Editorial Comments

What have we learned from the HOT (Hypertension Optimal Treatment) study?

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The Hypertension Optimal Treatment (HOT) Study was initiated in 1992 and tries to address two major issues in essential hypertension. The first is to establish the optimal therapeutic regime it is perfectly possible to lower blood pressure in the great majority of the hypertensive population. However, in order to obtain an adequate control of blood pressure, the percentage of patients needing the combination of two or more drugs increases to more than 60% [4].

The HOT Study was designed to evaluate the effect of sex, the presence or absence of other associated risk factors, and of the fact that the patient was or was not receiving previous antihypertensive therapy [4]. Furthermore, the percentage of patients attaining the expected level of control for diastolic blood pressure was significantly higher in elderly (above 65 years of age) than in younger (ages between 50 and 64) hypertensives [4]. Another interesting aspect of this study is the higher percentage of patients attaining the lowest level of diastolic blood pressure in United States as compared to Europe [7].

The drop in blood pressure was independent of age, sex, the presence or absence of other associated risk factors, and of the fact that the patient was or was not receiving previous antihypertensive therapy [4]. Furthermore, the percentage of patients attaining the expected level of control for diastolic blood pressure was significantly higher in elderly (above 65 years of age) than in younger (ages between 50 and 64) hypertensives [4]. Another interesting aspect of this study is the higher percentage of patients attaining the lowest level of diastolic blood pressure in United States as compared to Europe [7].

The data obtained in the HOT Study show that the presence of an elevated serum creatinine does not impede the expected fall in blood pressure in order to attain the goal of control. However, they also show that in the presence of an elevated serum creatinine (>1.5 mg/dl, n=468), the number of drugs needed to obtain that control is significantly higher [8].

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In summary, this trial has clearly shown that it is possible to obtain an adequate control of diastolic blood pressure in the great majority of the hypertensive population. Furthermore, renal function does not influence this possibility, although combined therapy seems to be particularly prevalent in the presence of renal failure.

References

Comprehensive HLA matching

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Optimal use of HLA typing for kidney transplant selection is still a matter of discussion. Analysis of data gathered by large registries such as the UCLA Tissue Typing Laboratory [1], Eurotransplant [2], and the Collaborative Transplant Study [3] have repeatedly confirmed the beneficial effect on graft survival of matching between donor and recipient. Taking into consideration antigens belonging to the HLA-A, -B and -DR loci, it is clear that the zero ABDR mismatch (MM) provides the best survival rates as compared to other configurations. However, one of the features of the HLA system is its enormous polymorphism. The consequence is that even in very large organizations possessing a significant pool of donors and recipients, these optimal matches can be provided only for 10 [1] to 22% [2] of the patients. For the others, only partially matched organs can be given. One of the enigmas of transplantation immunology is the existence in these patients bearing a mismatched kidney of a subset of subjects displaying an unexpected prolonged graft survival. The role of the HLA system in this paradox has never been clearly understood.

A method that has been used to clarify this issue was to individualize specific antigens in the recipient that conferred either a good or a bad prognosis to the graft and reflecting a state of responsiveness of the recipient toward his graft. Similar analysis performed in the donor was considered to reflect either a peculiar immunogenicity of the graft or its capacity to generate suppressor mechanisms. As a marker of non-acceptance in the recipient while of good prognosis in the donor, the influence of the DR6 antigen, originally described by the group of Leiden, became the illustration of this paradigm [2]. In our centre, the beneficial influence of the DR5 both in the donor and the recipient was found [4]. Subsequently, many studies reported the impact of several antigens on graft survival. The presence of an immunogenic HLA molecule on donor cells in the context of the HLA type of the recipient constituted the basis of the taboo concept [2]. This led workers to conceive that some combinations were detrimental, as opposed to others which were non-immunogenic and thus permissible [5]. The obvious consequence of these observations was the possibility of offering partially matched organs to an additional set of patients without jeopardizing the graft. However, prospective applications for matching in clinical transplantation was difficult in view of the lack of a clear definition of the molecular basis standing behind this state of unresponsiveness.

