UNIVERSITE BIOLOGIE BRETAGNE SANTE LOIRE

Année 2019/2020

FICHE SUJET DE THESE

Sujet N° (à remplir par l'ED) :	FINANCEMENT : ☒ Demandé ☐ Acquis	Origine du financement :
Titre de la thèse : **TEMRA CD8 in Human Kidney Transplantation**		3 mots-clés : Kidney Transplantation CD8 T cell Migration
Unité/équipe encadrante : **CRTI Team4**		
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Contexte socioéconomique et scientifique (env. 10 lignes) :
As it remains the best therapeutic option for end stage renal disease and as current immunosuppression poorly influences chronic rejection, favoring long-term kidney graft survival requires the design of innovative therapeutics adapted to patient's own risks. A better understanding of the immune response resulting from the chronic antigenic stimulation triggered by allogeneic graft is needed to identify novel biomarkers that may anticipate risk of allograft injury and to identify innovative therapeutic targets to prevent graft failure. The diagnosis of T cell and Antibody dependent rejection (ABMR) is currently made on invasive biopsies scores of endothelial and inflammatory lesions and specific biomarkers that characterize endothelial and CD16 dependent cytotoxic immune cells activation have been identified. Besides the involvement of humoral response and NK cell, we have accumulated evidences of the involvement of TEMRA CD8 in the long-term graft outcome. We demonstrated that TEMRA CD8 not only promote inflammatory response upon donor-specific TCR stimulation but also mediate antibody-dependent cellular cytotoxicity. Moreover, the high frequency of TEMRA CD8 in kidney transplanted patients is associated with a 2-fold higher risk of graft dysfunction. These prove the potential of TEMRA CD8 cells to be 1) novel biomarkers that may anticipate risk of allograft injury and 2) targeting option for innovative therapeutics to prevent graft failure. **This project is aiming to address these important clinical needs through the identification of specific mechanisms involving Memory re-expressing CD45RA (TEMRA) CD8 T and CD16 dependent humoral responses.**

Hypothèses et questions posées (env. 8 lignes) :
We have accumulated evidence that the frequency of TEMRA CD8 impacts the graft outcome and that TEMRA CD8 exhibit innate-like features, including the expression of CD16 and other KIR molecules. TEMRA CD8 could not only represent a risk factor of kidney graft failure but also be per se a potent mechanism promoting intragraft inflammation and kidney graft rejection. The *in situ* detection of TEMRA CD8 within kidney biopsy would constitute a strong and convincing argument to support the need to control TEMRA CD8. Taking advantage of the large number of kidney biopsy already classified, the use of advanced multicolor Immuno histological chemistry (OPAL technology) and state-of-the-art Artificial Intelligence based algorithm to characterize the cellular infiltration and localization, we will decipher the composition of the graft infiltration. We will identify the chemokine leading to the recruitment of TEMRA into the graft and we will investigate the regulation of TEMRA CD8 function by the local inflammatory micro-environment.

Grandes étapes de la thèse (env. 12 lignes) :

In situ characterization of renal TEMRA CD8 infiltrate in kidney transplantation and auto-immunity. Opal™ is a method for multiplex fluorescent immunohistochemistry in formalin-fixed paraffin-embedded (FFPE) tissue. The method involves detection with Opal reactive fluorophores that covalently label the epitope which allow antibody removal without disrupting Opal fluorescence signal and prevent antibody cross reactivity. In collaboration with the MicroPICell core facility, we will setup a 6-color staining procedure to simultaneously detect CD3, CD8, CD16, CD56, CX3CR1 and Granzym B in FFPE kidney biopsies with different phenotypes and we will compare the cellular infiltrate with those of biopsies of ANCA-patients.

