J Am Soc Nephrol 14: 541–543, 2003

Circulating Permeability Factors in the Nephrotic Syndrome: A Fresh Look at an Old Problem

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The identification and characterization of circulating factors involved in the development of abnormal glomerular permeability to plasma proteins in the nephrotic syndrome has long been one of the holy grails of nephrology. Originally proposed by Shaloub in 1974 (1), such factors have often been presumed to be of T cell origin. The beneficial effects of immunosuppressive agents, particularly those with a selective action on T lymphocytes, and the association of nephrotic syndrome due to minimal change disease (MCD) with lymphoid neoplastic diseases have been cited as indirect evidence in support of an immune cell origin for putative permeability factors (PF) (2). A recurrence of glomerular disease after renal transplantation has also been claimed as evidence for the presence of circulating PF (3,4). However, progress in the identification of the molecular nature and specific cellular origin for PF and characterization of the constituents of the glomerular capillary wall that represent the target(s) of PF has been painstakingly slow. Several candidate PF have been identified to date; most have only been partially characterized biochemically (2). Different PF appear to be involved in the abnormal glomerular permeability seen in MCD and focal and segmental glomerulosclerosis (FSGS), the two lesions causing nephrotic syndrome that have attracted the greatest attention as possibly caused by the action of putative circulating PF (2). Some of the PF thus far identified are not entirely specific for any particular glomerular lesion causing the nephrotic syndrome. Most studies of PF have involved complex, *in vitro* bioassays, often of uncertain sensitivity, specificity, and reproducibility. One glaring deficiency of the available bioassays for PF is that they cannot directly distinguish and separately analyze the effects of permeability promoting factors and the influence of inhibitors of permeability promoting substances. Changes in either or both of the permeability promoting and inhibitory factors could account for the PF activity of a given specimen of serum in an *in vitro* functional bioassay. Indeed, factors inhibiting glomerular permeability appear to be present in normal serum and can also be found in the urine of nephrotic patients (5,6,7). These may, in many instances, be a special class of regulatory HDL apolipoproteins.

One important aspect of this subject relates to uncertainties concerning the relationship of serum levels of PF activity to the clinical response to treatment of the native disease. Serial studies of PF activity before, during, and after treatment, with or without a decline in proteinuria, would help to clarify the potential role of PF in nephrotic proteinuria and perhaps shed light on the mechanism of action of remission inducing agents. Such a study has been conducted by Cattran *et al.* and is reported in this issue of the *Journal of the American Society of Nephrology*. (8) These investigators sought to determine whether changes in the circulating levels of PF activity (as measured by a bioassay) would correlate with remissions of proteinuria in patients with steroid-unresponsive nephrotic syndrome due to FSGS concomitantly treated with Cyclosporin or a placebo. A subset of patients enrolled in a randomized controlled study of the effect of Cyclosporin on steroid-resistant FSGS were serially analyzed using a bioassay for PF activity, using the change in volume of isolated rat glomeruli subjected to an oncotic gradient (P_{alb} assay) (9). This assay had previously been shown to demonstrate abnormally elevated PF activity in a high percentage of patients with idiopathic FSGS, and also high levels of PF detected by the P_{alb} assay roughly, but inconsistently, predicted recurrence of FSGS in renal allografts (10,11). The study by Cattran *et al.* clearly demonstrated that Cyclosporin treatment had no effect on circulating PF activity levels. The circulating levels of PF before, during, and after Cyclosporin or placebo treatment were not different. Of equal importance was the observation that the levels of PF activity did not change with remission or relapse of proteinuria. Similar findings of a lack of correlation between circulating PF activity and the magnitude of proteinuria and the response to steroid or plasma exchange treatment have also been reported by other investigators (7,12).

On the surface, these findings would seem to contradict the long-held view that putative PF are synthesized by and released into the circulation by immune cells (T-cells), thought to be sensitive to the immunosuppressive action of Cyclosporin (and other calcineurin inhibitors). However, as pointed out by the authors, these findings do not exclude a potential role for PF in the pathogenesis of proteinuria in FSGS. First, the bioassay may lack the requisite sensitivity to detect threshold-like changes responsible for a pathogenic effect. Second, local intraglomerular processes, sensitive to the cellular effects of Cyclosporin, may be responsible for the decline in proteinuria observed in Cyclosporin-treated patients. Thus, Cyclosporin could be regarded as a locally acting, anti-proteinuric agent not a systemically acting immunosuppressant agent. Whether the intraglomerular effects of Cyclosporin are mediated by inter-

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1046-6673/1402-0541
Journal of the American Society of Nephrology

DOI: 10.1097/01.ASN.0000051640.04273.75

ference with the action of PF locally cannot be directly determined from these studies, but *in vitro* studies have supported this hypothesis. A third possibility is that the P_{alb} bioassay is not measuring the effects of a single or predominant permeability-promoting substance, but is rather evaluates the complex interaction of several permeability promoting and inhibitory factors, the balance of which have differing effects *in vivo* and *in vitro*. Of course, all of these possibilities, as well as others not yet conceptualized, could be resolved by the identification and molecular characterization of the putative permeability promoting and inhibitory factors operating in normal and pathologic serum samples (13). Such an accomplishment would be quickly followed by substance-specific assays. Perhaps the application of proteomics to the dilemma will help to clarify the present situation.

