The anti-CD40 auto-antibody: a biomarker or a factor for the permeability of recurrent focal segmental glomerulosclerosis?

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Primary focal segmental glomerulosclerosis (FSGS) leads to end-stage renal disease in a high proportion of cases. Recurrent idiopathic (rFSGS) after renal transplantation is frequent (30-100%) and can lead to rapid allograft failure. This severe prognosis is caused by an inability to identify the physiopathology and development of specific and successful treatments, despite the use of high-dose immunosuppressives (1).

Different studies suggest that a circulating factor (CF) plays a key role in the pathogenesis of rFSGS. This hypothesis is supported by case series, which show that adsorption of plasma proteins or plasmapheresis reduces proteinuria in these patients (2). However, the exact nature of CF remains still unknown. Two biochemical properties have been proposed: (I) a molecular weight ranging between 30-50 kDa and (II) a high affinity for the protein-A columns, which are used during immunoadsorptions (2,3). To date, several molecules have been suggested as CFs but without reasonable certainty. The lack of reliable models for rFSGS makes problematic the identification and evaluation of molecules triggering podocyte injuries and the development of rFSGS.

Recently, the soluble urokinase receptor (suPAR) has been proposed as an initiating factor for rFSGS (4). However, its overexpression in other diseases such as infections or inflammations, not associated with nephrotic syndromes (5), suggests that suPAR does not trigger rFSGS by itself, but rather cooperates as co-factor in its initial phase or in the development of such disorder. Yet, another intriguing hypothesis proposes undefined alterations in the 3D structure of suPAR from rFSGS patients. As a result, refolding of suPAR could modify its spatial conformation and expose some residues with strong antigenic capacity.

Recently, it has been proposed that auto-antibodies, such as anti-actin, anti-adenosine triphosphate synthetase, anti-nephrin, and anti-protein tyrosine phosphatase receptor type O, could act as initiating factors as they can alter glomerular permeability when injected into animals (6). The idea that an auto-antibody could play a role in the physiopathology of this disease introduced the use of rituximab (RTX), an anti CD20 monoclonal antibody, in the treatment of rFSGF by deleting B lymphocytes synthetizing auto-antibodies, though its effectiveness is being questioned (7). Delville *et al.* have identified a panel of auto-antibodies that could predict rFSGS before transplantation (8). This group used an integrative bioinformatics approach in a high-density protein array followed by validation using an independent enzyme-linked immunosorbent assay (ELISA). Among multiple auto-antibodies detected in the sera of patients with rFSGS, they propose a pathogenic role for patient-derived anti-CD40, which drives to podocyte injury and proteinuria in an intricate way.

From sera of 20 patients with FSGS (ten with rFSGS and ten without recurrence of after renal transplantation), Delville *et al.* have identified a large panel of 789 auto-antibodies present in patients with rFSGS, with high density protein microarrays suggesting that rFSGS occurs in the context of autoimmune alteration despite limited extra-renal manifestations. The main problem was to identify a relevant antibody involved in the physiopathology of this disease without exploring their overall potential effects. After using filtering criteria, including glomerular expression, functional relevance in inflammation and kidney injury, a panel of ten auto-antibodies was selected (CD40, SNRBP2,

FAS, PTRO, P2RY11, RXRA, CCL19, MYLK, APOL2 and CGB5). Based on ELISA titers and ROC analyses, Delville *et al.* found that anti-CD40 could be a potential biomarker for rFSGS since it tended to decrease during the phase of remission in rFSGS patients after RTX plus cyclosporine treatment. The authors concluded that the anti-CD40s, compared to other antibodies, revealed a maximal sensitivity as predictor of rFSGS risk, even when used alone (AUC: 0.77; CI: 0.63-0.92). However, the role and the presence of the other auto-antibodies remain unsolved.

To explain the development of auto-antibodies against CD40 (widely expressed in lymphoid cells), Delville *et al.* speculated on the *de novo* exposition of a particular cryptic peptide that could become antigenic during the development of the disease, leading to the production of auto-antibodies. They found two β-hairpin peptides (34-NSQCC… and 64-ESEF…) between two anti-parallel beta-strands into the structure of CD40. The flexible folding of these peptides make them particularly exposed and easily recognizable by anti-CD40s from rFSGS patients. CD40 is not expressed in normal kidneys but in podocytes of glomeruli affected with FSGS. So, strong CD40 staining was observed only in patients with FSGS or rFSGS (n=2) but not in normal human kidneys. This suggests that a trigger factor is required to induce the expression of CD40 in podocytes, potentially mis-folded or not, for further development of CD40 auto-antibodies. This idea is supported by the identification of a blocking antibody during native conditions for FAST analysis, which can mask the cryptic epitope, suggesting that CD40 would be expressed in injured podocytes. Alternatively, podocyte damages could induce an aberrant folding in other proteins adopting some antigenic properties for anti-CD40 antibodies. In addition, anti-CD40 antibodies from rFSGS induced an alteration in human podocyte structure with actin redistribution; contrasting to anti-CD40s with no rFSGS after renal transplantation. This cytoskeletal reorganization was inhibited by either monoclonal CD40-blocking antibodies and by monoclonal suPAR blocking antibodies, or by an inhibitor of αVβ3 suggesting that the suPAR/β_3-integrin system cooperates with rFSGS anti-CD40s to induce podocyte injury *in vitro*.

The role of anti-CD40 on other cells, such as B cells, is also interesting as it may participate in the large panel of anti-auto-antibodies identified initially in the study. One hypothesis that remains to be demonstrated is that this antibody could stimulate B cells to induce the release of other auto-antibodies. Experiences in mice show that two intravenous injections of CD40 antibodies from rFSGS patients induced proteinuria in mice on day 8. Amazingly, this effect was strengthened with a supplementary intravenous suPAR injection and inhibited by CD40-blocking antibodies. However, anti-CD40 had no effect in mouse $CD40^{-/-}$ whereas suPAR induced proteinuria with anti-CD40 antibodies from recurrent FSGS, but not with those of non-recurrent FSGS. It is also possible that anti-CD40 cross-reacts with suPAR to enhance its effect and perhaps to induce a conformational activation. Therefore, it is not unconceivable that, in the pathogenesis of the disease, the main target of this auto-antibody was suPAR (or other molecules associated) instead of CD40. The true is that these evidences pointing towards a cooperative role between suPAR and anti-CD40 auto-antibodies in the development of rFSFS but not totally clarifies the primary cause of the disease.

The identification of the causal agent of rFSGS (the CF) is a real challenge often called: the "Holy Grail" of nephrologists. If accomplished, it could improve graft survival and enable the development of customized treatments. In addition, CF could be a biomarker for disease and, would help to select patients with a high risk of recurrence before or after renal transplantation and/or help to evaluate the efficiency of treatment. The work of Delville and colleagues provides new and robust information about the basis of the development of FSGS. A larger cohort of patients will be required to validate these results and to evaluate the specificity of the CD40 antibodies. The origin of the production of anti-CD40 is enigmatic but the idea of a potential mis-folding of proteins from rFSGS patients (suPAR or others) is truly fascinating and compels to trace the causal link to the origin of the disease.

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