New insights into the molecular mechanism of the action of diuretics

Rainer Greger
Physiologisches Institut der Albert-Ludwigs-Universität, Freiburg, Germany

The mechanisms of Na\(^+\), Cl\(^-\) and H\(_2\)O transport along the various nephron segments are depicted in Figure 1. These schemes have all evolved from functional studies involving a large spectrum of methodologies [1,2]. According to these schemes the mechanisms by which diuretics act in the kidney have been elaborated [3] and, in fact, in many instances the diuretics have been used to clarify the mechanisms depicted in Figure 1. All diuretics inhibit the absorption of Na\(^+\), Cl\(^-\) (HCO\(_3^-\)) and water by their interaction with luminal uptake mechanisms. Although it has been shown that diuretics can also exert effects on tubule cells from the peritubule side at high concentrations, these effects are probably not of pharmacological relevance [4]. Diuretics are kidney specific, not because they interfere with proteins specific for the kidney, but because they are secreted into the lumen. This secretion and the water absorption along the nephron contribute to the high free concentrations of diuretics in tubule lumen. The mechanism whereby diuretics are secreted and hence accumulated has been unravelled very recently [5–9] and is depicted in Figure 2. Diuretics are taken up across the basolateral membrane by an anion- (e.g. furosemide, thiazides) or cation- (amiloride, triamterene) exchanger [5,6]. The exit step into the lumen is not quite clear. It cannot be excluded that this occurs via ATP-driven pumps such as those described for the secretion of bile acids in the canalicu lar membrane of the hepatocyte [10]. This would be in agreement with old observations that a collapse in lumen volume increase in almost every cell and is lar membrane of the hepatocyte [10]. This would be found in almost every tissue. It participates in regu-

occurs and can now explain previously poorly understood genetic diseases.

In the proximal tubule inhibitors of carbonic anhydrase can be used to interfere with HCO\(_3^-\) absorption. Their effect is usually not very marked because the ensuing acidosis limits their inhibitory effect, and because more distal nephron segments, by increasing the absorption at these sites, blunt the natriuresis and diuresis. Genetic defects in carbonic anhydrase II lead to osteopetrosis connected with proximal-tubule-type renal tubular acidosis [12]. Another possible target for inhibition of proximal tubule absorption is the NHE-3 Na\(^+\)/H\(^+\) exchanger (Figure 3A). Its sensitivity and selectivity towards amiloride and ethylisopropylamiloride is rather poor [13]. However, very recently a new derivative benzylguanidino amiloride has been shown to block this cotransporter with a very high affinity [14].

The Na\(^+\)2Cl\(^-\)K\(^+\) cotransporter of the thick ascending limb is depicted schematically in Figure 3B [15,16]. This protein is closely related to the Na\(^+\)Cl\(^-\)cotransporter of the distal tubule [17]. However, it is of clinical relevance that there exists no overlap in terms of pharmacology; loop diuretics bind only to the Na\(^+\)2Cl\(^-\)K\(^+\) cotransporter but not to the Na\(^+\)Cl\(^-\)cotransporter, and thiazides bind only to latter but not to the former. The Na\(^+\)2Cl\(^-\)K\(^+\) cotransporter exists in two major forms. One is the colonic type and is found in almost every tissue. It participates in regulatory volume increase in almost every cell and is involved in the basolateral uptake of Cl\(^-\) in exocrine glands. This type of cotransporter has an affinity to diuretics (azosemide > bumetanide > furosemide) different from that present in the thick ascending limb (bumetanide = torasemide > piretanide > furosemide = azosemide) [4,18]. The other type of Na\(^+\)2Cl\(^-\)K\(^+\) cotransporter appears to be specific for the kidney. Regulation of the Na\(^+\)2Cl\(^-\)K\(^+\) cotransporter is complex. Current evidence suggests that it is upregulated, with activation of protein kinase A, when cytosolic Cl\(^-\)-activity falls and cells shrink [19–22]. Whether all three signalling pathways converge or whether cell shrinkage is the common denominator is currently being studied.

Mutations of this membrane protein can lead to Bartter’s syndrome type I, resembling the same symp-
The basic processes of Na\(^{+}\), Cl\(^{-}\) (HCO\(_3\)\(^{-}\)) and H\(_2\)O absorption in the proximal tubule (PT), thick ascending limb of the loop of Henle (TAL), distal tubule (DT) and collecting duct (CD). Circles with ATP, \((Na^{+} + K^{+})\)-ATPase/H\(^{+}\)-ATPase; circles, carrier systems; arrows, ion channels/ conductances; CAI, carbonic anhydrase inhibitor; CA, carbonic anhydrase; Furos., furosemide; HCTZ, hydrochlorothiazide; Aldo, aldosterone; R/MR, mineralocorticoid receptor. In all nephron segments the basic pump mechanisms is identical: \((Na^{+} + K^{+})\)-ATPase, the luminal uptake mechanisms (Figure 3) are divergent. Diuretics usually interfere with these luminal Na\(^{+}\) uptake mechanisms.

Fig. 1. Secretion of diuretics. Circle with ATP, \((Na^{+} + K^{+})\)-ATPase; circles, carrier mechanisms; Furos., furosemide; SFA, short fatty acid. Diuretics are taken up by the organic anion (cation) transporter [5,6]. Luminal secretion is possibly via ATPases [10]. These specific secretory processes ensure, together with water absorption, that diuretics accumulate in the lumen fluid.
type I. Given that the macula densa cell Na\(^{+}\)\(2\)Cl\(^{-}\)K\(^{+}\) cotransporter is also affected hyper-reninism should also prevail after adequate volume substitution [26,27]. Type II will also be severe. Here, however, K\(^{+}\) losses will probably be less because the primary defect is one of the ROMK channel. The hyper-reninism will depend exclusively on the degree of volume contraction. Type III might be less marked in terms of general symptoms (cf. above), because Cl\(^{-}\) can also leave these cells via the KCl exit (Figure 1). Hyper-reninism might also be less marked than that of type I because these cells would not completely lose their sensory function. All three types will be different from Gitelman’s syndrome (cf. below) because in this syndrome renal Ca\(^{2+}\) excretion is diminished, whereas it is enhanced in Bartter’s syndrome [12,23,28].

