Indirect recognition of allo MHC peptides—potential role in human transplantation

Bruno Watschinger
Department of Medicine, Renal Division, University of Vienna, Vienna, Austria

Introduction

The introduction of cyclosporin in the early 1980s led to a dramatic improvement of transplant survival rates mainly through a reduction of acute rejection episodes early after transplantation. The long term decline in graft function, however, was not significantly influenced as indicated by a virtually unchanged half life of 7–8 years for kidney transplants [1]. Thus a main focus of transplantation research remains to elucidate the mechanisms that lead to this perpetual long term loss of kidney allograft function. It is now believed that both immunological as well as non-immunological factors ‘injure’ the allograft resulting in functional and morphological changes known as chronic allograft failure/rejection [2].

The direct and the indirect pathways of allorecognition

The central immunological event that initiates allograft rejection is the recognition of allo-MHC-molecules by recipient T-cells [3,4]. The allorecognition process was long considered to occur exclusively in a direct fashion, where T-cells recognize intact allo-MHC-molecules on the surface of donor cells. Then, in the early 1980s, Lechler and Batchelor [5] suggested an additional route of T-cell recognition of alloantigen. They observed that MHC-incompatible rat kidney allografts, that were dendritic-cell-depleted after being ‘parked’ in intermediate hosts could be rejected in certain donor/recipient combinations after retransplantation.

A decade later major advances in the field of biochemistry, e.g. the discovery of the structure of MHC class I and II molecules, new insights into antigen processing and presentation, and the ability to synthesize peptides corresponding to known sequences of MHC molecules, provide insights into the mechanisms underlying this second way of allorecognition. The indirect pathway of allorecognition resembles that of nominal antigens (e.g. viral peptides), with the main difference that peptides of allogeneic origin are presented by self antigen presenting cells (APCs) (Figure 1). These peptides are derived from donor MHC molecules, presumably after being shed from the allograft into the recipient’s circulation. The molecules are internalized by the recipient’s APCs, processed and presented on the cell surface by self MHC class II molecules. The T-cells of the recipient are activated after recognizing this combined structure of a foreign peptide presented in the groove of a self MHC molecule [6,7]. In the case of indirect allorecognition the activated CD4+ T-cells mediate the effector mechanisms of allograft rejection (cell-mediated cytotoxicity, antibody-dependent cytotoxicity, delayed-type hypersensitivity reactions/inflammation and natural killing) by providing the necessary cytokines for the activation of CD8+ T-cells, B-cells, macrophages and natural killer cells (Figure 1).

Recent work by several investigators using synthetic, MHC derived peptides indicates that this particular way of allorecognition occurs in animals and in humans. DeKoster et al. [8] were first to show that human T-cells recognize processed allo-MHC in a self restricted manner. T-cell clones primed by a HLA-DR3 derived peptide were able to proliferate to allo-MHC (HLA-DR3) molecules on HLA-DR3/DP3 cells.

Liu and Suciu-Foca [9] studied a larger number of different synthetic peptides and demonstrated that human responder T-cells primed in vitro by allogeneic DR1 expressing cells recognize and proliferate to synthetic MHC peptides derived from the hypervariable domain of the DR1*0101 molecule when presented by self APCs. Liu’s work brought about another important observation: the frequency of T-cells taking part in the indirect mode of presentation is about 100-fold lower than that of T-cells recognizing the whole MHC molecule directly [10]. This must be kept in mind when one interprets results of clinical studies that investigate the indirect mode of presentation in humans.

What is the role for the indirect pathway of allorecognition in transplant recipients?

Allopeptide specific T-cells can be found in the blood of transplant recipients. Lymphocytes from HLA-A2...
mismatched renal allograft recipients proliferated in vitro to two of five overlapping peptides derived from HLA-A2 [11]. In another study, Susskind et al. have shown that CD4+ cytolytic T cells of transplant patients are able to recognize and to be sensitized by HLA Class I allopeptides of donor origin [12].

Similar to a large number of animal studies demonstrating a role of the indirect pathway of allorecognition in vivo during rejection of skin and vascularized allografts [13–15], there is mounting evidence that it may also play a role in human organ transplantation. But studies in patients are still complicated by a variety of problems. First, we lack an appropriate assay to detect the relatively low frequency of all alloreactive T-cells activated by the indirect pathway. Given the complexity of the HLA system and differently processed class I and class II MHC peptides, it is clear that an undefined number of variable peptide/MHC complexes can activate different sets of T-cells. At the moment we only test for T-cell proliferation to a limited number of arbitrarily synthesized peptides. Thus we may miss additional T-cell clones, which are also activated through the indirect pathway and may underestimate the contribution of the indirect pathway to the total alloimmune response. In addition Liu et al. [16] found a 10- to 50-fold higher frequency of allopeptide reactive T-cells within the rejecting organ, which remains undetected when looking at the frequency in the systemic circulation. This methodological problem might be a reason for undetectable or seemingly low precursor frequency of alloreactive T-cells in transplanted patients [17,18]. Nevertheless there is increasing evidence for an important role of the indirect pathway during rejection episodes.

What is the role for the indirect pathway of allorecognition in acute allograft rejection?

The occurrence of the indirect pathway during acute allograft rejection has been demonstrated earlier in rat heart and kidney transplant models [13,14,19]. First data supporting an activation of the indirect pathway of allorecognition during human allograft rejection were recently published. In human cardiac allograft recipients undergoing acute rejection, Liu et al. [20] were able to detect circulating T-cells, specific for a DR1 peptide in DRβ*1101 recipients of hearts from DRβ*0101 donors. They further demonstrated that these cells can be found prior to and during acute and chronic rejection. In another series investigating 32 cardiac allograft recipients with different MHC class II donor/recipient combinations, episodes of acute rejection were strongly associated with T-cell proliferation to synthetic peptides corresponding to the hypervariable regions of the mismatched HLA-DR antigens [16]. The T-cell response was restricted by a single HLA-DR antigen and directed against one immunodominant peptide, but during subsequent rejection episodes, the second HLA-DR antigen was similarly able to elicit allopeptide reactivity. They found that during repetitive rejection episodes, the alloreactive T-cell repertoire changes, suggesting an intermolecular spreading [16]. This has to be kept in mind, when strategies to interfere with the presentation of allopeptides will be developed. It may not be sufficient to target a single MHC molecule or else a particular MHC/peptide combination to prevent activation of the alloreactive T-cell repertoire.
What is the role for the indirect pathway of allore cognition in chronic allograft rejection?

A significant role of the indirect pathway of allore cognition has long been discussed for chronic rejection, but indirect evidence for its presence in chronic allograft rejection in humans was only recently published. Its involvement was suggested in studies looking at precursor frequencies of directly primed T-cells, soluble HLA antigens, and proliferation to synthetic MHC-peptides. In limiting dilution analysis Mason et al. [21] studied the precursor frequencies of alloreactive T-cells directly primed against the donor MHC antigens in kidney transplant patients, who had lost their graft. In a significant number of patients, who lost the graft more than 2 years post-transplantation, directly primed alloreactive T-cells could not be detected. Thus a role for an alternate rejection mechanism, e.g. the indirect pathway was suggested. Reed et al. [22] examined heart transplant patients and found an increased risk for the development of transplant-related coronary artery disease for patients with persistent donor-derived soluble HLA antigens for more than 26 weeks post-transplantation. These donor MHC molecules, shed from the graft and present in the circulation of the recipient, could be a source for the activation of the indirect pathway of allore cognition and constitute a continuous immunologic stimulus for the perpetuation of chronic allograft rejection. Further support for a role of the indirect pathway is provided by Vella et al. [23] who studied synthetic peptides and reported that T-cells from renal transplant recipients with signs of chronic rejection proliferated to synthetic allopeptides that corresponded to the mismatched HLA-DR molecules of the donor in 82% of patients, while only a minority of patients without chronic rejection showed proliferation. In heart transplant recipients chronic rejection, defined by the presence of coronary artery vasculopathy, was associated with persistent (up to 3 years after transplantation) allopeptide specific T-cell-responses and both inter- and intramolecular epitope spreading [24].

Concluding remarks

Although it is still too early to define its exact contribution to acute and chronic human allograft rejection, a considerable importance of indirect allore cognition in allograft rejection is suggested by an increasing number of human and animal studies [25,26]. The biological role of the indirect mode of peptide presentation is further underlined by recent findings demonstrating that synthetic nonpolymorphic and polymorphic peptides (derived from MHC class I and class II molecules) and other non-MHC-peptides can be used as immunomodulating agents (reviewed in [26–28]). It would be an intriguing idea to target this indirect presentation process therapeutically and use peptides to interfere with the T-cell and/or antigen presenting cell action/interaction in humans. Indeed, one such peptide (residues 75–84), derived from HLA-B27 has already been tested in a small phase II clinical trial in kidney allograft recipients. It was shown that this peptide is able to reduce peripheral blood NK cell cytotoxicity, but the mechanisms leading to the allele non-specific inhibition of alloimmune response are still unknown [29].

Until we can use synthetic peptides as immunotherapeutics to prevent or treat allograft rejection in humans we need to elucidate further the mechanisms of cytosolic protein degradation, peptide transport systems, intracellular peptide assembly and peptide binding and gain more insight into possible immunodominant peptide epitopes that could serve as suitable targets for a possible and economically reasonable peptide therapy. If we are able to resolve these issues in the future, peptide therapy could help to achieve better long term survival rates in organ transplantation and to reach the ultimate goal of transplantation medicine, i.e. tolerance induction.

References

15. Benichou G, Takizawa AP, Olson AC, McMillan M, Sercarz EE. Donor major histocompatibility complex (MHC) peptides

Nephrol Dial Transplant (1999) 14: 11–16

Regulation of vitamin D action

Alex J. Brown
Renal Division, Washington University School of Medicine, St. Louis, USA

The vitamin D endocrine system plays a central role in calcium and phosphate homeostasis. The active form of the vitamin, 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] increases blood levels of these minerals through actions on the intestine, bone and kidney. 1,25(OH)₂D₃ also can act on many other tissues and cell types to alter the cellular proliferation rate or state of differentiation, to regulate other endocrine systems, and to modulate immune function. These actions have suggested a multitude of potential therapeutic applications for 1,25(OH)₂D₃. Unfortunately, the calcemic activity of 1,25(OH)₂D₃ has prevented its use in most cases. New analogues with more selective actions may not only offer new therapeutic approaches, but may provide new insights into the factors that control vitamin D action. This review examines our current understanding of the mechanism of 1,25(OH)₂D₃ control of gene transcription, discusses the factors that can influence these actions, and contrasts the effects of the natural vitamin D hormone to those of vitamin D analogues in order to illustrate the importance of some of these factors.

