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Autoantibodies against podocytic UCHL1 are associated with idiopathic nephrotic syndrome relapses and induce proteinuria in mice

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ABSTRACT

Idiopathic steroid sensitive nephrotic syndrome (INS), the most frequent childhood nephropathy, is thought to be mediated by a circulating soluble factor that reversibly affects the renal protein sieving. The efficiency of rituximab therapy recently highlighted the involvement of B cells. Here we studied the involvement of a specific immunoglobulin G (IgG) in the disease. After plasma fractionation by size exclusion chromatography, a detachment of cultured podocyte was observed with one IgG-containing fraction from 47% patients in relapse, 9% of patients in remission and 0% of controls. Podocyte protein lysates were immunoprecipitated by IgG from those plasma fractions identifying a list of 41 podocyte proteins after proteomic analysis. Five podocyte targets were selected on statistical and biological criteria. Specific antibodies were tested and only anti-Ubiquitin Carboxyl-Terminal Hydrolase L1 (UCHL1) IgG led to podocyte detachment. UCHL1 was mainly found inside the podocyte but also weakly expressed on podocyte cell surface. Incubation of either anti-UCHL1 IgG or plasma fractions with recombinant UCHL1 prevented podocyte detachment. Plasma levels of anti-UCHL1 IgG were significantly increased in relapsing INS patients compared to patients in remission and controls. Proteinuria correlated with anti-UCHL1 IgG level at various stages of the disease. Purified patient anti-UCHL1 antibodies induced proteinuria and podocyte foot effacement in mice. Altogether, these results identified UCHL1 as a target podocyte protein of autoantibodies in a set of relapsing patients and support a causative role of anti-UCHL1 autoantibodies in the development of INS.

1. Introduction

Idiopathic steroid sensitive nephrotic syndrome (INS) is the most frequent childhood nephropathy in the world. INS is characterized by a massive proteinuria due to the increase of albumin urinary clearance by 1000-fold. This acquired disease with an acute onset specially affects the pediatric population between 1 and 9 years of age [1]. Morphological changes in the kidney are restrained to podocyte foot process effacement, which is only detectable with ultra microscopy [2]. Numerous hypotheses have been raised to explain the massive proteinuria in idiopathic nephrotic syndrome. A morphological change in the kidney exclusively limited to the foot process effacement is suggesting a primary intrinsic glomerular dysregulation [3]. Nevertheless, the full reversibility of proteinuria and foot process effacement [2] within a few days of oral prednisone [4] as well as the efficiency of immunosuppressive

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Abbreviations

Abs	antibodies
BSA	Bovine Serum Albumin
EDTA	Ethylene-Diamine-Tetra-Acetic Acid
FCS	Fetal Calf Serum
FITC	fluorescein isothiocyanate
HPLC	High Performance Liquid Chromatography
HRGEC	Human Renal Glomerular Endothelial Cells
HSP	Henoch Schonlein Purpura
INS	Idiopathic Nephrotic Syndrome
MCD	Minimal Change Disease
NAMPT	Nicotinamide phosphoribosyltransferase
PBS	Phosphate Buffer Saline
SND1	Staphylococcal nuclease domain-containing protein 1
TCP1	T-complex protein 1 subunit alpha
UCHL1	Ubiquitin Carboxyl-Terminal Hydrolase L1

drugs targeting T and B cells to prevent relapses [1] also support the podocyte damage as a secondary event originating in a primary immune disease.

The identification of the HLA DQ locus as the chromosomal locus of steroid sensitive INS [5] also strongly suggests that INS might not be a kidney disease but a primary immune disease. Indeed, cyclophosphamide and rituximab, that seriously impact the survival of B cells, are the only treatments leading to long lasting remission after withdrawal in a significant subset of patients with a steroid dependent disease [6,7]. Moreover the number of memory B cells, especially switched memory B cells is predictive of relapse after B cell depletion following rituximab therapy [8].

This work was supported by the hypothesis that INS was a primary B cell disease mediated by a specific circulating antibody. Consistently, plasma from patients have been shown to modify the morphology of cultured podocytes *in vitro* [9]. As the morphological changes only affect podocytes during relapse, the aim of this work was to identify antibodies (Abs) directed against podocyte targets in the blood of INS patients at relapse.

