Possible new perspectives for our understanding of nephrotic syndrome recurrence

Caroline Hervé¹ and Jacques Dantal²

¹INSERM U643, ITERT and ²Service de Néphrologie, Immunologie Clinique, 30 Bd Jean Monnet, CHU Nantes, 44093 Nantes, France

Keywords: nephrotic syndrome; permeability factors; podocyte; recurrence

The identification and characterization of circulating factors that alter glomerular permeability in idiopathic nephrotic syndrome (INS) remain the subject of intensive research in nephrology. The existence of such factors was initially evoked by Shalhoub in 1974 [1] and, although they are thought to be of T-cell origin, their precise nature remains elusive. INS recurs immediately after kidney transplantation in 30% of patients with corticosteroid- or cyclosporin A-resistant INS [2]. The rapidity of this recurrence and the beneficial effects of plasmapheresis [2] and/or immunoadsorptions [3], together with several cases of materno-fetal transmission of glomerular abnormalities [4], lend credence to the involvement of plasmatic factors. In addition, the beneficial effects of corticosteroids and/or immunosuppressive therapies on the initial disease and the T-cell abnormalities recently reported in steroid-sensitive INS [5] indirectly support a T-cell origin.

Hypothetical plasmatic factors

Major discrepancies have been revealed in the results of studies on the putative plasmatic factor(s) performed in vitro or in vivo in rodents. These discrepancies could be related to the origin of the biological fraction tested [i.e. cortico-sensitive disease, recurrence after transplantation, genetic variant of focal and segmental glomerulosclerosis (FSGS)] and/or to the sensitivity or specificity of the experiments in question. Studies of patients with steroid-sensitive minimal change disease using stimulated cultured T cells, T-cell hybridomas or serum have described hypothetical plasmatic factors. Nevertheless, the specificity of these factors is poor and no firm conclusions could be drawn. Currently, the best characterized candidate in these steroid-sensitive patients is haemopexin [6]. The activity of this 100 kDa haem-binding protein correlated tightly with INS evolution (remission/relapse). Moreover, haemopexin has been shown to reduce the anionic charges of the filtration barrier [7] and to have protease activity [8].

Other groups have studied the putative FSGS factor following INS recurrence post-kidney transplantation. The first reported approach consisted of injecting different plasmas or plasma fractions into rats (intravenously and/or intraperitoneally). These reports were encouraging as they demonstrated the transfer of (an) albuminuric factor(s) in vivo [3,9]. Nevertheless, the results were difficult to reproduce and a clear lack of specificity was demonstrated [10]. These negative results could have been due to a low final concentration of a putative factor, species specificity and/or the presence of an antagonist/neutralizing factor in normal rat serum. The second approach consisted of using isolated rat glomeruli in vitro. Using this technique,
pre-transplantation plasma or fractions from INS patients were shown to increase the albumin permeability of normal glomeruli but did not seem to be specific to the disease (i.e. 30% of membranous glomerulonephritis patients) [11]. Greater risk of recurrence was reported for patients in whom pre-transplant sera induced a high albumin permeability [12], but the predictive value of this particular test was not a consistent finding [11]. This bioassay was used in an attempt to detect and identify the plasmatic factor but, despite the use of various samples, no biochemical characterization was performed.

**Genetic contribution**

More recently, major progress in our knowledge concerning the mechanisms of INS has come from the identification of some key proteins of the podocyte slit diaphragm. These proteins, which are inter-related, are critical for congenital and early childhood onset nephrotic syndrome [13,14]. Their role is to maintain the integrity of the slit diaphragm and thus its permselectivity [15]. Late recurrence of INS reported in transplanted children with congenital nephrotic syndrome of the Finnish type was elegantly demonstrated to be related to an immunization against nephrin inherent to normal kidney grafts [16]. However, the difference between the intrinsic ‘genetic’ form and the extrinsic ‘immunological’ form due to an extra-renal factor of nephrotic syndrome is not clear-cut. Recurrence of nephrotic syndrome after kidney transplantation is observed in 35% of patients with the ‘immunological’ form, but also in 8% of patients with podocin mutation [17]. Moreover, the involvement of plasmatic factors in the recurrence of congenital nephrotic syndrome cannot be totally excluded, as an increase in albumin permeability was detected with the serum of these patients, both before and after transplantation [18]. The pathophysiological mechanisms underlying INS and its recurrence after kidney transplantation are more complex than the simple hypothesis of an albuminuric factor. They probably involve disequilibria between permeability-inducing as well as protective factors, rendering their identification more difficult.

**Podocyte culture techniques**

The successful transfection of primary cultures of human or mouse podocytes with a temperature-sensitive gene construct represents a new and practical tool to study podocyte biology [19,20]. Differentiated podocytes express the typical markers of *in vivo* podocytes, including key proteins of the slit diaphragm such as synaptopodin, P-cadherin, nephrin, podocin and CD2AP. This cell line can thus be used as a new tool to examine the effect of nephrotic plasma directly, with a particular focus on the organization of the slit diaphragm complex.

