Editorial Comments

Why do loop diuretics cause hypokalaemia?

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Introduction

The treatment of hypertension by diuretics is always fraught with the complication of the induction of hypokalaemia [1]. Hypokalaemia is in fact ranked as one of the primary complications of chronic diuretic treatment [2]. During the long history of the development of new diuretics it has always been a primary goal to avoid this complication and to advertise any new diuretic as ‘K+ saving’, ‘K+ neutral’, or, at the least as to be ‘less K+ wasting’. The present review is aimed at the clarification of such claims. No loop diuretic at this stage can prevent K+ losses. This is based on several lines of evidence. However, it should also be stated that our current understanding of the function of the thick ascending limb of the loop of Henle permit the prediction that loop diuretics with reduced K+ losses appear a feasible new development. One of the key losses of K+ are through one specific type of pH-sensitive K+ channel, the so-called renal inhibitable Na+ channel [11]. Hence the driving force for K+ secretion will be increased to the same extent that Na+ is absorbed. This is the mechanism by which K+ excretion is increased by increased secretion of aldosterone and following an increase in distal tubule flow rate [8] and by which it is abolished after administration of amiloride or triamterene [11,12].

The determinants of renal K+ excretion

Unlike the renal excretion of Na+ and Cl−, the excretion of K+ can vary enormously between a few percent of the filtered load and more than 100% [8]. Most regulatory mechanisms affect the loop of Henle, the distal tubule, and the collecting duct [4,5,8]. Major determinants are the dietary intake of K+, acid–base disturbances, changes in aldosterone secretion, and tubule flow rate. Hyperkalaemia increases and hypokalaemia decreases K+ excretion [8]. Alkalosis tends to enhance and acidosis usually reduces K+ excretion [4,6,8]. Aldosterone enhances K+ excretion to the same extent that Na+ absorption is increased [9]. These three factors are interdependent in a complex way. Hyperkalaemia, for instance, has a direct stimulatory effect on distal tubule K+ secretion resulting from an increase in distal tubule flow rate and from direct stimulation of aldosterone secretion [10]. Most of these effector mechanisms affect the principal cells of the collecting duct [8].

A schematic view of how K+ is secreted by these cells is depicted in Figure 1. The luminal and the basolateral membranes of these cells are equipped with various types of K+ channels [5,6]. The amount of K+ secreted is determined by the following:

1. The conductance of the luminal membrane (enhanced by alkaline pH, enhanced Ca2+, elevated plasma K+, regulation by hormones such as arginine vasopressin).

2. The driving force, i.e. the difference between the luminal membrane voltage and the Nernst potential for K+ (ca. −90 mV). The luminal membrane is depolarized by Na+ entry via the amiloride-inhibitable Na+ channel [11]. Hence the driving force for K+ secretion will be increased to the extent that Na+ is absorbed. This is the mechanism by which K+ secretion is increased by increased secretion of aldosterone and following an increase in distal tubule flow rate [8] and by which it is abolished after administration of amiloride or triamterene [11,12].

How do loop diuretics influence renal K+ excretion?

Loop diuretics inhibit Na+ and Cl− absorption in the thick ascending limb of the loop of Henle [13]. This has a direct effect on the K+ handling by this nephron segment. The luminal membrane hyperpolarizes towards the Nernst potential for K+ [13]. Hence, K+ recycling via luminal K+ channels ceases. Concomitantly the transepithelial lumen-positive voltage collapses and the net K+ absorption, occurring under control conditions, is abolished. Therefore loop diuretics have a direct kaliuretic effect in the thick ascending limb itself.

Loop diuretics paralyse the macula densa segment [14,15] and hence increase renin secretion. This leads
The clinical relevance of loop diuretic induced hypokalaemia

The above discussion has clarified that hypokalaemia is an unavoidable consequence of the saliuresis produced by loop diuretics. The clinical experience shows that to some degree hypokalaemia is invariably seen after loop diuretics or thiazides [1,2]. The exact degree of hypokalaemia depends on dose and duration of treatment. The degree of hypokalaemia will also depend on confounding mechanisms, which have been discussed above: (1) if dietary K⁺ intake is too low, hypokalaemia will develop fast and will be severe; (2) if alkalosis develops this will aggravate renal K⁺ loss; (3) inappropriately high doses of the diuretic will increase K⁺ losses by direct and indirect (hyper-reninism!) mechanisms.

The basic mechanisms are identical for all currently used loop diuretics: frusemide; piretanide; bumetanide; torasemide [1,13]. Differences between the substances have not been found with respect to their proximal secretion [18] and hence their tubule accumulation. However, the extrarenal pharmacokinetics of the four substances vary considerably [1]. If these differences are taken into account in chronic treatment the net saliuretic and kaliuretic effects are probably similar [1]. If hypokalaemia requires clinical interference the dose of the used diuretic should be reconsidered; K⁺ substitution and/or a combination of the loop diuretic and inhibitors of Na⁺ channels such as amiloride or triamterene are useful. As discussed above (Figure 1) these substances inhibit K⁺ secretion to the extent that they reduce Na⁺ absorption in the principal cell.

Are there new perspectives?

The functional characterization and cloning of ‘renal outer medullary’ (ROM) K⁺ channels [3,19] makes it feasible to design new and maybe specific inhibitors of these K⁺ channels. The claim that glibenclamide may be a prototype for these inhibitors [20] has not been verified in our laboratory, since the effect is seen only at therapeutically inacceptable concentrations [21]. Specific ROMK inhibitors would also act as loop diuretics, because K⁺ recycling across the luminal membrane of the thick ascending limb is a prerequisite for Na⁺ and Cl⁻ absorption by this nephron segment [13]. In fact, we have originally suggested the concept of Na⁺⁺Cl⁻K⁺ cotransport, because Ba²⁺, a non-specific inhibitor of K⁺ channels, inhibited Na⁺ and Cl⁻ absorption [7].

References

Antigens in experimental models of membranous nephropathy: are they involved in human disease?

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Key words: Heymann nephritis; immune complexes; membranous nephropathy; podocyte

Introduction

Human idiopathic membranous nephropathy (iMN) is the most common cause of nephrotic syndrome in adults. Although the pathogenesis is unknown, it is somehow related to formation of subepithelial glomerular immune deposits. Very similar lesions and clinical course can be reproduced in experimental animals. In 1959 a form of membranous nephropathy was induced by injection in the rat of homologous renal cortex (Heymann nephritis, HN) [1]. Since then the pathogenetic aspects of this disease have been thoroughly investigated.

This editorial comment will briefly discuss whether the neprhotogenic epitopes identified in membranous experimental models can be relevant for human disease.

Rat antigens

A 330-kD tubuloglomerular glycoprotein (gp330) is the main pathogenic antigen (Ag) of rat HN [2–4]. Its function has been recently ascertained: gp330 is a member of the low-density lipoprotein/alpha2-macroglobulin-receptor family [5]. Among other rat tubuloglomerular Ads, whose structural and functional relationship with gp330 is still indistinct, a 90-kD glycoprotein (gp90) with dipeptidyl-peptidase IV activity (DPP IV) can cause transient membranous-like glomerular aspects [6–8].

Other animal antigens

Studies performed in the mouse and in the rabbit have evidenced other forms of membranous nephropathy induced by intravenous injection of antibodies (Abs)
raised against brush-border Ags. Molecular identification of these epitopes has allowed researchers to find the same glycoproteins as those discovered in the rat [4]. However, while DPP IV plays a definite nephritogenic role, gp 330 has no glomerular expression and pathogenic significance in these animals. Furthermore, neutral endopeptidase (NEP), also referred to as enkephalinase (m.w. = 85 kD), a rabbit glomerular glycoprotein coexpressed on tubular cell surface, has been identified as an additional pathogenetic Ag [4].

These animal models of membranous nephropathy have been less extensively studied than rat HN; however, they are worth stressing because they point out the evolutionary preservation of tubuloglomerular Ags provided with nephritogenic activity.