Transcriptional regulation by 1,25(OH)₂D₃

The mechanism for control of gene transcription by 1,25(OH)₂D₃ has received a great deal of attention during the past decade. The basic steps involved are shown in Figure 1. The central player in modulating the actions of 1,25(OH)₂D₃ is the vitamin D receptor (VDR). This protein is a nuclear transcription factor that binds 1,25(OH)₂D₃ with high affinity and undergoes a conformational change that enhances its interaction with another nuclear protein, retinoid X receptor (RXR). It is the VDR/RXR heterodimer that binds to specific DNA sequences (vitamin D response elements or VDREs) in the promoter region of target genes. The bound heterodimer attracts coactivators that initiate the assembly of the RNA polymerase II holoenzyme in the preinitiation complex. The exact order of this assembly following VDR activation has not been established, but direct interactions of the

Correspondence and reprint requests: A. Brown, Renal Division, Washington University, Department of Internal Medicine, Campus Box 8126, 660 S. Euclid Ave, St. Louis, MO 63110-1093, USA.

Nephrol Dial Transplant (1999) 14: Editorial Comments
the interaction of the receptor with other transcriptional components.

**Control of ligand availability by serum vitamin-D-binding protein (DBP)**

The concentration of ligand in the target cell that is available for VDR binding will be determined by the rate of uptake into the cell and the rate of catabolism within the cell. In vivo, the rate of clearance of the ligand from the circulation will be an important factor. Clearance rate and uptake are known to be controlled to a large degree by the serum vitamin-D-binding protein or DBP. This serum globulin binds all natural vitamin D metabolites with relatively high affinity and is present in a large molar excess. Other proteins, serum albumin and lipoproteins, can bind vitamin D metabolites as well. Under normal conditions nearly all circulating vitamin D compounds are protein bound. Direct measurements have shown that more than 99% of the circulating 1,25(OH)_{2}D_{3} is protein bound. DBP appears to perform two major functions in controlling the availability of vitamin D compounds. First, DBP has a great influence on vitamin D pharmacokinetics. Bound metabolites (and analogues) are less susceptible to hepatic metabolism and subsequent biliary excretion. Thus, vitamin D analogues with low DBP affinity have a higher fraction in the unbound state and have been shown to have greater accessibility than those with low DBP affinity. DBP has been shown to reduce the fraction of free 1,25(OH)_{2}D_{3} and reduce its biological activity in cell culture. Increasing the amount of serum (DBP) blunted the activation of keratinocyte 24-hydroxylase (Figure 2). Analogues with low DBP affinity are cleared more rapidly from the circulation. Second, DBP limits the accessibility of vitamin D compounds to the target cells. Studies by Bikle and Gee [3] have shown that DBP reduces the fraction of free 1,25(OH)_{2}D_{3} and reduces the percentage of free 1,25(OH)_{2}D_{3} in the medium. When they calculated the percentage of free 1,25(OH)_{2}D_{3} for each of these data points, and replotted the activity data, they found that the induction of the 24-hydroxylase was dependent on the concentration of free, not total, 1,25(OH)_{2}D_{3} (Figure 3). Analogues with low DBP affinity have a higher fraction in the unbound state and have been shown to have greater accessibility than 1,25(OH)_{2}D_{3} to target cells both in vitro and in vivo. Overall, DBP acts as a sink for vitamin D compounds and likely plays an important role in guarding against vitamin D intoxication. With respect to 1,25(OH)_{2}D_{3} and its analogs, those with high DBP affinity have long half-lives and are delivered slowly to target cells, while those with low DBP affinity can achieve higher cellular levels for a much shorter duration.

An example of how these altered pharmacokinetics can influence the biological profile of a vitamin D analogue is the selectivity of 22-oxa-1,25(OH)_{2}D_{3} (OCT) on parathyroid hormone (PTH) secretion. On the one hand, the DBP affinity of OCT is 500 times lower than that of 1,25(OH)_{2}D_{3} resulting in more rapid clearance and a lower maximal circulating level of the analog following injection. On the other hand, the peak levels of OCT within the parathyroid glands

---

**Fig. 1.** Transcriptional control of gene expression by 1,25(OH)_{2}D_{3}. The diagram shows the key steps involved in transcriptional regulation by 1,25(OH)_{2}D_{3}: (i) Ligand binding to the vitamin D receptor (VDR), (ii) heterodimerization of VDR with retinoid X receptor (RXR), (iii) binding of the VDR/RXR complex to the vitamin D response element, and (iv) recruitment of components of the RNA polymerase II (Pol II) complex, including direct interactions with coactivators (CoA) and transcription factor IIB (B).

VDR with TFIIB and with several coactivators, including steroid receptor coactivator 1 (SRC1), receptor interacting protein 140 (RIP140) and nuclear coactivator 62 (NeCoA-62), have been reported. While our current knowledge of the control of gene transcription by 1,25(OH)_{2}D_{3} is presented in Figure 1, the mechanism will likely be much more complex than the simple model shown here. A more complete treatment of 1,25(OH)_{2}D_{3}-mediated gene transcription can be found in recent reviews [1,2].

There are a number of factors that determine the amount of transcriptional activity produced by vitamin D compounds. These are illustrated in Figure 2. Perhaps the most important determinants of vitamin D action are the intracellular levels of the ligand and the VDR content, which together will determine the amount of activated, liganded receptor produced. The factors that affect the ligand availability and VDR levels are discussed below. In addition, there is now evidence that structural differences in vitamin D analogues may produce distinct ligand-dependent conformational changes in the VDR which could influence
Fig. 2. Regulation of vitamin D action. The factors that influence the control of gene transcription by 1,25(OH)₂D₃ are shown. Details of these processes are discussed in the text.

Fig. 3. Influence of DBP on 1,25(OH)₂D₃ induction of the vitamin D 24-hydroxylase. Keratinocyte 24-hydroxylase was induced by 1,25(OH)₂D₃ in the presence of various concentrations of serum. The left-hand panel shows the 24-hydroxylase activity in response to total 1,25(OH)₂D₃ in the medium. Free 1,25(OH)₂D₃ was measured under each condition and the 24-hydroxylase activity was replotted against free 1,25(OH)₂D₃ (right-hand panel). The data illustrate that free, rather than total 1,25(OH)₂D₃ is the biologically active form. From Bikle et al. [3].

and intestine are greater than those of 1,25(OH)₂D₃, but decrease quickly following clearance of the analog. This 'pulse' of OCT produces only transient increases in intestinal calcium absorption and bone mobilization [4], but the same short exposure to the parathyroid glands produces a prolonged suppression of PTH gene expression [5]. Thus, the differences in the duration of the calcemic responses vs PTH suppression lead to a greater effect of OCT on the parathyroid glands.

**Catabolic inactivation**

An important attenuator of vitamin D action in target cells is catabolic inactivation. In all target cells, the major route of catabolism is oxidation of the side chain of the molecule. This is catalyzed by a single enzyme, the vitamin D 24-hydroxylase, and involves successive oxidations of carbons 24 and 23 followed by oxidative cleavage of the side chain to form calcitriolic acid, the final water-soluble metabolite of 1,25(OH)₂D₃. This enzyme is highly inducible by 1,25(OH)₂D₃ and its analogues and acts as an attenuator of vitamin D action. An example is shown in Figure 4. In their study, Zhao et al. [6] assessed the anti-proliferative effect of 1,25(OH)₂D₃ and an analogue, KH1060, on the breast cancer cell line, MCF-7. Both compounds inhibited [³H]thymidine incorporation into these cells in a dose-dependent manner, although the analogue was more active. The effectiveness of these compounds was enhanced significantly when the 24-hydroxylase was blocked by the cyto-
Fig. 4. Influence of catabolism on the biological activity of vitamin D analogue. Left, the antiproliferative effects of 1,25(OH)<sub>2</sub>D<sub>3</sub> and its analog KH1060 on MCF-7 breast cancer cells was measured in the absence or presence of the cytochrome P<sub>450</sub> inhibitor, ketoconazole. Blocking the catabolic enzyme (24-hydroxylase) enhanced the antiproliferative activity. Right, the ratio of the ED50 in the presence (+keto) vs absence (−keto) of ketoconazole. The activity is enhanced by ketoconazole in all cases, but the variation between analogs likely reflects differences in their rates of catabolism. From Zhao et al. [6].

Vitamin D receptor regulation

Another very important determinant of transcriptional activity in a target cell is the content of the vitamin D receptor (VDR). The content of the VDR will be determined by both the rate of synthesis and the rate of degradation. Both of these are under regulation. In addition, the VDR may be bound to calreticulin, a calcium-binding protein that recognizes the nuclear translocation motif in many steroid receptors, and rendered inactive (see Figure 2). The regulation of VDR levels has been studied in detail for many target cells both in vivo and in cell culture. Many of the regulators are cell- or tissue specific and there may be differences between species. A thorough review of this topic is beyond the scope of this article.

The most ubiquitous regulator of the VDR is 1,25(OH)<sub>2</sub>D<sub>3</sub>. Ligand-dependent upregulation can occur through at least two mechanisms. In select tissues (e.g. parathyroid glands and kidney, but not intestine) 1,25(OH)<sub>2</sub>D<sub>3</sub> upregulates VDR mRNA, possibly indirectly through changes in calcium. However, in virtually all cell types, 1,25(OH)<sub>2</sub>D<sub>3</sub> increases VDR protein through ligand-dependent stabilization. Studies in vitro [7] have shown that the presence of 1,25(OH)<sub>2</sub>D<sub>3</sub> greatly increases the cellular half-life of the VDR. In vivo evidence for ligand-dependent stabilization comes from the study of Denda et al. [8] which noted that the decreased VDR in the parathyroid glands of uremic rats was highly correlated with the circulating levels of 1,25(OH)<sub>2</sub>D<sub>3</sub>. Administration of low doses of 1,25(OH)<sub>2</sub>D<sub>3</sub> or the analogue OCT to uremic rats restored VDR levels. Importantly, VDR mRNA was not different in these groups suggesting that, under these conditions, the regulation of parathyroid VDR levels was through ligand stabilization.

