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Editorial Comments

Leptin—a new hormone of definite interest for the nephrologist

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Introduction

The obesity (*ob*) gene protein, known as leptin (from Greek *leptos* 'thin'), was cloned in 1994 by Friedman *et al.* [1] and regulates food intake and energy expenditure in animal models. Leptin which is exclusively produced in the adipocytes, circulates in the blood with ~50% in free form and the remainder attached to binding proteins. Leptin reaches the brain by a saturable transport mechanism via the blood–brain barrier and, via direct effects on the hypothalamus, decreases appetite and increases metabolism. Markedly elevated leptin levels have been reported in patients with obesity, who seem to be insensitive to the endogenous leptin production. It has been proposed that in obese patients there is a defective transport of leptin across the blood–brain barrier indicating a resistance to the central actions of leptin. Treatment with recombinant leptin induces dramatic 40% weight reduction in mice [2], but no results are as yet available regarding the weight-reducing effect of recombinant leptin treatment on obese humans.

Leptin in chronic renal failure

Protein–calorie malnutrition is a common problem in patients with advanced chronic renal failure (CRF) and is associated with increased morbidity and mortality. Numerous factors contribute to malnutrition, but reduced food intake due to anorexia is probably the most important. The mechanism(s) causing decreased appetite in uraemia are not obvious. However, in 1997 a number of studies demonstrated that CRF patients with [3–6] and without [3,6,7] ongoing dialysis treatment have markedly elevated serum leptin without an increase in body fat mass. Since leptin is thought to be an inhibitor of appetite, by restraining the synthesis and release of neuropeptide Y, it has been speculated that elevated serum leptin could contribute to anorexia and poor nutrition in patients with renal failure [7]. Since protein binding may be enhanced by renal failure, it could be argued that the elevated serum leptin demonstrated in the present study are due to an excess of bound forms of leptin rather than free bioactive forms. However, a recent study has shown that elevated leptin in patients with CRF are due to an increase in the free bioactive form of leptin rather than in bound forms [4].

Causes of hyperleptinaemia in chronic renal failure

As the kidneys are important in clearing several other polypeptide hormones such as insulin, parathyroid hormone, and glucagon, it seems reasonable to surmise that leptin also accumulates in the case of renal failure due to reduced renal clearance. However, elevated serum leptin are not a universal finding in advanced CRF [8], and the exact cause of elevated serum leptin in uraemia is not well understood, although there are data suggesting that a decrease in glomerular filtration rate [4,6,9, P. Stenvinkel, unpublished data], inflammation [3] and hyperinsulinaemia [8,10] may affect serum leptin levels in CRF. Leptin, insulin concentrations, and body weight, are interrelated and there is a direct correlation between insulin and leptin concentrations in non-uraemic [11] as well as uraemic patients [8,10]. It has been shown that long-term (72 h) insulin infusion stimulates leptin secretion in humans [12]. Another possible reason for elevated leptin levels in renal failure is chronic inflammation [3]. It has been demonstrated that cytokines, such as TNFα and IL-1, induce both an increase in leptin mRNA concentrations and anorexia in animals [13], and this suggests that elevated leptin concentrations may be one of the mechanisms by which anorexia is induced during inflammatory conditions.

Consequences of hyperleptinaemia in chronic renal failure

As leptin-receptor antagonists are likely to be developed for clinical use, it would be of obvious

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clinical importance if hyperleptinaemia could be proved to contribute to uraemic anorexia. However, for leptin to suppress appetite in CRF patients we have to assume that leptin is present mostly in its unbound bioactive form and that there is a normal transport of leptin across the uraemic blood–brain barrier. Young *et al.* [7] have demonstrated a significant negative relation between the plasma leptin/body fat ratio and the dietary protein intake in dialysis patients, and this is consistent with the concept that leptin contributes to malnutrition. Moreover, data presented by Heimbürger *et al.* [3] suggest that elevated serum leptin may induce anorexia in uraemic patients with an ongoing inflammatory process. Accordingly, at present there is some evidence indicating that elevated leptin concentrations may mediate anorexia in CRF, but it is obvious that further studies are necessary to substantiate this suggestion.

Anorexia, one of the major symptoms of uraemia, is only partly corrected by dialysis. Even if the majority of leptin circulates in the unbound form, the size of leptin (16 kDa) suggests that leptin will not be cleared by ordinary synthetic dialysis membranes, and accordingly several studies have demonstrated that leptin concentrations are not reduced by haemodialysis [4–6]. However, it remains to be tested whether or not high-flux synthetic membranes with greater clearance of large molecules will decrease serum leptin and improve appetite. In contrast to the unchanged serum leptin concentrations observed after the initiation of haemodialysis, leptin concentrations increase markedly in patients treated by CAPD [3]. Recently, Heimbürger *et al.* [3] demonstrated that 12 months of CAPD treatment was associated with a marked increase in the body fat content. It has been suggested that the continuous carbohydrate load in CAPD increases body fat mass and consequently serum leptin [3]. However, it remains to be proved whether or not elevated leptin concentrations contribute to the low eating drive in CAPD patients despite their great need for protein and calories [14].

In addition to its effects on appetite, leptin also increases energy expenditure by a stimulatory effect on the sympathetic nervous system [for a review see 15]. Ikizler *et al.* [16] have demonstrated that haemodialysis patients have an elevated energy expenditure, and it could be speculated that elevated leptin levels will be one factor contributing to a negative energy balance in uraemia. The kidney has been shown to express leptin receptors, and it has been demonstrated that leptin in high doses increases diuresis and natriuresis in rats without significant effects on renal blood flow and glomerular filtration rate, suggesting a direct tubular effect [15]. The effects of leptin on insulin metabolism are complex. Whereas it has been demonstrated that leptin acutely increases insulin sensitivity in rats [15], Emilsson *et al.* [17] reported that leptin receptors are present in the pancreas and that leptin, in a dose-dependent manner, inhibits pancreatic insulin secretion in mice. In accordance with this finding, results in predialysis patients indicate that when the level of circulating serum leptin is markedly elevated in relation to the per cent fat plasma insulin does not continue to increase [8]. This may imply that markedly elevated serum leptin impair the pancreatic insulin secretion and that hyperleptinaemia could be a factor contributing to impaired glucose tolerance in uraemia. As suggested by Haynes *et al.* [15], some of the peripheral actions of leptin may act as a signalling mechanism to activate compensatory mechanisms for the potentially deleterious effects of an increased body fat mass. Interestingly, Shek *et al.* [18] recently demonstrated that chronic increases in CNS leptin concentrations in rats increase heart rate and blood pressure, suggesting a possible role of leptin in obesity hypertension. One might therefore speculate that if obese and uraemic patients with hyperleptinaemia are resistant to the facilitative effects of leptin on insulin sensitivity and sodium excretion but not resistant to the stimulatory effects of leptin on the sympathetic activity, then this explains why sodium sensitive hypertension and insulin resistance are such frequent accompaniments of both obesity and uraemia. Thus, leptin has multiple actions that are potentially relevant not only to the control of eating behaviour but also to cardiovascular regulation.

A new and fascinating aspect of the putative peripheral actions of leptin are the recently described proliferative effects of leptin on haematopoietic stem cells suggesting a role for leptin in erythropoiesis. Actually, there might be a synergy between leptin and erythropoietin, as discussed by Bennet *et al.* [19]. The bone marrow contains adipocytes in which the *ob* gene is expressed. It has been speculated that the fat cell content of human bone marrow reflects the requirement for leptin in active haematopoiesis [19]. This may mean that in clinical states with anaemia and insufficient production of erythropoietin, such as CRF, other haematopoietic factors, such as leptin, will be increasingly important in stimulating erythropoiesis [20].

Summary and perspectives

The recent discovery of the *ob* gene product leptin has markedly increased our understanding of the complex physiological system that regulates satiety and eating behaviours. Moreover, as leptin receptor isoforms have now been reported in several peripheral organs, it may be conjectured that leptin, besides having central effects, also has pleiotropic action in the peripheral organs. This suggests that the functional role of leptin appears to extend beyond the regulation of feeding and metabolism to include other organs and some biological functions of definite interest for the nephrologist. Indeed, recent findings indicate that besides regulating appetite leptin may play a role in sympathico-activation, insulin secretion and sensitivity, renal sodium handling and haematopoiesis. Probably other actions of leptin will also soon be discovered. It is possible that leptin-receptor antagonists will be avail-

able for clinical use in the future. It is therefore important to determine whether or not hyperleptinaemic uraemic patients are resistant to the central and peripheral actions of leptin by analogy with the end-organ resistance to other hormones (such as insulin and PTH) documented in renal failure.

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T lymphocyte-derived cytokines in experimental glomerulonephritis: testing the Th1/Th2 hypothesis

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The pathogenesis of glomerulonephritis can be conceptually divided into immunologic and inflammatory phases (Figure 1). Immune responses to foreign or self antigens result in formation of nephritogenic lymphocytes, antigen–antibody complexes, and autoantibodies which initiate an inflammatory cascade in the kidney [1]. The consequence of these immunologic and inflammatory processes is glomerular and interstitial fibrosis.

T lymphocyte-derived cytokines regulate the cellular and humoral arms of the immune response to nephritogenic antigens and modulate the inflammatory events which ensue (Figure 1). The discovery of T helper lymphocyte subsets (Th1 and Th2) that differ in their cytokine secretion patterns and effector functions has provided a model for understanding how cytokines regulate pathologic immune and inflammatory responses [2]. We will briefly summarize the Th1/Th2 hypothesis, how it relates to experimental glomerulonephritis and highlight recent studies which question this hypothesis.