**Human findings**

After such experimental evidences, several researchers have investigated the possibility of similar pathogenetic mechanisms in human iMN. Anti-brush-border Abs were occasionally found in the sera or renal tissue of patients affected by the nephropathy [9,10]. Nevertheless, most investigators were unable to confirm their presence in larger series of patients [11,12].

Another wave of studies on the role of tubuloglomerular Ags in human iMN has arisen after the molecular identification of renal nephritogenic epitopes in experimental animals. Among the most suggestive results it may be mentioned that an Ag of molecular weight 400 kD (gp400), cross-reactive with rat gp330, has been demonstrated on the brush border of human tubules [13]; this molecule is able to induce a model of membranous nephropathy if injected in the rat; in addition a sequence homology of rat gp330 with the glomerular epitopes (gp90, gp85) are able to induce a model has a nephritogenic role only in the rat [19], while in other animal species different tubular coexpressed of membranous nephropathy if injected in the rat; in other animal species different tubular coexpressed if different from initial immune deposits [14]. However, while DPP IV plays a definite nephritogenic role of such brush-border-related podocyte Ags, because renal biopsy is usually performed late in the course of human iMN, when deposits are probably rather different from initial immune reactants.

On the other hand, *in situ* nephritogenic human Ags should not necessarily be coexpressed at tubular and glomerular level. Fukatsu *et al.* [16] raised a monoclonal Ab reactive with a human podocyte protein (m.w. = 56 kD). Fine subepithelial glomerular deposits were provoked by perfusion of isolated human kidney or by monkey immunization with this monoclonal Ab; nevertheless, severe and persistent lesions like those of full-blown human iMN could not be induced.

Another realistic possibility concerns the absence of *in situ* pathogenic Ags from the normal human glomerulus. This is supported by the finding that immunoglobulins eluted from deposits of human iMN usually fail to react with normal renal tissue [17].

Neo-expressed nephritogenic molecules (viral epitopes, inflammatory cytokines) could appear on plasma membrane of human glomerular cells (podocyte?) and participate in the formation of immune deposits [18] under stress situations. Ronco *et al.* [17] found that a 280-kD glycoprotein, usually absent from rat glomerulus, is clearly evident in membranous deposits of HN.

**Conclusions**

The pathogenetic Ag(s) of human iMN is still elusive. *In situ* formation of gp330-related immune complexes has a nephritogenic role only in the rat [19], while in other animal species different tubular coexpressed glomerular epitopes (gp90, gp85) are able to induce membranous nephropathy. Interestingly, these molecules are present in human normal kidney as well, but a role of them in the development of human iMN has not been demonstrated.

This could be due to the following events: (1) masking of the investigated epitopes by immunoglobulins in excess in renal deposits; (2) presence of nephritogenic tubuloglomerular Ags different from those involved in experimental models; and (3) pathological autoimmune action of podocyte specific (non-brush-border-related) Ags.

More probably the failure to identify the Ag(s) of human iMN is the result of its absence from normal glomerular cells. This supports the current opinion that a main pathogenetic Ag similar to rat gp330 does not exist in human iMN; in all likelihood we have to face a broad spectrum of nephritogenic Ags. Nevertheless the conceptual importance of the experimental models of membranous nephropathy remains exceptional, because of the discovery of pathogenic Ags located at renal level and able to form *in situ* nephritogenic immune complexes.

Further studies on human renal tissue are necessary to analyse early glomerular deposits and their antigenic constituent(s). Transplanted kidney affected by *de novo*
Fig. 1a,b. Immunohistochemistry of human membranous nephropathy (stage II). Cryostat sections were incubated with monoclonal antibodies specific for: (a) human anti-DDP IV (immunoperoxidase; ×250); (b) human anti-NEP (enkephalinase)/CALLA (alkaline phosphatase; ×250). Both Ags are intensely expressed along the epithelial side of the glomerular capillary wall and on the tubular brush border; however, no definite staining of typical subepithelial glomerular deposits can be shown.

or recurrent membranous nephropathy might be a good tool, considering the greater probability of early diagnosis in this condition.

The molecular identification of pathogenic IC could lead to specific therapeutic approaches, by removing nephritogenic Ags or blocking the relevant circulating Abs by means of immunological manipulations [20].

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Lipodystrophy in MCGN type II: the clue to links between the adipocyte and the complement system

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Introduction

It is gratifying when the advance of medical and scientific knowledge illuminates pathogenetic mechanisms for clinical syndromes which previously have been unexplained curiosities, especially when it can be demonstrated that careful study of a rare syndrome has revealed a general principle of biology and pathology. The association between partial lipodystrophy (PLD) and mesangiocapillary glomerulonephritis type II (MCGN II) is one such rare syndrome. The link between these two conditions, and the associated overactivity of the alternative pathway of complement, has baffled nephrologists for many years.

In the last few years, evidence has accumulated that dysregulation of the alternative pathway of complement may be causally related to adipocyte destruction, and possibly also to nephritis, by hitherto unsuspected mechanisms; and that the complement system plays an important role in normal adipose tissue biology.

PLD, nephritic factor (NeF) and MCGN II

In PLD, subcutaneous fat is permanently lost from the face and upper body, often acutely and sometimes following a viral infection such as measles. Patients with PLD typically have dysregulated activation of the alternative pathway associated with the presence of nephritic factor (NeF), an IgG autoantibody. NeF binds to the rate limiting C3 convertase enzyme of the alternative pathway (C3bBb), and protects it from dissociation by factor H, a regulatory protein that normally acts to control the rate of alternative pathway activity. A proportion of patients with PLD also develop MCGN II, although the PLD and MCGN II may be dissociated in time by several years. There are a few reports of NeF in apparently healthy individuals.

The adipocyte as a source of complement components

In 1989 Rosen and colleagues from Harvard reported that adipsin, a major protein product of mature adipocytes, is highly homologous to factor D, a serine protease whose only known function is the cleavage of factor B in the alternative pathway \cite{1}. This suggested to us a mechanism for the link between PLD and NeF and led to the experiments described in the next section. Subsequently the Harvard group showed that adipsin and factor D are identical, that adipose tissue is a major source of factor D \textit{in vivo}, and that adipocytes also produce C3 and factor B and activate the alternative pathway \textit{in vitro} and \textit{in vivo} \cite{2}. More recently, yet another link between the adipocyte and the complement system has become apparent with the description of an adipocyte-specific product which is highly homologous to C1q \cite{3}. The possible function of these complement components in adipocyte biology will be considered below.

NeF, adipocyte lysis and factor D expression

We have reported that NeF-containing sera or IgG induce complement-dependent lysis of adipocytes \textit{in vitro} \cite{4,5}, indicating that when there is dysregulation of the alternative pathway the production of comple-
ment components by the adipocyte becomes deleterious for the cell, which effectively contributes to its own destruction. There are regional differences in factor D expression which broadly mirror the regional distribution of adipocyte loss in PLD [5], possibly indicating that high-level factor D expression is required for adipocyte loss to occur. Whether interindividual variation in factor D expression can account for the fact that PLD only occurs in a proportion of patients with NeF remains undetermined. Further, it is unknown why adipose tissue loss occurs so acutely; the association with viral infection suggests that cytokine release might play a role, for example by upregulating complement component expression by adipocytes.

**Alternative pathway dysregulation and nephritis**

The demonstration that NeF can induce lysis of adipocytes, and therefore may be causally related to adipocyte loss in PLD, has implications for MCGN II: could NeF lead to injury of renal cells [6]? Two pieces of evidence suggest that this could indeed be the case.

First, human intrinsic renal cells express complement components [7]. This has been shown most clearly for C3 and C4, which are synthesized and secreted by renal tubular epithelial cells, mesangial cells and glomerular epithelial cells. This expression is upregulated by proinflammatory cytokines, and also in various forms of nephritis. Factor B is also expressed in the kidney, but there is little information on factor D.