The Na\(^{+}\)Cl\(^{-}\) cotransporter of the distal tubule is shown in Figure 3C. It is obvious that it is closely related to the Na\(^{+}\)\(2\)Cl\(^{-}\)K\(^{+}\) cotransporter discussed above. The Na\(^{+}\)Cl\(^{-}\) cotransporter is present in various tissues but little is known on its function in these tissues. It is blocked with high affinity by thiazides [17].

Inhibition by thiazides leads to well-known effects and complications very similar to those caused by loop diuretics. There is, however, one important difference. While loop diuretics cause hypercalciuria, thiazides cause enhanced absorption of Ca\(^{2+}\). This is explained by two mechanisms (Figure 1): (i) thiazides increase cytosolic calbindin and this facilitates transcellular Ca\(^{2+}\) absorption [29], and (ii) thiazides reduce cytosolic Na\(^{+}\) concentration thus enhancing the driving force for Ca\(^{2+}\) exit via Ca\(^{2+}\)/Na\(^{+}\) exchange. Gitelman’s syndrome can now be explained as a genetic defect of the Na\(^{+}\)Cl\(^{-}\) cotransporter. The symptoms and signs resemble those of thiazide treatment [12,23,28].

The \(\alpha\)-subunit of the Na\(^{+}\) channel, present in the collecting duct and probably also in the late proximal tubule [30] is depicted in Figure 3D [31]. The actual Na\(^{+}\) channel, also called the epithelial Na channel (ENaC), is probably made up of four subunits: two \(\alpha\), one \(\beta\) and one \(\gamma\). Its function is closely controlled by aldosterone [32]. This type of channel is also present in other epithelia lining the outer ‘surfaces’ of our
body: lung, colon, sweat gland ducts, salivary gland ducts, etc.

To the extent that Na⁺ absorption is enhanced in the collecting duct (e.g. via mineralocorticoid effects), K⁺ secretion, and that of H⁺, will also be increased [33]. The ENaC channel is blocked with high affinity by amiloride and triamterene [32,34]. It is downregulated by aldosterone antagonists such as spironolactone. Several inborn defects can alter ENaC function. In glaucoctroid remidiable aldosteronism (GRA) overabsorption of Na⁺ occurs because the cortisol synthesis is defective at the level of the β11-hydroxylase. Mineralocorticoids are formed in excess, because, in view of low circulating cortisol, adrenocorticortropic hormone (ACTH) is secreted and turns on the production of corticoids with mineralocorticoid function. In cases of disturbed 11β-hydroxy-steroid dehydrogenase, be it caused by liquorice abuse, other inhibitors or genetic defects, glaucoctroids cannot be degraded to cortisol in mineralocorticoid target tissues. The excessive concentrations of cortisol in these tissues then exert a mineralocorticoid effect and cause enhanced Na⁺ absorption and K⁺ secretion (pseudohyperalderstonism). In cases of inborn mineralocorticoid-receptor defects the opposite occurs, Na⁺ is lost excessively (pseudohypoaldosteronism). Finally, the ENaC channel can also be genetically defective. Interestingly, defects in the β- and γ-subunit can lead to a ‘gain of function’ mutation, i.e. more channels are present and/or they show an increased activity. This gives rise to Liddle’s disease with overabsorption of Na⁺, loss of K⁺ and salt-dependent hypertension (pseudohyperaldosteronism) [35]. Other mutations of ENaC lead to a ‘loss of function’ with Na⁺ losses (pseudohypoaldosteronism) [32].

Our understanding of the various ion transporters in renal tubule segments has increased exponentially during the past few years. Most of the transporters which have been characterized on a functional basis over the last 25 years have now been identified. This enables us to understand the effect of diuretics at a truly molecular level and to characterize tubule transport defects which up until now have only been described empirically. Future work will have to concentrate on the detailed mechanisms whereby these transporters are regulated.

Acknowledgements. Work in the author’s laboratory has been continuously supported by Deutsche Forschungsgemeinschaft Gr 480.

References
Therapeutic uses of heparinoids in renal disease patients

Gary E. Striker

Laboratory of Renal Cell Biology, Department of Medicine, University of Miami School of Medicine, Miami, FL, USA

Introduction

The single most conspicuous feature of progressive glomerular diseases is an increase in extracellular matrix, i.e. scarring. There is often an associated increase in the number of cells in the mesangial regions, leading some to conclude that mesangial cell proliferation plays an important role in the development and progression of glomerulosclerosis. This supposition has been confirmed by recent findings of the presence of increased mesangial cell turnover in some human diseases and in a large number of experimental animal models, using either histochemical methods or autoradiographic techniques [1]. However, ourselves and others have shown that proliferation and glomerulosclerosis are not necessarily linked [2]. Nonetheless, these data provided the rationale to examine drugs, which decrease proliferation and extracellular matrix synthesis for their potential therapeutic value. Drugs that would be clinically useful should be orally available, reasonably specific, and free of undesirable side effects.

The purpose of this editorial is to review the data on one class of compounds that fit these requirements, namely sulfated oligosaccharides, including modified fractions of heparin.

Experimental and human data in renal disease

The demonstration that fibrin appeared to be a major component of crescentic glomerulonephritis in experimental animals and man, led to the supposition that abnormalities in coagulation played an important role in acute glomerulonephritis. Based on this hypothesis, there were many attempts to utilize heparin or heparin-like compounds in the treatment of glomerulonephritis (GN). Encouraging results in experimental animals led to attempts to use heparin in the management of rapidly progressive glomerulonephritis [3]. These trials were stopped due to the side effects of heparin therapy, namely the appearance of severe bleeding as a result of anticoagulation. Subsequently it has been generally agreed that coagulation is very likely a secondary event in GN [1].

More recently, heparin and various fractions of heparin have been shown to decrease proliferation of mesangial cells, bind many growth factors, serve as an electron sink, and influence lipid metabolism [4]. As these properties of the heparin, and fragments thereof, have become more evident and heparinoids with reduced, or no, anticoagulant activity were prepared, investigators have again begun studying their use in models of kidney and vascular disease.