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The Th1/Th2 hypothesis

Studies in mice, rats, and humans suggest that CD4+ T helper lymphocytes differentiate into at least

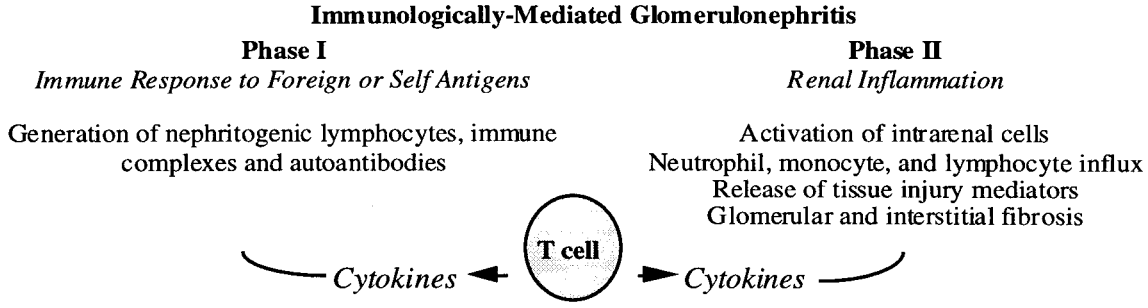

Fig. 1. Pathogenesis of immunologically-mediated glomerulonephritis. T cell-derived cytokines regulate both phases of disease. These phases are likely to overlap temporally and physically.

two subpopulations based on their pattern of cytokine production [2,3]. Th1 lymphocytes secrete interleukin-2 (IL-2), interferon-γ (IFNγ) and tumour necrosis factor-β (TNFβ or lymphotoxin). Th2 cells, on the other hand, secrete IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13 (Figure 2). Both cell types originate from the same precursor lymphocyte (Th0) and their differentiation is determined by cytokines present at time of antigen recognition. The presence of IL-12 and IFNγ favor the Th1 phenotype while IL-4 promotes generation of Th2 lymphocytes.

Th1-derived cytokines are considered to promote tissue injury by mediating delayed-type hypersensitivity (DTH) reactions, cytotoxic T lymphocyte generation, macrophage activation and production of complement fixing antibodies [2,3]. In contrast, Th2-derived cytokines appear to protect against tissue injury by deviating antibody production towards IgE and non-complement fixing subclasses of IgG [2,3]. They also suppress DTH reactions and counteract IFNγ's actions on macrophages.

Th1 and Th2 subsets are reciprocally regulated [2,3]. IFNγ inhibits the differentiation and proliferation of Th2 cells resulting in a dominant Th1 response. On the other hand, IL-4 and IL-10 inhibit IFNγ production by Th1 lymphocytes. Th1- and Th2-type cytokines are expressed in rat and mouse kidneys during the course of glomerulonephritis [4,5]. It is therefore suggested that a balance between Th1 and Th2 lymphocytes determines the outcome of immune-mediated glomerulonephritis [6].

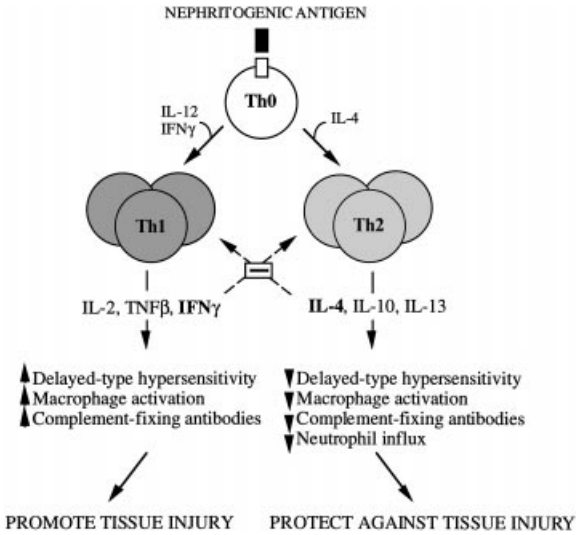

Fig. 2. The Th1/Th2 paradigm in immunologic renal injury.

The Th1/Th2 hypothesis and experimental glomerulonephritis

Several studies have provided evidence that IL-4 protects against antibody-mediated glomerulonephritis. By comparing the intensity of nephritis between IL-4 gene-knockout and wild-type mice, IL-4 was found to be an endogenous inhibitor of neutrophil influx and subsequent glomerular inflammation in the heterologous phase of passive murine anti-glomerular basement membrane (anti-GBM) nephritis [5]. It was also observed that Brown Norway rats, which exhibit increased IL-4 production and decreased DTH responses, are resistant to glomerular injury associated with the autologous phase of accelerated rat anti-GBM nephritis [7]. Tam *et al.* reported that exogenous recombinant IL-4 partially blocks development of accelerated anti-GBM nephritis in Sprague–Dawley rats possibly by inducing renal expression of the IL-1 decoy receptor [8]. In a murine model of crescentic glomerulonephritis, Kitching *et al.* found that IL-4 treatment alone is not sufficient for ameliorating disease but that a combination of two Th2-type cytokines, IL-4 and IL-10, suppresses DTH responses and crescent formation [9]. Studies in lupus-prone mice carrying the *lpr* gene demonstrated that IL-4 deviates immune response towards a Th2 phenotype (reduced titres of complement-fixing autoantibodies) and protects against nephritis [10].

IL-4, however, does not always protect against glomerular injury. Peng *et al.* found that MRL/*lpr* mice deficient in IL-4 have reduced rather than exaggerated lymphadenopathy and end-organ disease [11]. Overexpression of IL-4 in mice results in B cell hyperactivity, autoantibody formation, and glomerulonephritis characterized by proteinuria, glomerular hypertrophy, and progressive glomerulosclerosis [12]. IL-4 has also

been shown to promote polyclonal B cell activation and glomerulonephritis in graft-versus-host disease and in $HgCl_2$-induced autoimmune syndrome [13].

Few studies have directly addressed the role of IFNγ in immunologically-mediated glomerulonephritis. IFNγ appears to be essential for development of lupus-like nephritis in MRL/*lpr* lupus-prone mice by switching autoantibody production to complement-fixing isotypes [14]. Huang *et al.* observed that IFNγ-neutralizing antibodies partially block proteinuria and crescent formation in murine nephritis [15].

In contrast, we found that albuminuria and glomerular pathology in accelerated murine anti-GBM nephritis are enhanced in the absence of IFNγ [16]. This controversy is further emphasized by studies showing that IFNγ mediates suppression of $HgCl_2$-induced autoimmune renal disease in Brown Norway rats and inhibits mesangial cell proliferation in anti-Thy1 nephritis [17,18]. In other disease models, such as experimental autoimmune encephalomyelitis and collagen-induced arthritis, clinical and pathological manifestations are worse in mice lacking IFNγ or IFNγR than the wild-type group [19–21]. In fact, mice lacking IFNγ exhibit uncontrolled splenocyte proliferation in response to mitogen or alloantigen and are resistant, rather than permissive, to induction of long-term allograft acceptance [22,23].

Summary

CD4+ Th lymphocytes play a key role in orchestrating the immune response to nephritogenic antigens. Although the Th1/Th2 hypothesis has served as a framework for studying immune regulation, it does not necessarily apply to all models of experimental glomerulonephritis. Cytokines, predicted by the Th1/Th2 hypothesis to either promote or prevent injury, may have dual functions in the immunopathogenesis of renal disease. IFNγ which activates macrophages may play a critical role in downregulating activated T cells. Conversely, IL-4 which has important anti-inflammatory actions also promotes polyclonal B cell activation. Like all other hypotheses, the Th1/Th2 paradigm was written to be tested, or, as aptly stated by Italo Calvino, '… if the model does not succeed in transforming reality, reality must succeed in transforming the model …'.

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Diabetic kidney disease: the role of growth factors

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Introduction

Growth factors have attracted attention in several areas of diabetes mellitus including conceivable effects on the pathogenesis of diabetic kidney disease. In a recent review article the published evidence for a connection between changes in various growth factors: growth hormone (GH), insulin-like growth factors (IGFs), transforming growth factor β (TGF-β), epidermal growth factor (EGF), platelet derived growth factor (PDGF), tumour necrosis factor α (TNF-α), fibroblastic growth factors (FGFs) and the development of renal changes in diabetes was covered [1]. The present review attempts to cover the most recent evidence for a causal interrelationship between growth factors and the development of diabetic kidney disease with focus on two of the above mentioned growth factor systems. Accordingly, the first part of the review presents evidence for a pathogenic role of the GH/IGF system in the development of diabetic renal changes with emphasis on the renoprotective effects of long-acting somatostatin analogues and GH-receptor antagonists. Secondly, evidence is presented for a causal role for TGF-β in the pathogenesis in diabetic kidney disease.

The GH/IGF axis in diabetic kidney disease

During the last decade attention has been drawn towards GH and IGF-I as mediators of the characteristic features of early diabetic kidney disease, i.e. renal hypertrophy and hyperfunction. Pronounced changes in the renal GH/IGF-I axis have been demonstrated both in short- and long-term experimental diabetes [2]. Accordingly, experimental diabetes in dwarf rats with isolated GH and IGF-I deficiency is associated with a lesser renal and glomerular hypertrophy than observed in diabetic control animals with intact pituitary [3]. In addition, diabetic dwarf rats with a diabetes duration of 6 months, display a smaller rise in urinary albumin excretion (UAE), indicating that GH and IGF-I may be involved in the development of the specific diabetic kidney changes [4]. Furthermore, the initial increase in renal size and function in experimental diabetes is preceded by a rise in renal IGF-I, IGF binding proteins (IGFBPs) and IGF-II/mannose-6-phosphate receptor (IGF-II/man-6-P receptor) concentration [2]. Moreover, specific changes occur in the renal GH binding protein (GHBP) mRNA, IGF-I receptor mRNA and IGFBP mRNA expression in long-term diabetes [2]. Finally, renal effects of potent inhibitors of the GH/IGF-I axis (i.e. long-acting somatostatin analogues and GH-receptor antagonists) have given further evidence for a role of GH and IGF-I in diabetic kidney disease.