An opening came from investigations made to widen the scope of permissible mismatches by reducing the HLA polymorphism. One of the approaches [5] was to define histocompatibility in terms of public determinants by applying results from studies of class I HLA antibodies directed toward multiple specificities.

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and defining CREGs (cross reacting groups). The analysis of the primary sequence of each antigen belonging to these CREGs displays the existence of residues specific for each of them. These sequences represent highly conserved regions of HLA class I antigens. In order to reduce DR antigens, public determinants represented by supertypic antigens DR52, DR53, and DR51 encoded by DRB3, DRB4, and DRB5 genes respectively were used. Surprisingly, a retrospective study showed that matching using these simplified determinants yielded results comparable to those obtained by using conventional antigens [1]. Thus minimization of the HLA polymorphism by this approach was clinically relevant and could lead to a considerable increase of the number of patients that could benefit from a suitable transplant.

Do receptors for these simplified determinants exist? What could be the intracellular mechanisms that are triggered by them? These questions can now be addressed in the light of the recent description of various inhibitory processes potentially involved in allorecognition. Despite the fact that they do not yet provide practical answers, their understanding could modify in the future our view of matching. One of the mechanisms that could operate is the release by the graft of inhibitory HLA molecules. Soluble HLA class I molecules of donor origin have been identified in the serum of patients with an allograft [6]. They are endowed with the capacity to inhibit cell-mediated lympholysis (CML) by inducing the apoptosis of alloreactive CD8 cytotoxic T lymphocytes (CTL). This phenomenon represents a novel process contributing to tolerance induction [6]. The comprehension of the mode of action of soluble HLA has been further facilitated by the use of synthetic peptides corresponding to functionally important HLA regions. Peptides specific for alleles and for conserved regions of the α2 domain of HLA class I molecules (Bw4/Bw6 epitopes) have been constructed. Both have also been shown to be effective in blocking CML.

Another mechanism that has been individualized in this peculiar setting is the binding of the peptide to two members of the heat shock protein (HSP) family that are constitutively expressed (HSC 70) or heat-inducible (HSP 70) [7]. It is similar to those of cyclosporin and FK 506 on the immunophilins. Thus these soluble HLA molecules can induce tolerance by at least two mechanisms: apoptosis and inhibition of HSP-induced cellular activation. In addition, this set of studies focused attention on the amino-acid residues 60–84. These are endowed with an additional function in alloreactivity, since this region has been involved in recognition by killer cell inhibitory receptor (KIR). These receptors constitute a novel mechanism implicated in downregulation of allorecognition [8]. KIR were initially found on NK cells and their ligands were class I antigens present on the target. These KIR behave as a regulatory feedback that reduces the action of the lytic system. Specific KIR were detected for several HLA-C antigens (p53 receptor) and for HLA-B antigens (p70 or NKB1 receptors). The Bw4 motif and more recently the Bw6 motif have been identified as one of their targets. Later on, KIR were identified on T cell. Then, systematic studies using CML combinations in which responder and stimulator share specific HLA antigens started. Some inhibitory combinations have already been identified. Among them, the sharing of the Bw4 epitope conferred a marked inhibition [9]. Thus sequences exist in the HLA molecules that are common to many antigens and block the effector mechanisms involved in rejection. This is illustrated by the observation made in clinical bone marrow transplantation in which signal transduction by KIR appears to provide a protective effect for the HLA mismatched graft [9]. Similar inhibitory receptors have been described for DR antigens [10]. Whether these receptors will help us to better understand the DR5 and DR6 effects remains a question to answer.

Our current understanding of some mechanisms involved in the DR effect is based upon observations made after planned blood transfusion in humans [11] and after marrow infusion in animals [12]. A number of studies have indeed found that one HLA haplotype or DR-matched pretransplant blood transfusions improve kidney graft prognosis in human [11]. Likewise, administration of donor bone marrow sharing one DR antigen also increases kidney graft survival in monkey [12]. In this particular model, appearance of veto cells has been described. One hypothesis that has been put forward for the DR shared pretransplant blood transfusion is the downregulation of recipient CD4+ cells induced by donor’s peptides presented by matched class II molecules [11]. In a study evaluating the production of the immunosuppressive cytokine IL-10 during MLR, we found that DR-sharing combinations were characterized by a release of IL-10 exerting a maximal control of gamma interferon, which is a pivotal cytokine of the rejection process [13].

