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Pre-transplant levels of ficolin-3 are associated with kidney graft survival

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Abstract Ficolin-3 is an initiator of the lectin complement pathway. The complement system is a mediator of the pathophysiology of graft rejection in kidney transplantation, but the role of ficolin-3 in this process is unknown. Using a prospective study design, 527 kidney transplanted patients were included. 97 blood donors served as controls. Ficolin-3, C4 and C3 were measured in pre-transplant as well as in control serum samples. In controls, deposition of ficolin-3, C4, C3 and the terminal complement complex (TCC) was measured in an assay based on acetylated albumin as matrix. The ficolin-3 levels correlated with the serum levels of C4 and C3. The serum levels of ficolin-3 correlated with the deposition of ficolin-3, C4, C3 and TCC. Survival analyses showed that high pre-transplant serum levels of ficolin-3 were associated with decreased graft survival. These results suggest an important role of ficolin-3 in the pathophysiology of kidney graft rejection.
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1. Introduction

The role of the adaptive immune system in graft rejection after kidney transplantation is well established and remains a target for immunotherapy. However, mechanisms related to the innate immunity — and particularly the complement system also play a major role in graft rejection. Deposition of activated complement factor C4 (C4d) in the peritubular capillary in kidney grafts is a widely accepted marker of antibody mediated kidney graft rejection suggesting an involvement of the classical antibody dependent complement pathway in this process [1]. Intriguingly, ficolin-3 from the lectin pathway of complement has also been shown to be deposited in kidney allografts alongside peritubular C4d deposition [2].

Activation of the complement system is mediated via three different routes: the classical, the lectin and the alternative pathways. All three pathways converge in the activation of

Abbreviations: HR, hazards ratio; MBL, mannose-binding lectin; MASPs, MBL/ficolin associated serine proteases; ROC, receiver operating characteristic analysis; R-PE, R-Phycoerythrin; SPS, sodium polyanethole sulfonate; TCC, terminal complement complex

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C3 and subsequently C5, with generation of C5a and further generation of the terminal complement complex (TCC). In humans, five recognition molecules of the lectin pathway have been described: mannose-binding lectin (MBL), ficolin-1 (M-ficolin), ficolin-2 (L-ficolin), ficolin-3 (Hakata antigen or H-ficolin) and collectin-11 (CL-11 or CL-K1) [3,4]. The five recognition molecules show structural homology, including an N-terminal domain that contains cysteine residues, a collagen-like domain with typical Gly–Xaa–Yaa repeats of varying length and a C-terminal so-called calcium-ion dependent carbohydrate recognition domain for MBL and CL-11 and a fibrinogen-like recognition domain for the ficolins [5,6]. The molecules are further assembled into larger multimeric structures of several hundred kDa, composed of four or more trimers [6,7]. The collagen-like domains can associate with MBL/ficolin/CL-11 associated serine proteases (MASPs) that activate the lectin pathway of the complement system [8]. Of the five, ficolin-3 is the most abundant serum component with a median concentration in healthy Caucasians of 25 μg/mL, followed by ficolin-2 (5 μg/mL) and MBL (1 μg/mL), respectively [9–11]. Serum levels of ficolin-1 and CL-11 are very low (<0.5 μg/mL) [12]. A hierarchy in the complement activation capacity between the lectin pathway initiators exists, as ficolin-3 has the strongest potential, MBL and ficolin-2 are intermediate, and ficolin-1 has the weakest potential [13]. The genes encoding MBL, CL-11, ficolin-1, -2 and -3 are named *MBL2*, *COLEC11*, *FCN1*, *FCN2* and *FCN3*. *MBL2* is expressed in the liver, *COLEC11* ubiquitously, *FCN1* in bone marrow derived cells, *FCN2* in the liver, and *FCN3* in the liver and in lungs [3,6].

Recently we reported that low MBL levels and *MBL2* variant genotypes are associated with decreased graft survival in non-HLA immunized patients transplanted with a kidney from a deceased donor [14]. This is in good accordance with the well known ability of MBL to sequester apoptotic and damaged endothelial cells and shows that MBL is important for kidney graft survival under certain conditions.

Because ficolin-3 has been shown to be deposited along with C4d in kidney allograft [2] suggesting that additional mechanisms other than antibody mediated activation may lead to C4 deposition and downstream complement activation associated with inflammation and tissue damage, we hypothesized that ficolin-3 might be one of the initiating factors involved in kidney rejection. Therefore, we investigated the pre-transplant level of ficolin-3, C4 and C3 in kidney transplanted patients and controls. Moreover, in the controls we investigated whether the serum concentration of ficolin-3 correlated with complement deposition on a matrix that allows ficolin-3 binding.

2. Materials and methods

2.1. Patients and study design

Patients from the Transplantation Centers of Copenhagen at Herlev and Rigshospitalet University Hospitals, who underwent a single organ renal transplantation with an organ from either a deceased heart beating donor or a living donor from November 2002 until the end of 2009 were included in the study. During this period, 557 patients were transplanted. 30 patients were excluded, because of missing serum samples, which gave a total of 527 patients included in the study. The following data were collected: donor age, donor gender, donor cytomegalovirus (CMV) status, whether the donor was deceased or living, recipient age at the time of transplantation, recipient gender, HLA immunization status in recipients, number of HLA-A, B and DR mismatches, cold ischemia time and treatment with mycofenolate mofetil. The clinical records concerning CMV status, cold ischemia time and treatment with mycofenolate mofetil were only available from 426, 372and 366 of the 527 included patients, respectively. HLA immunization was defined as >5% panel reactive antibodies in a complement-dependent cytotoxicity (CDC) assay, determined on the latest serum sample within three months. The study period was from 2002 until the end of 2010 hence more than a year after the last patient was included. The results of the serum level measurement of ficolin-3 from the kidney transplanted patients were merged with the clinical data from the Danish Nephrology Registry and data from Scandia Transplant.

2.2. Ethics

The project was approved by the Capital Region of Denmark and the Danish National Data Protection Agency (reference number: 2007-58-0015) and was performed according to the Declaration of Helsinki and adheres to Declaration of Istanbul.

2.3. Samples

Pre-transplant serum samples were collected within three months before transplantation and routinely stored at −20 °C until analysis. Serum samples from 97 healthy controls were collected and stored at −80 °C in order to preserve the complement activation capacity.

2.4. Assay for measurement of serum levels

The samples were analyzed in an in-house bead based Luminex assay which is similar to our previously published beadbased MBL-assay [15] except for the antibodies employed.

Briefly, antibodies were coupled to empty Luminex xMAP-beads according to the protocol (April 2007) downloaded from www.luminexcorp.com, except for small modifications. The antibodies used were mouse monoclonal anti-ficolin-3 (FCN334), an antibody produced in-house, which has been widely used on the ELISA platform [9] and rabbit polyclonal anti-C4c and anti-C3c antibodies from Dako. In all assays the same antibody was used both for capture and detection. The antibodies were biotinylated for use in the detection step. In addition, because of the potential problem with heterophilic antibodies and rheumatoid factors we also included beads coated with antibodies without specificity towards human antigens in order to detect possible false positive results.

Each serum sample was diluted in 1:3200, 1:25600 and in 1:204800 and due to great variation in the serum levels of the measured proteins they were incubated separately in these dilutions and merged again after incubation with the detection antibodies. A calibration curve made of a serum-pool consisting of four donors with known levels of ficolin-3, C4 and C3 was incubated on the same plate. Finally, streptavidin-R-PE was added and the samples were analyzed by the Luminex-machinery and the results were analyzed by StarStation 3.0 software.

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