Definition and types of heparinoids

Definitions

Considerable confusion has arisen in the literature in the nomenclature of heparin derivatives and heparinoid...
like molecules. This may be due to the fact that heparin is a large molecule with a protein core to which are attached glycosaminoglycan side chains of variable composition. The glycosaminoglycan side chains are most often prepared by limited nitrous acid cleavage, followed by chromatographic separation. Because of the heterogeneity of the chemical composition of the glycosaminoglycan side chains of heparin, the composition of the side chains may vary considerably between different preparations. This makes it difficult to make comparisons between different isolates. A further complication is that the product marketed as heparin is extracted, from either beef or pork tissues and may contain other proteoglycans, the most abundant being chondroitin sulfate. Thus, there is considerable variation between individual lots in terms of biological activity and exact chemical content. For this reason, the material is characterized by international units based on its anticoagulant activity, not on its molecular mass. For obvious reasons, the degradation products of commercial heparin share this uncertainty as to chemical composition. In addition, heterogeneity between different commercially available products is further accentuated by the fact that low molecular weight heparins (LMWH) are modified chemically in somewhat different ways to either promote or decrease their anticoagulant properties.

Heparin derivatives

These preparations consist mostly of side chains composed of highly sulfated saccharides, and contain most of the anticoagulant activity. They also include the regions containing growth factor and extracellular matrix molecule binding sites.

Plant extracts

Following the elucidation of the structure of heparin glycosaminoglycan side chains, there were identified other sources of oligosaccharides, namely those found in the plant world. A xylan oligosaccharide, with an approximate molecular mass of 4–6 kDa, was isolated from beech trees. Following sulfation, it was found to have little anticoagulant activity, but the sulfated oligosaccharide inhibited thrombus formation. This compound, called pentosan polysulfate or SP54, has been in clinical use since the 1960s [5]. This compound has often been called a heparinoid. We prefer the term sulfated oligosaccharide, referring to its general chemical properties, since the term heparinoid carries the implication of an anticoagulant action. In fact PPS has 1/15th the anticoagulant activity of unfractionated heparin.

Recently, we have found that PPS shares some of the non-anticoagulant features of unfractionated heparin, and adds others, making it a potentially interesting therapeutic agent [5]. In addition, it has been shown to be effective as an oral agent. The oral form is an approved drug for the treatment of interstitial cystitis, a painful bladder condition associated with thickening of the bladder wall due to smooth muscle hyperplasia and scarring.

Finally, PPS has the additional advantage that there is less biological variation between individual batches. It is marketed under the commercial name Elmiron®.

In vitro data

Mesangial cells

Heparin and heparin derivatives, decrease proliferation of vascular smooth muscle cells [1,6]. We developed mesangial cell lines from normal C57/B6 mice that retain many phenotypic characteristics of the in vivo situation, including extracellular matrix turnover [7]. They produce a large amount of type IV collagen and a lesser amount of type I collagen, as well as matrix metalloproteinases (MMP) and tissue inhibitors of matrix metalloproteinases (TIMPs).

We examined the responses of these mesangial cells in vitro to unfractionated heparin and compared it to PPS [8]. In response to heparin or PPS, mouse mesangial cells exhibited a sharp decrease in cell proliferation without any change in viability. As noted above, there was variation between heparin lots, but not between different PPS preparations. Collagen types I and IV decreased in the media and cell layer following treatment with PPS, but not with heparin. There was an increase in MMP2 following treatment with both heparin and PPS. Furthermore, both drugs altered the TIMPs profile. TIMP 3 was released in the medium instead of being bound to the MMPs on the cell surface. These data suggest that heparinoids may alter extracellular matrix turnover, partly by increasing collagen degradation, and may therefore be useful in vivo. These data also point out that heparin and PPS have different effects on mesangial cells. It is not yet established how PPS modifies the mitogenic activity of mouse mesangial cells.

Mesangial cells from diabetic NOD mice

There is still considerable debate as to whether mesangial cells in diabetic animals behave like normal cells when they are exposed to high glucose concentrations. We previously showed that diabetic non-obese diabetic (NOD) mice develop glomerulosclerosis [9]. We also found that mesangial cells isolated from diabetic NOD mice (D-NOD-MC) exhibited a stable phenotypic switch in culture, consisting of increased secretion of IGF-I and an enhanced growth rate [9]. We measured collagen deposition and the activity of MMP and TIMPs in diabetic and non-diabetic NOD-MC. Total collagen degradation was markedly reduced in D-NOD-MC and they had a constitutive decrease in MMP-2 and nearly unmeasurable MMP-9 activity. This was at least partially due to decreased mRNA levels. TIMP levels were slightly decreased in D-NOD cells. Thus, it is possible that similar changes occur in other models of IDDM in animals or in humans, and possibly in other types of renal disease. This question
is a current topic of research in our laboratory. Since PPS treatment is associated with a increase in the secretion of MMPs in normal mesangial cells [8], we postulated that it may also increase MMPs in the diabetic cells, providing another avenue for a net decrease in collagen deposition. We found that PPS treatment increased MMP2 activity and released TIMP3 into the medium in the diabetic cells. Thus, PPS treatment appears to result in a net decrease in excess collagen deposition, which is one of the chief features of glomerulosclerosis in diabetic nephropathy.

**Chronic progressive glomerular diseases, in vivo studies**

Several studies suggest that glomerulosclerosis is decreased by heparin, either intact or non-anticoagulant, and by sulfated oligosaccharides [10]. This was first shown in the rat ablation model in which the subcutaneous administration of non-anticoagulant heparin treatment reduced proteinuria and glomerular lesions [11]. In the habu venom model, which is characterized by mesangial and endothelial cell proliferation, subcutaneous heparin treatment also decreased the number of cells in the mesangial region [6]. Similarly in the aminonucleoside nephrosis model there is a decrease in the glomerular lesions following subcutaneous heparin treatment [12]. Interestingly, the investigators showed that the decrease was not mediated by a change in glomerular haemodynamics. Others have also shown a reduction in cell proliferation and progression of the lesions in a model of diffuse glomerulonephritis in the rat thy-1 model, in which the lesions are largely mediated by the influx of activated macrophages [13].

Several recent experimental studies report on the use of heparin-derived glycosaminoglycans (GAG). GAG delay the onset of albuminuria in streptozotocin (STZ) Sprague Dawley diabetic rats [14–17]. These data suggest that heparin and heparin-derived GAG decrease local glomerular lesions through local effects on glomerular cells, rather than through modification of renal haemodynamics. One of the current theories entertained by investigators in the field is that heparin and LMWH replace the lost heparan sulfate at the surface of cells. These data and our own in vitro observations led us to investigate the potential therapeutic value of PPS.