Manipulation of the altered GH/IGF axis in diabetic kidney disease

Long-acting somatostatin analogues

The potential role of somatostatin and its analogues in the treatment of human diabetes and long-term diabetic complications is based on the probable benefit of suppressing elevated circulating GH levels in order to minimize their deleterious effects on diabetic metabolism and development of the vascular and functional long-term diabetic changes. Native somatostatin has too short a biological half-life to allow its use in clinical therapy, however, the development of new long-acting somatostatin analogues (octreotide and lanreotide) have made experimental and clinical trials possible. In short-term experimental diabetes 7 days treatment with octreotide or lanreotide from diabetes onset is able to fully inhibit the initial renal hypertrophy through an inhibition of kidney IGF-I accumulation [5,6]. If initiation of octreotide treatment is postponed 3–9 days after diabetes induction, the diabetic renal hypertrophy is only partly inhibited, indicating that early intervention with somatostatin analogues is important [7].

In long-term experimental diabetes, 6 months of octreotide treatment from the day of diabetes onset was followed by significant reductions of increase in kidney weight, kidney IGF-I levels, and UAE when compared to untreated diabetic rats [8]. A recent study from our group aimed at examining the effect of 3 weeks treatment with octreotide and an ACE-inhibitor (captopril) either alone or in combination following 3

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months of untreated diabetes [9]. No effect was observed of octreotide, captopril or the combined treatment on kidney IGF-I levels, however, octreotide treatment alone and in combination was followed by a significant reduction in kidney weight compared to placebo treated diabetic rats [9]. Further, the combined treatment of octreotide and captopril was followed by a significant reduction in UAE compared to placebo treated diabetic rats giving evidence for a beneficial effect of the combined treatment with octreotide and an ACE-inhibitor on manifest experimental diabetic renal changes [9].

In clinical studies acute infusion (2–3 h) of octreotide to patients with insulin-dependent diabetes mellitus (IDDM) induced a reduction in renal plasma flow (RPF) and glomerular filtration rate (GFR), concomitantly with decreased plasma GH and glucagon levels [10]. In long-term studies octreotide administration for 12 weeks in 11 IDDM patients induced a significant decrease in the elevated GFR; in addition, total kidney volume was reduced. Three of the patients were re-examined 12 weeks after cessation of octreotide treatment, and their GFR had risen to the level at the start of the study [11]. No clinical studies have yet appeared on the possible long-term effects of somatostatin analogues on UAE in IDDM patients.

GH-receptor antagonists

By recognizing the potential role that GH and IGFs may play in various pathophysiological conditions, including diabetic nephropathy, a series of highly specific antagonists of the GH action has been developed for the potential therapeutic use. Initially, it was shown that alteration of single amino acids in the third α-helix of bovine (b)GH (residues 109–126) results in a functional GH antagonist. *In vitro* experiments show that the group of GH antagonists binds to the GH receptor with the same affinity as native GH, but *in vivo* a phenotypic dwarf animal characterized by low circulating IGF-I levels and a proportional body composition develops when the GH antagonist is expressed in transgenic mice. Recently, studies have reported renoprotective effects of GH antagonists in long-term diabetic transgenic mice that express these GH antagonists (bGH-G119R or hGH-G120R) [12–14]. Compared with transgenic diabetic mice expressing wild-type bGH or bGH, the diabetic mice expressing GH antagonists showed lesser glomerular damage [12,13], no increase in total urine protein [12], no glomerular hypertrophy [14] and no increase in glomerular α1 type IV collagen mRNA levels [14]. Interestingly, the inhibitory effects of GH antagonists in transgenic mice were seen without alteration in glycemic control as similar levels in blood glucose and Hb_{Alc} were seen in the different diabetic animals expressing wild-type bGH, GH antagonists or bGH [12,13]. Studies including exogenous administration of GH antagonists to diabetic animals models are needed to elucidate the usefulness of the GH antagonists as a potential therapeutic agent.

The transforming growth factor β (TGF-β) axis in diabetic kidney disease

TGF-β is unique among growth factors in its ability to modulate extracellular matrix production and both glomerular mesangial and epithelial cells increase synthesis of extracellular matrix proteins (i.e. protoglycans, fibronectin, type IV collagen and laminin) in response to TGF-β. Recent studies have suggested the TGF-β system may play a role in the pathogenesis of diabetic nephropathy. High glucose concentrations increase TGF-β1 mRNA levels in both cultured mesangial cells and proximal tubular cells *in vitro* [15,16]. Further, the elevated glucose levels *in vitro* stimulate TGF-β gene expression and bioactivity, cellular hypertrophy and collagen transcription in proximal tubules [16]. In addition, interesting experiments have been published recently on changes in renal TGF-β in early experimental diabetes *in vivo*. In a study performed in STZ-diabetic rats, an increase in glomerular TGF-β mRNA was reported as early as 24 h after the onset of hyperglycaemia with a sustained increase for up to 2 weeks [20]. Increased renal levels of TGF-β mRNA in the early renal hypertrophy have also been described both in non-obese diabetic (NOD) mice and diabetic BB rats [18]. Finally, a sustained increase in glomerular TGF-β mRNA levels is seen in long-term STZ-diabetic rats with a diabetes duration for up to 6 months [19,20]. Two clinical studies have been published dealing with changes in the renal expression of the TGF-β system [21,22]. Increased TGF-β immunostaining was initially described in glomeruli obtained from diabetic subjects [21]. This observation was confirmed and further extended in a recent study, in which increased glomerular and tubulointerstitial levels of all three TGF-β isoforms were reported in diabetic nephropathy, but also in several other conditions characterized by accumulation of extracellular matrix [22]. Further, a positive correlation between TGF-β, fibronectin and plasminogen activator inhibitor-1 levels in glomeruli and tubulointerstitium was reported [22]. These studies support the hypothesis that glucose induced rise in renal TGF-β expression and peptide in the kidney may be responsible for some of the renal changes that precede the development of diabetic nephropathy.

Manipulation of the altered TGF-β axis in diabetic kidney disease

Neutralizing antibodies

Data supporting the hypothesis that the glucose induced rise in renal TGF-β expression and peptide may be responsible for some of the renal changes in diabetic nephropathy have been published in recent elegant studies using neutralizing antibodies. *In vitro* the glucose mediated increase in type IV collagen synthesis in mesangial cells is dependent on the autocrine action of TGF-β1 as neutralizing antibodies to

TGF-β attenuate the rise [23]. Further, in a recent study *in vivo*, administration of a neutralizing TGF-β 1,2,3 antibody to STZ-diabetic mice for 9 days attenuated the elevated renal TGF-β1 and TGF-β Type II receptor mRNA levels and reduced both the diabetes associated renal/glomerular growth and enhanced renal expression of collagen IV and fibronectin [24].

Angiotensin converting enzyme (ACE) inhibition

It has been suggested that activation of the renal TGF-β system in diabetes may be mediated, besides a direct stimulatory effect of hyperglycaemia *per se*, through activation of the renin–angiotensin system, as exposure of mesangial cells to angiotensin II *in vitro* stimulates the expression of TGF-β and extracellular matrix proteins [25]. Further a recent study examined the effect of an ACE-inhibitor (captopril) on high-glucose induced changes in the TGF-β system and growth in LLC-PK_1 cells, a porcine kidney cell line analogous to the proximal tubule cell [26]. In this cell system high-glucose increased TGF-β1 mRNA, TGF-β Type I and II receptor protein expressions and cellular hypertrophy, while cellular mitogenesis was inhibited. Captopril dose-dependently decreased TGF-β Type I and II receptor protein expressions and cellular hypertrophy, increased cellular hyperplasia while TGF-β mRNA was unchanged [26]. A recent study from our group aimed at examining the possible effect of ACE-inhibition on the intrarenal changes in the various TGF-β isoforms (TGF-β 1,2,3) and TGF-β receptors (Type I, Type II, Type III) in experimental diabetes *in vivo* [27]. STZ-diabetic and non-diabetic rats were treated for 2 and 4 weeks with enalapril or placebo. Enalapril partially prevented the diabetes associated renal hypertrophy and fully the increase in 24 h UAE. Further, enalapril-treatment decreased the glomerular levels of TGF-β Type I and Type III receptor isoforms to values below non-diabetic control level while treatment decreased the glomerular TGF-β Type II receptor level to almost undetectable levels. The glomerular expression of the TGF-β isoforms were not dramatically influenced by treatment. These findings suggest that the TGF-β axis operates through a complex intrarenal system that may be a significant mediator of the renal changes observed in experimental diabetes. Moreover, ACE-inhibition has pronounced inhibitory effects on the elevated levels of the TGF-β receptors required for intracellular signaling through this growth factor system. These findings suggest a possible new mechanism of action for ACE-inhibitors.

Concluding remarks

The knowledge we have today indicate that growth factors, through a complex system may be responsible for both early and late renal changes in experimental diabetes. Further insight into these processes may be useful in the future development of new antagonists useful in the treatment of diabetic kidney disease.

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Are all patients with idiopathic focal segmental glomerulosclerosis (FSGS) created equal?

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Since the initial description of FSGS in 1956, controversies have been the constant companions of this disease. It is beyond the scope of this editorial to cover the entire area of FSGS but we will review the issues related to pathological variants as well as the influence of age, gender, race, and treatment response on outcome. Most of the clinical data is based on patients with the 'classic' lesion so this needs definition. Histologically this consists of an area of adhesion between a peripheral capillary loop of the glomerular tuft and Bowman's capsule, with progressive replacement of this area with matrix material that gradually increases in size [1]. This leads to the characteristic pathology seen on light microscopy, a discrete segmental solidification of the glomerular tuft most commonly found close to the vascular pole.