The second piece of evidence comes from studies of the consequences of the dysregulated alternative pathway complement activation which results from deficiency of the regulatory protein factor H [6]. In a variety of Yorkshire pig and in rare human cases, absence of factor H is associated with a renal lesion with very similar morphology to MCGN II. Further, there is an intriguing case report of a patient whose serum contained a monoclonal lambda light chain which interacted with factor H in vitro and prevented its action, allowing unregulated alternative pathway activation, and whose renal biopsy showed features of MCGN type II [8].

Thus, overactivity of the alternative pathway for three entirely separate reasons (NeF protecting C3Bb from the effects of factor H; genetic factor H deficiency; or altered factor H function) has the same consequence for the kidney, strongly suggesting that it is the complement activation per se which induces the nephritis. Whether this arises by a mechanism similar to that postulated for adipocyte loss in PLD remains unproven.

**Complement and normal adipocyte biology**

The expression of complement components by adipocytes may explain PLD and may have implications for MCGN II. These rarities are of interest to nephrologists, but are presumably the result of loss of the normal regulatory mechanisms. What does this tell us about the normal adipocyte: why should adipocytes express proteins whose role was thought to be mainly concerned with host defence? The answer is suggested by the demonstration that the protein C3a, which is generated when C3 is cleaved, has potent activity as an acylation stimulating protein, promoting the esterification of fatty acids into triglyceride. C3a increases membrane transport of glucose into adipocytes and increases the activity of diacylglycerol acyltransferase: both effects markedly increase the rate of triglyceride synthesis [9].

The physiological role of the adipocyte is to act as an energy store, accumulating triglyceride in times of plenty for use in times of need. Adipocytes express all the components required for the local generation of C3a, which can then act to mediate this storage function. Factor D expression is increased in fasting or catabolic states and decreased in various experimental models of obesity; thus the adipocyte may modulate its capacity to activate the alternative pathway according to the need for triglyceride storage or release. The C1q-related protein exclusively expressed by adipocytes [3] also seems to play a role in energy storage by these cells, and is similar to a hibernation-specific protein isolated from the plasma of Siberian chipmunks!

The expression of phylogenetically ancient complement proteins by adipocytes strongly suggests that these proteins are integral to the physiological function of these cells. This is a previously unsuspected role for the complement pathway, and one illustration of a growing appreciation that complement may have local roles in various tissues in addition to the well-known systemic actions [10].

**Conclusions**

Adipocytes express key complement proteins, and the alternative pathway of complement probably plays an important role in normal adipocyte biology. When this pathway is dysregulated by the presence of NeF, fat cell destruction can result, leading to the rare clinical syndrome of PLD. MCGN type II may be associated with PLD and since intrinsic renal cells also express complement components, a similar mechanism of complement-mediated injury may be responsible: this hypothesis is supported by the occurrence of a similar form of nephritis when the alternative pathway is overactive for other reasons. Careful study of this relatively rare form of nephritis and its clinical associations has opened up an area of much broader biological relevance.

**References**


Direct and indirect pathways of alloantigen recognition: relevance to acute and chronic allograft rejection

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Introduction

The design and effectiveness of strategies to promote long-term graft acceptance requires a fundamental understanding of the mechanisms underlying acute and chronic rejection. In this review we discuss the two pathways of allorecognition, direct and indirect, and suggest that the direct pathway plays the major role in the early weeks after transplantation, and that the indirect pathway may contribute to the process of chronic rejection. The results of in vitro and in vivo experimental models will be discussed, together with a limited amount of clinical data.

Pathways of alloantigen recognition

Direct alloantigen recognition

Molecular basis. One of the most striking features of the T cell response provoked by MHC incompatible cells is its vigour. This is illustrated by the mixed lymphocyte reaction in vitro, and by allograft rejection in vivo. Indeed it was the very strength of the allore- sponse that led to the discovery of MHC molecules. The strength of this response was accounted for by the uniquely high precursor frequency of T cells with specificity for allogeneic MHC molecules. Two hypotheses have been proposed to account for these observations (reviewed in [1]). The first is referred to as the multiple binary complex hypothesis, and proposes that T cells with direct allo specificity, like MHC-restricted T cells, have dual specificity for both peptide and the foreign MHC molecule. Given the extreme diversity of the peptides bound and displayed by MHC molecules at the cell surface, a single foreign MHC molecule can give rise to several hundred distinct peptide: MHC complexes, each recognized by a distinct clone of alloreactive T cells. The alternative hypothesis was proposed a few years later and is referred to as the high determinant density hypothesis. This envisages that the alloreactive T cell's specificity is for the foreign MHC molecule, with little or no specificity for bound peptide. If this is the case, it is argued, that the determinant density available to the alloreactive T cell is several logs higher than that available to an antigen-specific T cell, because all the MHC molecules of any given isotype (e.g. HLA-DR) can serve as ligands for the alloreactive T cell. Consequently T cells whose receptors have low, medium, and high affinity for the allogeneic MHC molecule can contribute to the alloreponse, thereby expanding the frequency of responding cells.

T cells normally recognize protein antigens as peptides presented by self major histocompatibility (MHC) molecules (MHC restriction). In contrast, the direct pathway of allorecognition results from an interaction between a T cell's antigen receptor (TCR) and a foreign MHC molecule: peptide complex (Figure 1). At face value this appears to break the rules of self MHC restriction; however, the two hypotheses to account for the high precursor frequency of alloreactive T cells can be accommodated within the context of self MHC restriction. This is easiest to envisage where responder and stimulator MHC molecules are similar, sharing conserved sequences in the exposed TCR-
Direct allorecognition involves the recognition by T cells of the polymorphism of the MHC alloantigens displayed at the surface of donor dendritic cells. No other cells intervene in this initial step of the direct pathway. Indirect allorecognition involves the recognition of donor alloantigens (primarily allogeneic MHC molecules) in the same way as any ‘nominal’ protein antigen, i.e. as processed peptides associated with self (recipient)-MHC class II molecules. Following activation of ‘indirect’ T helper cells, allospecific effector cells may be stimulated in the same localized environment.

Fig. 1. Direct allorecognition involves the recognition by T cells of the polymorphism of the MHC alloantigens displayed at the surface of donor dendritic cells. No other cells intervene in this initial step of the direct pathway. Indirect allorecognition involves the recognition of donor alloantigens (primarily allogeneic MHC molecules) in the same way as any ‘nominal’ protein antigen, i.e. as processed peptides associated with self (recipient)-MHC class II molecules. Following activation of ‘indirect’ T helper cells, allospecific effector cells may be stimulated in the same localized environment.

Contacting surface of the molecule, differences in the peptide-binding groove allow binding and display of different sets of peptides. The alloresponse is thus directed to the multiplicity of different peptides bound by the MHC molecule. When the exposed surfaces of the responder and stimulator MHC molecules are substantially different, the alternative, high determinant density hypothesis may provide a better explanation for the observed strength of the alloresponse. In order to reconcile this with self MHC restriction it only needs to be suggested that a small fraction of T cells whose receptors were selected for self MHC recognition cross-react, by chance, with a foreign MHC structure.

The distinction between antigenicity and immunogenicity

In the mid 1970s Bretscher and Cohn [2] first proposed the idea that lymphocyte activation required two qualitatively distinct signals. These ideas were translated into an in vivo model by Lafferty et al. [3] in a series of thyroid allograft experiments. They made the important observation that rat thyroid tissue that had been cultured for a period in vitro was not rejected, despite expressing the same alloantigens that led to the rejection of a fresh allograft. These studies were extended in a rat renal allograft model by Lechler and Batchelor [4], who observed that depletion of donor bone marrow-derived ‘passenger’ cells from an allograft led to its acceptance in some MHC-incompatible strain combinations, in the absence of any immunosuppression. In particular they showed that the specialized antigen-presenting cell population, dendritic cells, from the donor were central to the initiation of allograft rejection. These findings highlighted the fact that tissues could be antigenic (capable of being recognized by T cells) without being immunogenic (capable of initiating T cell activation). This is illustrated by the passenger cell-depleted kidney grafts that expressed abundant donor MHC alloantigens, but did not provoke a sufficient magnitude of response to induce rejection. The immunogenicity of a tissue appears to correlate with its content of specialized antigen-presenting cells.