The mechanism for degradation of the VDR has only recently been addressed. Masuyama et al. [9] documented the direct interaction of the VDR with SUG1, a component of the proteasome. The proteasome is a large complex that is known to degrade many cellular proteins, especially those that have been targeted for destruction through derivatization by ubiquitination. Using proteasome inhibitors, Masuyama et al. confirmed that the VDR is degraded by the proteasome. The role of SUG1 binding in this process is currently under investigation, but it is clear that its binding to the VDR is 1,25(OH)<sub>2</sub>D<sub>3</sub> dependent. Thus, ligand binding both protects the VDR from degradation and promotes association with a proteasomal subunit. It has been proposed that SUG1 may target the VDR for degradation following the initiation of gene transcription (Figure 5). The ability of vitamin D analogues to promote SUG1 interaction and/or stabilize the VDR is an important area of future investigation.

Post-translational modification of the VDR

The VDR is a phosphoprotein and ligand binding has been shown to promote hyperphosphorylation of the receptor. Phosphorylation occurs at several loci and is mediated by various protein kinases. Phosphorylation
extracts, but more recent studies have shown that addition of fractionated uremic plasma ultrafiltrate to cultured cells blunts the transcriptional activity of 1,25(OH)\textsubscript{2}D\textsubscript{3}. It has been postulated that components in the uremic serum react covalently with the VDR at or near the DNA-binding domain.

**Ligand-dependent VDR conformation**

Crystallographic data indicate that a conserved \(\alpha\)-helix near the C-terminus of steroid receptors undergoes a dramatic conformational shift upon ligand binding. This region of the receptor, the AF2 domain, is critical for transcriptional activity and has been shown to directly interact with other components of the transcriptional complex including coactivators and TFIIB. Removal of the AF2 domain eliminates the transcriptional activity of 1,25(OH)\textsubscript{2}D\textsubscript{3} without affecting ligand binding. However, Peleg et al. [11] have characterized several vitamin D analogues that are active even with receptors lacking the AF2 domain. These analogues, with the 20-epi configuration, appear to induce a distinct VDR conformation as evidenced by their ability to partially protect the VDR from proteolysis. The unique VDR conformation induced by these analogs promoted a tighter association with RXR and a VDRE as shown in Figure 6. These findings emphasize the importance of ligand structure on the conformation and activation of the VDR.

**Regulation of transcriptional cofactors**

Vitamin D action could also be controlled by changes in the nuclear levels of the other components of the transcriptional complex. However, nearly all of these proteins act as cofactors for other steroid receptors
and transcription factors, and altered levels would not affect the vitamin D system exclusively. A recent report demonstrated reduced RXR levels in hyperplastic parathyroid glands [12] of uremic rats which could reduce the VDR-mediated inhibition of PTH gene expression and contribute to PTH overproduction in this disorder.

Summary

The control of gene transcription by vitamin D compounds is initiated by binding to the VDR, which enhances the receptor’s ability to heterodimerize to RXR, interact with response elements in target genes and attract components of the transcriptional initiation complex. A number of factors are capable of influencing this process, including (i) the rate of uptake and catabolism of the ligand, (ii) the nature of the conformational change induced by a specific ligand, (iii) the cellular content of the VDR, (iv) post-translational modifications of the VDR and (v) the availability of other transcriptional components. Vitamin D analogues may affect these factors differently to 1,25(OH)2D3 to produce unique biological profiles that can be exploited for therapeutic use.

References

9. Masuyama H, Dowd DR, Brown AJ, MacDonald PN. Proteasome-mediated degradation of the vitamin D receptor (VDR) and the potential involvement of a 1,25(OH)2D3-dependent interaction between the VDR AF-2 domain and SUG1. J Bone Miner Res 1997; 12: S122

Molecular mechanisms of vitamin D hyporesponsiveness in renal failure

Raymond Vanholder and Griet Glorieux

Nephrology Division, Department of Internal Medicine, University Hospital, Gent, Belgium

Key words: calcitriol, uraemia, vitamin D

Introduction

Calcium homeostasis depends on two major hormone systems. Parathyroid hormone (PTH) and calcitriol (1, 25(OH)2D3), the active form of vitamin D, interact to balance calcemia; they influence intestinal calcium uptake, renal calcium losses, and calcium exchange at the bone. Calcemia itself influences PTH secretion.

In the classical model [1] both a rise in blood calcium and calcitriol cause a negative feed-back on PTH release, whereas both PTH and calcitriol cause a rise in blood calcium. PTH also induces an increase of calcitriol.

In renal failure this subtle balance is disturbed, when active kidney mass is lost; the capacity of the kidney to produce calcitriol from precursors by the activity of the enzyme 1α-hydroxylase is reduced in parallel with renal function. Consequently, serum calcium and calcitriol decrease, parathyroid hormone secretion is enhanced, and a new equilibrium is found, however,
at the expense of a loss of the calcium content in the bone. The end-point is hyperparathyroidism; the degree of this PTH hypersecretion may, however, largely differ from patient to patient, and within the same patient, from time-point to time-point in his/her evolution.

This imbalance is further affected by phosphate; renal failure causes phosphate retention, which in turn causes serum calcium to decrease, with the above-mentioned consequences on calcitriol and PTH. In addition, phosphate itself also decreases calcitriol and increases PTH directly or indirectly [2].

Curiously enough, several studies point to the fact that at the initiation stage of chronic renal failure, without supplementation of vitamin D analogues, calcitriol levels remain normal; in spite of this, PTH is elevated [3]. This data suggests the development of a certain degree of vitamin D resistance, as both the metabolic degradation of calcitriol and the inhibition of PTH secretion are regulated by calcitriol. These findings have, however, not consistently been confirmed [2,4], but this might be attributed to differences in patient selection, e.g. with regard to kidney function.

Several additional arguments point in the direction of calcitriol resistance in renal failure. Already in association with moderate defects of renal function, intestinal absorption of Ca<sup>2+</sup> is decreased and whole-body retention of Ca<sup>2+</sup> is increased [5]. In response to increasing supplements of calcitriol, urinary calcium remains lower in early renal failure, compared to healthy subjects [3]. Also in rats with moderate renal failure (reduction of GRF <50%), PTH increases compared to sham-treated rats with normal renal function, in spite of identical serum calcitriol levels [6].

All these data suggests that the response to calcitriol is depressed, even if the fall in calcitriol levels normally occurring with renal failure can be prevented. Calcitriol exerts its biological actions through binding with the calcitriol receptor, which has two functional domains, one hormone binding and one DNA-binding. The DNA-binding domain has similarities to that of other steroid hormone receptors (e.g. glucocorticoid, oestrogen, thyroid, and retinoic acid receptors). After complexation of calcitriol with the vitamin D receptor (VDR), binding occurs with the DNA of vitamin-D-responsive elements (VDREs—e.g. osteocalcin, osteopontin, 9K calcium binding protein gene), which are present in vitamin D responsive genes.

Impaired response to vitamin D at the receptor level could be the consequence of: (i) decreased binding of calcitriol to the VDR; (ii) decreased expression of the VDR; (iii) decreased binding affinity of the vitamin D–VDR complex to the VDREs (Figure 1).

What is the evidence that one or more mechanism play a role in uraemia? If this were the case, hyperparathyroidism would not be prevented, even if adequate calcitriol levels can be obtained.

**Decreased binding of calcitriol to the VDR**

This issue has been studied repeatedly. In none of these studies could a decrease of binding affinity be demonstrated.

**Decreased calcitriol receptor content**

It is of note that under normal conditions calcitriol upregulates its own receptor, i.e. increasing serum calcitriol induces an increase of VDR. VDR content in uraemia has most frequently been studied in parathyroid glands. A decrease in VDR of parathyroid glands has been demonstrated in the rat [7], the dog [8], and in men [9]. Similar data were also reported for human peripheral blood mononuclear cells [10] and rat intestine [11].

Szabo et al. could not confirm this decrease in VDR expression in parathyroid glands of uraemic patients [12]. The reason for this inconsistency remains unclear. One of the reasons could be that in the studies by Szabo et al., protease inhibitors were used in the experimental set-up, preventing proteolytic degradation of the receptor during its preparation from the uraemic sample. This approach had not been followed in earlier studies. However, later studies, where proteolysis was prevented in a similar way as in the study by Szabo et al., nevertheless also demonstrated a decrease of VDR [7].

Another possible explanation for these divergences, is that the expression of VDR depends on the type of parathyroid hyperplasia, which may be nodular or diffuse. Hyperplastic parathyroid of the nodular type contain substantially less VDR than glands of the diffuse type [13].

Even if the parathyroid glands from the experiments by Szabo et al. contain a similar number of VDR, with or without renal failure, the normal upregulation in response to calcitriol is inverted in the uraemic
condition [12]. Parallel data were reported by Koyama et al. in duodenum of rats with chronic renal failure [14].

Several authors have studied the levels of VDRmRNA in renal failure, but a decrease could not be demonstrated [12,14,15]. Also the changes in VDRmRNA in response to calcitriol administration were similar in the uraemic and in the normal condition. Infusion of uraemic ultrafiltrate to rats resulted in a rise of VDRmRNA in spite of a fall in VDR [15]. These data suggest that uraemic toxins inhibit VDR synthesis at the post-transcriptional level.

Decreased binding affinity of VDR with VDRE

In earlier studies based on DNA–cellulose chromatography it has been suggested that interaction of VDR with DNA was inhibited in chronic renal failure [11]. Binding affinity of the hormone receptor for DNA was also reduced when the receptor was preincubated with uraemic ultrafiltrate [16]. Again, these findings were not uniformly confirmed [17].

Hsu et al. used the electrophoretic mobility shift assay to compare the ability of VDRs from normal and renal failure rats to bind to the osteocalcin gene VDRE [18]. DNA binding capacity was reduced by 50% in renal failure rats. In transfected JEG-3 cells, calcitriol-induced reporter gene expression was blocked by uremic ultrafiltrate [18].

Using the same electrophoretic assay, Sawaya et al. observed a significant reduction in kidney calcitriol–receptor complex binding to mouse osteopontin VDRE [6]. This defect occurred already with moderate renal failure despite normal calcium, phosphorus, calcitriol and VDR concentrations.

If uraemic compounds inhibit VDR binding to its target genes, it could be expected that 24-hydroxylase activity would be decreased in renal failure. This enzyme is responsible for the transformation of active calcitriol to 1,24,25(OH)3D3; its synthesis is a VDR-receptor-mediated process [19]. Uraemic ultrafiltrate indeed reduced production rate of 1,24,25(OH)3D3 in kidney homogenates by ± 50%, in the presence of both 1 µM and 25 nM calcitriol [16].