1. Long-term outcome—is there a difference between children and adults?

In the clinical realm it was thought initially that outcome in patients with FSGS was different in children compared to adults. This belief was reinforced by the study of Newman *et al.* in the 1970s, [2]. However, subsequent observations including our most recent, long-term follow-up have shown a complete remission rate with treatment of 25–40% in both age groups and have suggested the apparent differences in outcome between children and adults may have been the lack of a trial of therapy in the latter [3–5]. Even in the elderly the response rate is in the same range although the lesion is seen less frequently [6]. Recently remissions in up to 60% of patient have been reported in both age groups with more vigorous treatment including the use of prolonged cytotoxic drugs combined with high dose corticosteroid therapy but the risks are also noted to be higher. [7,8]

2. Race and natural history of focal segmental glomerulosclerosis?

Racial bias in terms of both incidence and outcome is another area of interest. Korbet *et al.* [9] has suggested an increased prevalence of the disease in blacks, in his report of 340 patients with nephrotic range proteinuria and some regional registries of ESRD support this contention [10]. Alternative explanations to a racial predisposition to the development of this disease include local population demographics, referral filter bias, and failure to separate primary from secondary

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causes. The latter could easily occur in registry reports. The other important issue in regards to race is whether the disease behaves differently in the black population. Data from the same United States Renal Data Systems indicates that FSGS is over represented as the cause of renal failure among African-Americans compared to Caucasian children, suggesting a more aggressive disease in that population but this could be explained by the higher incidence. Regional but more detailed data presented by Inguli *et al.* supports the contention that the disease is more severe [11]. They demonstrated that 78% of black and Hispanic children with this lesion progressed to end ESRD compared to only 33% of their Caucasian population. Since the groups were comparable in demographics, observation, time and treatment, they suggested that other factors such as race might contribute to this poor outcome. However, the data that prognosis is influenced by race is not unanimous since Rydel *et al.* did not find this factor to be predictive of ESRD in their study of 81 adults [4]. Certainly Agarwal *et al.* in their report on a large group of Asian patients from India, the majority with the classic lesion found their response to treatment and long term outcome similar to the white population. [12]

It is possible that the differences in outcome may be linked to the frequency of the specific type of FSGS lesion that develops in blacks versus whites. A recent abstract by Hogan-Malton *et al.* found that their black patients were more likely to have the collapsing variant and be less responsive to treatment. [13] Thus, the question of a racial effect on FSGS has not been resolved.

3. Gender and natural history of focal segmental glomerulosclerosis

An interesting and relatively unexplored factor is the influence of gender on outcome and its potential interaction with age. Most reported series in children show a 1:1 male to female incidence rate, but adult series tend to show a 2:1 ratio. [9] Similarly it is difficult to ascertain from the literature whether progression is influenced by gender alone since the current published data is retrospective, treatment protocols vary widely and the observation periods are often short. In our own recent long-term review the incidence was equal in children by gender as was the rate of complete and partial remission and chronic renal failure but the incidence in the adult population was 2 males to every female and the outcome was different i.e. the complete/partial remission rate favoured women 2:1 and the chronic renal failure group was the opposite i.e. 2 males to every female. [5]

4. Variants of glomerular morphology

The recent spate of articles describing the morphologic variants of FSGS needs to be placed into the context of our primary question. The variants that have been described include the position of the lesion within the glomerular tuft, (tip type) the presence or absence of hypercellularity and the state of the surrounding glomerular tuft (collapsing type).

The 'glomerular tip lesion' originally described by Howie and Brewer was thought to behave differently from classic FSGS with a higher response rate to steroid treatment [14]. This observation has not been confirmed in a recent review of this area. [15] Thus at present it would be difficult to assign a favorable prognosis based solely on the position of the sclerotic lesion. This conflicting data is partially related to the difficulty in determining the specific location of the lesion in this focal and segmental disease since serial sectioning of the tissue has frequently demonstrated a surprising variation even within a single glomerulus. This problem might also apply to the cellular type. Endocapillary as well as extracapillary epithelial hypercellularity usually in a focal and segmental distribution characterize this variant. Initially it was thought to be associated with a poorer prognosis but it is now apparent that this outcome is not uniform and the most recent data suggests that perhaps this is an early phase of FSGS. The last variant, the collapsing type of FSGS was described originally over 10 years ago and is characterized by the 'implosive retraction' of the endocapillary space as opposed to its classic expansion by hyaline and matrix material. The distinguishing clinical features described by Detwiler were the persistence of high grade proteinuria the poor response to corticosteroids, the rapid downhill course to ESRD and its predominance in the black population [16]. It is interesting to note that only five of the 14 patients in their initial series were given treatment and a response was seen in 20%, not so different from the classic rate. Certainly this type of down hill course is not peculiar to the collapsing variant. Brown *et al.* [17] described this rapid deterioration in renal function in 11 patients with the more classic FSGS lesion several years ago. Their emphasis was not on the pathology but on the clinical observation, similar to Detwiler group, that persistent heavy proteinuria (>10 g/day) predicts a very poor prognosis. Our review would confirm this finding but add the proviso that corticosteroids should still be tried since a complete remission may occur and lead to an excellent long term prognosis.

At this point it seems reasonable to assume that the pathological lesions seen in idiopathic FSGS are similar but not identical reactions to injury from a variety of unknown causative agents, superimposed, perhaps, on a genetic susceptibility [18].

5. The splitters versus the lumpers

In summary if your penchance is for splitting, the options for explaining the differences seen in FSGS are virtually unlimited and include many more factors than the ones discussed. This is probably related to the fact that the response to therapy is based not only

on the particular initiating agent but also on the underlying demographic factors and others yet to be determined. On the other hand if lumping is closer to your approach there is no evidence to suggest that any of these variations have such a distinct prognosis that outcome can be 100% predicted. This means to treat or not to treat based on one or any combinations of these parameters is currently unwise. At this stage we must then conclude that at least at a practical therapeutic level all FSGS should be considered equal.

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Controversial association of left ventricular hypertrophy and the *ACE* I/D polymorphism—is the mist clearing up?

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Introduction

Recent years witnessed a growing recognition of the genetic principles of cardiovascular disorders. First, the origins of hypertension, coronary artery disease, left ventricular hypertrophy, or progressive loss of renal function were demonstrated to be multifactorial and polygenic (i.e. resulting from interaction between environmental factors and multiple genes). Next, molecular variants of candidate genes, i.e. genes with the potential to modulate these cardiovascular phenotypes, were uncovered. Some of these genetic variants were found to be associated with altered levels of circulating proteins or enzymatic activities, e.g. angiotensin converting enzyme (*ACE* I/D), angiotensinogen (M235T), or fibrinogen (G-455A) polymorphisms. Given the (patho)physiological functions of respective proteins, it appeared attractive to associate these genetic variants specifically with more complex cardio-

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vascular disorders. However, what was meant to be a short cut to resolve the molecular etiology of cardiovascular disease quickly turned into a rocky ride spiked with controversial observations.

The *ACE* I/D polymorphism

The insertion/deletion (I/D) polymorphism of the *ACE* gene represents a most prominent genetic variant subjected to this ongoing debate. At present, it is undisputed that the I/D polymorphism in intron 16 of the human *ACE* gene is related to 14–50% of the interindividual variance of serum ACE activity (for references see recent review [1]). Furthermore, increased levels of ACE activity were found in lymphocytes and cardiac tissue of individuals carrying the *ACE* deletion allele [1]. Unfortunately, the consensus on the *ACE* I/D genotype ends at this point. In particular, the associations of this polymorphism with a variety of cardiovascular disorders were inconsistently observed when multiple populations were investigated [1].

Left ventricular hypertrophy and *ACE*

For the nephrologist, left ventricular hypertrophy (LVH) is an important prognostic marker since the condition is frequently encountered in patients with renal failure or hypertension. In particular, LVH is accompanied by a markedly enhanced risk of heart failure, myocardial infarction, and death. Thus, it may be of relevance to study the *ACE* I/D polymorphism in the context of LVH and/or renal failure [1,2]. However, the first question to ask is whether such analysis is meaningful given the experimental data on the role of ACE in LVH. In this regard, it is well established that *ACE* gene expression is induced in experimental and clinical pressure overload LVH [1]. Likewise, cardiac ACE activity and cardiac angiotensin I to angiotensin II conversion rates were found to be enhanced in hearts with LVH [1]. Further, angiotensin II, has been demonstrated to be a powerful stimulus of cardiac protein synthesis and growth [1]. Moreover, ACE inhibition has been shown to ameliorate cardiac hypertrophy independently from effects on cardiac afterload [1]. Finally, isolated cardiac ACE inhibition (via intracoronary infusion of an ACE inhibitor) improved diastolic function in patients with chronic LVH. Thus, given the experience with ACE in clinical and experimental pressure overload hypertrophy, it seemed plausible indeed to investigate the role of the *ACE* I/D polymorphism as a genetic modulator of left ventricular mass. Even more striking support of this idea comes from molecular genetic studies in inbred animals. In particular, the chromosomal locus of the *ACE* gene was found to be linked to left ventricular mass (independently of blood pressure) in a cross between Brown Norway and New Zealand hypertensive rats.

ACE I/D and left ventricular hypertrophy

A review of the recent literature (Medline search) allowed to identify 23 studies dealing with the association of the *ACE* I/D polymorphism or ACE activity with left ventricular mass and hypertrophy [2–11, additional 14 references are quoted in the review article [1]: 49–55, 57–61, 63]. Of these, 20 studies were in favour of such association. However, while 20:3 may be a clear cut result in a competitive ballgame, this score does not necessarily resolve a scientific problem. Furthermore, the game might have been unfair since negative studies suffer from publication bias (positive findings have a higher chance of being published) such that even more advanced arithmetics by means of meta analysis may be inconclusive.