**Experimental animal models**

**5/6 nephrectomy in rats**

PPS (100 mg/kg/body weight/day) was administered orally to Wistar male rats (Bobadilla et al. personal communication). The treatment commenced 1 week after 5/6 nephrectomy, and continued for 30 days. Proteinuria in the PPS treated group did not differ from non-nephrectomized rats. In contrast, proteinuria in the untreated 5/6 nephrectomy group was 40 mg/day. In addition, histologic lesions in the interstitium and glomeruli was markedly decreased in the animals treated with PPS. Interestingly, the intraglomerular capillary hydrostatic pressure and single nephron GFR were normal in the PPS treated group.

**ROP/Os mice**

We recently described an accelerated model of diabetic glomerulosclerosis in ROP Os/+ mice [18]. These mice have an inborn reduction in nephron number and are susceptible to the development of glomerulosclerosis [2, 19]. Diabetes was induced with multiple STZ injections. The mice were maintained for 12 weeks without insulin treatment. At sacrifice there were severe glomerular lesions affecting the glomeruli, as well as the interstitium and tubules. One group of mice received PPS in the drinking water. We found a decrease in the amount of mesangial sclerosis in the treated mice as well as a diminished amount of type IV collagen mRNA in the glomeruli as measured by RT–PCR.

**PTFE grafts in haemodialysis patients**

**In vitro studies**

Vascular access failure is a major cause of morbidity in patients undergoing maintenance haemodialysis and results in large expenditures. Stenosis is the underlying cause of loss of patency in the majority of failed grafts and appears to result from proliferation of smooth muscle cells and an accumulation of extracellular matrix. To determine whether this process was amenable to pharmacologic intervention and/or prevention, we obtained material occluding the vascular access from seven patients undergoing surgical revision of the graft [5]. In all seven patients the outgrowth contained predominantly smooth muscle-like cells, which produced a large amount of type IV and type I collagen. Treatment with PPS inhibited cell proliferation and significantly reduced the accumulation of type I and type IV collagen. This was associated with an increase in metalloproteinase-9 (MMP-9) and a shift of tissue inhibitor of metalloproteinase-3 (TIMP-3) from the cell layer into the medium. These data suggest that PPS may have a favorable effect in patients with a PTFE graft by decreasing cell proliferation and collagen deposition.

**Clinical trials utilizing heparin fractions**

There are several studies in patients with diabetic nephropathy, each with a small number of subjects. In one early study, injection of unfractionated heparin or a low molecular weight heparin fraction decreased the albumin excretion rate in 35 patients with IDDM [20]. Complications led to discontinuation of the study in four patients, and 12 noted discomfort or bleeding at the injection site, including six treated with the low
molecular weight heparin fraction. When the treatment was discontinued, proteinuria returned in all patients.

In a more recent study, oral sulphated glycosaminoglycans were administered in a crossover study in 12 insulin requiring NIDDM patients who were hypertensive and had either microalbuminuria or overt proteinuria. The authors noticed a modest decrease in albumin excretion rate, most apparent in those in the treatment arm of the first crossover period [21,22]. Interestingly, the proteinuria did not rebound after the treatment was discontinued in those patients observed during the placebo period of the crossover.

Finally, danaparoid sodium, another low molecular weight fraction of heparin, was administered subcutaneously to nine IDDM patients with overt diabetic nephropathy [23]. This product, which was injected daily, largely consists of heparan sulfate but also contains chondroitin sulfate. In this crossover study, the duration of each crossover period was 6 weeks. A statistically significant decline in the albumin/creatinine excretion rate and the urinary protein/creatinine ratio followed the treatment period.

Future perspectives

Taken together these data suggest that sulphated oligosaccharides, including heparan sulfate(s) and low molecular weight heparin(s) may play a role in the treatment of sclerosing kidney diseases, including diabetic nephropathy, at least on a short-term basis. Many questions remain unanswered in the use of these preparations, and it remains to be seen whether the decrease in the albumin excretion rate, following their short-term administration, signals a sustained decrease. Such an event would only be significant if it were associated with a decrease in the incidence of end-stage renal disease.

We have chosen to study another sulphated oligosaccharide, PPS, which has often been compared to heparinoids. Our in vitro and in vivo animal experimental data suggest that PPS has the unique and specific effect on the extracellular matrix of returning the degrading activities of smooth muscle cells towards the normal range. Importantly, one of the changes seen in smooth muscle cells exposed to high glucose concentration and in glomeruli isolated from diabetic mice, is a decrease in the turnover of extracellular matrix. This effect is reversed by PPS. Thus, PPS may directly reduce the deposition of scar tissue in the glomeruli and blood vessels of patients with diabetic nephropathy.

References

Cyst formation in ADPKD: new insights from natural and targeted mutants

Albert C. M. Ong

Institute of Molecular Medicine, University of Oxford, Oxford, UK

The 31st American Society of Nephrology meeting in Philadelphia (25–28 October 1998) provided a unique opportunity to catch up on the fast moving field of research into the pathogenesis of autosomal dominant polycystic kidney disease (ADPKD). Firstly, from the analysis of germline mutations in PKD1 and PKD2 patients, the genetic basis underlying cyst formation in ADPKD is now clear. Secondly, the consistent findings of somatic PKD1 mutations in PKD1 cyst epithelia is changing our concepts as to how cysts arise in the first place. Finally, the characterization of PKD1 and PKD2 knockout mice has led to unexpected insights into when and how cysts arise in different tissues.

The spectrum of mutations in PKD1 and PKD2

Detailed mutational analysis of the PKD1 gene has proved difficult especially in the region of the gene which is duplicated. Nevertheless, most of the duplicated region has now been analysed using methods which exploit subtle differences between the HG (homologous genes) and PKD1 sequences [1,2,3]. These studies have given us a better idea of the spectrum of mutations present in PKD1 families. First of all, they have revealed that the majority of mutations detected are private, indicating a high rate of new mutation. Secondly, most of the mutations are stops or frameshifting changes which would inactivate the gene. Thirdly, no mutation ‘hot spot’ or genotype-phenotype correlation has been clearly identified in the region surveyed so far.

Like PKD1, the majority of germline changes in PKD2 are unique inactivating change but unlike PKD1, mutational analysis of the whole of PKD2 has been much more rapid [3]. Evidence for a mutational ‘warm spot’ (in proximity to a polyadenosine tract) has been proposed but like PKD1, no clear genotype-phenotype correlation has been shown, at least for renal disease severity [4]. Taken together, these results suggest that the first step leading to cyst formation in PKD1 and PKD2 involves the inactivation of one functional copy of the corresponding gene.