We have now entered an era of comprehensive examination of alloreactivity. There is no doubt that the key of our understanding of the mysterious permissible and taboo mismatches lies in the molecular factors governing rejection. This evolving knowledge of the alloreactive response will certainly allow us to extrapolate the previous epidemiological data to a future donor selection.

References

Molecular mechanisms of immunosuppressive chemical agents recently introduced in clinical transplantation protocols

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Introduction

Most of the chemical immunosuppressants currently used in human transplantation were not designed for this purpose. The details of exactly how they exert their immunosuppressive effects are still largely unknown. This ignorance not only reduces the selective targeting of the drug but makes it difficult to avoid side-effects. Nevertheless, these new drugs represent a significant advance in clinical immunosuppression and they have helped to increase our knowledge of lymphocyte activation that leads to graft rejection. But a better understanding of these pathways is still needed to improve the efficacy and safety profiles of these drugs and to design new immunosuppressants with synergistic actions. This short review is restricted to the drugs that are being developed for use in clinical transplantation. Other molecules might also be very promising for elucidating the molecular pathways following allogenic activation, but at present they are not developed for use in transplantation, and hence will not be discussed.

Tacrolimus (FK 506)

FK 506 was shown to prolong allograft survival in animals just 10 years ago. Since that time it has been registered in several countries for primary immunosuppression in liver and kidney allografts. As a primary immunosuppressive drug, FK 506 is more effective than cyclosporin (CsA, Sandimmun) but it has yet to be shown that it is more potent than the microemulsion CsA (Neoral) and that it decreases the rate of chronic rejection. Clinically, the most striking difference between FK 506 and CsA is that FK 506 can reverse steroid-resistant acute rejection [1]. Initially it was licensed for this purpose. This effect was indeed unexpected and the reason for the difference between cyclosporin A and FK 506 remains unclear, considering the way the two drugs act, and given their similar nephrotoxicity profiles.

Both CsA and FK 506 bind to immunophilins (CsA to cyclophilin A and FK 506 to FKBP 12) and both inhibit the peptidyl prolyl cis–trans isomerase activity of the binding molecules. Although these enzymes assist in the de novo folding of nascent proteins during translation and may function as chaperones in protein trafficking and secretion, the inhibitory actions of CsA and FK 506 are not sufficient to explain the immunosuppressive action. One of the main, but possibly not the only, target inhibited by these cyclophilinA/CsA and FKBP 12/FK 506 complexes is calcineurin, a serine threonine phosphatase that plays a pivotal role in the transcription of cytokines after T-cell activation [2]. Calcineurin has two subunits, the catalytic calcineurin A (CNA) and the regulatory calcineurin B (CNB) that must bind the Ca^{2+}/calmodulin complex in order to be functional after T-cell activation. This binding releases an autoinhibitory domain from CNA, resulting in the activation of this phosphatase. Cyclophilin/CsA and FKBP 12/FK 506 complexes bind to an overlapping region of CNA that also interacts with CNB, and this binding inhibits allosterically the catalytic site of the enzyme but not the binding of substrate to the enzyme [3]. Inhibition of this enzyme is of the utmost importance, because
activated calcineurin acts on essential transcriptional factors required for T-cell activation.

Most studies have focused on the nuclear factor of activated T cells (NFAT) which has two components, a T-cell-specific cytoplasmic subunit, NF-ATc, and a nuclear subunit, NF-ATn, composed of c-fos and c-jun (related to AP1). Stimulation of T cells results in increased intracellular calcium, and this activates calcineurin. The activated enzyme dephosphorylates NF-ATc and this transcription factor is then translocated into the nucleus, where it associates with AP-1 on their respective DNA binding sites [4]. These sites are present on the promoter of various cytokines, and the binding of NF-AT is crucial for the transcription of several cytokines including IL-2. The inactivation of calcineurin in T cells by FK 506 and CsA results not only in the inhibition of the migration of NF-ATc to the nucleus, but also in the inhibition of other transcription factors involved in cytokine transcription. The activation of jun N-terminal kinase (JNK) in T cell is calcineurin dependent and both FK 506 and CsA inhibit the activation of jun [5].