It may be worthwhile, therefore, to return to the pathophysiology of LVH and the renin–angiotensin system. The general perception is that LVH is a consequence of arterial hypertension, aortic stenosis, or obesity in order to normalize cardiac wall stress. Neurohormonal mechanisms, e.g. the renin–angiotensin system, are thought to modulate this process [10]. It has to be kept in mind, however, that the renin–angiotensin system is normally under strict negative feedback inhibition. Thus, genetically enhanced expression of singular components of the angiotensin II forming cascade may or may not be of physiological relevance. Given this information, it may be questionable if the *ACE* I/D polymorphism by itself has enough biological significance to substantially increase left ventricular mass in otherwise healthy subjects. In fact, the impact of this genetic marker may be too small to be detectable in groups of <100 subjects. Likewise, M-mode echocardiograms carried out during the 70s without 2D guidance may be too inaccurate to uncover subtle differences. In this respect it may be of interest that the largest negative trial on the association between LVH and the *ACE* I/D polymorphism revealed positive associations between *ACE* DD genotype and cardiovascular phenotypes that are easier to determine namely blood pressure levels or hypertension status, that are prominent risk factors for LVH [1].

The impact of a slightly activated renin–angiotensin system may be more pronounced when the heart is under stress or the renin–angiotensin system is also activated for other reasons. Thus, chronic arterial or pulmonary hypertension, aortic stenosis, hypertrophic or dilatative cardiomyopathy, myocardial infarction, renal transplantation, or polycystic kidney disease may help to precipitate the consequences of the *ACE* polymorphism. In fact, the majority of positive association studies between the *ACE* DD genotype and elevated cardiac mass are based on patients with these conditions [1–11]. In other words, the *ACE* DD genotype appears to play a permissive role in the development of LVH when the cardiac growth machinery is activated. An impressive example for this hypothesis is brought about by most recent data from Montgomery *et al.* [11]. The authors studied in a blinded fashion

young healthy subjects before and after a rigorous exercise protocol. Only those participants who carried the *ACE* deletion allele displayed an increase of left ventricular mass as estimated by echocardiography, electrocardiography, or BNP measurements [11]. Thus, the *ACE* genotype may act only under specific conditions suggesting an interaction between altered haemodynamics, ACE, and/or other genetic cofactors in the modulation of left ventricular mass. In agreement with this notion are observations of Pinto *et al.* and Ohmichi *et al.* who both found pathological remodeling early after myocardial infarction, predominantly in those subjects with the *ACE* DD genotype [1,5].

The hypothesis that the system needs to be stressed to uncover implications of cardiac *ACE* receives further indirect support from two experimental lines of evidence. First, low concentrations of angiotensin I (the substrate of ACE) resulted in similar angiotensin I to angiotensin II conversion rates in healthy individuals with various *ACE* genotypes [1]. However, high doses of angiotensin I related to augmented angiotensin conversion rates and more pronounced blood pressure responses in subjects with the *ACE* DD genotype [1]. Thus, conditions that go along with high renin or angiotensin I levels such as physical exercise, acute myocardial infarction or heart failure may enhance the implications of the *ACE* DD genotype (or elevated ACE activity) (Figure 1). Second, preliminary data from Pinto *et al.* suggest that transgenic rats with high levels of cardiac *ACE* expression have normal (or even smaller) hearts as long as these animals are housed under physiological conditions [12]. However, cardiac growth and diastolic dysfunction were augmented in the same *ACE* transgenic rats when the animals were stressed by abdominal aortic banding and subsequent cardiac pressure overload [12].

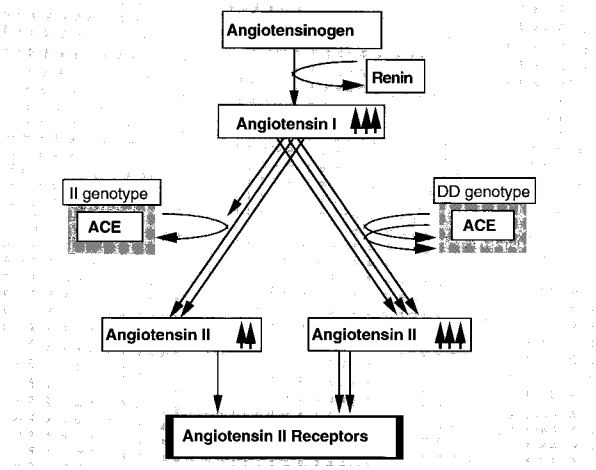

Fig. 1. ACE genotype and angiotensin II generation. The implications of the *ACE* I/D polymorphism may depend on the overall activity of the renin–angiotensin system. In particular, the DD genotype may result in enhanced local angiotensin II production when high concentrations of angiotensin I are available for conversion by ACE. This hypothesis is based on investigations demonstrating enhanced angiotensin II generation and blood pressure responses during high dose angiotensin I infusion in subjects with the *ACE* DD genotype. Furthermore, an augmentation of cardiac growth was observed in carriers of the DD genotype who were hypertensive, renal transplant recipients, or patients with myocardial infarction or aortic stenosis. Finally, transgenic rats with high cardiac ACE activity displayed enhanced LVH following abdominal aortic banding.

Nevertheless, current convincing evidence that demonstrates the role of the *ACE* I/D polymorphism in the development of cardiac hypertrophy is lacking and potential mechanism(s) responsible for the enhanced cardiac growth in subjects with the *ACE* DD genotype remain to be elaborated. In this respect, a molecular geneticist cannot escape the principles of pathophysiology since molecular genetic association studies may only provide hypotheses rather than proof for biological effect. Finally, the clinical usefulness of the *ACE* DD genotype as a molecular marker of LVH appears to be rather limited unless we learn how ACE increases left ventricular mass and whether ACE related cardiac growth is good or bad for the respective individual.

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Cardiac disease in the diabetic dialysis patient

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Diabetes mellitus causes more end-stage renal disease in the western world than any other disease category. Study after study has shown that diabetics have a poorer survival than their non-diabetic ESRD counterparts. About half of this excess mortality can be attributed to cardiovascular causes. It is already known that diabetes is an independent risk factor for coronary artery disease and cardiac failure in non-uraemic populations [1].

Prevalence of cardiac disease in the diabetic on dialysis

Few clinical studies have focused on diabetic dialysis patients; most studies combine diabetics and non-diabetic patients together, or exclude diabetic patients. Surprisingly little is known about the natural history and risk factors of cardiac disease in diabetic ESRD. There is a dearth of basic information, such as the prevalence of left ventricular disorders and symptomatic cardiac disease at the start of dialysis therapy, when in pre-dialysis life they begin, the rate of progression of these disorders, the incidence of symptomatic cardiac disease of new onset, the lethality of cardiac events occurring while on dialysis therapy and the nature of cardiac risk factors, specific to diabetics with end-stage renal disease. There is a strong temptation to assume that the cardiac problems of diabetic ESRD patients relate to factors in their predialysis life about which little can be done when they reach dialysis. This assumption is probably not justified. For example, Manske *et al.* randomly assigned insulin-dependent diabetic patients with ESRD and significant coronary artery stenosis (with normal ejection fraction, without typical anginal pain) to medical or surgical management. In 8.4 months of follow-up 15% of the surgical group had a cardiovascular end-point, compared with 77% in the medically-managed group [2]. Data like these suggest that much could be done to prevent or treat cardiac disease in diabetic ESRD.

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Results of the Canadian Study

We studied a prospective inception cohort of 433 patients who survived for an average of 41 months, collecting clinical data monthly, and echocardiographic data yearly [3]. At inception of dialysis diabetic patients ($n=116$) had more echocardiographic concentric LV hypertrophy (50% vs 38%, $P=0.04$), ischaemic heart disease (32% vs 18%, $P=0.003$) and cardiac failure (48% vs 24%, $P<0.00001$) than non-diabetics. Among diabetics, older age was associated with baseline concentric LVH, systolic dysfunction, and cardiac failure; anaemia was associated with LV dilatation; smoking was associated with symptomatic ischaemic heart disease. There was a very striking association between left ventricular morphology and the presence of cardiac failure at inception of dialysis. Compared to those with normal LV dimensions and contractility, the odds of cardiac failure were 5.4 ($P=0.02$) in those with concentric LV hypertrophy, 13.7 ($P=0.02$) in those with LV dilatation but preserved systolic function, and 26.7 ($P=0.006$) for those with systolic dysfunction. After starting dialysis diabetic patients had similar rates of progression of echocardiographic disorders and new cardiac failure as non-diabetics, but higher rates of new ischaemic heart disease (RR 3.2, $P=0.0002$), overall mortality (RR 2.6, $P<0.0001$) and cardiovascular mortality (RR 2.6, $P<0.0001$). Mortality was higher following admission for clinically-diagnosed ischaemic heart disease (RR 1.7, $P=0.05$) and cardiac failure (2.2, $P=0.0003$), suggesting that these events are more lethal in diabetics. Among diabetic patients older age, LV hypertrophy, smoking, clinically-diagnosed ischaemic heart disease, cardiac failure, and hypoalbuminaemia were independently associated with mortality. This study confirms the widely held impression that the burden of clinically

manifest cardiac disease in diabetic patients starting ESRD treatment is huge. It is well known that silent coronary artery disease is very common in diabetics, especially those with ESRD. As such this study probably underestimates the burden of cardiac disease in diabetic ESRD. It very clearly suggests that the amount of cardiac morbidity and mortality on dialysis cannot be fully attributed to cardiac disease present at inception; the 'extra' mortality seems to be mediated via ischaemic disease, rather than quicker progression of cardiomyopathy while on dialysis therapy. Indirectly these data suggests that diabetes accelerates further the 'accelerated atherosclerosis' of uraemia. Of equal importance, this study suggests that are many treatable risk factors such as anaemia, smoking, LV hypertrophy, and hypoalbuminaemia.

