OTKA NN 110909

Studies on the role of complement in antibody-mediated rejection of kidney transplants

Final closing report, 2015-2018

This collaborative study on the role of complement in late antibody mediated rejection of kidney allografts was conducted by multiple researchers of two universities, the Semmelweis University in Budapest, and the Medical University of Vienna. Collaborating departments and groups were:

I. Hungary:

Semmelweis University Budapest, 3rd Department of Medicine, Research Laboratory

Zoltán Prohászka (Co-PI in Hungary), Blanka Mező, Dorottya Csuka, Ágnes Szilágyi

II. Austria:

1. *Department of Paediatrics and Adolescent Medicine, Division of Paediatric Nephrology:*

- Krisztina Rusai (Co-PI in Austria), Thomas Müller-Sacherer, Christoph Aufricht

2. *Department of Internal Medicine III, Division of Nephrology and Dialysis:*

- Georg Böhmig, Gregor Bartel

3. *Department of Pathology*

- Sabine Kain, Heinz Regele

4. *Biobank of the Clinical institute of Laboratory Medicine*

- Helmut Haslacher, Thomas Perkmann

The aim of our project was to determine the role of complement system in the development of ABMR after kidney transplantation, in particular, to understand it's relationship with C4d positive or negative feature on histology. The study was conducted and finished according to the plans.

1, Screening of 741 previously kidney-transplanted patients for donor specific antibodies (DSA) was carried out by the Austrian partner during the first year of the project. 634/741 patients turned out to be DSA negative, 107/741 patients were identified as de novo DSA positive. Male-to-female ratio was 61/46 in the DSA positive group. A database was built (among others) with the following parameters of the patients: age, gender, previous medical history, baseline disease, laboratory parameters, immunosuppressive therapy (type and duration) and type and titre of DSA.

2, All of the 107 de novo DSA positive patients underwent kidney biopsy to screen for the presence of C4d (by immunohistochemistry), and to determine the stage of antibody-mediated rejection in the given patient.

3, DNA, serum, plasma and urine samples of the 107 DSA positive patients and selected controls have been collected and were transferred to Budapest for genetic and complement analysis.

4, Immunoassays for the determination of complement proteins and activation products were adjusted or developed, or purchased during the first year, and screening assays for the determination of constituents of the complotype were also developed and adjusted.

5, The DNA samples of the participants were examined for the following SNPs: CFB (L9H, R32W, R32Q), CFH (rs3753394, Y402H, E936D), C3 (R102G) and MCP (IVS9-78G<A) by restriction fragment length polymorphism (RFLP) or sequencing and in the case of some samples by both methods to validate the assay. Allele and genotype frequencies of eight polymorphisms were identified in the studied genes. As deduced from genotype data, a total of 46 individuals carried the H3 kidney disease risk haplotype of the CFH gene (43 in heterozygous form, 3 in homozygous form). Frequency of H3 risk haplotype was 0.25. In C3, G allele of rs2230199 (p.R102G) was found with a frequency of 0.152. In CFB, frequency of A allele of rs4151667 (p.L9H) was found to be 0.043, of T allele of rs12614 (p.R32W) was 0.12, and of A allele of rs641153 (p.R32Q) was 0.09. Frequency of A allele of MCP rs1962149 (IVS9-78 G/A) was found to be 0.364 in the studied population. Neither of the SNPs showed divergence form the Hardy-Weinberg equilibrium. These results were presented at the national immunology meeting in Velence (October, 2015).

6, To determine whether and to which extent the detection of complement components, (split) activation products and additional complement activation markers in the blood and urine of DSA-positive kidney transplant recipients is able to predict the presence of intra-graft complement activation (C4d deposition in transplant capillaries) and the diagnosis of ABMR in concomitant protocol biopsies, detailed complement biomarker measurements were done. Eighty-three of 107 DSA-positive recipients underwent protocol biopsies and were tested for plasma and/or urinary levels of complement proteins (C1q, C4, C3) and activation products (C4d, C3a, C5a, C5b-9). Forty-seven of the recipients were diagnosed with ABMR and 21 showed capillary C4d staining. While ABMR (and C4d) was associated with DSA binding strength [IgG mean fluorescence intensity versus ABMR; area under the curve (AUC) in receiver operating characteristic (ROC) analysis: 0.76], tested complement markers did not have any additional predictive value (AUC 0.49-0.56). There were, however, tight correlations between urinary levels of complement activation products and proteinuria (protein/creatinine ratio versus C4d, rho=0.48; C3a, rho=0.45; C5a, rho=0.66; sC5b-9, rho=0.45; p<0.001). These results were presented at the International Complement Workshop in 2018 (Santa Fe, USA), and the manuscript is submitted to the American Journal of Transplantation.

7, The determination of complement C4 gene copy number variations (CNV) in the 211 kidney transplant recipients were performed by TaqMan real-time PCR (RT-PCR) assays. The aim was to analyze if low C4 gene copy numbers might be associated with C4d negative stain on histology, in case of ABMR patients. The relationship between serum concentration of C4 and C4 CNVs (sum of C4A and C4B gene copy numbers) has been confirmed, although we did not find any connection to the development of antibody mediated rejection, with or without C4d-deposition in the peritubular capillaries. In addition it was noted that ABMR-positive patients with positive C4d-staining have less often C4BQ0 (C4B CNV=0/1) status (p=0.046). Interestingly, C4 CNV is marginally correlated with the mean fluorescence intensity (MFI) of C3b-binding donor-specific antibodies (DSAs), but not with the MFI of C4d-binding DSAs. The manuscript summarizing these determinations is currently prepared and will be submitted in 2019.

8, Intellectual property protection aspects

Basically, this study turned out to be a negative study. Despite the known association between C4d deposition in the peritubular capillaries and ABMR, no additional diagnostic or prognostic utility of complement biomarker determination in plasma or urine was obtained. Therefore, the results were not considered for patenting or transfer for industrial utilization.

9, Summary evaluation and publishing of the results

This study was a collaborative study, in which collection of patient's samples and clinical data, together with statistical evaluation was done in Vienna, and complement analysis was performed in Budapest. All of the planned determinations were performed, and the results about complement analysis (genotypes, complement biomarkers in plasma and urine) have been published at local and international immunology and complement meetings. The baseline characteristics of the ABMR cohort and controls, the clinical context and immunological background was described in 'Hypertonia és Nephrologia', in 2017. However, due to the maternity leave of the Co-PI, Krisztina Rusai, in 2018, the finalization and final complex evaluation of study data (together with clinical context), allowing the preparation of manuscripts, is slower than usual. We were able to finalize the manuscript about the lack of diagnostic and prognostic utility of complement biomarkers in ABMR, and this ms is now under evaluation at Americal Journal of Transplantation (B. Mező, A. Helios, G. A. Böhmig, F. Eskandary, M. Wahrmann, G. Bond, H. Regele, N. Kozakowski, P.F. Halloran, K. Rusai, and Z. Prohászka: Complement Markers in Blood and Urine– No Diagnostic Contribution in Late Silent Antibody-Mediated Rejection. Am J Transplant., submitted). We are currently preparing a second manuscript about the association of C4 gene copy numbers, ABMR, C4d-staining and complement biomarker data (in the same cohort of ABMR patients). A third manuscript will also be prepared in 2019 about the complotype and complement biomarker data. Therefore, we will kindly as the evaluation panel for a second re-assessment of our project, after 2 years of the closing date.