VDR functions primarily as a heterodimer with retinoid X receptor (RXR), at least in the case of the stimulation of gene transcription. Since both VDR and RXR can bind to direct repeats of the sequence AGGTCA [20], the question can be raised whether uraemic toxins will chemically modify VDR alone, RXR alone, or the two of them together. Patel et al. demonstrated that the inhibitory effect of uraemic toxins on the formation of the VDR–RXRz–VDRE complex is due to a modification on the VDR alone, and not of the RXRz [21].

VDR and the immune system

An often neglected functional aspect is that calcitriol also shows immunomodulatory effects. Anti-

proliferative, prodifferentiating, and immunosuppressive actions have been demonstrated.

Macrophages are potential targets for immunomodulatory action of 1,25(OH)2D3. Following treatment with 1,25(OH)2D3, macrophages exhibit enhanced antimicrobial action. 1,25(OH)2D3 regulates cytotoxic cells and biosynthesis of immunologically active humoral substances [22]. The question arises whether the uraemic milieu also blunts this aspect of response to vitamin D. Recently we could demonstrate that uraemic ultrafiltrate obliterated the calcitriol-induced CD14-expression on the surface of isolated monocytes in culture [23]. Preliminary data suggest that a similar inhibitory action is observed with respect to the differentiation of the promyelocytic leukemia cell line, HL-60, to differentiate (unpublished results).

Conclusions

End-stage renal disease is characterized by a state of relative calcitriol resistance. The following mechanisms play a possible role: (i) lower expression of VDR, at baseline and in response to calcitriol; (ii) diminished VDR binding to VDRE. One of the consequences is decreased breakdown of calcitriol. VDR and VDRE play an important role in the hormonal regulation of Ca/P metabolism and homeostasis of the bone, but may also play a role in other systems, e.g. the immune system.

References

10. Martinez J, Olmos JM, de Francisco ALM, Amado JA, Riancho...
Glomerular lesions of diabetes mellitus: preventable and reversible

Michael W. Steffes

Department of Laboratory Medicine and Pathology, Medical School, University of Minnesota, Minneapolis, USA

Glycaemia and the development of diabetic nephropathy

Although preceded by a series of observations documenting advanced lesions of the glomerulus in the diabetic patient [1,2], Ruth Østerby of Aarhus University first emphasized in man the early development of glomerular basement membrane (GBM) widening and mesangial expansion [3,4]. She and colleagues measured these structures in successive renal biopsies, demonstrating convincingly the progressive nature of diabetic renal disease, even early in its course. Furthermore, she worked towards unifying the underlying pathophysiologic mechanisms of diabetic nephropathy stressing the accumulation of basement-membrane-like material. Although widening of the GBM and expansion of the mesangium may not reflect identical biochemical or biophysical processes, they do evolve similarly in the diabetic environment—as reflected by studies in identical twins in which GBM width and mesangial expansion were increased uniformly in the diabetic twin [5]. Importantly, these morphometric measurements of glomerular lesions enabled quantitative comparisons among normal and non-diabetic subjects, and those subjects treated with insulin or islet/pancreas transplantation both in rats [6–8] and in man [9–12].

Euglycaemia and the prevention or reversal of diabetic nephropathy

With animal models, islet transplantation or optimum insulin therapy altered the course of the structural and functional lesions of diabetic renal disease [6–8]. Applied to rodent models, the time for reversal encompassed 2–6 months—a duration much shorter than the time for efficacy demonstrated in people; however, studies in rats must account for the short time span available over the life of the rodent. This problem is especially complicated in diabetic rats because of the minimum of 6 months of diabetes necessary to establish most glomerular lesions [8]. Thus these time demands within an animal of relatively short life span reduce the questions that may be answered. Nevertheless, much important work has been accomplished in rodents demonstrating the feasibility of subsequent studies in man. Of importance are those issues raised above concerning the resiliency of glomerular lesions, once established, to reversal with implementation of
normoglycaemia. The animal studies demonstrated convincingly the fundamental efficacy of normoglycaemia in preventing and/or reversing the lesions of diabetic nephropathy.

For prevention or reversal of early glomerular lesions in man, 4–5 years of optimal glycemic control were necessary to yield efficacy in patients with transplanted kidneys [9,10]. The more advanced structural lesions resisted reversal or amelioration with pancreas transplantation alone, at least after the initial 5 years of follow-up [11]. Overall, these studies demonstrated quite convincingly that euglycaemia established at relatively early stages of disease greatly affected the development and/or progression of human diabetic renal disease.

With the large body of work on the progression of functional markers of diabetic nephropathy an array of both structural and functional parameters of diabetic nephropathy may now be used to evaluate the efficacy of various therapeutic modalities. Specifically, the observations of reduction of albuminuria in the Diabetes Control and Complications Trial (DCCT) [13] were elaborated in the DCCT nephropathy paper [14]; however, the relatively short duration of diabetes in the DCCT subjects (i.e. most being less than 5 years at the start of the DCCT, relative to the 15–25 years of diabetes necessary to allow nephropathy to produce advanced lesions—see below) compromised opportunities to demonstrate improvement of other parameters of diabetic nephropathy (e.g. blood pressure and glomerular filtration rate estimated by two different techniques).

**Glomerular lesions: the course of development and the time required for reversal**

Since the structural and functional lesions of diabetic nephropathy evolve over the first 2–3 decades of disease, macroalbuminuria (e.g. >300 mg/24 h) in type I diabetic patients will present during the second (and later) decade(s) of disease. The increase in GBM width becomes evident during the first 10 years followed by a measurable expansion of the mesangium [3,4,15]. Of importance, the expansion of the mesangium characterizes the lesions that threaten renal function. Therefore in those patients evolving to macroalbuminuria the GBM width and mesangial expansion will clearly reach abnormal proportions as microalbuminuria progresses [16,17].

From the information outlined above one might expect that the effort to reverse a chronic pathophysiological process would require years to produce demonstrably beneficial results. Therefore to alter the course of albuminuria in the DCCT, 5 or more years of lowered hemoglobin A1c (HbA1c) were necessary before a clear benefit of intensive management became apparent [13,14]. Since a rising albuminuria reflects progression of structural lesions [16,17], one would anticipate a similar time course to demonstrate reversal of the early expansion of the mesangium. The results of Bilous et al. [9] with pancreas transplantation on average 4–5 years after kidney transplantation indicated clearly that early lesions could be beneficially altered by the establishment of euglycaemia.

The question of reversing the more advanced mesangial lesions was addressed with pancreas transplantation to type I diabetic patients with their own kidneys and advanced diabetic nephropathy by both functional and structural criteria [11,12]. Of importance, the mesangial expansion was marked, and of greatest interest concerning the reversibility of extra cellular matrix constituents, the GBM was widened greatly. Thus any evidence of reversibility would emphasize strongly that even very long-lived proteins in their native state or following post-synthetic modification may return towards normal molecular structure and concentration following prolonged exposure to a normal or near-normal metabolic environment—best estimated by HbA1c. With the long duration of diabetes in the recipients and their advanced renal lesions, it was not surprising that the lesions remained unchanged 5 years after pancreas transplantation, as first reported [11]. Yet, in keeping with the prolonged time course necessary for the development of glomerular lesions and significant abnormalities in the renal function characteristic of this pancreas transplant recipient population, the 10-year results demonstrated a clear benefit of euglycaemia after pancreas transplantation in reversing nearly all lesions [12].

The time course (5–10 years) of the response to euglycaemia with pancreas transplantation reflects the need to clinically implement optimal glycemic control over a course of years in each patient or patient population. Thus, intensive glycemic control or pharmaceutical agents directed specifically at the moderate-to-advanced functional and structural manifestations of diabetic nephropathy will require treatment for periods approaching a decade before any clear benefit may be demonstrated. Alternatively these optimistic findings of reversibility of the advanced lesions compel those managing diabetic patients to work as aggressively as possible to prevent the original development and progression of the structural and functional renal lesions of diabetes.

**References**

Glomerular lesions other than amyloidosis in patients with familial Mediterranean fever

Fatouş Yalçınkaya and Necmiye Tümer

Department of Pediatric Nephrology, Faculty of Medicine, Ankara University, Ankara, Turkey

Introduction

Familial Mediterranean fever (FMF) is an autosomal recessive hereditary disease which primarily affects Jews, Armenians, Arabs and Turks [1,2]. The disease was first outlined by Siegal in 1945 and characterized by recurrent and self-limited attacks of fever, usually accompanied by peritonitis, pleuritis, arthritis or erysipelas-like erythema [3]. The development of amyloidosis is the most common renal complication of FMF that leads inevitably to chronic renal failure [4–7]. The frequency of amyloidosis differs among various ethnic groups and depends on whether patients are taking colchicine [1,2]. It has been well documented that the presence of amyloidosis determines the prognosis of the disease. However, in addition to amyloidosis other renal lesions have been described in the patients with FMF and it seems that these renal lesions have been relatively neglected [6–13].

The spectrum of renal involvement other than amyloidosis in FMF

Soon after the first well documented cases of FMF were published it became apparent that renal involve-
glomerulonephritis, and rapidly progressive glomerulonephritis were reported in patients with FMF even in the absence of amyloidosis [6,9–12]. In addition, some of the non-amyloid glomerular lesions described in the patients with FMF were associated with vasculitis [6,7,12–14]. Furthermore, the coexistence of a variety of vasculitic syndromes with FMF was noted to be more frequent than in general population [13,14].

Possible pathogenesis

The biochemical basis of FMF is still unknown but the gene responsible for the disease was cloned recently by two independent teams [15,16]. The gene product called pyrin/marenostrin was reported to be a protein that is responsible for limiting the intensity of inflammation. It was suggested that FMF-associated mutations and the resultant structural changes of the protein interfere with the normal pyrin/marenostrin mediated negative feed back mechanism, thus favouring the onset and persistence of inflammation [15–17]. Although the identification of the FMF gene advanced the understanding of the regulation of acute inflammatory responses, the pathogenesis of the disease is still obscure. Based on clinical and laboratory findings several pathogenetic mechanisms have been suggested. Circulating immune complexes, complement consumption, increased concentrations of immunoglobulins during attacks and return to normal after the acute attacks support the notion that an immune reactant mechanism underlies FMF [6].