Similarly, FK 506 and CsA prevent the translocation of NFkB into the nucleus following T-cell activation [5]. NFkB is found in the cytoplasm of unactivated T cells associated with its inhibitor I-kB. When these cells are activated I-kB is phosphorylated and dissociates from NF-kB, allowing NFkB to migrate into the nucleus, where it is involved in the transcription of various cytokines. FK 506 and CsA inhibit certain I-kB kinases, so preventing the dissociation of NFkB from its inhibitor.

The targeting of various transcription factors in T cells explains that these drugs prevent the transcription of many cytokines including IL-2, IL-3, IL-4, IL-5, IFNγ, TNFα, GM-CSF and CD-40L. But all the cytokines are not downregulated; for example, IL-10 transcription is not affected by FK 506 or CsA. The targeting of NF-kB by FK 506 and CsA also explains the inhibition of the synthesis of IL-2 and IL4 receptors in activated T and B cells. However, calcineurin inhibition may be incomplete in vivo; for example, it has been shown that CsA only reduces calcineurin activity by 50% in lymphocytes from renal transplant patients [6].

Some of the molecular pathways common to CsA and FK 506, and possibly involving calcineurin inhibition, might be detrimental to the induction of tolerance. These compounds inhibit the apoptosis that follows activation of T cells (AICD: activation induced cell death) by decreasing the transcription of Fas-ligand, inhibiting various transcription factors involved in apoptosis (e.g. NUR 77 and MEF2), and by increasing the synthesis of bcl-2 [7].

These drugs also modulate cyclic-AMP-dependent signalling, decreasing the activities of cAMP dependent protein kinase A and of the cAMP-responsive element binding protein (CREB) [8]. These actions could modulate the immunosuppressive properties of the drugs and may be involved in their diabetogenic and nephrotoxic effects.

These biochemical pathways do not discriminate between the actions of CsA and FK 506. One of the most significant differences between them may be in their effects on the TGF β pathway. CsA increases the synthesis of TGF β in various cells, including T lymphocytes, endothelial and renal cells [9], whereas FK 506 does not. TGF β acts as an immunosuppressant by interfering with the cyclin-E/cyclin kinase 2 complex involved in the cell cycle, and thus inhibiting cell proliferation. However, a sustained elevation of TGF β may stimulate smooth-muscle proliferation, excessive matrix deposition, activate the endothelin gene, and so cause pathological fibrogenesis, HTA and chronic rejection. It has also been reported that FK BP12 is associated with type 1 receptors of the TGF β family and that FK 506 decreases this interaction [10]. However, the functional significance of this interaction remains to be determined. In clinical practice, HTA seems to be less frequent in patients treated with FK 506 than in transplant patients given CsA and it has been reported that the kidney grafts in FK 506 treated patients have an extended half-life, suggesting less chronic rejection.

It is also suspected that part of the immunosuppressive action of FK 506 is mediated via the activation of glucocorticoid receptors (GR). Untransformed GR, which do not bind to DNA, are heteromeric structures containing the hormone binding receptor and the heat shock proteins (hsp), hsp 70 and hsp 56. Human hsp 56 is also an immunophilin that can bind FK 506 and rapamycin. FK 506 potentiates GR-mediated transcription by increasing the hormone-binding affinity of the GR and GR translocation [11]. This could explain the steroid-sparing effect of FK 506, and could participate to the reversal of acute rejection produced by FK 506. However, a new GR heterocomplex has recently been discovered that contains cyclophilin 40 (Cyp 40) along with hsp 90. Cyp 40 binds CsA and like FK 506, CsA increases GR transactivation [11]. In addition, both FK 506 and CsA inhibit the multidrug resistance pump (MDR) competing for the drug-binding sites of P-glycoprotein, thereby increasing the intracellular concentration of drugs [11]. Hence the interaction of FK 506 with the steroid transduction pathway does not seem to account for its unique ability to reverse established acute rejection.