Susceptibility of PKD1 to new mutations

Given the prevalence of PKD1 in the population and the high rate of new mutation, a question that has arisen is why the human PKD1 gene should be so susceptible to new mutations. Preliminary evidence for two interesting hypotheses have been presented. The first proposes that a unique structural feature of PKD1 (a long polypyrimidine tract in intron 21) is what makes the gene more susceptible to mutation [5]. In theory, this tract could promote triplex DNA strands to form, leading to an increased error rate by several mechanisms. The second hypothesis proposes that gene conversion events (between the HG loci and the PKD1 sequences) during recombination could lead to an increased mutation rate [6].

Each hypothesis gives rise to a logical prediction. If the first mechanism is operative, further mutational analysis should reveal a clustering of mutations around intron 21, particularly of gene deletions. If the second mechanism were true, then a high frequency of identity between PKD1 mutations and the corresponding HG sequences should be observed. Evidence for the latter should be forthcoming with a more complete analysis of the duplicated region of PKD1 and the HG loci. These mechanisms may also help to explain why there is an apparent high rate of somatic mutation at PKD1 (see below).

Somatic PKD1 mutations in PKD1 cysts

There is now a consensus that the mutational mechanism in both PKD1 and PKD2 is likely to be a ‘loss of function’ mechanism. However, while the presence of a germline mutation is a necessary first step, it is clearly insufficient to trigger cystic behaviour in any given susceptible cell, especially given the observed focal and sporadic nature of cyst formation. This has in turn led to the idea that an additional event: a second step, is required for cysts to form.
The nature of this additional event has been the subject of detailed study [7,8] and some debate [9]. Attractive evidence has been put forward to suggest that this trigger may involve inactivation of the normal polycystin protein by somatic mutation: a ‘two hit’ model, better known among researchers of tumour biology. Using loss of heterozygosity (LOH) analysis of PKD1 (a technique utilizing polymorphic microsatellite markers flanking the PKD1 gene), two groups found LOH involving the normal PKD1 gene in ~20% of PKD1 cysts analysed [7,8]. In addition, a somatic mutation consisting of a 2 base pair deletion inactivating the normal PKD1 gene was detected in one cyst, implying that other smaller changes inactivating PKD1 might be present in LOH negative cysts [8]. More recently, similar changes have been reported in PKD1 liver cysts [10].

Perhaps the most striking feature of these findings is that, where it was possible to distinguish between the normal and mutant PKD1 alleles, somatic mutations invariably occurred on the background of the normal allele. At first glance however, these results appear to conflict with several published studies showing prominent polycystin-1 expression in PKD1 cystic epithelia [9]. One possible explanation that may reconcile the observations of somatic mutations and persistent polycystin-1 expression is that what is being detected in cyst epithelia is inactivated or non-functional protein. This would be the case if the majority of germline and/or somatic mutations turn out to be missense. Analysis of the whole PKD1 gene in individual PKD1 cysts would help clarify the full spectrum of somatic PKD1 mutations. However, given the present difficulties in analysing PKD1 and the similarity in phenotype between the two diseases, a more feasible approach might be to examine PKD2 cysts for somatic mutations in PKD2.

**Somatic inactivation in PKD2 knockout mice**

Similar to the human disease, heterozygous PKD2 knockout mice (PKD2+/−) develop kidney and liver cysts [11]. Strikingly, mice heterozygous for an unstable PKD2 allele (which undergoes somatic inactivation by intragenic homologous recombination to form a true null allele) showed many more cysts than mice heterozygous for just a stable PKD2 null mutation. Consistent with this, cysts that develop in PKD2+/− mice showed no polycystin-2 expression by immunohistochemistry. These results contrast with our preliminary findings in human PKD2 cystic tissue [12] but the PKD2+/− mice develop relatively few cysts and further analysis may reveal that some cysts express polycystin-2, where somatic missense changes have occurred. Overall, these results nevertheless provide strong support for a ‘two hit’ model of cystogenesis in PKD2.

Whilst a cystic phenotype was not unexpected in these mutant mice, there was a surprising difference in the rate and timing of cyst formation between different organs. Like PKD1−/− mice [13], PKD2−/− mice had numerous kidney and pancreatic cysts in utero but no liver cysts [14]; liver cysts were however observed in PKD2−/− mice with increasing age [11]. Although the full phenotype of PKD1−/− mice has not yet been reported, these observations suggest that the function of both polycystin proteins lies more in maintaining biliary ductal organization than in their formation. They also imply that the process of cyst formation not only occurs during development but may also occur throughout adult life.

In the light of the current evidence, a ‘two hit’ model for cyst initiation seems plausible. Such a model is attractive because it could account for the focal nature of cyst formation and the phenotypic variability seen between affected individuals within the same family. However, there may be other mechanisms influencing disease severity in ADPKD, albeit less well defined. These include the effect of modifier genes and mutant polycystin proteins on expression of the disease phenotype.

**Infantile-onset PKD1 and modifier genes**

Very rarely, severely affected infants or children may be born to a mildly affected parent with PKD1. Many such cases have been described and a striking difference between these ‘early onset’ cases and the more typical adult-onset cases is both the number and the phenotype of renal cysts (mainly glomerular) found in these individuals. It is interesting to note that the increase in disease severity is confined to the kidney. These cases also resemble homozygous PKD1−/− mice, in terms of their renal phenotype although so far, there have been no reports of severely affected offspring born to two affected parents with ADPKD. Typically, the child or foetus carries the same stable mutation as the affected parent [15]. Nevertheless, a high recurrence risk (~45%) has been reported in the subsequent offspring of the affected parent, sometimes with different partners. These findings strongly suggest that other inherited factors (so called ‘modifying genes’ which may be transmitted from the affected parent) can modify the rate of cyst formation. If the ‘two hit’ model is correct, these genes might specifically alter the rate of somatic mutation at PKD1. Alternatively, these genes could encode allelic variants of other proteins which might modulate the activity of polycystin-1 by protein–protein interactions in a cystic pathway or complex.