It is not known whether the presence of non-amyloid glomerular diseases with FMF is coincidental or causal. But a detailed review of the presentation, clinical course and histopathologic results of the reported cases in the literature favours a causal relationship. Although the exact pathogenesis of the development of non-amyloid glomerular lesions in the patients with FMF is unknown recent evidence suggests that an immunologic mechanism may play an important role in most of them, including acute post-streptococcal glomerulonephritis, type II rapidly progressive glomerulonephritis and IgA nephropathy [6,9,10]. In addition, Said and Hamzeh [11] suggested that the presence of mesangial IgA, IgM and C3 deposits that were observed in the patients with FMF and renal involvement may represent an immunoreactant mechanism. Another possible explanation is the entrapment of these substances as a result of mesangial dysfunction in clearing and processing immunologically-irrelevant macro-molecular aggregates. The frequent association of mesangial proliferation also offers good supportive evidence for an immunological process underlying the development of non-amyloid renal lesions in the patients with FMF. As FMF can be described as a disease of defective inhibition of inflammation, resulting from the mutations of the gene, it can be speculated that patients with FMF might have an exaggerated response to certain antigens that sometimes exceeds the clearing and processing capacity of the glomerulus and facilitates the development of these non-amyloid glomerular diseases.

Conclusion

The observations reported from different countries indicate that the patients with FMF are prone to exhibit a variety of glomerular diseases other than amyloidosis. Although amyloid renal disease is definitely common in FMF patients, there are no good epidemiological studies showing that non-amyloid glomerular diseases are also more frequent in the patients with FMF than in the general population. An important source of confusion is the interpretation of persistent albuminuria as a sign of amyloidosis in the absence of histologic proof. It is pertinent to point out that the actual frequency of non-amyloid glomerular lesions in patients with FMF depends largely on the number of kidney biopsies performed in patients with abnormal urinary findings.

Colchicine treatment was clearly shown to ameliorate the course of FMF and to prevent the development of amyloidosis [1,2]. A review of the literature reveals that some of the FMF patients were not taking regular colchicine treatment when they developed non-amyloid glomerular diseases [5–12]. It remains to be seen whether regular colchicine treatment that prevents acute attacks and amyloidosis, may prevent the development of non-amyloid glomerular diseases in the patients with FMF. Finally, it is important to emphasize that non-amyloid glomerular diseases should be considered in the differential diagnosis of the patient with FMF and renal involvement.

References

Does cyclosporin have a role in the treatment of membranous nephropathy?

Claudio Ponticelli and Margarita Villa
Divisione di Nefrologia e Dialisi, IRCCS Ospedale Maggiore di Milano, Milano, Italy

Introduction

Idiopathic membranous nephropathy (IMN) is an immune-mediated glomerular disease, usually associated with a nephrotic syndrome. The renal prognosis may be variable but a recent review of the available studies, including nephrotic and non-nephrotic patients, reported that renal survival at 10 years was 0.65 [1]. It is likely, however, that the prognosis is even worse in nephrotic patients, as the risk of renal failure is correlated to the severity and the duration of proteinuria [2]. Nephrotic patients are also exposed to an increased risk of cardiovascular death [3] as well as to intravascular thrombosis and other potential complications [4]. Thus, although many clinicians still adhere to the dogma that IMN should not be treated, it is our opinion that any treatment that is able to modify the natural course of this disease in patients with the nephrotic syndrome is welcome.

Treatment with corticosteroids and/or cytotoxic agents

Corticosteroids have been used in IMN for many years. A meta-analysis of the available controlled trials concluded that, at least at the doses used in the few available randomized trials, corticosteroids neither improve the probability of remission of proteinuria nor protect from renal function deterioration [1]. Controlled studies showed that the administration of cyclophosphamide or chlorambucil for 1 year or more may cause consistent reduction of proteinuria in a certain number of patients (see review in [4]) although admittedly prolonged treatment with alkylating agents theoretically exposes the patient to disquieting long-term side-effects.

The results of three multicentre trials made in Italy showed the effectiveness of a schedule based on alternating cycles of methylprednisolone and chlorambucil every other month for 6 months. A first study compared the effects of methylprednisolone and chlorambucil and those of symptomatic treatment in patients with IMN and nephrotic syndrome. At 10 years, 92% of treated patients versus 60% of untreated controls were still alive without dialysis. Treated patients spent 58% of their time without nephrotic syndrome versus 22% of controls [5]. A second study compared methylprednisolone and chlorambucil, and methylprednisolone alone given at the same cumulative dose. At the end of a mean follow-up of 54 months, 64% of patients given methylprednisolone and chlorambucil versus 38% of patients given methylprednisolone alone were without nephrotic syndrome, the difference being significant [6]. In a third trial methylprednisolone and chlorambucil were compared with methylprednisolone alternated with cyclophosphamide [7]. The probability of attaining as a first event complete or partial remission of the nephrotic syndrome was similar (82 vs 93%), the risk of relapse was also similar (30 vs 20%). Thus, the use of steroids alternated with either chlorambucil or cyclophosphamide may favour remission of the nephrotic syndrome and may protect renal function in the long term. It is worth remembering that in more than half of responders, remission of proteinuria develops weeks or months after the treatment was completed [5–7].

Results with cyclosporin

In the last few years cyclosporin has been used in a number of proteinuric glomerular diseases including...
minimal-change disease, focal and segmental glomerular sclerosis, and lupus nephritis. Several non-controlled trials have been conducted also in IMN. A first report of De Santo et al. [8] reported complete remission of proteinuria in four of five patients who had failed to respond to a 6-month therapy with methylprednisolone and chlorambucil. Unfortunately the follow-up was quite short; moreover it is possible that in some patients remission was a late response to methylprednisolone and chlorambucil. Meyrier [9] reported the data of the Sandoz file. Complete remission was seen in 14 of 73 adults treated with cyclosporin and partial remission was obtained in another 18 patients. Gausch et al. [10] reported a decline of proteinuria from a nephrotic to a non-nephrotic range 2–4 weeks after treatment in 10 of 14 patients with IMN. Rostoker et al. [11] obtained complete remission of proteinuria in four of 15 nephrotic patients treated with cyclosporin and another seven had reduction of proteinuria from a nephrotic to a non-nephrotic range. In a cross-over study, Ambalavanan et al. [12] assigned 14 patients to receive a 3-month course with cyclosporin or enalapril. Enalapril reduced blood pressure but had no effect on proteinuria; cyclosporin showed a reduction of proteinuria of more than 50%. After stopping cyclosporin proteinuria rose to pre-treatment values but reduced again when cyclosporin was reintroduced. Cattran et al. [13] assigned 17 patients with severe proteinuria and/or renal dysfunction to placebo or to cyclosporin for 1 year. Proteinuria significantly decreased in the treated group; moreover the decline in creatinine clearance was more rapid in the placebo than in cyclosporin-treated group. Taken together, these data show that cyclosporin may be effective in favouring the remission of the nephrotic syndrome in 50 to 60% of patients. Reduction of proteinuria usually occurs within a few weeks, so that if urinary protein excretion does not modify within 3–4 months it is unlikely that cyclosporin will be effective. As in other glomerular diseases, the nephrotic syndrome reappears in most cases after the drug is stopped. With the exception of the small trial conducted by Cattran et al., little information is available on the effects of cyclospo- rin on prevention of renal functional deterioration. However, when repeat biopsy was performed, as in the study of Ambalavanan et al. [12], it was shown that cyclosporin did not prevent continuing autoantibody formation in MN. It is not clear whether the addition of small doses of corticosteroids improves the effectiveness of cyclosporin in patients with IMN.

**Problems with the use of cyclosporin**

Cyclosporin is a potentially nephrotoxic drug and may expose the patient to hypertension and to the risk of progressive renal failure. This risk is dose-dependent and age-dependent [14]. It is particularly increased in patients with elevated plasma creatinine and with tubulointerstitial lesions at initial renal biopsy [15]. Thus patients with a creatinine clearance lower than 60 ml/min, and/or severe arterial hypertension and/or severe interstitial fibrosis and tubular atrophy at renal biopsy should not be treated with cyclosporin. For those patients who do not have contraindications to the use of cyclosporin it is mandatory to monitor plasma creatinine carefully during treatment in order to prevent the development of so-called cyclosporin nephropathy which consists of arteriolar changes and irreversible interstitial fibrosis. The higher the increase in plasma creatinine under cyclosporin the higher the risk of irreversible nephrotoxicity. The risk of cyclosporin nephropathy is about 10–12% for increases of plasma creatinine of about 30%, it is around 50% when plasma creatinine doubles over the baseline and approaches 100% for higher increases of plasma creatinine [14]. To be on the safe side, whenever plasma creatinine rises by more than 30% over the baseline, cyclosporin should be stopped for at least 1 month. Administration of the drug may be resumed if plasma creatinine returns to normal or to values not higher than 10% over the baseline.

**Practical recommendations**

Cyclosporin should probably be considered as a second-line treatment in patients with MN and severe nephrotic syndrome. In fact, those patients who respond to a 6-month course with methylprednisolone and either chlorambucil or cyclophosphamide may maintain remission for years or even indefinitely. There is good evidence that the responders maintain normal renal function over time. Patients who relapse often respond similarly as during the first course. It is therefore rational to retreat them if they had a good response to a first treatment. On the other hand, more prolonged treatment is presumably unsafe in those patients who did not respond to a 6-month course of steroids alternated with an alkylating agent. Thus, if no response is observed, one should consider administration of cyclosporin. We suggest, however, waiting at least 12 months after the cessation of the steroid–cytotoxic treatment before beginning treatment with cyclosporin, in order to avoid an useless treatment for late-responders. Moreover, it is preferable that patients are not submitted consecutively to two immuno-suppressive treatments in order to reduce the risk of side-effects.

The starting doses of cyclosporin should not exceed 4 mg/kg/day with the new microemulsion cyclosporin, which has a better bioavailability than the old formulation. Blood levels should be checked in order to assess whether the patient is a good or a poor absorber of cyclosporin. Initially, through blood levels may be kept between 75 and 200 ng/ml, as assessed by the monoclonal assay. Once again the need of a careful monitoring of renal function and blood pressure under cyclosporin treatment must be stressed. If no reduction of proteinuria is seen within 3 months cyclosporin may be stopped, it being unlikely that the drug would exert an antiproteinuric effect with a more prolonged admin-
Angiotensin converting enzyme inhibitors and angiotensin receptor (AT1) antagonists: either or both for primary renal disease?