The cause of cardiac problems in the diabetic

There is considerable evidence for a specific diabetic cardiomyopathy, characterized by ventricular thickening beyond that expected for blood pressure levels. For example, the Framingham Study found that female diabetic patients had larger LV wall thickness, end-diastolic diameter and relative wall thickness than non-diabetic patients, [4]. In our study, diabetic patients had a higher baseline prevalence of concentric LV hypertrophy than non-diabetic patients; this was especially true in female diabetics. It is not known whether this 'diabetic cardiomyopathy' is preventable by good glycaemic control, although this would seem to be an attractive hypothesis. The limited data linking LV hypertrophy to outcome in ESRD are not fully consistent. Weinrauch *et al.* have reported that echocardiographic abnormalities were better predictors of outcome than clinical ischaemic heart disease or congestive heart failure [5]. On the other hand, there was no association between LV hypertrophy and myocardial infarction or sudden death in the large, prospective, German multicentre study reported by Koch *et al.* [6].

A number of other authors have reported that smoking is harmful in diabetic patients with advanced renal impairment [7]. Smoking is a major cardiovascular risk factor in non-uraemic diabetic patients and cessation has been suggested as the single intervention with the biggest impact on survival. It has been speculated that malnutrition is responsible for much of the excess mortality of diabetic patients with ESRD [8]. The strong association between hypoalbuminaemia and mortality seen in our study supports this viewpoint. We saw no independent association between serum cholesterol levels and mortality in this study. On the other hand, diabetic patients dying from myocardial infarction had higher total cholesterol, LDL cholesterol, LDL/HDL ratio, and apolipoprotein B levels than survivors in the German study alluded to above [9]. Other factors reported as cardiovascular risk factors in diabetic ESRD include poor glycaemic control [10], lower dialysis intensity, and accumulation of advanced glycosylation end-products. It not known with any degree of certainty whether peritoneal dialysis or haemodialysis is associated with longer survival in diabetic ESRD.

Patient management

Smoking cessation, optimal glycaemic control, aggressive blood pressure control, ACE inhibition, aggressive treatment of dyslipidaemia and very careful monitoring for malnutrition, and avoidance of severe anaemia are the cornerstones of preventive management in the pre-dialysis phase. There is very little evidence to suggest that the management of symptomatic cardiac disease of diabetics should be different to non-diabetics. The balance of evidence suggests that coronary arteriography is indicated for most diabetic transplant candidates. We feel that serial echocardiography is useful and influences clinical management; however, our viewpoint is likely to be skewed, and it is highly unlikely that a trial comparing a policy of serial echocardiography versus no echocardiography will ever be performed. When diabetic patients reach ESRD we feel, in the absence of controlled data to suggest the opposite, that preventive and symptomatic management should not differ substantially from the pre-ESRD phase. The need for high quality, prospective epidemiological studies and randomized intervention trials of cardiac disease in ESRD is acute; this is more true in diabetic ESRD than other major cause disease category.

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Why so much disparity of PD in Europe?

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If the choice of dialysis method were based solely on accepted, strictly medical, criteria a similar percentage of patients should receive the respective type of treatment in economically similar countries. And yet 20 years after the introduction of ambulatory peritoneal dialysis (PD) for treating ESRD patients, the extent of its use differs greatly between countries. Thirty-eight per cent of patients in Canada, and only 6% in Japan, are treated by PD [1]. Despite well-known imperfections in data collection, mainly because they depend on voluntary reporting to national and European registries, it appears that there are even differences between western European countries: in 1995, 45% of dialysis patients in Britain and only 7.9% in Germany were treated by PD [1]. These percentages have remained constant for years (Figure 1) and reflect true differences in the selection of treatment modalities [2]. There are also regional differences in some countries. In northern Italy, approximately 15% of patients are treated by PD, and only 2% in Sicily [3]. In France, similarly, there are large differences in the use of PD, and the percentage of patients treated by PD can vary from 0% to 22% between different towns in the same region [4]. In contrast, PD is used to the same extent throughout the whole of Germany and in Switzerland, with the exception of the canton of Valais (Switzerland), where PD is seldom used [5].

There are psychological and social reasons, as well as medical ones, to explain these disparities. The way that care-givers and society view the so-called autonomous dialysis methods is important and depends in part on guidelines, which are themselves largely determined by economic factors. Here, we analyse the role of these different factors in influencing the choice of treatment modality.

Medical factors

At the end of the 1970s, PD was seen as an alternative to chronic haemodialysis (HD), which had a proven efficacy. The many initial complications led numerous medical teams to view PD as an inferior method ('second class treatment by third class physicians') and therefore abandoned its use. Some doctors still reject this method categorically, although many of the technical problems have been overcome and thus there are now far fewer failures. Nevertheless, although the incidence of peritoneal infection is now low, peritonitis due to intra-abdominal pathology is a major complication, particularly in the elderly: this problem may carry weight when physicians make their choice, a choice which is rarely explained.

In theory, the choice between two dialysis techniques is only an issue if they result in the same survival and quality of life. There has been no truly prospective, randomized trial, involving a large population of patients of similar age, sex, associated pathology and medical treatment before dialysis, but treated using different methods of dialysis under equivalent conditions (which are very difficult to verify). No such a study has been performed, and is unlikely ever to be performed, for many, mostly ethical reasons. The comparison of PD and HD has thus been based on retrospective single or multi-centre analyses, or on data from registries. These studies suffer from sampling bias and their conclusions are sometimes divergent. However, it is clear that after 3–5 years, very few patients are still treated by PD, the limits of this method being reached when the dose of dialysis it can provide is no longer sufficient [6]. Thus, in the 1990s, PD must be viewed not as a potential replacement for HD, but as an alternative to HD during the early years of treatment [7].

There is still much debate over the indications, which are based on medical background and underlying renal disease, for particular dialysis methods. PD has major advantages for very young children and HIV-infected patients. However, there are very few patients in these categories throughout western Europe. This cannot therefore explain the observed differences of the percentage of patients on PD between regions and countries. PD is regarded by some as being ideal for elderly patients, because it provides haemodynamic stability and permits treatment of the patient in his/her own home [8]. Others disagree and prefer HD. It is only in the over 65 age-group that the number of new dialysis patients per million population continues to increase annually, to a similar extent throughout the European Union. Thus, this factor is not responsible for the differences shown in Figure 1, even in Britain, where the number of elderly patients treated is consistently lower than in neighbouring countries. Possibly, the high incidence of diabetes in Scandinavian countries

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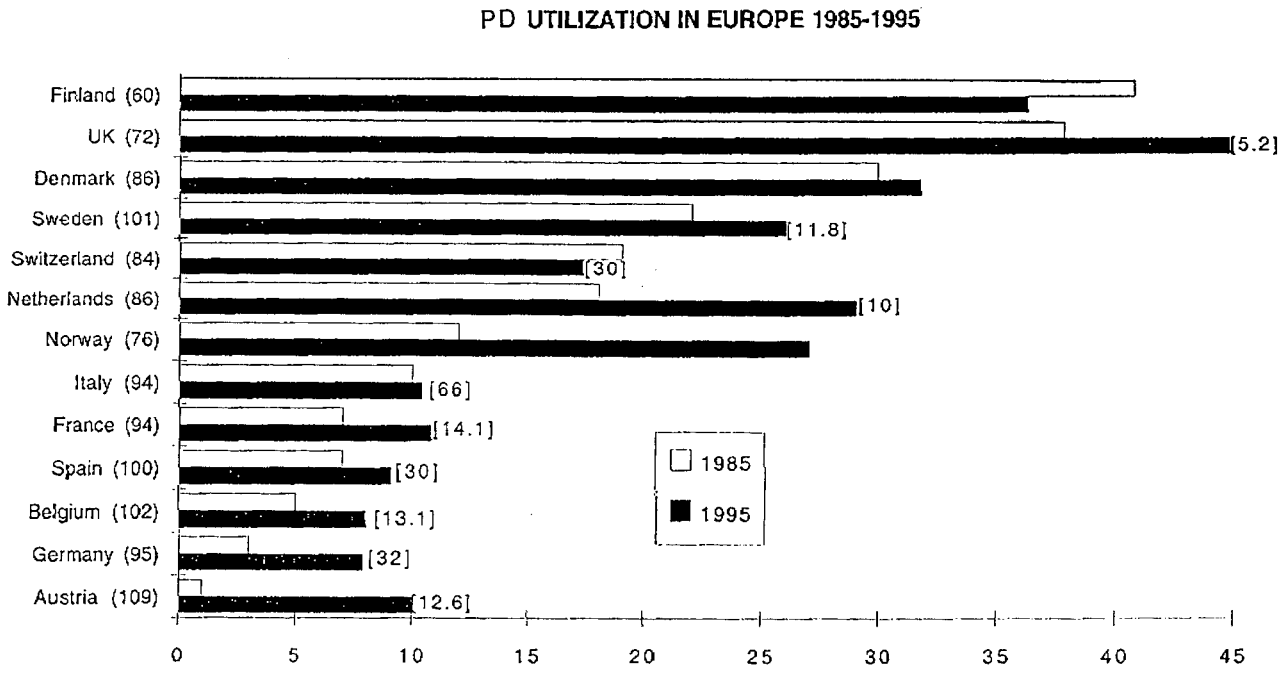

Fig. 1. Percentage ESRD patients treated by PD in Europe, 1985–1995 [2]. (), Incidence of end-stage renal failure per million population 1995, [], Nephrologists per million population 1995.

may be responsible for the high prevalence of PD. It has been claimed that PD is the best method for treating diabetic patients, although there is now some debate about whether this is actually the case [9]. In fact, it is clear that most choices of therapy are based largely on non-medical criteria.