Recognition without activation can lead to T cell tolerance; antigen presentation by tissue parenchymal cells

Bretscher and Cohn’s original two-signal model of B lymphocyte activation [2], was similarly predicted for T cells [5]. Signal 1 alone failing to activate should
negatively signal a lymphocyte and make it refractory to subsequent activation (anergic), whereas signal 2 should prevent the refractory state and lead to full activation. The delivery of signal 1 refers to the engagement of the antigen specific T cell receptor (TCR/CD3 complex) with allogeneic MHC or antigenic peptide plus self MHC. For full T cell activation and prevention of anergy a second signal must be supplied by the molecular interaction between members of the B7 family present on antigen presenting cells (APC) with their T cell ligand CD28 [6]. Successful orchestration of an alloimmune response depends upon the interaction of CD4\(^+\) helper cells with cells capable of supplying both signals. In man, only three populations of cells, dendritic cells, monocytes/macrophages, and B lymphocytes constitutively express B7 and MHC class II molecules. Parenchymal cells induced to express MHC class II molecules by pretreatment with IFN-\(\gamma\) do not support activation and may result in anergy of naive and in some but not necessarily all, primed CD4\(^+\) cells.

Relevance to T cells with direct allospecificity

In a rodent model, in vitro primed, donor-specific, direct pathway alloreactive T helper cells were shown to effect acute graft rejection when adoptively transferred into irradiated recipients that had been transplanted with an allogeneic kidney. Rejection occurred only in the presence of donor-derived dendritic cells [7]. However, in animals bearing an established graft, the renal parenchymal cells were unable to reactivate the alloreactive T cells. This indicates that whilst direct alloreactive T cells play a dominant role in acute rejection, T cell hyporesponsiveness can occur following dendritic cell loss.

Direct alloreactive CD4\(^+\) and CD8\(^+\) T cells are primed in the spleen and draining lymph nodes following the migration and maturation of donor dendritic cells [8]. Effector functions are carried out by CD8\(^+\) cytotoxic T lymphocytes (CTL) within the graft without necessarily further input from CD4\(^+\) Th cells. Th cells can initiate antibody production at sites distant from the allograft by allospecific B cells. Delayed-type hypersensitivity (DTH) responses may be orchestrated by Th cells which have infiltrated the graft having left the recipient lymphoid tissue. The eventual replacement of donor dendritic cells by recipient dendritic cells [9] and the apparent paucity of cells capable of stimulating direct allorecognition left within the graft is likely to account for the development of hyporesponsiveness in T cells with direct allospecificity.

Clinical data

In recipients receiving immunosuppression, episodes of acute rejection become less frequent and destructive with the passage of time following transplantation, whilst the progression of chronic rejection continues.

Recently in our own laboratory, limiting dilution techniques specific for the direct pathway have been utilized to estimate recipient:anti-donor Th and CTL frequencies in patients who have progressive chronic rejection. Using donor-derived splenic APCs, donor-specific hyporesponsiveness was observed against both donor MHC class I and II antigens in approximately 50% of recipients (Hornick et al. submitted). Similar findings have been reported for renal transplant recipients [10]. These findings are important since they indicate that in man, as in rodents, the prolonged residence of an allograft can induce donor-specific hyporesponsiveness in T cells with direct allospecificity in a proportion of patients. This has potential implications for graft monitoring, outcome, and adjustment of immunosuppression. These findings also suggest that chronic rejection can progress despite such hyporesponsiveness.

Chronic rejection appears to result from both immunological and non-immunological mechanisms. The importance of HLA matching, the influence of previous acute graft rejection, the occurrence in allogeneic as compared to syngeneic grafts, as well as more tangible experimental evidence [11–13], all points to a determining role for alloreactive T cells. Direct allorecognition is likely to play an important part in the initiation of chronic rejection as the early post-transplant period is dominated by tissue damage caused by episodes of acute rejection. However, chronic rejection may occur in the absence of previous episodes of acute rejection [14] and the observations of direct pathway hyporesponsiveness in patients with chronic rejection argues against this pathway as being a driving force in its progression.

The contribution of the indirect pathway to allograft rejection

Passenger cell-depleted rat kidney allografts. Evidence for a second route of allorecognition was provided by retransplantation experiments in the rat model described above. Lechler and Batchelor observed that MHC incompatible kidney allografts depleted of indigenous dendritic cells (by 'parking' kidneys in intermediate hosts) were permanently accepted without immunosuppression, in certain donor/recipient combinations, but in others suffered rejection [15]. Given that no immunogenic donor-derived cells were present in these retransplanted grafts, they proposed that rejection of these grafts resulted from the sensitization of T cells with indirect allospecificity. Indirect allorecognition mirrors the normal mechanism of T cell stimulation by nominal antigens. Alloantigens shed from a graft are internalized, processed, and presented as peptides in the context of recipient MHC class II molecules (Figure 1). Whilst T cells sensitized by the direct pathway might initially dominate the rejection process occurring in non-immune recipients, T cells sensitized by indirect allorecognition might contribute substantially to continuing graft damage after the allograft has lost its dendritic cell population [15].
In vivo—animal models

In a murine system, Benichou et al. [16] showed that T cells which had been sensitized by allogeneic splenocyte infusions or skin grafting proliferated to synthetic peptides derived from the polymorphic regions of the \( \alpha \) and \( \beta \) chains of the allogeneic class II MHC molecules presented by host APC. Peptide immunization of allogeneic MHC antigens has been shown to hasten the rate of graft rejection for skin and kidney allografts [17,18]. In another study using skin grafts from MHC class II-deficient knockout mice, rejection in a self-restricted CD4\(^+\) -dependent manner has been demonstrated [19]. Further importance of indirect alloresponses is suggested by the downregulation of T cell responses following thymic administration of allogeneic MHC-derived peptides, leading to prolonged survival of subsequent renal allografts. Such peptides could not have affected the direct pathway and suggests that indirect presentation is critical to the rejection process [20].

In vitro

The first demonstration utilizing human material that indirect mechanisms were capable of stimulating an alloresponse was provided by Liu et al. [21] utilizing an in vitro system whereby the responder's T cells were sensitized in mixed lymphocyte culture with allogeneic DR1-expressing cells. The frequency of T cells engaged in the indirect recognition of synthetic DR1 peptides, was found to be about 100-fold lower than T cells participating in direct recognition of intact DR1 molecules. Such data does not necessarily imply dominance of direct pathway mechanisms, as there is the potential for the generation of a multiplicity of epitopes derived from MHC molecules, particularly when several MHC incompatibilities are expressed by the stimulator cells. The strength of the indirect response would therefore be the sum of all indirect frequencies estimated for each peptide epitope. It thus becomes clear that a failure to demonstrate an indirect alloreactive frequency might reflect the insensitivity of the assay system and the methodology used [22]. This challenges the precept that direct allorecognition dominates the rejection process in its early stages just because high precursor frequencies are produced in vitro. The precise cellular origins of donor antigens are likely to be of little significance, as donor antigens are processed and presented by recipient APC, the important factor being the quantity of donor antigen. Class I MHC antigens may be of greater importance than class II antigens, being expressed in all cells of a tissue and thus more common in the long-term life-span of the allograft [23].

Influence of HLA class II matching

As discussed above, the most efficient 'indirect' presentation occurs when the MHC alloantigen is co-expressed with the responder MHC class II restriction element on the same cell surface. Precisely this situation arises when an allograft is matched for one or more MHC class II alleles. Whether this exaggerates or diminishes the indirect response in vivo is a matter of conjecture. It can be assumed that class II matching will favour the sensitization of indirect pathway T cells by donor dendritic cells. However, it can also be argued that class II matching will favour the induction of tolerance in T cells with indirect allospecificity due to the effects of indirect presentation by MHC class II-expressing graft parenchymal cells that lack expression of costimulatory molecules. HLA class II matching has been reported to be associated with an increased incidence of rejection of corneal allografts, which are known to be rejected by predominantly indirect pathway T cells. In solid organ transplantation it is difficult to separate the beneficial effects of class II matching due to reducing the activity of direct pathway T cells, from the possible beneficial effects of indirect presentation by parenchymal cells.