Kevin McLaughlin¹ and Alan G. Jardine²

¹Renal Unit, Royal Infirmary and ²Department of Medicine and Therapeutics, Western Infirmary, Glasgow, UK

Introduction

The activity of the Renin-Angiotensin System (RAS) may contribute to progression of renal diseases as a result of its effect on arterial blood pressure and intraglomerular pressure. In addition, there is evidence that RAS may also act via non-haemodynamic mechanisms such as mesangial cell mitogenesis and by influencing the balance of accumulation and degradation of extracellular matrix in mesangial cells and interstitium which may contribute to the development of glomerulosclerosis [1,2]. The benefits of reducing RAS activity in patients with diabetic nephropathy and non-diabetic renal disease have been demonstrated in prospective trials in which angiotensin-I-converting enzyme inhibitors (ACEI) were shown to have a renoprotective effect greater than that afforded by strict blood pressure control alone [3,4]. With the introduction of angiotensin II receptor antagonists (AT1-ra), there is now a choice as to the method of RAS manipulation and recent editorials in this journal have reviewed the role of the RAS within the kidney and highlighted some of the similarities and differences.
between ACEI and AT1-ra [5–7]. We would like to add to the debate regarding the merits, or otherwise, of these drugs in clinical practice and highlight the potential role of molecular biology in future clinical decision making.

Limitations of ACE inhibition

ACE is not specific to the RAS and is involved in the breakdown of several other products, such as bradykinin. Elevated levels of bradykinin is probably the mechanism for the ACEI-related side effects such as cough and anaphalactoid reactions [8]. Cough occurs no more frequently with AT1-ra than with placebo [9]. While ACE is the principal method by which angiotensin I (AI) is converted to angiotensin II (AII), this step can also be carried out by other enzymes. Non-ACE formation of AII is not blocked by ACEI and may be important in maintaining tissue AII levels. In the heart, for example, cardiac chymase activity contributes to formation of AII and is unaltered by ACEI. Indeed, the effect of the non-ACE pathways may actually be exaggerated during treatment with ACEI as a result of high levels of AI and AT1 receptor up-regulation. It is not clear, however, if the non-ACE conversion of AI to AII is of clinical significance although there is evidence that AII levels gradually rise with time after starting ACEI [10]. AT1-ra, by contrast, offer the possibility of ‘total blockade’ of the effects of AII at the AT1 receptor that cannot be achieved by ACEI.

Limitations of AT1 receptor blockade

The results of prospective clinical trials evaluating the role of AT1-ra in renal disease are not yet available and their efficacy, in this respect, remains unproven. Similarities in the mode of action (i.e. reduced AII-mediated activity) of AT1-ra and ACEI may suggest that their clinical effects will be comparable. There are several reasons, however, why this may not be the case. In rats the beneficial effect of ACEI on renal haemodynamics is partly through bradykinin-mediated dilatation of the efferent arteriole [11]. This accounts for the fall in intraglomerular pressure (and the initial fall in glomerular filtration rate [GFR] and protein excretion [12]) after ACEI. In animal models AT1-ra do not appear to produce the fall in proteinuria that is observed with ACEI although they have a similar effect on the attenuation of glomerulosclerosis [13]. In the absence of salt and water depletion, AT1-ra do not have this acute haemodynamic effect on the human kidney and do not produce an initial fall in GFR [14,15]. A study of 13 patients did, however, observe a reduction in proteinuria with AT1-ra despite no change in GFR [14]. The relative contribution of haemodynamic and non-haemodynamic mechanisms to the renoprotective effect of ACEI is not known, although the reduction in proteinuria appears to correlate with the degree of attenuation of GFR reduction. Increased bradykinin levels may also have important non-haemodynamic benefits by increasing the activity of metalloproteinases which degrade extracellular matrix.

In addition to actions on AT1 receptors, AII also stimulates AT2 receptors. It may be premature to speculate on the importance of AT2 receptor stimulation as their clinical role in healthy humans and disease states has not been fully elucidated. In healthy humans AT2 receptor expression in the kidney appears to be low. Stimulation of these receptors does, however, appear to have an effect of lowering blood pressure (possibly by a central mechanism involving reduction in sympathetic nervous system activity) which may contribute to the antihypertensive effect of AT1-ra. This dual antihypertensive action may limit the dose of AT1-ra such that maximally tolerated doses (as judged by blood pressure response) may be below the doses required to produce additional renoprotection (resulting from blockade of the ‘local’ RAS). Within the kidney AT2 stimulation has an antinatriuretic effect and may reduce degradation of extracellular matrix leading to accumulation in the glomerulus [7,16]. The local effect of AT1-ra is dependent upon the distribution of AII receptor subtypes and considerable interspecies variation in distribution has been demonstrated [17]. As a consequence of this the effects of AT1-ra seen in animal studies, e.g. regression of left ventricular hypertrophy [18], may be more difficult to demonstrate in humans as a result of the increased expression of AT2 receptors in human myocardium [19,17].

ACEI and AT1-ra alone or in combination?

AT1-ra should not be seen solely as an alternative to ACEI. Some patients do not achieve satisfactory blood pressure control (or renoprotection) on maximal doses of ACEI and the combination may, therefore, allow further RAS blockade while overcoming some of the concerns of using either agent in isolation. Additional RAS blockade has been confirmed by a further increase in plasma renin levels [20] and seems not to have adverse clinical or biochemical effects. Animal models, however, have not found the combination to be better than either drug alone [21]. Further work is required to study the expression of AT1 receptor in disease states and to examine the possibility that some of the actions of AT1-ra may be due to AT2 receptor stimulation. If the actions of AT1-ra were found to be mediated partly by AT2 receptor stimulation then the reduction in angiotensin II levels caused by ACEI may be a possible explanation for the failure to demonstrate clinical synergy by concomitant use of both agents.

Trials involving AT1 receptor antagonists

A recently published trial comparing AT1-ra and ACEI in patients with cardiac failure [ELITE] suggested that
AT₁-ra are better tolerated [22]. There was also a suggestion of superior efficacy of AT₁-ra although this may be explained partly by a difference in compliance if ACEI were associated with increased side-effects. A study on the effect of AT₁-ra on cardiovascular end-points is underway (Losartan Intervention for Endpoint Reduction—LIFE) as are separate studies into the effects of Ibersartan (the Collaborative Trial Group) and Ibersartan (The RENAAL study) in delaying progression to end-stage renal failure in patients with type II diabetes and established nephropathy. The Ibersartan and the Losartan studies will also evaluate the effect of AT₁-ra on cardiovascular events in a group at high risk of developing cardiovascular complications but they do not consider the effect of preventing the progression from microalbuminuria to overt nephropathy, as was shown recently for ACEI [23]. Unless trials directly comparing AT₁-ra to ACEI (± combined treatment) are carried out, the indication for AT₁-ra over ACEI, with the exception of intolerance to the latter, will remain elusive.

The role of molecular biology

Factors influencing RAS activity may determine the rate of progression of renal disease. RAS activity is under genetic control and some studies have suggested an effect of polymorphisms of the angiotensinogen and ACE genes on the rate of progression of diabetic and non-diabetic renal disease [24–28] while others have failed to observe such an effect. Additionally, there is some evidence that ACE genotype may influence response to treatment with ACEI [29]. Organisers of AT₁-ra studies have an ideal opportunity to incorporate molecular biology into their overall design. Studies of this size could provide a great deal of information in terms of identifying factors related to disease progression and therapeutic response to AT₁-ra. Most studies involving the genetic polymorphisms of RAS components are retrospective and non-interventional and studies of this size and design are urgently needed. Polymorphisms of the AT₁ receptor gene have been described and, although they have not been extensively studied, synergy between these and polymorphisms of the ACE gene on the risk of myocardial infarction has been suggested [30]. Thus, if information was available as to the natural history of disease and response to treatment as a function of genotype, it may be possible to predict individual patient response to RAS blockade at various points, such that a choice between ACEI and AT₁-ra (neither or both) could be made.

Summary

At the present time we cannot assume that the proven benefits of ACEI on renal disease will be reproduced by using AT₁-ra. With potentially differing modes of activity of these drugs, they cannot be seen as interchangeable and ACEI should remain the drug of choice in patients with progressive renal disease unless they are not tolerated. It is possible that AT₁-ra may offer additional advantages in some patients or that synergy exists between the two agents, but this view will remain entirely speculative unless proper trials are conducted. Despite the results of the ELITE study [22], the uncertainty regarding the use AT₁-ra in cardiovascular disease mirrors that of renal disease. This issue is obviously of relevance to the nephrologist in view of the spectrum of cardiac disease that accompanies chronic renal failure, such as left ventricular hypertrophy and cardiac failure, which provide multiple indications for manipulation of RAS. Despite their renoprotective effect, previous studies on ACEI [3,4] have not shown an overall reduction in mortality and this issue needs to be addressed in addition to renoprotection in studies comparing AT₁-ra and ACEI.

References

9. Faison EP, Snively DB, Thiyagarajan B, Nalson EB. The incidence of cough with angiotensin II receptor antagonist (Ang II RA), losartan, is significantly less than with angiotensin converting enzyme (ACE) inhibitors (ACEI) and is similar to that of placebo. Am J Hypertens 1994; 7: 34A
16. Vaughan DE, Lazos SA, Tong K. Angiotensin II regulates the


Hypertension in haemodialysis patients: who cares?

Evert J. Dorhout Mees
Oude Zutphenseweg 3, Vorden, The Netherlands

Once upon a time, when no effective treatment was available for hypertension, the conviction prevailed that high blood pressure was an inescapable fate and that lowering it might even be harmful. German doctors found a nice expression for this comforting view: ‘Erfordermisschobdruck’ (hypertension of necessity).

Since then, the devastating effects of hypertension have been amply documented. What is more, large trials have shown such decreases in morbidity and mortality after antihypertensive treatment, that this may be considered one of the most important successes of modern medicine.

Hypertension in the uremic patient

In 1960 Belding Scribner initiated chronic haemodialysis treatment for terminal renal failure, until then a fatal condition. Incidentally, his first patient also had malignant hypertension, which was cured by controlling his extracellular volume. It became clear, that controlling blood pressure is an essential part of dialysis treatment and that this could be achieved in the large majority of patients without the use of drugs, by salt restriction and ultrafiltration during dialysis sessions.

