeyoung Chae[4], Caroline Bouzin[5], Eliano Bonaccorsi Riani[2,3], Jérome Duisit[6], Pierre Gianello[2], Catherine Behets-Wydemans[1], Benoit Lengelé[1,6]
[1]Pole of Morphology, Institute of Experimental and Clinical Research, UCLouvain, Brussels, Belgium, [2]Pole of Experimental Surgery and Transplantation, Institute of Experimental and Clinical Research, UCLouvain, Brussels, Belgium, [3]Department of Abdominal Surgery and Transplantation, Cliniques Universitaires Saint-Luc, Brussels, Belgium, [4]Endocrinology, Diabetes and Nutrition, Institute of Experimental and Clinical Research, UCLouvain, Brussels, Belgium, [5]Imaging Platform (2IP), Institute of Experimental and Clinical Research, UCLouvain, Brussels, Belgium, [6]Department of Plastic and Reconstructive Surgery, Cliniques Universitaires Saint-Luc, Brussels, Belgium

Background: Diabetes is currently treated by insulin injection. However, its ideal cure, pancreas transplantation, is limited by donor shortage and side effects of a lifelong immunosuppressive treatment. Aiming to overcome these limits, we used tissue engineering techniques in order to create a decellularized spleen matrix (DSM), thereafter seeded with B-cells, to regenerate a functional, biocompatible and transplantable graft.
Methods: Rat spleen grafts were harvested on the celiac trunk and decellularized by detergents perfusion. DSM was characterized by DNA, collagen, elastin, GAGs and residual SDS quantification, as well as by classical histology and immunohistochemistry. PrestoBlue Cell Viability Assay evaluated cytotoxicity after 5 days static culture of MIN-6 cells, seeded on DSM patches. Biocompatibility was analyzed after subcutaneous implantation of DSM or native tissue; the infiltration of CD68 and CD3 cells was assessed by IHC at 14 & 30 days. Finally, whole DSM were recellularized with HUVECs and MIN-6 cells, then cultured in a perfusion bioreactor for 5 days. MIN-6 cells function was evaluated with Glucose-stimulated insulin secretion (GSIS) test.
Results: Spleens were successfully decellularized by detergents perfusion, becoming white while preserving their 3D architecture. The reduction of 99% of DNA amount and histology confirmed the complete cell removal and the preservation of the micro-architecture. IHC for collagens I & IV, fibronectin, laminin and ECM proteins assays showed the preservation of the main ECM components. The low amount of SDS residues (<1%) and the unchanged cell viability emphasized the non-toxicity of the DSM. Biocompatibility was confirmed by a lesser infiltration of CD68 cells in DSM than in native tissue. DSM cultured in a bioreactor allowed HUVECs engraftment to the vascular wall, and the formation of MIN-6 cells clusters into the DSM, while preserving their insulin release during a perfused GSIS.
Conclusion: Vascularized DSM can be obtained by perfusion-decellularization while retaining its macro- & microarchitecture. ECM components are also preserved, with a good biocompatibility and without any cytotoxicity. Moreover, DSM could be a potential vascularized scaffold for pancreatic regeneration.