The identification and mapping of these loci would thus provide valuable insights into the mechanism/s governing the initiation and rate of cyst formation. The influence of genetic background in suppressing or accelerating the rate of cyst formation has been convincingly demonstrated in a recessive mouse model of cystic disease (pcy) and two modifying gene loci mapped [16]. The availability of PKD1 and PKD2 mutant mice now opens up the possibility of other loci specifically modifying cyst formation in ADPKD being
PKD1 knockout mice: phenotypic differences between del4 and del34 mutants

Two different mouse mutants with targeted deletions of Pkd1 in exon 4 (del4) and exon 34 (del34) have been described [13,20]. The full description of heterozygous mice carrying either change is incomplete but homozygous mice bearing either Pkd1 mutation develop kidney and pancreatic cysts. Interestingly, del4 mice appear to have a more severe phenotype than del34 mice suggesting that a truncated polycystin-1 protein (containing all of the extracellular N-terminal region) could be functional [20]. The demonstration of such a protein is awaited but if this were the case, it could also be predicted that del4 heterozygous mice would have more cysts than del34 heterozygous mice. An alternative explanation is that these observed differences are the result of differences in genetic background between these two mutants. These observations also contrast with the absence of any clear correlation so far between disease severity and the position or nature of PKD1 mutations in man.

In summary, analysis of germline PKD1 and PKD2 mutations has shown that a necessary first step in cyst formation in ADPKD is the loss of one functioning polycystin protein. The evidence for somatic mutation of the normal PKD1 gene in individual PKD1 cysts and for somatic mutation of Pkd2 in the cysts of PKD2+/− mice suggests that loss of the corresponding normal polycystin protein may be the rate-limiting second step determining the acquisition of a cystic phenotype. These two observations provide a rationale for gene replacement as a therapeutic option. Nevertheless, the timing of such therapeutic intervention is clearly important in view of the different times at which kidney and liver cysts arise. Finally, the influence of genetic modifiers which can alter the rate of cyst formation is increasingly recognized. The identification of such genes could lead to powerful alternative therapeutic strategies to halt cyst initiation or expansion in ADPKD.

References

NIMA (Non-inherited Maternal Antigens) versus NIPA (Non-inherited Paternal Antigens) and tolerance of human kidney graft

Etienne Dupont

Department of Immunology and Transfusion, Erasme Hospital, Brussels, Belgium

In the December 3, 1998 issue of the New England Journal of Medicine [1], Burlingham et al. report on improved kidney graft survival in patients \((n = 205)\) transplanted with sibling donors bearing non-inherited maternal antigens (NIMA) \((n = 95)\) compared to non-inherited paternal antigens (NIPA) \((n = 110)\) in nine centres during the 1966–1996 period. This retrospective study involves patients from the pre-cyclosporin and the cyclosporin era. Graft survival at 5 years \((86\% \text{ vs } 67\%)\) and at 10 years \((77\% \text{ vs } 49\%)\) was significantly better \((P = 0.006)\) in the NIMA group in which survival achieved in HLA identical sibling was obtained. An unexpected observation made in this group of patients was that early reversible rejection crisis occurred more frequently than in NIPA subject, suggesting that neonatal exposure to maternal antigens had primed the immune system of the recipient. Thus, as proposed in the classical Medawar’s [2] studies, results obtained in this study are compatible with the hypothesis that tolerance to the HLA antigens could have been induced during intrauterine life. The concept of NIMA and NIPA evolved a decade ago in Leiden [3,4] during studies trying to define acceptable mismatches that could be provided to those patients who had high levels of anti-HLA antibodies in response to previous transplants, pregnancies or rejection of a graft. Such patients are difficult to transplant because cross-match with potential donors is almost always positive [4]. During a systematic study conducted to assess to which HLA antigens they had preferentially formed antibodies, it was found that 50% of the patients did not form antibodies against NIMA as compared with NIPA against which more than 85% were sensitized. This observation became the basis of a novel concept to consider NIMA as an acceptable mismatch for these hyperimmunized patients thus allowing to enlarge the pool of possible donors [4,5]. However, its extrapolation at the cellular level for matching purposes provided conflicting results. In the parent to child setting, there was no improvement of results in patients having received a kidney from their mother compared to those having received a kidney from their father [6–8]. Why do results obtained with sibling kidneys show a difference? The authors propose that presensitization of the mother to the antigens of her child could have disrupted some tolerance mechanism through passenger leukocytes [1] infiltrating the graft. They also take argument of the finding in another study of a higher graft loss from female kidneys than from males [9]. In a recent study evaluating 669 cadaveric recipients who had determination of the HLA type of the mother, an improvement of survival rate of grafts with a mismatched antigen identical to the NIMA as opposed to non-NIMA was found [10]. Confirmation of these observations would indicate that inducible tolerance in human is achievable and that efforts should be made to provoke it. However, it must be noted that data of the study suggest that the NIMA effect seems to be lessened since the introduction of cyclosporin and that putative mechanisms involved in this phenomenon are yet unclear. Influence of anti-idiotypic antibodies to anti-HLA antibodies to NIMA that could inhibit the tolerance to the HLA antigens could have been induced during intrauterine life. The concept of NIMA and humoral response associated with rejection and induction of TH2 memory cells are invoked but this is merely speculative. The study of Burlingham also has consequences for selection of living donors in kidney transplantation. Figure 1 shows possible segregation patterns of the haplotypes in a family in which potential donor 2 who shares haplotype d with the mother could be provided to those patients who had high levels of anti-HLA antibodies in response to previous transplantation. Figure 1 shows possible segregation patterns of the haplotypes in a family in which potential donor 2 who shares haplotype d with the mother could
be selected. Furthermore, in view of the recent use of haploidentical donors in stem cells transplantation [11], it would be important to evaluate if a similar effect is observed in this type of treatment for which patients often lack an HLA identical donor. Likewise, implementation of cadaver kidney transplantation programme with such acceptable mismatches could also be envisaged.

References


Haemolytic–uraemic syndrome—the experience in Argentina

Horacio A. Repetto

University of Buenos Aires, Buenos Aires, Argentina

Introduction

Haemolytic–uraemic syndrome (HUS) was first described and given its name by Swiss haematologists [1]. The first observations on children, most of whom had reversible acute renal failure, came from Argentina [2]. Subsequent experience showed that the incidence of this type of thrombotic microangiopathy (TMA) was higher in Argentina than in any other part of the world. Up to 1993, >5500 cases have been registered in Argentina, the incidence being 2.9/100 000 children, of <15 years of age (Nephrology Committee of the Argentine Society of Pediatrics), and these numbers may even be an underestimate.

Epidemiology

The clinical experience in Argentina had shown that >90% of the patients had a diarrhoeal prodrome which was bloody in 76% of cases [3]. Subsequently it was confirmed that these were due to verotoxin-producing *Escherichia coli* [4]. The proportion of gastroenteritides associated with Shiga toxin is ~23% in Argentina, much higher than the 0.6–2.4% reported from other parts of the world.