What we do know is that the PF associated with steroid-sensitive MCD and steroid-resistant FSGS appear to have dissimilar characteristics. In MCD lymphocyte-derived vascular permeability factors (VPF), acting both on skin and glomerular capillaries, have been identified in supernates of Concanavalin-A stimulated cultures of T cells and also from T cell hybridomas derived from patient with steroid-sensitive nephrotic syndrome (SSNS) believed to be due to MCD (14,15). The production of these factors is apparently stimulated by IL-2, IL-12, and IL-15 and inhibited by Cyclosporin, glucocorticoids, IL-4, IL-10, and IL-13 (2). Unfortunately, the specificity of these VPF for MCD is poor and the ability of VPF to reproducibly influence glomerular permeability *in vivo* is not convincing. The best-characterized PF in SSNS is Hemopexin, a 100-kD heme-binding acute phase reactant (16-18). The levels of Hemopexin activity in sera increase with relapse of SSNS. Hemopexin has been shown to reduce the density of anionic sites in the lamina rara interna of glomeruli (18). Alpha-2 macroglobulin is a naturally occurring inhibitor of Hemopexin. Vascular endothelial cell growth factor (VEGF) has also been investigated as a possible PF in SSNS (19). However, like the P_{alb} assayed PF studied by Cattran *et al.*, VEGF levels in the serum and urine of nephrotic patients are not different in remission or relapse (20). In addition, VEGF does not increase glomerular permeability *In Vivo*. Heparanase, an enzyme digesting Heparan sulfate and produced by immune cells, is an attractive candidate PF because it would degrade the anionic sites in the capillary wall so critical for maintenance of the charge-selective barrier to protein filtration that is disturbed early in the development of proteinuria in SSNS due to MCD (21). Investigations of the role of heparanase as a PF are ongoing, no conclusions can be drawn at present.

The PF associated with FSGS have been the subject of intense investigation and provide a useful background for the studies described by Cattran *et al.* FSGS can be regarded as a prototypical disorder in which circulating PF ought to be operative. As many as 40% of patients with idiopathic FSGS will develop a recurrence of a similar disease after renal allotransplantation, sometimes as soon as a week after implantation of the new organ (3,4). The pretransplant levels of PF, as measured in the P_{alb} bioassay, are sometimes predictive of recurrences of FSGS in renal allotransplants, but this is not a consistent finding (10,11). Godfrin, Dantal, Soulillou, and coworkers (11,22,23) have described a <100-kD PF, measured by a bioassay based on variation in the volume of isolated glomeruli subjected to an oncotic gradient (similar to the P_{alb} assay of Sharma and Savin). The glomerular volume variation (GVV) activity is taken as a measure of PF activity. GVV binds to Protein-A columns but is not an Ig. Treatment of patients with recurrent FSGS in ren al allografts with plasma exchange or Protein-A immunoadsorbent columns reduces GVV activity (23). Decreased GVV activity was associated with decreased proteinuria, but pretransplant GVV activity levels did not reliably predict recurrences (11). Other investigators have demonstrated that treatment of patients with native FSGS by plasma exchange or immunoadsorption has limited effects on proteinuria (12).

Savin, Sharma, and colleagues have identified a 30- to 50-kD glycoprotein PF in patients with FSGS using the same assay (P_{alb}) used by Cattran *et al.* This PF is weakly anionic, heat-labile, and sensitive to protease (5,8,9). This PF is capable of inducing reversible proteinuria in rats, and its action is inhibited by Cyclosporin and cyclooxygenase inhibitors *in vitro* (24,25). It also inhibits nitric oxide production from isolated rat mesangial cells, indicating a pluripotential action. (26) As mentioned previously, the levels of this PF pre-transplant are predictive of recurrence of FSGS in the renal allograft.

Somewhat surprisingly, a similar PF activity has been observed in patients with a genetic form of FSGS due to mutations in the Podocin gene (7). A recurrence of proteinuria developed in two of five children with the Podocin gene mutation and FSGS after renal transplatation in association with high levels of PF activity pretransplant. The recurrences responded to plasma exchange with a decline in PF activity. In these patients, urinary loss of PF inhibitors were believed to be operative in pathogenesis of proteinuria as mixing nephrotic urine with nephrotic plasma markedly decreased the PF activity.

Naturally occurring circulating inhibitors of permeability promoting substances are increasingly recognized (5,6,7). Some of these may be special class of proteins, such as apolipoprotein G or J, but other as yet poorly characterized substances may also possess PF inhibitory activity.

The array of confusing and sometimes conflicting data concerning circulating PF activity in various lesions found to underlie the nephrotic syndrome requires one to be cautious in ascribing a specific pathogenic role to particular molecular entities until they have been further characterized in terms of *in vivo* action and the cellular targets identified. The results of Cattran *et al.* (8) primarily serve to shift the focus of attention away from sites of production and metabolism and circulating levels of putative PF and toward achieving a better understanding of the local actions of permeability promoting factors on the constituents of the permeability barrier of the glomerulus and how naturally occurring inhibitors of permeability potentially influence these actions. Progress in this area of investigation will likely continue to be slow until greater success has been achieved in the isolation and molecular characterization

of permeability promoting and inhibiting substances. Early results of attempts to isolate in pure form from normal and pathologic serum substances that promote permeability *in vitro* are encouraging (13).

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See related article, "Serial Estimates of Serum Permiability Activity and Clinical Correlates in Patients with Native Kidney Focal Segmental Glomerulosclerosis," on pages 448–453.