In the following years an unexpected development was observed in the dialysis community: cardiovascular mortality remained unacceptably high and continued to be the leading cause of death among dialysis patients. Hypertension also persisted in the majority of patients, despite the increasing use of ever more sophisticated antihypertensive drugs. Nevertheless relatively little attention is given to this vital subject. Textbooks on dialysis devote not more than 5–8% of their pages to hypertension and cardiac complications. In addition, a change of opinion among investigators can be observed in recent years, on two most crucial subjects.

Is hypertension associated with poor survival?

The first is that the importance of hypertension as a risk factor and major cause of death in dialysis patients, although demonstrated previously [1–3] is being questioned by investigators who could not find a relationship between blood pressure and survival.
Secondly, it is being suggested that the pathophysiology of hypertension in dialysis patients is different from essential hypertension and that unknown factors, special for the uremic state, play a dominant role. In particular the pathogenetic role of expansion of blood volume and extracellular volume, once widely accepted, is again being doubted. Although these two matters are closely related, particularly when it comes to prevention and treatment, I will only address the first one here.

The suspicion that hypertension has little to do with the poor prognosis of dialysis patients is indeed widespread, particularly in the US. As a result, not a single paper or session devoted to blood pressure control was accepted at the NKF meetings or at the ISN congress in the past year [4]. As an extreme consequence, the Health Care Financing Administration withdrew blood pressure data from the Haemodialysis Core Indicator data set. The reason for this unfortunate decision is probably the paucity of longitudinal studies demonstrating that blood-pressure control reduces morbidity and mortality [5].

Indeed in some studies a clearcut relationship between blood pressure and survival was not found. Others, who did observe increased mortality with high blood pressure levels also reported more mortality in patients with blood pressure below a certain point (so-called J curve) [3].

Elsewhere in this issue Salem reports a 2-year multicentre follow-up on 649 haemodialysis patients [6]. Surprisingly, hypertension was associated with a better survival.

This raises intriguing questions. Is hypertension only harmful without renal disease but beneficial in dialysis patients? Do we witness a rebirth of the ‘Erfordernisshochdruck’ concept? It is obviously not possible to explain the existing controversies from the available data in the literature, but commenting on some details of this study may be helpful.

In a previous report on the same patients after 1-year follow-up [7], the relative risk ratio (RR) of hypertension vs normotension was 0.38 for black patients (who constituted 89% of the group) but in whites this risk was 3.38 a 9-fold difference. In the present 2-year follow-up, this difference has decreased but blacks were still better off with hypertension (RR 0.56), while whites had a RR of 1.4. Still the suggestion remains that hypertension during dialysis treatment may not be harmful in blacks, while it is well known that in the absence of renal disease hypertension is much more devastating in blacks than in whites. Indeed, blacks had a better survival in this study.

Paradoxically better survival in patients with high blood pressure—are confounding factors the explanation?

How can these apparent contradictory facts be reconciled? Hypertension is a long-term process and observations during dialysis describe a relatively late phase. In addition blood pressure often decreases when severe heart disease is present. This has been a plausible explanation for the J curve. The fact that with multivariate analysis, serum albumin level had a very significant relation with survival while the low RR of hypertension lost the significance seen with univariate analysis [6], may also suggest that the normotensive patients were not generally in good shape. Unfortunately, the absence of any data (e.g. chest X-ray and echocardiogram) in this study preclude validation of these speculations. Anyhow, the assumption that a simple linear relationship between blood pressure and mortality is also present in short-term studies would neglect the complicated nature of these problems.

Another remarkable fact in Salem’s study [6] is that most of the hypertensive patients (81.5%) used antihypertensive drugs, while none of the normotensives took any. This implies that the antihypertensive drugs were completely ineffective, not a single patient thus treated becoming normotensive. Despite this, the author speculates that the drugs may have improved the prognosis by ameliorating excessive blood pressure swings.

That drug treatment in haemodialysis patients decreases blood pressure variation has never been demonstrated, while the opposite is reported in most textbooks.

As remarked earlier, studies proving an adverse effect of hypertension and an improvement of prognosis with adequate treatment are few. In my opinion this is related to the fact that drug treatment is not often successful in dialysis patients as is also apparent in this study [6].

Is volume control the answer?

In contrast, an impressively long survival, far exceeding any other reported series, was achieved in a large group treated without drugs but (like the historic first patient) with meticulous volume control [8]. That such a large difference would be due to ethnic differences seems highly unlikely. Why should cardiovascular pathophysiology be different? Reluctance to accept that it is indeed possible to treat hypertension and thereby prevent cardiovascular complications by adequate volume reduction may stem from the present-day preoccupation of the medical profession with ‘controlled trials’. Some results, particularly when they are as striking as those of the Tassin investigators, and in addition fit with known (but neglected) pathophysiological principles [9], do not need a control group. To include a control group would imply leaving a large number of patients untreated for some years, which would be unethical.

When investigators still call for prospective studies it should first be established which treatment is applied. It is not at all certain that hypotensive drugs can bring down blood pressure for long periods in hemodialysis patients. In contrast this goal can be achieved by volume control, but this method needs intense, ongoing individual care for the patients, for which much more doctor’s time is needed than is customary [10].
Conclusion

In conclusion it would be regrettable if readers would conclude from reports such as Salem’s [6] that lowering elevated blood pressure is unnecessary and harmful. Fortunately, the authors do not draw that conclusion. But decisions like that of the HCFA are a sign on the wall. Let all of us beware, the secret of real improvement is: more care [10].

References

2. Ritz E. Hypertension and cardiac death in dialysis patients should target blood pressure. Semin Dial 1993; 6: 227–228


Haemodialysis for French diabetic patients

Eli A. Friedman

Department of Medicine, State University of New York, Brooklyn, New York, USA

For 20 years, disparity between dialysis survival in Europe and the US has been a vexing concern. Especially difficult to understand is the nearly miraculous longevity reported in Tassin, France by Laurent, Calemard and Charra [1]. In a stirring 1983 retrospective analysis of 373 haemodialysis patients, survival was 75% at 10 years and 65% at 15 years [1]. Setting aside a potentially key criticism, the authors classified their patients as ‘an unselected population.’ However, only 15 (4%) of the Tassin subjects had diabetes while another 15 (4%) had systemic disease. What happened to uraemic diabetic people in Tassin who were not accepted for dialytic therapy? They died, uncounted in any survival statistics.

Previously, I rebutted the assertion that the Tassin dialysis subset was representative of the renal-failure population at large on the grounds that the low prevalence of co-morbidity, especially diabetes, must be the result of strict selection. It follows, I deduced, that the purportedly distinctly superior survival was a misperception: the product of comparing nonequivalent cohorts in France and the US [2].

Apologetists for the American style of uraemia therapy take as a ‘given’ that the country that invented maintenance haemodialysis, continuous ambulatory peritoneal dialysis and kidney transplantation is incapable of delivering treatment at a standard uniformly practiced in less-rich nations. The reality that European treatment rates are half that in the US, meaning that sicker and older patients, those most likely to die, are grossly excluded, was ignored. Instead, the ‘American Tragedy’ was explained by defective dialysis, due to dialysers reuse or inordinately short dialysis treatment times. Worry over application of American ‘short dialysis’ spread to Europe [3]. Troubled by substandard quality in dialysis care, the National Kidney Foundation, supported by an ‘unrestricted educational grant’ from AMGEN Inc., Thousand Oak, CA, USA, conducted a broad extensive review termed the Dialysis Outcomes Quality Initiative (DOQI) [4]. Virtually every aspect of the dialysis process from the establishment of a vascular access to management of anaemia and metabolic bone disease is spelled out in DOQI advisories. Not covered, however, is the patient referral process that may exclude elderly, diabetic and minority group members.

Elsewhere in this issue, Chantrel et al. 1 lament the high mortality in type II diabetic haemodialysis patients treated in Strasbourg. Chantrel et al. report that 27 of 84 (32%) of type II diabetic patients begun on dialysis died in a mean follow-up of 211 days. Strict comparison of this outcome with the 1-year survival of 78% for diabetic dialysis patients in the US is flawed.
Mycophenolate-update after it has come of age

Josep M. Grinyó
Servei de Nefrologia, Hospital de Bellvitge, Cuitat Sanitària i Universitària de Bellvitge, Universitat de Barcelona, Spain

Mycophenolate mofetil (MMF) is the morpholino-monophosphate dehydrogenase, that depletes the cell of guanosin nucleotides. Mycophenolic acid selectively inhibits the proliferation of T and B lymphocytes, the production of antibodies, and the generation of cytotoxic T lymphocytes. Furthermore, depletion of guanosine nucleotides results in the inhibition of glycosilation of adhesion molecules, which might interfere with exclusion of the first 90 days by the US Renal Data System [5]. Nevertheless, it is evident that Chantrel et al. had to cope with extensive undertreatment of hypertension as well as absent planning for the provision of a vascular access and elective initiation of uremia therapy. Had this cohort of diabetic patients been intermixed with the Tassin selectees, the slope of survival curves would have been bent downward. In other words, the best method for ensuring superior survival is to treat patients less likely to die.

Other components in the Chantrel et al. report reflect universal problems in dealing with diabetic azotaemic patients: (i) Distinguishing acute from chronic kidney failure may be difficult. (ii) Pulmonary oedema induced by hypoproteinaemia is readily confused with congestive heart failure. (iii) The misconception that ketoadosis is restricted to type I diabetes when it is actually more prevalent in type II diabetes [6,7]. (iv) Ophthalmoscopy is inadequate screening for the detection of diabetic retinopathy missing 25% of cases detected by retinal photography [8].

There is another important message communicated by Chantrel et al., that bias against accepting diabetic patients for uremia therapy continues in France today. How else can the authors’ statement that although diabetes accounts for 40% of their patients entering dialysis, other dialysis facilities in France admit only 15.7% with diabetes [9]. Either renal failure induced by diabetes has an extraordinary epidemiology in France, varying widely from city to city, or criteria for acceptance for dialysis are applied unequally. From introspective studies in other countries, particularly the UK [10,11], it is clear that the treatment acceptance rate for diabetic renal-failure patients is a correlate of governmental policies and economic pressures [12].

Returning to the issue raised at the outset, admiration is justified for the wonderful life extension effected by the Tassin team. High-quality haemodialysis does facilitate rehabilitation minimizing the need for erythropoietin and antihypertensive drugs. Emulating carefully performed dialytic therapy is a desirable objective for all clinical nephrologists striving for excellence in patient care. Improving the outcome of dialysis in diabetic patients is a complex and elusive goal worthy of all of us. Chantrel et al. are to be congratulated for their candor in recounting the complexity, stress and disappointment that is usual when trying to deliver uremia therapy to diabetic patients afflicted with life-threatening extrarenal co-morbidity.
the recruitment of lymphocytes to the sites of inflammation in rejected allografts. MMF has been studied in the prevention and treatment of acute rejection in renal transplantation and preliminary experiences have been reported in the management of late allograft dysfunction.