One explanation for the different in vivo effects of FK 506 and CsA could be the specific action of FK 506 (and rapamycin) in regulating the intracellular calcium-release channels. FKBP 12 is physically associated with the ryanodine receptor (RyR) and with the inositol 1,4,5-triphosphate receptor (InsP3R). Calcineurin is physiologically associated with IP3-FKBP 12 and RyR-FKBP 12 receptor complexes and this interaction can be disrupted by FK 506 (and rapamycin), resulting in the loss of the Ca2+ oscillations thought to be necessary for T-cell activation [12].

Graft rejection depends on an influx of lymphocytes from the circulation into the graft in response to locally secreted chemotactic factors. There is evidence that FK 506 not only inhibits the production of lymphocyte...
chemotactic factors, such as IL8, but also prevents the migration of lymphocytes from healthy donors and liver allograft recipients [13]. FK 506 may act by inhibiting a general mechanism of chemotaxis via its ability to inhibit the protein kinase-C-mediated signaling pathway involved in the actin polymerization and cytoskeletal reorganization that is a prerequisite for cell migration. The effect of FK 506 on endothelial and lymphocytes adhesion molecules is more controversial, but the inhibition of lymphocyte recruitment by FK 506 might contribute to the ability of this drug to reverse graft rejection.

We have summarized some of the new possible actions of FK 506, but we do not know which of them occur in vivo, and which of them are important for its unique clinical profile in organ transplantation. It has been suggested that the renal toxicity of FK 506 results from the inhibition of calcineurin, and the recent discovery of a new isoform of FKBP (FKBP 51) in mice, which is restricted to T cells and which mediates calcineurin inhibition, may lead to the design of more lymphocyte specific drugs [14]. The limitation of such compounds could be that part of the FK 506 renal toxicity (but not CsA toxicity) is due to the activation of NF-κB in fibroblasts and mesangial cells, resulting in increased IL-6 production in the kidney [15].

**Sirolimus (rapamycin)**

Although rapamycin (RAPA) and FK 506 are structurally related macrolide antibiotic compounds, they cause immunosuppression by very different mechanisms. Whereas FK 506 inhibits T-cell proliferation in the G0–G1 phase, RAPA prevents the progression of T lymphocytes and other cells from G1 to the S phase and, in contrast to FK 506, blocks both calcium-dependent and calcium-independent signalling pathways in T and B cells.

RAPA binds to the same immunophilin as does FK 506, FKBP 12, to form a drug–immunophilin complex. This complex cannot bind to calcineurin and RAPA does not inhibit early T-cell activation or directly reduce the synthesis of cytokines. The target proteins of this complex were first identified in yeasts, and have been called TOR 1 and TOR 2. A mammalian RAPA effector protein (mTOR, FRAP, RAFT, SEP) has recently been identified [16]. A mutation in this protein confers resistance to RAPA. mTOR possesses a phosphatidylinositol-3 kinase domain but this activity is not inhibited by RAPA. On the other hand, activated mTOR undergoes autophosphorylation, and this is blocked by RAPA. Although neither the functional properties nor the proximal target of mTOR have yet been determined, RAPA is known to affect several critical biochemical events in the middle to late G1 phase of the cell cycle, but there is still much to do to tie up the many loose ends of this puzzle [16].

RAPA inhibits the activation of 70-kDa S6 kinase (p70S6k), an enzyme implicated in the regulation of various cellular processes that are critical in the cell cycle. Because the RAPA-FKBP complex does not inhibit the activation of p70S6k in a cell-free system, RAPA may act upstream of p70S6k by inhibiting other kinases or activating phosphatases. The result is that RAPA inhibits at least two substrates, the S6 ribosomal protein thought to enhance protein synthesis, and the cAMP-responsive element modulator (CREM) that is inducing the transcription induction of the proliferating cell nuclear antigen (PCNA) gene [16]. PCNA is an obligate processivity factor for DNA polymerase δ, and is required for the progression of cells into the S phase. Inhibition of p70S6k might also be involved in the RAPA-mediated inactivation of the initiation factor eIF-4E binding protein 4E-BP1, which appears to play a crucial role in the control of mRNA translation.

