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EDITORIAL COMMENTARY

The phenomenon of focal segmental glomerulosclerosis post-transplantation—a one-hit wonder?

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Abstract Steroid-resistant nephrotic syndrome (SRNS), also termed focal segmental glomerulosclerosis (FSGS), is one of the most difficult conditions for the nephrologist to manage, particularly when the disease recurs post-transplantation. This syndrome is extremely interesting from the biological perspective as the non-genetic form is most likely caused by an as yet unknown disorder of the circulating plasma. This elusive 'plasma factor' has been the focus of researchers for several decades. Many hypotheses have been proposed and tested, but none have yet passed the test of clinical utility. However, the search appears to be narrowing, facilitated by landmark discoveries in the molecular properties of the glomerular filtration barrier, as well as by improved experimental tools. In the therapeutic/clinical setting, the targeting of specific molecules in treatments has improved, of which one example is treatment with specific monoclonal antibodies. In this context, our report on the effects of tumor necrosis factor-alpha (TNF-α) on podocytes is instructive as it demonstrates that this cytokine can have directly deleterious effects on podocytes in vivo and that this effect can be targeted clinically, potentially halting or reversing the disease process. As with all thought-provoking research, this result raises several interesting questions. Is TNF-α the elusive 'plasma factor' or is it one of several? Does it directly affect the glomerular filtration barrier, or does it modulate the immune response? And could this technique be used as a cell-based assay for disease activity? Our report adds another potential candidate to the growing list of candidates that need to be tested in a wider population of well-phenotyped patients with SRNS.

Keywords Steroid resistant nephrotic syndrome · Focal segmental glomerulosclerosis · Plasma factor · TNF-α · Podocyte · Proteinuria · Cytokine · Cytoskeleton

Background

Focal segmental glomerulosclerosis (FSGS) is now known to be caused by a single gene mutation (in one of at least 26 genes currently known) in 18–40 % of affected children, depending on the cohort tested [1]. In those without a known gene mutation, the risk of recurrent disease post-transplantation is high—up to 50 % for a first graft—and perhaps even higher if genetic causes are comprehensively screened for. An interesting case report that was published this year demonstrated the primacy of the circulating factor hypothesis in this situation: an FSGS recipient developed severe recurrence, the dysfunctioning graft was removed and re-transplanted into a non-FSGS recipient, and the graft subsequently recovered full function with no proteinuria [2].

Therefore, recurrent FSGS is the archetypal disease process that is driven by a circulating plasma factor, and the question remains whether this is one, or several factors, and how do we best approach this conundrum scientifically and clinically.

Putting tumor necrosis factor-alpha into the context of FSGS and the filtration barrier

In this issue of *Pediatric Nephrology*, Bitzan et al. [3] describe an important approach to studying this

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phenomenon in children. The authors treated cultured human podocytes with plasma from a patient with active recurrence of disease post-transplantation and showed distinct cytoskeletal changes which could be mimicked by tumor necrosis factor-alpha (TNF-α) and inhibited by blocking the TNF-α pathway. Interestingly, they report that the same patient responded to blockade of the TNF-α pathway with a sustained partial remission. The paper is salient because the authors use a cell-based assay to test a putative circulating factor, TNF-α, and the results illustrate the potential clinical importance of identifying this cytokine.

The story of the search for a circulating factor, or factors, in FSGS has been a long and difficult one, with many researchers over at least three decades foundering on the rocks of this particular conundrum.

As always in science, the search for the answer is limited by the technology and knowledge available at the time. Consequently, the various factors proposed over the years have reflected the prevailing trends in biology. For example, in the 1990s, many groups investigated circulating cytokine levels, and in more recent years powerful screening methods for biological samples have become available, together with an advancing knowledge of the intuitive target cell in this disease—the glomerular podocyte. These approaches have identified promising candidate targets, such as the soluble urokinase plaminogen activator (suPAR) [4], galactose [5] and cardiotrophin-like cytokine [6]. suPAR is a compelling candidate to consider, as it has been shown biologically to activate β3-integrin in podocytes and increase podocyte motility in vitro, as well as to cause proteinuria in a mouse model. Levels of circulating suPAR in patients with active FSGS were higher in the patient cohorts tested, although not universally, and this is of course the final test of any circulating factor—can it be used clinically to predict and ultimately reverse disease? The testing of wider populations and potential clinical trials will be the first step to providing an answer these questions in the very near future.

More accurate cell culture models are also important, and the availability of podocytes in culture has enhanced the tools available to study the disease [7]. This has led to the development of cell-based assays, such as the one used in the study of Coward et al. [8]. This approach seems to be particularly relevant for FSGS, a disorder of circulating plasma, where the collection and application of those samples to human podocytes appears logically to be the most directly relevant model of human disease activity one could currently achieve.