Psycho-social factors

In this context, one important factor is the experience of the doctors involved. In countries with adequate HD facilities, doctors and medical staff are often unfamiliar with PD, and many have never used it. This is particularly true of older physicians in academic institutions. Efforts have been made by the teams that pioneered PD, often supported by industry, to educate other physicians about its use. Despite this effort, its use is still relatively modest. In Europe there is no relationship between the number of nephrologists per million population and the proportion of patients treated by PD (Figure 1). For example, although there are many nephrologists in Italy, PD is used very little. Switzerland and Germany have similar numbers of nephrologists per million population (about 30), but, in Switzerland, 2.5 times more patients are treated by PD than in Germany (17.9% *vs* 7.9%). Nevertheless, conclusions in this area should only be drawn with caution, because the exact numbers and qualifications of doctors and nurses who manage ESRD patients are not known. Doctors may also be concerned that PD increases the workload of the care team. They will be obliged to be on call 24 h a day to deal with complications, particularly infections, which can be complex to treat. It is difficult to recruit devoted physicians and nurses to take care of PD patients, because both technical skill and competence in interpersonal relations are required. The use of PD may also complicate the functioning of HD units, whose back-up is vital to the success of any PD programme. Indeed, PD programmes cannot function independently. The difficulties encountered in running such treatment facilities may partly explain the treatment choices made by some nephrology department directors.

Do the patients themselves have differing views about the various treatment methods? In 20–30% of cases, ESRD patients are referred to the nephrologist late. They are therefore mostly treated by HD, and are unlikely to be taken off HD subsequently [10]. In non-urgent situations, when they have the possibility to choose, the decisions of the patients depend mostly on the information provided by their doctors. Indeed, the patient's confidence in the nephrologist has a larger effect on his decisions than most other considerations. Various medical teams have very definite, but divergent, opinions on treatment choices. Ignorance, or refusal to acknowledge the benefits of patient autonomy leads some doctors to rule out this therapeutic approach systematically. Therefore, nephrologists are largely responsible for some of the observed differences in prevalence of use. This fact is well illustrated in Paris where there are 11 university hospitals: 90% of patients treated by home HD come from one of these hospitals and 72% of patients treated by PD come from three others.

The patient's psychological profile, family environment, type of housing, distance from the HD unit (particularly important in rural areas) and desire to

continue working, all affect the patient's decision to take a more or less active role in treatment. Nevertheless, in Europe, the desire to continue working is becoming less important in the decision-making process, because the average age of patients at the start of treatment is close to the age at which retirement is proposed to them to reduce unemployment.

In some countries, patients receive an indemnity for agreeing to home treatment. The amount given is the same for all methods and is too small to influence the initial choice. However, in France, legislation makes it possible to pay nurses who treat patients by PD at home and this clearly influences the choices made by both doctors and elderly and handicapped patients.

Different mental attitudes also account for some choices. Many patients, particularly in southern Europe, choose to be passive when faced with illness. They feel that they have already paid high contributions for health insurance and they refuse to become directly involved in their own treatment. Doctors and medical teams must fight against the patient's unwillingness to take responsibility, but teaching unwilling patients how to treat themselves is extremely difficult. Attitudes are probably very different in Scandinavian countries. Strong family support and national solidarity may well be at least partly responsible for the success of PD programmes in these countries. These same factors contribute to the success of transplantation programmes, including those involving living donors.

Economic factors

The cost of PD is about 30% less than that of centre HD, regardless of country, health care system or funding body. This difference tends to lessen as larger quantities of dialysate and often, a cycler, are required for adequate PD. In Great Britain, a policy of strictly limiting the funds available for HD was and continues to be the reason for the great success of PD programmes. In France, the number of HD centres is limited for legal reasons, and free access to PD thus allows a large number of elderly patients to receive dialysis treatment. Indeed in 1992 in France, 28% of patients aged 75 and over were treated by PD compared to less than 10% in the total dialysis population [8]. The proportions were the other way around in the US. Such contrasts cannot be considered satisfactory and presumably reflect inappropriate choice of PD for some patients who should have access to HD.

In western Europe, PD has had to fight for its place because of regulations that are complex and vary greatly between countries. It appears that the greater the involvement of the public (as opposed to private) facilities in the provision of health care, the greater PD programmes. In Nordic countries and in Switzerland, 90% of health care is provided by public facilities and these countries make a great use of PD. In contrast, in Spain, the public sector provides only 45% of the available health care and less than 10% of patients are treated by PD.

In some countries, where health care is largely provided by the private facilities (Germany, Italy, France), there is no or unfavourable reimbursement by health financing organizations for PD. The availability of grants for patient training also differs greatly and is dealt with separately only in Germany, Spain and Switzerland. In France, there is no training reimbursement for PD, whereas there is one for home HD. The same applies in both France and Switzerland for medical fees and this situation makes it extremely difficult, if not impossible, for private nephrologists to prescribe PD [2,3].

The proportion of the treatment cost accounted for by consumables is much larger for PD (about 70%) than for HD (about 52%). Also, in countries where reimbursements are paid at a flat rate which depends only on type of treatment, it is easier for the nephrologist to recover his fixed costs, particularly his staff costs, and to increase his own profits, simply by increasing the number of patients treated by HD. In contrast, an increase in the number of patients treated by PD causes additional expenditure with proportionately less profit for infrastructure and staff. Doctors are probably aware of these considerations, whatever their professional situation, and this inhibits the development of PD programmes.

In an economic environment which has become increasingly difficult for health care, the considerable differences in the choice and cost of dialysis treatments in Europe cause problems and may become an issue which attracts government interest. Nephrologists cannot be indifferent to this scenario. Together with health financing organizations, they must try to improve the cost-effectiveness of techniques before seeking additional funds. It is their role as doctors to assure the best possible quality of treatment for their patients and it is their role as citizens to ensure the best possible use of available funds. They should therefore look with open minds at all new technologies, both in the phase of their evaluation and subsequent use. Inflexible attitudes can only damage attempts to provide the best treatment at the lowest cost. This is the only way to ensure that certain categories of patient, particularly the elderly, do not in the coming months and years, become victims of unequal access to dialysis treatment.

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Major histocompatibility complex-associated odours

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Introduction

Aside its basic biological function of self/non-self discrimination in the immune system the major histocompatibility complex (MHC) has been recognized as a possible source of individual specific body odours. Dating back to speculations by Lewis Thomas in 1975 [1] on the role of the extraordinary polymorphism of the MHC as background of an individual chemosensory identity, and to early observations of MHC dependent mate choice in inbred strains of mice systematic experimental studies by Yamazaki *et al.* [2] revealed a first evidence for H-2 related body odours in this species. Meanwhile a large number of animal studies with rodents and a series of field studies and experiments with humans have extended our knowledge of MHC related odour signals and substantiated the hypothesis of immunogenetic associated odourtypes. Its potential role in kin recognition, mate selection, and social perception is currently under debate.

MHC specific odours in rodents

The most convincing series of experiments were conducted with mice which were trained in olfactory discrimination between two congenic inbred mouse-strains, e.g. BALB/k (H-2^k) vs BALB/c (H-2^d) [3–5]. The trainings were performed in an odourized Y-maze in which the previously water deprived animals had to identify which of the two in the goal regions of the maze randomly presented odours was associated with a reinforcement: correct choice of one end of the maze was reinforced by a drop of water. Several reports of successful odour discrimination of congenic strains in different laboratories led to the conclusion that the H-2 complex is involved in the constitution of odour types. Moreover using the same experimental approach (i.e. training for odour discrimination) it could be shown that semiallogenic [6] and fully allogenic experimental bone marrow transplantation (BMT) between congenic strains of mice [4] change the odour type of the recipient in the way that old (recipient) and new (donor) odour components characterize the chimeras. In other words: with the BMT a donor-specific H-2 odour component was added to the recipient's odour profile. Therefore it was concluded that the haematopoetic stem cell is one source of the MHC-associated odour signature.

The biological significance of H-2 related odours was documented in three quite different paradigms. First of all the above mentioned unsystematic observations of H-2 dependent mate choices were tested under experimental conditions [7]. As it turned out the prior results could be replicated and moreover it was reported that the phenomenon is dependent on the odour imprinting from the parents which raised the pups. In this case mating preferences serve to avoid mating with mice that share the same MHC as the foster parents [8]. Secondly it was found that H-2 related odours are capable of inducing the so-called pregnancy block phenomenon. In the animal literature, pregnancy block is defined as odour-induced return to oestrous after insemination caused by confrontation with the scent of a male genetically different to the stud. The odour of H-2 different compared to H-2 similar potential new mates leads in a higher proportion of newly inseminated mice to this pregnancy block.

Finally a field study by Potts *et al.* [9] which was conducted under seminatural conditions reported the increase of H-2 heterozygous F-generations in populations of mice with well defined H-2 types on a genetically diversified background.

For rats Roser *et al.* [10] reported RT.1 related odour differences when they tested repeated olfactory explorations of urine odour samples from RT.1 congenic strains. In these habituation–dishabituation tests rats first reduced their olfactory exploration (defined by the time sniffing at the odour stimulus) with the number of recurrent identical odour probes. Intro-

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ducing an odour signal that differs from the scent to which the animal is adapted immediately increases the exploration time (dishabituation). By means of this technique RT.1 related odour types were documented. Furthermore the authors suggested that soluble derivates of MHC class I molecules in the serum of the animals could selectively bind characteristic odour molecules which are excreted in the urine [11].

MHC specific odours in humans

First evidence for HLA related urine odours was reported by our group [12]. We used a computer controlled olfactometer in which rats were trained to discriminate successively presented odours in a so called go–no go discrimination learning paradigm. Urine odour probes were collected from two groups of six subjects each. One group was defined by its common HLA-class I A1,–, B8,– notation (half of the subjects being homozygous, the other half heterozygous). The second group consisted of members without an A1,–, B8,– tissue type and differed among all of its members according to their HLA-class I pattern. After a cumulative discrimination training procedure the animals were able to identify the specific A1,–, B8,– HLA related odour and to discriminate it from that of HLA-different persons. Meanwhile field studies [13,14] and an experiment [15] revealed that the similarity in the HLA class I genotype of two persons influences social perception measured by ratings of familiarity, attractiveness or pleasantness. Similarity of the class I HLA in these studies was defined by the number of shared HLA class I alleles.