Progression through the cell cycle requires the sequential activation of cell-cycle-dependent kinases (cdk) and cyclin complexes. RAPA has no effect on protein levels of cdk2, cdk4, cyclin D, and cyclin E but decreases the kinase activity of cdk4-cyclin D and cdk2-cyclin E complexes [16]. Their activation in mid-to-late G1 phase involves elimination of the cyclin-dependent kinase inhibitor p27kip1 from these cdk/cyclin complexes. RAPA prevents the elimination of p27 and blocks the activation of cdk4/cyclinD and cdk2/cyclin E complexes. Consequently, downstream events are inhibited: hyperphosphorylation of retinoblastoma protein (Rb) and dissociation of the Rb–E2F transcription factor (Rb–E2F) complex. The decreased activity of the E2F transcription factors results in downregulation of the cell cycle proteins, cdc2, cyclin A, and of a serine threonine kinase required for transcriptional activity. Inactivation of Rb might also block other pathways involved in cell proliferation or differentiation such as inhibition of RNA polymerases 1 and 3.

RAPA also inhibits the transcription of Bel-2, a proto-oncogene that may be critical for cell cycle progression. The reduced expression of Bel-2 could also favour the apoptosis of activated lymphoid cells.

It has also been reported recently that RAPA prevents the CD28-mediated downregulation of IκBα, resulting in inhibition of the nuclear translocation of c-rel. c-Rel is a CD28 response element binding factor that causes sustained upregulation of IL-2 gene expression [16].

These various ways in which RAPA can interfere with the progression of cells from G1 to S phase explain why it is not an effective inhibitor of cytokine synthesis in vitro, but antagonizes the effects of cytokines and growth factors in activated T and B cells. The activity of RAPA is not limited to cells of the immune system and its other important action is to inhibit the proliferation of smooth-muscle cells stimulated with b-FGF and PDGF.

RAPA is now undergoing phase III clinical trials in kidney transplantation. Two major types of clinical trials are in progress. Some protocols take advantage of the synergy between CsA and RAPA, whereas others combine RAPA with other non-nephrotoxic drugs.
Mycophenolate mofetil

Mycophenolate mofetil is an ester prodrug of the active immunosuppressant mycophenolic acid (MPA). This drug acts at a late stage of lymphocyte proliferation, beyond the steps which are inhibited by either FK 506 or rapamycin. It is a non-competitive, reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH) [17]. IMPDH is critical for the de novo synthesis of purines during lymphocyte activation. MPA binds to the nicotinamide site on IMPD and mimics the inhibition of IMPDH by nicotinamide adenine dinucleotide (NAD) [18]. Most cells proliferate normally with only an intact salvage pathway, whereas T and B cells require an intact purine biosynthetic pathway. In addition, the type II isoform which might be predominant in activated lymphocytes is more sensitive to inhibition by MPA than the type I.

The effects of MPA are directly attributable to the depletion of guanosines nucleotides [17]. This results in decreases in the activities of PRPP synthetase and ribonucleotide reductase, so inhibiting DNA and RNA synthesis, a decreased transfer of mannose to membrane glycoproteins which inhibits the glycosylation of the adhesion molecule, VLA-4, and the ligands of selectins, and the decreased production of tetrahydrobiopterin, a rate-limiting cofactor required for NO synthesis. The inhibition of adhesion of lymphocytes and monocytes to endothelial cells could be of value for preventing and treating acute rejection episodes. Despite its apparent lymphocyte selectivity, MPA also inhibits the proliferation of arterial smooth-muscle cells in culture and this action could be interesting for preventing chronic rejection. MPA is now registered in several countries for the prevention of acute rejection episodes and can reverse acute rejection [19].