2. Material and methods

2.1. Definitions

All definitions were based on the internationally accepted criteria [1]. INS relapse was defined, by a proteinuria/creatininuria ratio either over to 40 mg/m^2/h or 0.20 g/mmol associated to a serum albumin <20 g/L. INS remission was defined by a level of proteinuria/creatininuria ratio either below 4 mg/m^2/h or 0.02 g/mmol and serum albumin >35 g/L. Steroid sensitivity was defined by the full remission of proteinuria within 4 weeks of oral prednisone at the dose of 60 mg/m^2/day. Biopsies were not performed in children <11 year-old and in adolescents when they were steroid sensitive.

2.2. Patients and samples

INS patients and control individuals were prospectively recruited at the Robert Debré Hospital in Paris between 2007 and 2014. This work was an ancillary study of the NEPHROVIR biobank that was approved by the local ethics committee. Pediatric patients were included after a written informed consent was signed by the parents. INS children were sampled if they were <18 year-old, at least 1 month from any steroid therapy and either 3 months from immunosuppressive therapy. All patients with a steroid resistant nephrotic syndrome were discarded from the study.

Control subjects were <18 year-old, had neither proteinuria nor a history of nephrotic syndrome, and they had never received steroids or immunosuppressive therapy. Additional patients served as control for the measurement of plasma anti-UCHL1 IgG Abs : Nine proteinuric children with Henoch-Schonlein Purpura were included at the Robert Debré Hospital, 8 proteinuric adults suffering from Idiopathic Nephrotic Syndrome with minimal renal changes and 8 healthy adults were included at the Xavier Bichat Hospital in Paris (Supplemental Table S1).

2.3. Plasma fractionation

Plasma aliquots of 500 μL were fractionated by size-exclusion chromatography through a Superdex 200 HR10/30 column (GE Healthcare Life Sciences) connected to a High Performance Liquid Chromatography (HPLC) AKTA-basic automated liquid chromatography system (GE Healthcare Life Sciences) and plasma fractions were eluted in phosphate buffer saline (PBS).

2.4. Incubation of plasma fractions and specific Abs on podocytes

The adherent immortalized human podocyte cell line AB8/13 kindly provided by Pr Moin Saleem (Academic and Children's Renal Unit, University of Bristol, UK) was cultured as previously described [10]. Podocytes were used for experimentations after 14 days at 37 °C and 1 day of Fetal Calf serum (FCS) starvation. Human Renal Glomerular Endothelial Cells (HRGEC) were cultured following manufacturer's instructions (ScienCell Research Laboratories) and used after 1 day of FCS starvation for experimentations. Podocytes or HRGEC cultured on coated plates with collagen type 1 (0.2 mg/mL) were exposed to either 50% of plasma fractions or anti-Ubiquitin carboxyl-terminal hydrolase isozyme L1 (UCHL1) IgG (Cusabio), anti-Erlin-2 IgG (LSBIO), anti-T-Complex Protein 1 subunit alpha (TCP1 alpha) IgG (Novusbio), anti-Staphylococcal Nuclease Domain-containing protein-1 SND1 IgG (LSBio) and anti-Nicotinamide phosphoribosyltransferase (NAMPT) IgG (Raybiotech) diluted in culture medium at various concentrations (2.5, 5, 10, 20 and 40 μg/mL) during 2 h. Podocytes or HRGEC morphology was then evaluated by light microscopy (Leica DM-IRB, Leica Microsystems).

2.5. Neutralization of plasma fractions and specific Abs

Plasma fractions were incubated with 1.5 μg of human anti-human IgG Fc (clone 6864, Bio-Rad AbD Serotec Ltd) or HuCAL Fab-dHLX-MH negative control antibody (Bio-Rad AbD Serotec Ltd) for 30 min at 37 °C and then added to the culture medium of podocytes during 2 h. Anti-UCHL1 IgG (Cusabio) at 40 μg/mL was preincubated for 30 min at 37 °C with increasing concentrations (60, 125, 250 and 500 μg/mL) of Bovine Serum Albumin (BSA) (Euromedex) or recombinant UCHL1 protein (250 and 500 μg/mL) produced and purified in our lab (see below).

2.6. Podocyte viability after incubation with plasma fractions

After stimulation with plasma fractions, detached and adherent podocytes were incubated with 0.01 M EDTA for 3 min at 37 °C, 5% CO2, then washed and stained using Annexin V Apoptosis Detection Kit I (BD Biosciences). Podocytes viability was assessed by flow cytometry using BD FACSDiva Software (FACSCantoII, BD Biosciences) and podocyte viability was analyzed using the FlowJo

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