Clinical aspects

The prevailing form of the disease in Argentina is the epidemic form, caused by the cytotoxin which is produced by certain strains of *E. coli*. Several treatment trials have been carried out in the acute stage of this form of HUS; three prospective and controlled studies were executed in children with the diarrhoeal form of HUS. One was performed at a time when intravascular coagulation was believed to be responsible for HUS [5]. The authors compared heparin against supportive treatment and found no benefit, while the frequency of intracranial haemorrhage was increased. Two later studies [6,7] were designed to validate reports on the beneficial effect of plasma infusion or plasmapheresis in the atypical form of HUS. In the controlled studies, however, patients treated with plasma infusion for 2 or 3 weeks had similar early and late outcomes to those of matched controls. Obviously, efforts to prevent the intestinal infection or the effect of the toxin must take priority if the burden from the disease is to be relieved.

Correspondence and offprint requests to: Horacio A. Repetto, University of Buenos Aires, Teodoro Garcia 2369-5A, 1426 Buenos Aires, Argentina.

© 1999 European Renal Association–European Dialysis and Transplant Association
Treatment of the intestinal infection with antibacterial drugs is apparently harmful and increases the risk of development of HUS [8], possibly because massive amounts of toxin are liberated from disintegrating bacteria.

**Preventive measures**

Three approaches may diminish the incidence of HUS. First, adequate information must be available to the public about the risk of consuming undercooked meat, unpasteurized cheese and raw milk. Investigators in Argentina noted that a high proportion of patients came from areas where unpasteurized milk is usually consumed [9]. Violation of food hygiene also explains outbreaks in nursing homes or day-care centres.

A second approach might be prevention through development of vaccines directed against *E. coli* or its toxins. Efforts in this direction are underway in some countries with a high prevalence of toxigenic *E. coli*.

A third approach is currently under study in Canada, i.e. administration of orally administered toxin-binding absorbents [10].

**Evolution and sequelae**

In Argentina, recovery from acute renal failure is seen in > 95% of children with the epidemic form of HUS. Haematological changes are seen only in the acute phase, and sequelae involving the central nervous system (CNS), pancreas, colon or other organs are infrequent. Chronic renal disease, on the other hand, is seen in 35–60% of affected children [11].

When large cohorts are followed for more than 1 year after the acute stage, three different outcomes are seen. Spizzirri *et al.* [12] observed 118 children who had HUS in early childhood after > 10 years of follow-up. The first group of 63% had normal urine analysis and creatinine clearance, a second group of 18% had persistent proteinuria with normal creatinine clearance, and a third group of 19% had different degrees of decreased glomerular filtration, generally associated with proteinuria and hypertension.

After recovery from the acute renal failure, patients may progress to terminal renal failure within 2–5 years, either without having recovered normal Ccr or, in the majority of patients, after having recovered normal serum creatinine levels or creatinine clearance, but exhibiting persistent proteinuria. This latter group arrive in terminal renal failure after an average of 10 years [13], but occasionally even after > 20 years.

Several findings in the acute stage are predictors of late renal prognosis. These are the presence and duration of anuria, hypertension persisting after correction of hypervolaemia and severe involvement of the CNS or intestine. These signs are indices of severe and widespread initial microangiopathy.

**Mechanism of progression**

Epidemic HUS constitutes a clinical model of one-shot disease, i.e. acute reduction of the number of functioning nephrons with subsequent progression to chronic renal failure by intrinsic mechanisms, depending on the number of nephrons lost. There are three lines of evidence to support this hypothesis. First, the histological lesion in the chronic stage resembles those found in experimental and clinical models of hyperfunction of residual nephrons, i.e. focal and segmental glomerulosclerosis with mesangial expansion and focal tubular atrophy and interstitial scarring [14].

Second, there is a maladaptive increase of glomerular filtration rate (GFR). For example, we studied 12 children with normal Ccr who had had HUS many years previously [15]. Four of the 12 were unable to increase inulin clearance after an acute protein load. Peak inulin clearance values after a protein load in the 12 patients were significantly lower than in normal children (84.9 vs 155 ml/min/1.73 m², *P* < 0.025). Third, there is a deficit of glomerular permselectivity. Some children who had completely normal clinical and laboratory findings years after the acute episode of HUS developed microalbuminuria [16], possibly a reflection of hyperfiltration in residual nephrons with increased filtration of macromolecules.

These considerations are important in the development of strategies to halt the late progressive decrease in renal function. Prospective studies currently are underway to evaluate the effectiveness of restriction of dietary protein intake and of the use of angiotensin-converting enzyme inhibitors. One question which remains to be answered is: which is the appropriate time to introduce these measures? Starting treatment once microalbuminuria appears may increase the chances of interfering successfully with progression.

**Transplantation**

The registry of the Argentine Society of Pediatrics reveals a prevalence of 16% of HUS among the 178 children entered into chronic renal failure, dialysis or transplantation programmes in 1996. HUS accounts for end-stage renal failure in ~20% of children requiring renal transplantation in our centre [17].

The first report of successful transplantation for HUS was published in 1972 by Cerilli [18], and later many centres confirmed the absence of recurrence of the disease in the graft, including in the substantial number of children with the epidemic diarrhoeal form transplanted in Argentina. This was contradicted by one report from Minneapolis [19]. To resolve this controversy, we reviewed the long-term course of 19 renal grafts in children with the ‘classic’ epidemic type with a mean follow-up of 5 years with a range of 1–11 years [20]. Survival of the grafts, maintenance of stable renal function, incidence of acute rejection and prevalence of proteinuria and hypertension was not different from those of a randomly selected control group of
The diagnostic trash bin of focal and segmental glomerulosclerosis—an effort to provide rational clinical guidelines

Alice Schmidt and Gert Mayer

Division of Nephrology and Dialysis, Department of Internal Medicine III, Währinger Gürtel, Vienna, Austria

In the age of evidence-based medicine, physicians are facing the task of integrating their individual clinical expertise and the best external evidence. To meet these goals for the treatment of a patient with proteinuria, normal renal function and the histodiagnostic diagnosis of focal and segmental glomerulosclerosis (FSGS) turns out to be rather difficult, as the published evidence, concerning the efficacy of immunosuppressive therapy, is extremely confusing. The chance of obtaining complete remission by steroid therapy has
been reported to range from 0 to 70% [1,2]. This extreme variability immediately suggests that maybe different disease entities are subsumed under a single histological term.