Sites of sensitization of indirect pathway T cells; co-operation with effector mechanisms of graft rejection

The delivery of T cell help requires physical proximity between the Th cell and the CTL or B cell. This usually occurs in the environment of the lymph node, following migration of dendritic cells carrying antigens that they have internalized in tissues, and that they present as peptides bound by MHC class I and class II molecules. This allows for Th and CTL to interact with the same APC surface, facilitating the delivery of T cell help at very close range. This kind of cell:cell collaboration has been described as a three cell cluster [24]. For sensitization of T cells with direct allospecificity, this is easy to envisage. However, assuming that either CTL, or delayed-type hypersensitivity (DTH) mechanisms are involved in effecting chronic rejection, it is necessary to envisage the delivery of T cell help occurring in the graft itself. Only here are cells expressing donor MHC alloantigens available, once the initial wave of emigrating donor dendritic cells has passed.

Indirect pathway in the human

Recent data derived in the context of acute rejection in recipients of heart grafts, utilizing synthetic peptides corresponding to the hypervariable regions of the mismatched donor HLA-DR antigens, have indicated an association between acute rejection and activation of the indirect pathway [25].

All evidence indicates that indirect allorecognition is important in the progression of the chronic rejection process; however, to-date there is no direct experimental evidence that Th cells with indirect specificity are functionally active in recipients with chronic rejection. Humoral mechanisms have long been thought to make important contributions to chronic rejection [26–28]. Although anti-donor alloantibodies may effect the graft directly, it is also possible that the allospecific B cells, with their enhanced capacity to capture and process alloantigens, are responsible for driving indirect pathway T cells.
Tolerance and immunosuppression

Divergent requirements for direct and indirect allorecognition activation and regulation indicate that there may be different susceptibilities to immunosuppression. Early experiments in the rat indicated that immunosuppressive protocols were more efficient at preventing rejection when dendritic cells had been depleted from the graft [4], but chronic rejection in humans appears unaffected by immunosuppression. This may indicate that T cells with indirect allospecificity may be resistant to cyclosporin A, possibly reflecting the continuous activity of recipient APC in stimulating such cells.

The acquisition of insights into the events that lead to T cell tolerance, and to the emergence of regulatory, rather than proinflammatory, T cells offers exciting possibilities for the future. Of considerable interest is the role played by Th2 cells and their signature cytokines which suppress Th1 function. Although no data exists in the human transplant recipient, it is tempting to speculate that routine immunosuppression does allow an ascendency of both direct and indirect allospecific Th2 cells. Once the frequency of alloreactive Th2 cells can be assessed in transplant recipients it may be possible to further develop strategies to induce a ‘self-reinforcing’ immune deviation of cytokine responses. Further attractive strategies include recipient pretreatment strategies using cells expressing donor alloantigens or peptides corresponding to donor MHC sequences coupled with antibodies or fusion proteins designed to interfere with the delivery of costimulation. If the hypothesis that T cells with indirect allospecificity play a key role in chronic rejection is confirmed, strategies to induce tolerance in such cells will be critical to long-term allograft survival.

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References


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Introduction

Bone mineral density (BMD) has an important genetic component [1,2]. In 1994 Morrison et al. [3] reported that polymorphisms at the 3′UT end of the vitamin D receptor (VDR) gene defined by the restriction enzymes Bsm I, Apa I, and Taq I are associated with the BMD. In the most widely used Bsm I polymorphism, the presence or absence of a restriction site in the 7th intron of the gene, yields the so-called ‘b’ and ‘B’ alleles respectively. Individuals with the ‘BB’ genotype show a higher bone turnover assessed by osteocalcin levels, and a lower BMD at the spine and femoral neck than ‘bb’ homozygotes. Heterozygotes ‘Bb’ individuals range in between [3]. Since the initial report of an association of VDR polymorphisms with BMD, many additional studies on various populations have reached divergent conclusions, with some investigators finding an association [4] while others not [5]. In studies showing an association, the magnitude of the BMD difference between genotypes was less than originally suggested, usually closer to one-half of a standard deviation in BMD [6] rather than a full standard deviation as originally proposed [3]. A recent meta-analysis including 16 studies from Australia, Europe, United States (African-Americans and whites) and Japan, found evidence favouring a modest effect of the VDR genotype on the BMD at the hip, spine, and radius, with a reduction in BMD of about 2% in ‘BB’ as compared to ‘bb’ genotype groups [7]. Thus, VDR polymorphisms represent one genetic factor affecting BMD but they only partly account for the genetic effect on bone mass.

Calcium intake has been shown to influence the genetic effect of the VDR polymorphism on BMD and bone turnover. Krall et al. [8] observed that the rate of bone loss was higher in ‘BB’ than in ‘bb’ postmenopausal women only when calcium intake was low (<500 mg/day). Moreover, in elderly women Ferrari et al. [9] showed that the bone loss at the lumbar spine and the calcium intake were correlated only in the heterozygous ‘Bb’ genotype. Finally, Dawson-Hughes et al. [10] demonstrated a lower intestinal fractional calcium absorption in ‘BB’ than in ‘bb’ individuals after dietary calcium restriction, a situation in which intestinal calcium transport is more dependent on vitamin D; this finding suggests functional differences at the intestinal VDR protein among the various VDR genotypes.

Age may also interact with the genetic effect of the VDR polymorphisms on the BMD. Riggs et al. [11] observed a much stronger genotype effect among younger than among older women at the hip, but there was no statistically significant genotype-age interaction at the spine. A similar finding has been reported in the meta-analysis study of Cooper and Umbach [7]. If this interaction is confirmed, the magnitude of the effect at younger ages is greater than the overall estimate indicates.

A T/C polymorphism at the first of the two potential translation initiation sites (ATG) of the VDR gene has been described [12]. Using PCR and the FokI restriction enzyme, the presence of the FokI site denoted by ‘I’, indicates that the first ATG is present predicting a longer VDR protein than in ‘F’ individuals where the first ATG is absent. Gross et al. [13] observed that the ‘ff’ genotype was associated with decreased BMD at the spine and with an increased rate of bone loss at the hip in postmenopausal Mexican-American women. This initial finding warrants further study in larger populations with subjects of diverse ethnic background.

VDR polymorphisms represent one genetic factor affecting BMD but current evidence suggests that the inheritance of bone mass is under polygenic control [14]. Type I collagen is the major protein of bone encoded by the COLIA1 and COLIA2 genes. A novel G→T polymorphism in a regulatory region of COLIA1 at a recognition site for the transcription factor Sp1 has recently been associated with bone mass and osteoporotic fracture in two populations of British women [14]. The unfavourable ‘Ss’ and ‘ss’ genotypes were ‘over-represented’ in patients with severe osteoporosis and vertebral fractures.

In summary, at least two candidate genes, namely VDR and COLIA1, have been implicated in the regulation of bone mass although the molecular mechanisms underlying this association remain to be defined. In addition, the interaction between these and other potential genes, as well as the interaction with environmental factors such as calcium intake, are not fully understood. Current efforts to elucidate these interactions might provide an useful model applicable to the analysis of other common diseases under polygenic control such as hypertension or diabetes.

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VDR polymorphisms in renal patients

The complex calcitriol-VDR regulates parathyroid cell proliferation and PTH synthesis [15,16]. The ligand-receptor complex binds to specific promoter regions of the PTH gene inhibiting transcription [15]. In a recent study by Carling et al. [17] the incidence of the ‘bb’ genotype was higher in 90 Swedish postmenopausal primary hyperparathyroidism patients than in the control population (60 vs 33.3%). As the ‘b’ allele has been linked with a decreased transcriptional activity or mRNA stability in a minigen preparation [3], reduced VDR expression in the parathyroids of ‘bb’ patients may hamper regulatory actions of calcitriol and may contribute to parathyroid tumorigenesis in these patients.