Putting TNF-α into the context of FSGS and the filtration barrier

All of these studies beg the question of whether there is one consistently expressed 'factor' that causes the disease (a 'one-hit wonder'), or several completely different factors causing the different phenotypic manifestations of this disease, or a diverse but related family of factors. The phenomenon of post-transplant recurrence of FSGS is arguably the most consistent manifestation amongst the diverse causes of FSGS in terms of phenotypic presentation. Recurrence occurs rapidly, within hours of engraftment, and is responsive to plasma exchange, although less consistently responsive to enhanced immunosuppression. And, although still controversial, there is a growing body of evidence indicating that recurrence is very rare, if not absent, in patients with pathogenic mutations in genes encoding structural podocyte proteins (apart from rare exceptions, such as the development of anti-nephrin antibodies in patients newly exposed to nephrin in a graft) [9].

The finding of enhanced TNF-α activity can be viewed in this context. Either this cytokine could be one of a number of factors involved and is pathogenic in a proportion of patients affected, or it may be the single factor that accounts for all patients with recurrence. Testing of the findings demonstrated in this study will need to be carried out on a broader section of disease, at different stages of disease activity and correlated with response to treatment. Given the number of false dawns seen in the past, it would also be instructive to consider whether enhanced levels of this cytokine could be a disease-related epi-phenomenon. For example, are the enhanced TNF-α levels detected a feature of the systemic inflammation usually associated with episodes of disease relapse, particularly in children? Does the anti-TNF therapy used in the illustrated case target the immune system rather than the podocyte?

Additionally, any 'factor' targeting the podocyte in vitro needs to be considered in terms of its ability to access the podocyte in vivo. To accomplish this, the 'factor' needs to breach the endothelial glycocalyx [10], the endothelial cell and the glomerular basement membrane. TNF-α is a 51-kDa homotrimer and therefore smaller than albumin, but its filtration properties would depend largely on its properties, such as shape, deformability and charge [11].

Clinical approaches to testing candidate factors

These are exciting times for researchers studying FSGS, and already clinical trials are underway to test new therapies based on experimental data derived from patients.

The FONT trial is testing two interventions: oral galactose and adalimumab, an anti-TNF-α humanized monoclonal antibody. To date, the trial has reported on 11 patients in an uncontrolled phase I study, although clearly any data on proteinuria improvement have to be considered as preliminary [12]. The case report in the Bitzan et al. study [3] falls within the same level of evidence, but it is important to

derive the observations from clinical experience that form credible hypotheses—and then go on to provide biological data to support this hypothesis, which this report does.

Clearly there are vulnerabilities to consider with this approach with respect to ensuring that the results can be interpreted with confidence. It is crucial to time, collect and store samples carefully (e.g. to maintain consistent 'activity') and to have the appropriate controls with accurately documented clinical data.

The controls used are essential. Ideally there would be a pairing of relapse and remission samples from the same patient and analysis of changes between those samples, such as changes in immunosuppression, lipids, albumin levels, as all such changes could produce off-target cellular effects and should—in the ideal situation—be controlled for (e.g. by using control samples from nephrotic patients with the same degree of nephrosis). The plasma exchange protocol should also be considered (e.g. between different centers) as the amount and type of anticoagulant added to the circuit or given to the patient can vary. Citrate chelates calcium, and this could affect the extracellular Ca^{2+} environment in the cell culture setting, leading to changes in ion channel behaviour (e.g. TRPC6), which would then alter the cytoskeletal readouts being studied.

All of these parameters should be considered to satisfy the reductionist scientific discipline needed to underpin the experimental data. However, my key point here is that if these aspects are satisfactorily addressed, this model system is by far the closest and best system we have at present to study this fascinating and unique human disease. This point is worth dwelling on for a moment. If we consider the model systems developed to study nephrotic disease, there are a number of well-used techniques, all with their own limitations. Mouse models range from toxins (e.g. puromycin, lipopolysaccharide) that target podocytes or the filtration barrier with variable specificity to cause glomerular damage, to podocyte-specific gene knockouts, of which many dozens have been reported. Ex vivo glomeruli have been used as a measure of water permeability when stimulated by patient plasma, but the system is technically challenging to set up and has not achieved the desired predictability in practice [13].

The next step in designing an FSGS disease model would be to use human plasma samples in vivo, in a mouse or rat, to induce the same disease phenotype clinically and histologically. This has not yet been consistently achieved, and there is a real possibility that it will never be consistently achieved. This is particularly true if the human factor involved does not have the equivalent receptor expression or biological activity in the rodent as in the human kidney. There are numerous known species differences in, for example, immune receptors and regulators (take complement regulators as one example) between mouse and man that limit the development of mouse models of human disease. A good illustration of this is the lack of an accurate mouse model for the Shigatoxin-associated hemolytic uremic syndrome (D + HUS). The toxin injected into mice causes non-specific interstitial renal damage, rather than specific glomerular microangiopathy, because of the lack of the cognate receptor in the mouse glomerulus [14]. Thus, the model used to mimic human disease should be carefully and critically considered.

Summary

As illustrated in this issue of *Pediatric Nephrology* by Bitzan et al. [3], the use of human disease plasma on the target human cell (the podocyte) in a disease that is so uniquely plasma driven is arguably the most powerful approach currently possible to discover the causes and mechanisms of FSGS. The approach taken by these authors may allow the development of cell-based assays that can be used to predict recurrence risk in patients pre-transplantation and provide valuable data on the disease mechanism. This study further advances both our understanding of FSGS and our management of this most difficult of conditions.

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