It is interesting to note that sometimes nephrologists base their diagnosis and medical management of patients with glomerular diseases on the histological classification, ignoring important clinical features. Many other members of the medical profession have realized for a long time that tissues in general only have a limited spectrum of reactions to the huge variety of insults they may be confronted with. In inflammatory hepatic diseases, histological evaluation of liver tissue is only one part of the diagnostic workup, and hepatologists rarely treat a patient based on the pathologist’s report. In nephrology, unfortunately, the situation is often completely different: the final diagnosis is often made near the morgue, i.e. in the basement of the hospital by a pathologist who all too often is left alone by the clinician with little or no information on the patient’s actual status or medical history. Therefore, one should accept the pathologist’s report for what it actually is: the description of an injury pattern rather than the diagnosis of a disease entity. If so, it quickly becomes evident that additional clinical information is important to arrive at the correct diagnosis.

If FSGS is a uniform pattern of injury, that results from a variety of insults, therapeutic regimens have to be individualized.

Rennke and Klein [3] proposed the differentiation between two major disease complexes, both of which are characterized histologically by FSGS on biopsy. Whereas the primary form is a disease of the glomerular capillary wall, possibly at the level of the visceral epithelium, secondary FSGS results from a variety of conditions that promote glomerular capillary hypertension and increased transcapillary flow rates (see Table 1). Clinically, both entities are proteinuric conditions. An increase of urinary protein excretion is caused by a disturbance of the glomerular permselective properties, which determine the ability of the glomerular filter to restrict the passage of macromolecules from the blood into Bowman’s space. Large and/or anionic molecules are filtered less readily than smaller and/or cationic compounds (glomerular size and charge selectivity). The initial event in primary FSGS is a loss of charge selectivity. If the disease is severe, size selectivity can also be impaired. In the latter patients, the rate of response to steroid therapy is markedly reduced and prognosis is worse [4,5]. In secondary FSGS, the initial damage is also a loss of charge selectivity. In addition, however, as proteinuria increases, size selectivity is impaired gradually over time in these patients. Treatment with an angiotensin-converting enzyme (ACE) inhibitor is able to stabilize renal excretory function only as long as proteinuria is due to an isolated charge selectivity defect as is the case in microalbuminuric patients with diabetic nephropathy. In later stages, appropriate therapy improves size selectivity, but proteinuria is only reduced and the speed of loss of excretory kidney function is slowed, but not prevented [6].

The clinical distinction between primary and secondary FSGS is difficult. It is nonetheless essential, since immunosuppressive therapy is indicated only in the former condition. A patient with rapid onset of a full blown nephrotic syndrome is likely to suffer from primary FSGS, whereas a slowly developing proteinuric condition in a patient with a typical medical history, that includes conditions summarized in Table 1, is more likely to have secondary FSGS. Evaluation of biopsy material also provides some distinguishing features. The ultrastructural finding of relatively mild segmental foot process fusion over the non-sclerosed segments with lesser degrees of visceral cell hypertrophy and hyperplasia is indicative of secondary FSGS. In a systematic study of podocyte alterations in primary and secondary forms of FSGS, d’Agati and co-workers noted that the mean percentage of the glomerular surface area affected by foot process fusion was significantly less in obesity (42 ± 24%) and reflux nephropathy (mean 25%) compared with primary FSGS (65 ± 23%) [7]. The greatest degree of foot process effacement was observed in the clinically most malignant forms of FSGS, the collapsing (82 ± 24%) and cellular (87 ± 21%) variants. Unfortunately, with regard to this parameter, remarkable overlap between the two forms is seen. Serum protein (and especially albumin) concentrations can additionally provide valuable information for the differential diagnosis. Some years ago, Praga et al. compared 19 patients with massive (5–10 g/day) proteinuria and normal serum albumin concentration (>35 g/l) and 16 patients with similar protein excretion but persistent hypoalbuminaemia (proteinuria 6–14 g/day, serum albumin concentration <30 g/l) [8]. In the normoalbuminaemic group, he found healed crescentic glomerulonephritis, reflux nephropathy, scarred IgA nephropathy, unilateral renal agenesis and obesity, whereas patients with hypalbuminaemia suffered from membranous glomerulonephritis or minimal change glomerulopathy. Treatment with captopril reduced proteinuria only in patients with normoalbuminaemia, indicating that

---

**Table 1. Classification of focal and segmental glomerulosclerosis**

1. Primary (idiopathic) FSGS
2. HIV- or heroin-associated FSGS
3. Secondary FSGS

**A.** With reduced renal mass such as in

- Oligomeganephronia
- Unilateral renal agenesis
- Renal dysplasia
- Reflux nephropathy
- Massive surgical ablation
- Renal allograft failure
- Any advanced renal disease with reduction in functioning nephrons

**B.** With initially normal renal mass such as in

- Diabetes mellitus
- Hypertension
- Obesity
- Cyanotic congenital heart failure
these patients actually suffer from secondary FSGS. Why these subjects maintain normoalbuminaemia despite heavy urinary protein loss, similar to what is seen in patients on continuous ambulatory peritoneal dialysis (CAPD), is unclear, but several hypotheses can be proposed. The most likely explanation is that tubular catabolism of albumin contributes to albumin loss in patients with primary FSGS. Consequently, hepatic albumin synthesis can no longer cope with such an additional burden and maintain serum albumin levels. The general failure of ACE inhibitors to reduce proteinuria in acute primary nephrotic states has been confirmed in animal studies [9]. Nevertheless, even in this condition, ACE inhibitors reduce urinary protein excretion in a certain proportion of patients [10]. We interpret this response as evidence that an additional component of secondary FSGS is present in these subjects. Reduction of proteinuria by ACE inhibitors can be used to quantify the degree of chronic non-specific damage. This estimate can provide useful information before immunosuppressive therapy is considered.

In summary, a variety of clinical parameters help to distinguish primary and secondary FSGS and therefore enable clinicians to identify those patients who are most likely to benefit from immunosuppressive therapy or administration of an ACE inhibitor. In patients with primary FSGS, the finding of selective proteinuria, i.e. of an isolated defect in glomerular charge selectivity, helps to identify candidates in whom immunosuppressive treatment has the greatest likelihood of success.

References