Interestingly, Tsukamoto et al. [18] and Fernández et al. [19] have analysed the frequency of the VDR polymorphism in two large haemodialysis populations. Both studies observed higher PTH levels in ‘bb’ compared to ‘BB’ patients, suggesting that VDR variants may be contributing factors to the development of parathyroid hyperplasia. In addition, ‘bb’ patients on dialysis may be protected against adynamic bone disease.

Renal transplant patients immunosuppressed with CsA, exhibit both a significant bone loss [20] and an increased rate of bone fractures [21]. We have recently analysed the influence of the VDR genotype in the pattern of bone loss after renal transplantation in 34 non-diabetic patients immunosuppressed with prednisone + CsA [22]. During the first 3 months there was a rapid decrease in BMD which was directly correlated with the magnitude of pretransplant secondary hyperparathyroidism. No influence of VDR polymorphism was observed during this period. By contrast, a significant increase of BMD between 3 and 12 months after transplantation was observed only in ‘bb’ patients, and their total bone loss and final bone turnover were significantly lower than ‘Bb’ or ‘BB’ patients. This different pattern of bone loss was independent of the prevailing PTH levels. The effect of VDR genotype on BMD and bone turnover may become more evident in patients challenged under conditions leading to bone mass loss as is the case of renal transplantation.

In fact, corticosteroids, which decrease intestinal calcium absorption, and CsA, which may increase bone turnover, are widely used in renal transplant patients.

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References

Target haematocrit during erythropoietin therapy

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Introduction

Neophyte renal physicians and nurses working in dialysis centres in 1997 may not realize what is missing when they look around. Prior to the availability of recombinant human erythropoietin (rHuEpo) therapy in 1989 it was typical to see several units of blood infusing during any shift of patients. Nowadays, transfusions are the exception, rather than the rule. In the US multicentre licensing trial of rHuEpo for 333 anaemic patients with end-stage renal disease (ESRD), erythrocyte transfusions were eliminated within 2 months of therapy, whereas 1030 transfusions had been administered within the 6 months before rHuEpo therapy [1]. In that trial the target haematocrit was 35 ± 3%. Patients experienced significant improvements in quality of life (particularly related to energy and activity level), and the incidence of vascular access thrombosis did not differ from a large cohort of patients not treated with rHuEpo. Blood pressure was noted to increase, or control to worsen requiring additional medications, in 35% of patients. In the 8 years that have followed this seminal report, many studies have confirmed these findings. In addition, several investigators have examined outcomes and organ system effects of various target haematocrits achieved with rHuEpo. Questions still remain, however, regarding the optimal haematocrit, including whether normalization of haematocrit would be beneficial and safe. In addition, certain subpopulations (e.g. the elderly, diabetics, or those with cardiovascular disease) may have different haematocrit requirements because of critical differences in end-organ oxygen needs. Finally, it is clear that various end-organs respond differently to amelioration of anaemia, further confounding the search for the optimal haematocrit. What is a desirable haematocrit for the brain may be less so for the heart or the dialysis vascular access.

What target haematocrit during rHuEpo therapy optimizes cardiovascular function?

The optimal haematocrit during rHuEpo therapy in ESRD patients should be one that maximizes survival, cardiovascular function, and activities of daily life, while minimizing risk. Cardiac disorders account for about 40% of deaths in ESRD patients [2,3]. Uncorrected anaemia produces a hyperdynamic state that contributes to the development of left ventricular hypertrophy (LVH) [4]. Correction of anaemia would be expected to remove any anaemic component contributing to eccentric LVH and left ventricular dilatation, while leaving unaffected contributions from fibrosis. Anaemia also limits myocardial oxygen supply, particularly in those with coronary artery disease, provoking angina pectoris or other ischaemic events. It is thus not surprising that anaemia along with hypertension contributes to left ventricular dysfunction, and the development of congestive heart failure [5]. Each decrease in haemoglobin of 1 g/dl increases mortality by 18% in dialysis patients [6].

Although correction of the anaemia associated with renal disease with rHuEpo produces a number of significant benefits, it is the effects on the cardiovascular system and on physical activity which determine much of the risk benefit ratio. During sustained rHuEpo therapy producing partial correction of anaemia, incomplete reductions in LVH and volume occur, as measured by echocardiography. The mean decrease in left ventricular mass (LVM) among 15 studies averaged 18% after a mean treatment time of 45 weeks as haematocrit was corrected from 18–22% to 29–35% [7]. Complete normalization of LVM rarely occurs, reflecting perhaps the residual effect of incomplete anaemia correction, access fistula–shunt flow, and myocardial fibrosis. Improvement in exercise-induced ST-segment depression following partial anaemia correction can also be demonstrated [8]. On the negative side, a hypertensinogenic effect of rHuEpo therapy occurs in renal failure patients, probably due to a loss of anaemic vasodilatation, with a reported increase in incidence over placebo-treated patients of 6–20% [7]. In certain subsets of patients, worsening of LVH can develop, particularly in those who develop hypertension [9].

Initial positive reports of a decrease in cardiovascular events in small numbers of rHuEpo-treated patients are now being reported based on larger populations [6]. Treatment with rHuEpo tends to decrease overall hospitalization [10]. Total costs of care for ESRD patients may also be reduced in the long term through the use of rHuEpo [11]. The positive results obtained to date from rHuEpo therapy have in general been...
achieved with haematocrits of 30–36%. Some studies have suggested that raising the haematocrit further to 40–44% increases benefit without increasing risk [12]. On the other hand, the Normal Hematocrit Cardiac Trial (NHCT), designed to examine the effects of normalizing haematocrit in haemodialysis patients with significant cardiac disease, was halted when the group randomized to the normal haematocrit had an increased incidence of death or non-fatal myocardial infarction (Goodkin, D., personal communication). Thus in the absence of clinical studies which define a sustained dose–response effect of haematocrit of 30–45% on hard end-points such as LVM, cardiac morbidity, mortality, and exercise capacity, the target haematocrit in patients with cardiac disease should be individualized between 30 and 38%.

**Does the level of haematocrit impact on quality of life, cognitive function, or brain function?**

Following the earliest reports of the efficacy of rHuEpo in improving anaemia, and decreasing transfusion requirements, it became apparent that many of the symptoms reported by ESRD patients, and attributed to uraemia, were in fact related to anaemia. Weakness, difficulty concentrating, fatigue, impaired sexual functioning, and poor exercise tolerance were a few of the symptoms that improved when the haematocrit was raised to 32–38% in the US multicenter trial [13]. Similar findings were reported from Canada, Europe, and elsewhere. More recently a phase IV study of over 1000 patients receiving rHuEpo in regular clinical practice showed a significant improvement in quality of life when even a modest increase of haematocrit to 30.1% was achieved [14]. Four of six domains of the Short Form-36 quality of life questionnaire showed improvement: vitality, which improved to the greatest extent, physical functioning, social functioning, and mental health. In addition, the Canadian Erythropoietin Study Group reported improvement in quality of life when haemoglobin was raised to 10.2 g/dl, but no further improvement in a group of patients with haemoglobin raised to 11.7 g/dl [15]. The study results were confounded, however, since the baseline physiological tests in the group randomized to the higher target haemoglobin were quite high, making it unlikely that any improvement could be demonstrated. By contrast, Eschbach et al. have demonstrated an improvement in quality of life and functional capacity when haematocrit is raised to 42% [12], and Moreno et al. have shown a direct relationship between the score on the Karnofsky functional scale and Sickness Impact Profile, and level of haematocrit, at haematocrits ranging from 29% to over 35% [16].