Chemical characteristics of MHC related odour signals

While behavioural experiments, studies on odour-transfer by experimental BMT, studies of mating preferences and of odour-induced pregnancy block strongly suggest the existence of MHC associated odour types, there is only scattered evidence for the chemical characterization of the volatile signals. Schwende *et al.* [16] used gas chromatography to describe a correlation between H-2 relatedness and the similarity of the chromatograms of volatile components of the respective urine odours of mice. Comparing chromatographic odour profiles of the congenic strains BALB/k (H-2^k) and BALB/c (H-2^d) with that of C3H (H-2^k) (BALB/k and C3H being different with respect to their genetic background but identical in their H-2) Eggert *et al.* [17] concluded that a small number of specific chemical components (two out of 55 in their study) as well as a pattern of a few ubiquituous components differing in their relative concentrations between inbred strains characterize the H-2 signature of the urine odours. They also identified eight (out of 55) components that are associated with the genetic background, i.e. describe odour differences between C3H and BALB/k. Moreover the results from Eggert *et al.* [17] point to the conclusion that the MHC and other autosomal genes interact in producing the specific odour of an inbred strain. Recently Singer *et al.* [18] again comparing congenic strains of mice (H-2^k vs H-2^b) reported that the MHC-determined urinary odour is composed of a mixture of eight volatile carboxylic acids, the relative concentration of which characterizes the specific odour profile. In this study no specific components were found. Thus, it remains controversial whether the MHC odour specificity is also signalled by a small number of specific chemical components besides dependence on a profile of different concentrations of ubiquituous odour compounds. This may even differ from inbred strain to inbred strain.

Models of MHC related odour production

There are at least three different hypotheses about the mode by which MHC specific odours are generated: (i) selective binding of odour molecules to soluble derivates of MHC class I receptors [11], (ii) MHC specific selective bacterial colonizing. Data from two studies with germ-free mice and rats are contradictory [19,20], (iii) Coexpression of odour producing genes lying in the MHC region. Results from studies with mutant mice differing only in single genes of the MHC [21] as well as studies with HLA-transgenic mice [22] present evidence that indeed the MHC genes themselves are involved in the odour production. Summarizing results of our own research and that of other authors we would propose the following integrative model (cf. Figure 1) in which soluble MHC proteins play a central role in the production of MHC-associated odours.

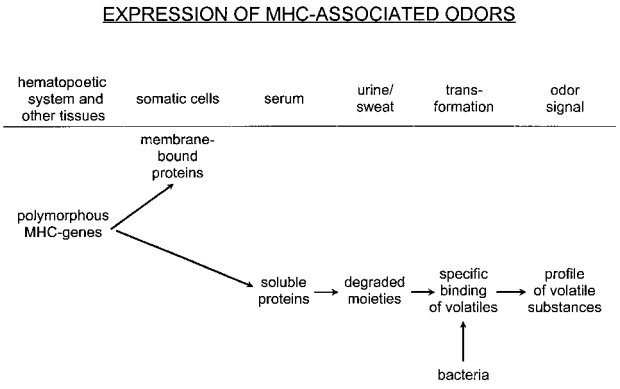

Fig. 1. Expression of MHC-associated odours. By means of experimental bone marrow transplantations in mice the haematopoietic system as well as other tissues and organs were identified as being the origins of MHC-associated odours. Immunological studies showed that soluble MHC proteins originating from these tissues can be found in serum. The intact molecules and degraded moieties of these MHC proteins occur in urine as well as in sweat. By an unknown process these specific peptides seem to be linked to specific profiles of volatile substances, which are detectable and can be described by means of gaschromatographic analyses.

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Non-steroidal anti-inflammatory agents in patients with a renal transplant

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In a recent letter to the Editor, Stoves and coworkers described in NDT a patient with a renal transplant, who developed acute renal failure following use of the non-steroidal anti-inflammatory drug ibuprofen [1]. The patient had obtained the drug as 'over-the-counter' medicine. Renal biopsy revealed tubulointerstitial nephritis. The authors concluded in their report that ibuprofen was probably the cause of this acute interstitial nephritis [2]. Stoves *et al.* suggested that major attention has to be paid to the increasing number of 'over-the-counter' preparations, which might include potential 'nephrotoxic' drugs for patients with a renal transplant. The authors further indicated, that in fact the differential diagnosis in renal functional impairment in the patient with a renal transplant has to include the use of non-steroidal anti-inflammatory drugs.

As the authors outlined in their letter, (allergic) tubulointerstitial nephritis due to non-steroidals is a well recognized side effect. I believe the renal community is aware of this potential hazard. One interesting issue, however, which derives from this letter, is

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whether patients with a renal transplant are at particularly high risk to the broad array of renal side effects following the use of non-steroidal anti-inflammatory drugs (NSAIDs), and which in most cases are not tubulointerstitial lesions.

What is the nature of NSAIDs induced renal impairment?

Non-steroidals can induce acute and chronic renal failure, as well as significant disturbances in salt and water homeostasis.

The acute renal functional disturbance can appear as haemodynamically mediated event or as the consequence of a tubulointerstitial nephritis with or without nephrotic syndrome [2,3]. These acute toxic or 'immunologic' side effects usually appear within days following the intake of the medication. In contrast, the long-term abuse of NSAIDs over years may lead to end-stage renal disease, due to progressive interstitial fibrosis [4]. The chronic abuse of NSAIDs can also lead to a significant impairment of some antihypertensive drugs and thereby aggravate blood pressure in patients with arterial hypertension.

Strong evidence exists that the NSAIDs exert their effects by the interference with the arachidonic acid metabolism. This polyunsaturated fatty acid is metabolized in the kidney by cyclooxygenases and lipoxygenases to prostaglandins, thromboxane, and leukotrienes. The predominant pathway in kidney tissue is the conversion of arachidonate to prostaglandins by the cyclooxygenases. NSAIDs act through the inhibition of cyclooxygenases. Under normal 'physiological' conditions, prostaglandins do not play a major role in the maintenance of renal function. If however, renal 'stress' develops, several renal functions become 'prostaglandin dependent'. Renal cortical and medullary blood flow, regulation of the glomerular filtration rate (GFR), renin release, and salt and water excretion are tightly linked to an intact prostaglandin system. Important clinical situations, which include renal 'stress' include hypovolaemia, congestive heart failure with low cardiac output states, hepatic cirrhosis, extensive diuretic therapy to name a few. In these clinical settings renal prostaglandin formation is increased and by their vasodilatory and diuretic actions, prostaglandins antagonize vasoconstrictor and salt retaining effects of angiotensin II, catecholamines, and vasopressin, which induce renal 'stress' [3]. The intact prostaglandin system maintains under these conditions normal renal function. Application of NSAIDs by patients to whom the outlined clinical states apply, will result in reduced renal perfusion and a decline in GFR with the possibility of development of acute renal failure. Fortunately, in most patients this functional impairment is reversible. However, dialysis and hospital care might become necessary. While the acute impairment of renal function following NSAIDs based on the described haemodynamic effects is well characterized, the pathophysiology of tubulointerstitial nephritis and the development of interstitial fibrosis is still incompletely understood. There is, however, recent experimental evidence that prostaglandins are involved in the repression of proinflammatory cytokines. Particularly the formation of chemokines molecules which regulate, the inflammatory cell influx in glomeruli and the tubulointerstitium in several renal diseases are suppressed by PGs [6,7]. The inhibition of prostaglandin formation ameliorates these repressor effects of prostaglandins and leads to sustained or enhanced expression of proinflammatory and profibrogenic mediators and may induce tubulointerstitial damage and fibrosis [8]. Thus, there is accumulating evidence that NSAIDs may induce proinflammatory and fibrogenetic effects in the kidney.

Is the transplanted kidney 'prostaglandin dependent'?

The described renal disturbance following NSAIDs can appear in otherwise healthy kidneys. There are, however, renal diseases where NSAIDs induce impairment of renal function, independent of the extrarenal 'stress' factors. The best studied and characterized renal disease is lupus nephritis. In lupus nephritis NSAIDs are used to reduce already compromised renal function [9]. This 'intrinsic' renal damage makes the kidneys 'prostaglandin dependent'. Additionally, experimental and clinical studies show that the reduction of renal mass with the resulting compensatory adaptation of GFR is also prostaglandin dependent, if the loss of mass exceeds 60% [10]. In many patients with a renal transplant loss of functioning renal mass is beyond 60%. Although naturally not extensively studied, the renal transplant may represent a prostaglandin dependent situation. Immunosuppression of most renal transplant patients includes cyclosporine A (CsA). The major functional renal side effect of CsA is the reduction of renal blood flow and GFR. Clinical and experimental evidence reveal that CsA reduces the formation of vasodilatory prostaglandins [11], but increases the formation of the vasoconstrictor thromboxane [12]. This results in a situation, where 'stress' adaptation by prostaglandins might be largely hindered.

Furthermore, CsA induces tubulo-interstitial fibrosis in renal transplants and initiates hypertension. The mechanisms of fibrosis are thought to involve increased transforming growth factor β (TGF-β) mediated collagen deposition [13].

The renal function of a patient with a transplant may thus represent a 'prostaglandin dependent' state as a result of the reduction in kidney mass. CsA can inhibit prostaglandins in the kidney [6]. This may lead to a state of relative prostaglandin deficit and would expose the kidney to a high risk of functional loss and development of fibrosis, once NSAIDs are added.

Even though this pathophysiologic theory has not been formally studied in the renal transplant patients (and will probably not be studied), I believe there is

substantial experimental and clinical evidence that patients following renal transplantation should not receive NSAIDs.

This might be of significant practical importance, since many transplanted patients suffer from different causes of pain, especially bone disease. The uninformed patient may easily choose an 'over-the-counter' NSAID, which may not only lead to the comparatively rare side effect outlined by Stove *et al.*, but also to the more or less well-noticed or unnoticed acute or chronic loss of renal function. The causes may include all the discussed mechanisms and consequences. I think not only the doctors and patients, but also the providers of 'over-the-counter' NSAIDs have to consider the transplanted kidneys at 'high risk'.

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