First, it has been reported that incubation of this immortalized podocyte cell line with normal or non-nephrotic plasma is associated with the development of long foot processes and the localization of nephrin, podocin and CD2AP at the cell surface [19]. Recently, Coward *et al*. [21] published significant data using these cells. They showed an abnormal distribution of slit diaphragm proteins after 48 h exposure to nephrotic plasma, with nephrin, podocin and CD2AP being retained within the cytosol. Previously, two key findings have come from human and animal models of nephrosis: it was shown that nephrin and podocin are localized away from the slit diaphragm in nephrotic patients and that there was a change in slit diaphragm proteins from a linear capillary loop pattern in normal glomerulus to a granular intracellular translocation in conditions of heavy proteinuria [22–24]. The experiments on podocyte culture are therefore able to reproduce *in vitro* what is thought to occur *in vivo* during nephrotic syndrome.

Immortalized podocytes are also unique tools for the analysis of intracellular mechanisms of podocyte differentiation and dedifferentiation. The maintenance of the differentiated form, observed with sera from patients in remission, is mediated within minutes through the suppression of calcium flow, via a kinase pathway. Moreover, recent studies showed that blockade of integrin-linked kinase prevented podocyte detachment [25] and that activation of a mitogen-activated protein kinase (MAPK) such as p38 was necessary for podocyte injury [26]. Thus, podocyte activation by kinases seems to play a crucial role in the dedifferentiation process. Another interesting observation made by Coward and colleagues [21] was that normal serum could reverse the delocalization of nephrin, strongly supporting the possibility of a defective or missing protective factor in nephrotic plasma.

**The nephrotic stage: a critical balance**

The hypothesis that nephrotic patients lack plasmatic factors essential for the maintenance of glomerular permeability was initially evoked by studies based on isolated glomerular experiments as well as the results obtained using immortalized podocyte cell lines. This hypothesis agrees with the failure to transfer albuminuria to animals using sera, plasma or immunoabsorption eluates and with the inability to reproduce these experiments due to the presence of natural factors that could reverse the effect of the transferred material. Further evidence for the loss of factor(s) necessary for the maintenance of glomerular permeability comes from the description of increased glomerular permeability in the isolated glomerular test using sera from FSGS patients bearing the NPHS2 gene mutation [18]. In the latter case, urine from nephrotic patients, but not from normal controls, blocks *in vitro* the induction
of glomerular abnormalities, strongly suggesting the loss of a protective factor in the urine of nephrotic patients.

Finally, is this hypothesis plausible with clinical observations? The mechanisms of action of plasma treatment are not clear: to our knowledge, ~60% of adult patients with FSGS recurrence present an initial complete or partial remission after such treatment, but no differences can be found with respect to the substitution fluids used (albumin vs fresh frozen plasma). Moreover, immunoabsorption onto protein A or Ig-Therasorb columns can give equivalent results without any protein substitution [2,28]. In addition, this effect is not specific for FSGS recurrence and was also reported in nephrotic syndrome of other origins [29]. Thus, these observations do not support a decrease of a protective factor and suggest that if these treatments could modify the balance between circulating factors, they act mainly by the removal of proteinuric factors. In the recurrent patients, the only widely accepted concept is the immediate recurrence of albuminuria, suggesting that the presence of a pre-formed factor is able to affect glomerular permeability of the kidney graft. The discrepancies concerning the predictive value of the effect of pre-transplantation sera on isolated glomeruli also need to be reconsidered. The defect of a protective factor is difficult to explain by a urinary loss process as these pre-transplanted patients are generally anuric and non-nephrotic. In addition, the high doses of cyclosporin delivered as the first-line therapy for patients with immediate FSGS recurrence could also protect the glomerulus from an extra-renal aggression [27] rather than only block the production of an albuminuric factor of T-cell origin. The non-specific effect is not specific for FSGS recurrence and was also reported in glomerular alterations induced by a human plasma factor. Nephron 1996; 74: 586–593


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Conflict of interest statement. None declared.

Acknowledgements. We thank Joanna Ashton for editing the manuscript and D. Sahali (Créteil, France) for critical reading of this manuscript.
Genetic determinants of albuminuria and renal disease in diabetes mellitus

Michèle M. Sale1,2 and Barry I. Freedman2

1Center for Human Genomics and 2Department of Internal Medicine/Section on Nephrology, Wake Forest University School of Medicine, Winston-Salem, NC, USA

Keywords: albuminuria; chronic kidney failure; diabetes mellitus; genetics

Introduction

Type 2 diabetes mellitus (T2DM) is increasing in epidemic proportions. The worldwide prevalence of diabetes was estimated to be 171 million cases in 2000 and is projected to rise to 366 million cases by 2030 [1]. Given current trends, the lifetime risk for developing T2DM is 30% in European Americans born in 2000, contrasted with 40% in African American males and 49% in African American females [2]. This global epidemic will clearly increase the development of diabetic nephropathy (DN) and cardiovascular disease (CVD) for decades to come. The dramatic change in prevalence of T2DM is clearly rooted in environmental shifts, with westernization leading to obesity, metabolic syndrome and hypertension. However, diabetes and its associated nephropathy and CVD strongly aggregate in families [3–5]. Here, we review our current understanding of the impact of genetic factors on the development of DN.

Dissection of the trait ‘diabetic nephropathy’

Reports evaluating the linkage and/or association of genes or genomic regions with DN have often yielded conflicting results. Discordant findings probably relate to true genetic heterogeneity, coupled with study design flaws such as population stratification between cases and controls, small sample sizes lacking in statistical power, and evaluation of inadequate numbers of polymorphisms in genes to determine their true involvement. However, the definitions of DN in each report typically differ, contributing to the confusion.