To define the ability of TEMRA CD8 to migrate toward non-lymphoid tissues in resting state and upon inflammation and to identify key molecules regulating the adhesion and the migration of TEMRA CD8. Our transcriptomic analysis of TEMRA CD8 highlight their potential to migrate to non-lymphoid tissue, especially under inflammation with a high expression of a and b chains of the LFA-1 integrin as well as CX3CR1 by TEMRA CD8. In addition to their role in lymphocyte trafficking, CX3CL1 and SDF1a chemokines have been shown to promote activation of human CX3CR1+ and CXCR4+ cells. **Studies directly comparing the trafficking potential of TEMRA and EM CD8 or identifying the mechanisms that dictate their trafficking into non-lymphoid tissues during inflammatory challenges have not been performed.** Several experimental procedures will be used to investigate the transmigration of purified CD8 subsets across a HMEC monolayer, the adhesion of CD8 on HMEC in resting and inflamed settings and time-lapse assay. We aim to use these experimental procedures to identify the chemokines that promote the transmigration of TEMRA CD8 from KTx across the endothelium barrier and the ligand-receptor interaction regulating their adhesion onto the endothelium in resting and inflamed settings. Selected knock-down TEMRA CD8 from HV and KTx will be generated to investigate the role of Gcnt1, LFA1 (a and b chains) and CX3CR1. The role of core-2 O-glycan in the binding of TEMRA CD8 from KTx to E- and P-selectin chimeric proteins will be assessed after short stimulation with IL-15 in the presence of BADG which blocks the incorporation of N-acetylglucosamine into O-glycans. The co-stimulatory role of SDF1a and CX3CL1 for CD8 subsets will be investigated by measuring effector functions (IFNg and TNFa secretion, degranulation marker CD107a and proliferation), migratory properties (cell polarization, migration speed and area covered by CD8 subsets), ATP release and cytosolic and mitochondrial Ca2+ flux. We will question the role of ATP release in the activation induced by SDF1a using selective inhibitors of P2X and P2Y receptors, as the role of ATP on human CD8 subsets function remains to be defined. The impact of chemokine/chemokine receptor polymorphism on migration of TEMRA CD8 will be studied in a genotype-phenotype follow-up analysis. Collectively, we will identify the key molecules regulating the migration of TEMRA CD8 and assess how selective targeting of specific molecules could prevent the migration of TEMRA CD8 in KTx.

Compétences scientifiques et techniques requises par le candidat (2 lignes) :
Strong immunological background, pro-active literature watch and team spirit.
Technical skills/ cell culture including cell purification, multi-parametric flow cytometry, time-lapse microscopy and knowledge regarding R-based analysis.

3 publications de l'équipe d'accueil relatives au domaine (5 dernières années) :

Yap M, Boeffard F, Clave E, Pallier A, Danger R, Giral M, Dantal J, Foucher Y, Guillot-Gueguen C, Toubert A, Soulillou J-P, Brouard S, Degauque N. Expansion of highly differentiated cytotoxic terminally differentiated effector memory CD8+ T cells in a subset of clinically stable kidney transplant recipients: a potential marker for late graft dysfunction. J Am Soc Nephrol. 2014 Aug;25(8):1856–1868. PMCID: PMC4116064

Tilly G, Doan-Ngoc T-M, Yap M, Caristan A, Jacquemont L, Danger R, Cadoux M, Bruneau S, Giral M, Guerif P, Nicol B, Garcia A, Laplaud DA, Brouard S, Pecqueur Hellman C, Degauque N. IL-15 Harnesses Pro-inflammatory Function of TEMRA CD8 in Kidney-Transplant Recipients. Front Immunol. Frontiers; 2017 Jun 30;8:778. PMCID: PMC5492498

Neel A, Bucchia M, Neel M, Tilly G, Caristan A, Yap M, Rimbert M, Bruneau S, Cadoux M, Agard C, Hourmant M, Godmer P, Brouard S, Bressollette C, Hamidou M, Josien R, Fakhouri F, Degauque N. B cell depletion therapy dampens CD8+ T cell response in ANCA-associated vasculitis. Arthritis Rheumatol. 2018 Oct 30. PMID: 30375745

Collaborations nationales et internationales :

Claire Pecqueur (CRCiNA ; Nantes)
Réseau FP7 VISICORT (WP leader)