Cognitive function and brain electrophysiology are highly sensitive to the level of haematocrit, and improve when the haematocrit is raised from the mid-twenties to 32–36% [17]. More recent studies suggest that raising the haematocrit to 42% from the commonly achieved target of 30% significantly improves brain function [18]. These findings are consistent with studies that demonstrate a relationship between haematocrit and oxygen delivery to brain tissue, with maximal level of oxygen delivery occurring within a haematocrit range of 40–45% [17]. It has recently been reported that neuronal cells carry the Epo receptor, and that Epo may be produced in brain and function in a paracrine fashion to protect against hypoxia-induced damage of neurons [20]. Whether exogenously administered rHuEpo is able to reach such receptors and also affect brain function independent of haematocrit level is an intriguing possibility. At first glance this would seem unlikely, since the molecular weight of rHuEpo (30 400 Daltons) far exceeds the molecular weight cut-off for the normal blood-brain barrier (500–750 Daltons). Uraemia as well as chronic hepatic disease is associated with significant alterations in the blood–brain barrier, however, at least for small solutes such as amino acids. Whether this would apply to larger substances such as rHuEpo is worthy of study.

**What is the relationship between haematocrit achieved with rHuEpo and exercise capacity/muscle function?**

Patients with renal failure demonstrate depressed exercise capacity compared to age-matched controls, and have decreased muscle strength and endurance. This is associated with low muscle mass and energy stores, and histological abnormalities [21]. Resting and exercise blood lactate levels are elevated, and oxygen consumption by muscle is decreased [22]. Anaerobic metabolism plays a more prominent role than in comparably anaemic controls. The ratio of phosphocreatine to inorganic phosphate is lower in ESRD patients, supporting decreased oxidative metabolism [23]. The aetiology of these alterations is unknown, but could be consequent to enhanced activity of the ubiquitin–proteasome pathway as reported in uraemia, acidosis, from cytokines released during dialysis, and infections [24]. Other factors such as poor nutrition, diabetic neuromuscular pathology, and physical inactivity may be involved.

An increase in haematocrit to the 30–36% range is associated with improvements in the parameters noted above. Cardiovascular function, oxygen transport, and exercise capacity are all augmented. Maximum voluntary contraction, force generation, and duration of muscle contraction all improve. Histological improvement in architecture and muscle fibre diameter have been reported. Resting and maximal exertion-induced blood lactate improve, as well as the ratio of phosphocreatine to inorganic phosphate, suggesting improved aerobic metabolism [22,23]. This is also reflected by increased oxygen consumption. The increases in maximal oxygen uptake per increment in haemoglobin in ESRD patients, however, are only half of those observed in subjects without renal disease [25], perhaps reflecting lower aerobic capacity in renal failure.
patients due to habitual physical inactivity or fixed limitations of oxygen extraction by diseased muscle.

It seems likely that a multifaceted approach will be needed to optimize exercise capacity and muscle function in ESRD patients. This includes normalization of acid–base balance, adequate nutrition, and a formal exercise programme. The optimal haematocrit for exercise efficiency and muscle function is at least 36% based on current evidence [26]. Lim [27] reported greater improvements of energy and work capacity in those whose anaemia was more completely corrected (haematocrit 35–40%) than in those only partially corrected. In addition, Barany et al. [28] found that exercise capacity did increase further with normalization of haemoglobin, but that the increase remained subnormal compared to rHuEpo-treated non-uraemic subjects.

Improvement in dialysis techniques are needed to decrease situations likely to contribute to poor exercise performance such as infection, under-dialysis, and depression. Additional research is needed in larger numbers of patients (1) to correlate functional, physical/psychological and histological parameters, (2) to optimize the beneficial effects of rHuEpo-induced rises in haematocrit, and (3) to better understand the complex interplay of uremic factors in the pathogenesis of the impaired exercise capacity and muscle dysfunction observed in these patients.

**Does haematocrit affect vascular access function?**

Our understanding of the relationship between haematocrit, rHuEpo dose, and vascular access patency is in evolution. In the US phase III multicentre trial, haematocrits increased from 22 to 32–38% on rHuEpo therapy, and the rate of vascular access thrombosis was lower than the rate seen among a large cohort of haemodialysis patients who did not receive rHuEpo [1]. However, similar increases in haematocrit through the use of rHuEpo in the Canadian multicentre trial showed a trend, although not statistically significant, toward increased access thrombosis [29]. When haematocrit was increased toward a target of 42% from an entry target of 30% in the NHCT involving some 1200 patients, a statistically significant increase in access thrombosis was noted (Goodkin D., personal communication). The reason for the increase in arteriovenous (AV) access thrombosis is unclear, as thrombosis did not correlate with haematocrit level. These observations are further limited by the fact that the patient population in the NHCT was older than the average US dialysis population, had major cardiovascular risk factors, and had a predominance of AV grafts. The mechanism of the increased rate of thrombosis is unknown. Possible contributing factors worthy of further study include improved bleeding times, potential erythropoietin activity as a mitogen for fibromuscular hyperplasia at the vein–graft anastomosis site, and hypotension in patients with severe left ventricular dysfunction. However, there does not appear to be an increase in access thrombosis when haematocrits are kept in the range of 30–36%.

**Conclusions**

When the initial phase III trials of rHuEpo were being planned, the haematologists involved recommended a target haematocrit in the normal range, while the nephrologists were concerned about adverse vascular effects at normal haematocrit levels. A compromise target of $35\pm3\%$ was selected for the clinical trials. When rHuEpo was approved by the FDA in the US, however, a target of 30–33% was delineated, for unclear reasons. There is still a widespread perception around the world that 30% is an adequate haematocrit target, maximizing benefits and minimizing complications, although some investigators recently suggested that a target haematocrit of 34–37% 'seems reasonable' [30]. A number of studies have since been published in abstract form, some referenced in this brief editorial, that indicate that quality of life, cardiac output, cognitive function and brain electrophysiology, amino-acid levels, maximum exercise capacity, sleep dysfunction, insulin sensitivity, and survival improve as the haematocrit rises toward the normal range. The real challenge is to determine the optimal haematocrit for a specific patient, that maximizes organ function, but does not cause adverse effects. The early termination of the NHCT suggests that a thorough knowledge of the physiological effects of higher haematocrits, coupled with an understanding of the pathophysiology of the organ abnormalities in individual patients is crucial if we are to achieve our goals of improved survival, decreased morbidity, and enhanced quality of life for ESRD patients. The currently available data suggest that no patient should have an haematocrit below 30%. Based on the beneficial effects on the cardiovascular system of partial correction of anaemia, as well as the improvements in quality of life, exercise capacity, and other organ function outlined in this review, we should not remain complacent by accepting haematocrits barely at 30% in any ESRD patient, but should strive to achieve haematocrits in the entire dialysis population of 33–36%. In order to achieve this goal, the haematocrit in some patients may at times rise above 36%. On balance, the benefits of maintaining the haematocrit above 30% at all times in all patients outweigh the possible risks of exceeding an haematocrit of 36% for short periods of time in some patients. Until the benefits and safety of a near-normal haematocrit have been clearly demonstrated, it is not recommended that it be maintained above 38%.

**References**

Introduction

A study evaluating patient survival in Lombardy, Italy, and in the United States has recently been published [1] using data taken from two registries, the Lombardy Dialysis and Transplant Registry (RLDT) [2] and the US Renal Data System (USRDS) [3]. The relative risk of mortality for the haemodialysis patients treated in Lombardy was 36% lower than that of the patients treated in the US. This difference was even greater in the 20–60-year-old age group. However, in the patients receiving peritoneal dialysis who were less than 60

Dialysis patient outcomes in Europe vs the USA. Why do Europeans live longer?

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years old, the relative risk of mortality was not statistically different.

**Country differences in dialysis treatments with mortality**

It is well known that haemodialysis patients in the US run a higher risk of death than those treated in Japan and Europe [4,5]; furthermore, patients receiving peritoneal dialysis in the US are at higher risk of death than those treated in Japan [5] and Canada [6]. However, the comparison of dialysis patient survival between Lombardy and the United States [1] is of particular interest because it is not only adjusted for age, diabetes, and gender, but also for differences in a number of comorbid conditions.