**MMF in the prevention of acute rejection**

The immunosuppressive potency of MMF has been clearly demonstrated in three large prospective, randomized, controlled trials in renal transplantation involving nearly 1500 patients [1–3]. The European trial of MMF for the prevention of allograft rejection [1], a double blind placebo-controlled study, demonstrated that MMF in conjunction with cyclosporin and corticosteroids significantly reduces the incidence of the composite end-point of biopsy-proven acute rejection/treatment failure (defined as graft loss, death or premature withdrawal from the study) at 6 months post-transplant from 56% for placebo-treated patients to 38.8% for MMF 3 g/day-and 30.3% for MMF 2 g/day-treated patients. This considerable reduction in rejection or treatment failure was consistent with the results obtained in the American [2] and Tricontinental [3] studies in which MMF was compared with the control drug azathioprine as a component of a triple therapy regimen including cyclosporin and corticosteroids. In addition, a pooled efficacy analysis for these three clinical trials at 1 year after transplantation [4] confirmed the ability of MMF to reduce the incidence of biopsy-proven acute rejection in comparison with placebo or azathioprine. The use of MMF resulted in a significant reduction of biopsy-proven acute rejection from 40.8% in the azathioprine/placebo-treated patients to 19.8% and 16.5% in MMF 2 g/day and MMF 3 g/day-treated patients, respectively. MMF also reduced the histological severity of rejection and consequently the need for antilymphocyte antibodies. Interestingly, at 1 year post-transplant, MMF significantly reduced graft losses due to rejection from 6.3% in the placebo/azathioprine group to 2.6% and 3.5% in the MMF 2/day and MMF 3 g/day, respectively.

This positive impact in the reduction of immunological failures persisted at 3 years after transplantation. In the Tricontinental study [5], using azathioprine in the control group, MMF reduced graft losses due to rejection from 9.9% in the azathioprine group to 5.8% and 3% in the MMF 2 and 3 g groups, respectively. Similarly, in the European study graft losses due to rejection decreased from 10.8% in the placebo group to 4.8% and 6.3% in the MMF 2 and 3 g groups, respectively [6]. In this study, an intent-to-treat analysis of patient and graft survival over 3-year follow-up showed that the cumulative incidence of graft loss (including graft loss as a result of death) for the MMF 2 g, MMF 3 g, and placebo groups were 15.2%, 18.8% and 22% respectively. Patient deaths in the respective groups were 7.3%, 8.2%, and 11.1%, being acute rejection the principal cause of graft loss in all groups. The differences in the 3-year graft loss rates (including death) for comparisons of MMF 2 g and MMF 3 g vs placebo were respectively 6.9% and 2.9%. Censoring for death, the differences in 3-year graft loss rates were 7.6% and 3.2%, respectively. For the MMF 2 g group, this represents a relative risk of 0.55 ($P = 0.04$), or a 45% reduction in graft loss compared with the placebo group. An analysis of the Tricontinental study also showed a trend in favour of MMF 2 g and MMF 3 g over 3 years in graft and patient survival compared with treatment with azathioprine [5]. The 3-year data from the European and Tricontinental study also confirmed the deleterious impact of early acute rejection on long-term patient and graft survival. In these two studies, patients who experienced a biopsy-proven acute rejection in the first 6 months post-transplant were 5 and 4 times, respectively, more likely to lose their graft to those who were free of such rejection.

At 3 years after transplantation the cumulative incidence of adverse effects of MMF [5,6] was similar to and consistent with the results previously reported [1–3]. MMF is associated with slight increases, in a dose-dependent manner, in gastrointestinal and haematologic adverse events as well as infections and malignancies. However, the cumulative incidence of cytomegalovirus invasive disease in azathioprine-treated patients from the Tricontinental study (6.8%) is higher than that observed in the MMF 2 g-treated patients (3.6%), and similar to that of MMF 3 g-treated recipients (8.1%) in the European study, suggesting that the increased incidence of opportunistic infections in MMF-treated patients cannot be only attributed to the administration of MMF but to the cumulative doses of conventional immunosuppressants. We have recently shown that by reducing the doses of cyclosporin and steroids in patients treated with 3 g of MMF, the incidence of cytomegalovirus disease remains similar to that observed in patients treated with conventional doses of cyclosporin and steroids, without an increased risk for rejection [7]. A similar observation is also true for the overall incidence of malignancies. It is higher in all groups from the Tricontinental study in comparison with that reported in the European study [5,6]. After 3 years the mortality rates and causes of death in the three therapeutic groups were similar in both studies. Vascular diseases were the most common cause of death followed by infection and cancer.

In summary, MMF is a highly effective immunosuppressant that greatly reduces the incidence of acute rejection early after transplantation and provides a sustained advantage for 3-year graft survival compared to the use of dual therapy with cyclosporin and steroids or triple regimens containing azathioprine, and with an acceptable safety profile. Accordingly, many transplant centers have replaced azathioprine for MMF in triple regimens or added MMF to dual therapies in the prophylaxis of acute rejection in renal transplanta-
tion. However, long-term observations are required to demonstrate the long-term benefits of this agent.

**MMF in the treatment of acute rejection**

High doses of MMF can successfully reverse acute renal allograft rejection in dogs [8]. In clinical studies, MMF has been shown to be effective for the treatment of acute refractory [9] or first [10] cellular allograft rejection. Because patients with acute cellular rejection episodes refractory to standard therapy with steroids and antilymphocyte antibodies are at high risk of losing their grafts, MMF was used in these cases in comparison with high doses of intravenous steroids in a prospective randomized trial. [9]. In this study, the proportion of MMF-treated patients who experienced a biopsy-proven or presumptive acute rejection or were classified as a treatment failure was significantly lower than in steroids-treated recipients (39% vs 64%) 6 months after the enrollment in the study. This resulted in a clinically significant reduction in the use of antilymphocyte agents from 24.7% in steroids-treated patients to 10.4% in MMF-treated patients subsequent to enrollment, and also the use of MMF was associated with a significant reduction, by more than 40%, in graft loss or death 1 year after the entry in the study. These positive results in the treatment of refractory rejection are in agreement with those recently reported on the utility of MMF in the treatment of first acute cellular rejection [10]. In this double-blind, double-dummy controlled study, renal allograft recipients experiencing the first biopsy-proven cellular rejection within 6 months of transplant were treated with MMF 3 g/day and intravenous steroids, or azathioprine (1–2 mg/kg/day) and intravenous steroids. In comparison with intravenous steroids, MMF decreased the subsequent use of antilymphocyte therapy (41.7% vs 16.8%), and the proportion of patients who lost their graft or died (14.8% vs 8.9%) at 6 months. In MMF-treated patients there was a trend for better renal function that may result from a more rapid and complete resolution of the rejection in these patients. This study has an extended follow-up to determine whether the use of MMF in the treatment of a first acute rejection will have an impact to reduce late graft loss and chronic rejection. However, in both studies MMF was associated with a higher incidence of adverse events. Cytomegalovirus tissue invasive disease was higher in MMF-treated patients in comparison with steroid-treated patients in the Refractory Rejection study (9.1% vs 1.4%), although it was similar in both groups in the Acute Renal Rejection study. In routine clinical practice, with less constraints than in study conditions, dose adjustments might help to reduce MMF side effects.

On the other hand, the combination of two agents effectively used in rescue therapy, such as MMF and tacrolimus, may constitute a promising association in the treatment of corticoresistant or refractory rejection [11].

**MMF in the management of chronic graft dysfunction**

In spite of improving 1-year graft survival, the introduction of cyclosporine has had a modest impact on graft half-life. It is well known that the leading cause of late graft failure is chronic transplant nephropathy, and both immunologic and non-immunologic factors may play a role in its pathogenesis. Cyclosporin causes a reversible dose-related renal vasoconstriction, a reduction in the glomerular filtration rate and may induce renal interstitial fibrosis contributing to chronic functional deterioration of renal allograft. Cyclosporin-associated renal fibrosis has been related to the overproduction of TGF-β, a fibrogenic cytokine. The different attempts to minimize cyclosporin nephrotoxicity, such as using low-doses of cyclosporin in patients treated with triple therapy or switching cyclosporin to azathioprine, are associated with an increased risk of acute rejection. MMF, that is a more potent immunosuppressant than azathioprine, may allow to reduce cyclosporin doses, while providing an adequate immunosuppression, in patients with chronic renal allograft dysfunction. In a short-term study. Weir et al. [12] have replaced azathioprine for MMF and reduced by 50% the doses of cyclosporin in patients with chronic transplant nephropathy and progressive deterioration of renal function. With this maneuver, the rate of loss in renal function and serum cholesterol levels significantly decreased in these patients 7 months after the introduction of MMF. These authors have also shown a significant reduction in the renal expression of TGF-β1 in patients with biopsy-proven chronic allograft nephropathy 1 year after the reduction of mean cyclosporin levels from 200 to 100 ng/ml [13]. In our center we have adopted a similar approach in patients with stable suboptimal renal function by adding MMF and reducing cyclosporin doses to achieve minimal levels between 40 and 60 ng/ml [14]. Six months after cyclosporin reduction a significant increase in the glomerular filtration rate and a decrease in TGF-β values were observed. Moreover, these TGF-β values positively correlated with cyclosporin levels. There were no rejection episodes after the immunosuppression change in the two trials. Taken together, the results of these two short-term studies suggest that the introduction of MMF allows a safe cyclosporin dose reduction that attenuates the reversible and hemodynamic component of cyclosporin nephrotoxicity, while decreases the production of TGF-β, which may lessen the fibrogenic capacity of this calcineurin inhibitor. Hence, the association of MMF and low-doses of cyclosporin might constitute a useful maintenance long-term immunosuppressive therapy. However, long-term, comparative, biopsy-controlled, studies are needed to assess the potential benefit of this immunosuppressive regime on the progression of chronic transplant nephropathy.

**References**

1. European Mycophenolate Mofetil Cooperative Study Group. Placebo-controlled study of mycophenolate mofetil combined
with cyclosporine and corticosteroids for prevention of acute rejection. Lancet 1995; 345: 1321–1325