**Factors possibly involved in the different survival rates**

Registry data should be used with caution when comparing dialysis patient survival because they may be affected by country differences in dialysis procedures or other factors. These differences may include the criteria for admission to renal replacement therapy (patient selection and the time of starting dialysis), gender and age distribution, baseline comorbid conditions, and the withdrawal rate due to kidney transplant; furthermore, patient compliance with different modalities of treatment may also differ between countries.

**Life expectancy of the general population**

The comparability of life expectancy in the general population must be evaluated: the 1988 life expectancy at birth in North America and Europe was similar (respectively 71 and 70 years for males, and 78 and 77 years for females), but was greater in Japan (75 years for males and 81 years for females). However, the relationship between the life expectancy of the general ‘healthy’ population and that of end-stage renal disease (ESRD) patients on dialysis is debatable.

**Admission to renal replacement therapy**

The selection criteria for admission to renal replacement therapy (RRT), access to dialysis facilities, and the registration of treated ESRD patients should also be considered. The rate of acceptance of ESRD patients for RRT varies widely in different countries: in 1992, 51 new patients per million of the population were recorded by EDTA, 98 p.m.p. by the Canadian Organ Replacement Register, 214 p.m.p. by the US Renal Data System, and 181 p.m.p. by the Japanese Registry [7]. The acceptance rate also varies from one European country to another: it is less than 40 p.m.p. in the countries of Northern Europe, and respectively 102 and 110 p.m.p. in Lombardy, Italy, and Austria [7].

The EDTA estimates may be less reliable because they depend on voluntary reporting, but all of the 44 dialysis centres in Lombardy supply data to the Lombardy Registry [2]. Treatment acceptance rates in the US have been running consistently above those in other countries and the proportion of elderly patients with diabetic and hypertensive ESRD is much higher.

One reason for the higher acceptance rate of elderly and diabetic patients in the US may be due to differences in the hidden selection criteria for admitting them to substitutive treatments on the part of nephrologists, and/or differences in the habits of general practitioners in referring patients with chronic renal failure to renal units regardless of their age or clinical condition. This may indicate that US nephrologists are more willing to give RRT to high-risk patients than their European counterparts, and so it is likely that frailer and sicker patients are accepted in the US.

The inclusion of comorbid conditions in the comparative analysis of Lombardy and the US reduced but may not have completely eliminated this selection bias and the observed difference in outcomes, since it has been hypothesized that patients living in a country with a higher acceptance rate are more likely to be affected by both the presence of comorbid conditions and greater disease severity.

In this respect, Friedman’s opinion is very provocative [8]. His suggestion is that the difference between the US and Europe in the ESRD treatment rate may be due to the fact that the rate of kidney failure is higher in the US and/or that the US inappropriately treat patients who ought to be left to die or who are not in renal failure and/or that Europe undertreats renal failure and thus allows a considerable percentage of uraemic patients to die because they are not given dialysis treatments [8]. Friedman’s final conclusion [8] is that ‘liberalizing European ESRD acceptance rates to equal those of the US, by including older and sicker patients would, I am certain, bring survival figures closer to those in America’.

It is possible that the number of treated ESRD patients dying during the first few weeks of RRT are underreported to some degree, but it is important to underline the fact that both the USRDS and RLDT prospectively collect the data relating to each new ESRD patient at baseline, and the fact that the evaluation of survival was started on day 30 partly overcomes this possible bias. When the same analysis was repeated (excluding deaths) for the first 90 and 180 days, the results of the comparison were not substantially different.

**Transplantation rate**

The use and selection of alternative ESRD treatment modalities, particularly the difference in the rate of transplantation among countries, could affect the results of these comparisons. It is well known that the patients chosen for transplantation are those with a long potential survival, leaving those with a
comparatively poor prognosis on dialysis. The ratio between the number of patients transplanted per year and those starting ESRD therapy varies widely from country to country ranging from 3% in Japan [9] to 25–29% in the US [10] and in Canada [11]; it also varies across Europe, currently it is 21% in Lombardy [2]. Furthermore, the relative risk of death is also higher in older patients who are less likely to be transplanted.

Treatment modality, adequacy, compliance, reuse, time of starting dialysis

When analysed according to dialytic modality, the relative risk of death between the Lombardy and the US Registries was significantly different only in the case of patients treated by haemodialysis aged less than 60 years. Peritoneal dialysis was a fairly uniform prescription in the late 1980s, with the great majority of the patients being treated with continuous ambulatory peritoneal dialysis with four exchanges of 2 litres per day. Moreover, the effect of residual renal function on patient survival is well recognized, and it is probable that this is greater than that of the total weekly Kt/V.

As far as haemodialysis is concerned, a large number of studies have shown that the level of the delivered dose of dialysis is closely associated with patient survival [12]. The dialysers used in Europe have a 20% larger surface area than those used in the US, and the duration of haemodialysis treatment is 23.5% longer in European patients [13]; in Japan the average duration of dialysis treatment is roughly 40% longer than in the US [9–13], and reimbursement varies, on the basis of its duration.

It is well known that the duration of haemodialysis is often reduced at the patients’ request, but the main reason is to cut the cost of the attending personnel. As a result, the treatment time is often no longer adequate from the depurative and cardiovascular points of view, and this increases the incidence of intradialytic hypotension and interdialytic hypertension. Hakim et al. [14] have reported a significant reduction in the mortality rate of US patients over the last few years, associated with an increase in Kt/V; and Held et al. [12] found an inverse relationship between Kt/V and mortality up to a Kt/V of 1.4 (an approximate log. linear relationship between decreasing mortality and increasing dialysis dose, with a risk of all cause mortality, that was 8% lower for each 0.1 increase in Kt/V from 0.8 to 1.4). However, lower doses of dialysis do not affect mortality as a result of one isolated cause, but rather as a result of a number of the major causes of death in the dialysis population.

The greater risk of mortality of younger haemodialysis patients in the US in comparison with Lombardy may be related to the wide range of dialysis doses (adequacy), which depend on the length of the dialysis session, blood flow, the surface and type of membrane, and the reuse of dialysers. The common use of unmodified cellulose membranes [15] and reused dialysers may contribute towards the higher risk of mortality in US patients [16], although the association between mortality and reuse overall was not particularly great.

Doctor/patient time

We need to put the time that doctors, nurses, and social workers spend taking care of the clinical and social needs of their patients in the Cox Model! A better relationship between doctors (and nurses) and patients could also improve patient compliance to dialytic treatment (in terms of duration and frequency), diet, and drugs. Intradialytic weight gain, and serum potassium and phosphate levels are also clear markers of compliance.

Family and social support

A very important point to take into account is the social context in which dialysis treatment is delivered. It is well known that the family and social support is extremely important: it greatly affects the quality of life of the patients in terms of vital needs (nutritional, motor, and mental rehabilitation) and transportation to and from the dialysis unit, the frequent smouldering depression and compliance to treatments’, diet, and drugs. In general, the familial and social support improves patients’ approach to life and therefore also to dialysis. However technologically advanced, no procedure can succeed unless it is performed in the context of humanized health care directed towards patient needs.

Conclusions

Any comparison involving different Registries must be interpreted with great caution because unmeasured differences in the characteristics of patients may have played a role.

There is evidence of a greater death rate among white US than Lombardy ESRD patients, even after having adjusted for age, gender, renal disease, and comorbid conditions. The relative death risk was also higher in older patients less likely to be transplanted. The higher death risk of US dialysis patients may be related to differences in the quality of haemodialysis therapy, since these differences were significant only for haemodialysis patients. The lower risk of mortality of haemodialysis patients in Lombardy may also be due to the presence of less severe disease, a higher dialysis dose, and the fact that dialysers are not reused. Clearly additional factors are also likely to play a part.

For international studies, the development of common standard data collection instruments are needed in all ESRD Registries, including the recording of comorbid conditions and their severity. However, we must also bear in mind patient characteristics. General medical care is a very strong determinant of

References


Patient outcome not only in the conservative phase of treatment of patients with chronic renal insufficiency [17], but also in the substitutive programme.