Other drugs

Two drugs, mizoribine and gusperimus (15-deoxyspergualin), have been used clinically only in Japan. Mizoribine is an imidazole nucleoside requiring phosphorylation to become a competitive inhibitor of IMPDH. Hence, MPA and mizoribine have different molecular mechanisms of action [20]. Mizoribine is less myelotoxic and hepatotoxic than azathioprine and is at least as immunosuppressive; it has therefore replaced azathioprine in Japan for the prevention of rejection.

Although 15-deoxyspergualin (DSG) is effective and used in Japan for treating acute rejection episodes, its mechanism of action is still something of a mystery [21]. DSG inhibits T and B cell maturation, the generation of cytokotoxic T lymphocytes, the production of antibodies by activated B cells, and certain functions of antigen-presenting cells such as peptide loading of MHC class I molecules. DSG binds to a member of the hsp 70 family and probably interferes with its function. Heat shock proteins are involved in a variety of intracellular activities, including protein folding, molecular chaperoning, peptide loading of MHC molecules, and translocation of proteins from the cytoplasm to the nucleus. DSG seems to prevent hsp 70 interacting with NFkB, so impeding the nuclear translocation of this factor and inhibiting the transcription of NF-kB-dependent genes such as IL-1, IL-6, IL-8, TNF, MCP, iNOS, IL-2R, and the κ light chain [21].

Brequinar and lefunomide are two other potential interesting immunosuppressive drugs, but their development for use in clinical transplantation has now been stopped.

Although most of the immunosuppressants currently in clinical development were discovered by screening programmes seeking new antibiotics or antitumour drugs, they have contributed a great deal to the unravelling of the complex biochemical pathways that mediate the immune response to an allograft. The growing body of new knowledge should make it possible to design new classes of drugs with greater specificity and fewer toxic effects.

References

Cyclosporin nephrotoxicity following cardiac transplantation

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Introduction

The capacity of cyclosporin to induce both reversible and irreversible renal dysfunction associated with morphological injury has limited its clinical use. The mechanisms responsible for this nephrotoxicity remain obscure, although the initial changes are haemodynamic and a consequence of the drug’s ability to vasoconstrict preglomerular resistance vessels. Whereas the native kidney is particularly sensitive to cyclosporin, the renal allograft appears protected from progressive injury. This observation may pave the way for future pharmacological or interventional therapies directed towards the preservation of renal function.

Cardiac transplantation

Chronic cyclosporin nephrotoxicity is responsible for a high (10%) prevalence of end-stage renal failure (ESRF) which occurs by 10 years after cardiac transplantation [1–3]. Still worse results are found following heart and lung transplantation with a 10% prevalence of ESRF within only 5 years [3].

Function

The renal vasoconstriction leads to renal hypoperfusion and salt and water retention associated with hypertension. Consistent with these events, there are increased numbers of renin granules present in the juxtaglomerular apparatus and in hilar arterioles, and increased expression of AT1 receptors [4].

After cardiac transplantation there is early loss of renal function (within 3–6 months) [5] which is dose dependent [2] and progressive [6] and the magnitude of this fall in GFR in the first year (or perhaps the first few months) appears to be the key indicator of poor renal outcome. Although in some cases withdrawal or dose reduction of cyclosporin may stabilize renal function [5,7], this is by no means the rule [6] and even if function remains stable, proteinuria and morphological injury can increase [2]. Similarly, irreversible loss of renal function is reported after liver, pancreas and bone marrow transplantation and in patients with uveitis, insulin-dependent diabetes, rheumatoid arthritis, and psoriasis [8], even with doses as low as 5 mg/kg/day. The method of cyclosporin administration may be relevant since GFR was reported to improve if cyclosporin was given once rather than twice daily to stable heart-transplant recipients [9]. It is probable that the relationship between progressive dysfunction and either dose or levels of cyclosporin is partly idiosyncratic.

Structure

Renal histology from native kidneys shows a variable amount of both global and segmental glomerular sclerosis [2,10] with surviving glomeruli tending to be larger, an appearance consistent with glomerular hyperfiltration and increasing proteinuria. In contrast to native kidneys, glomeruli in renal allografts are spared. These changes appear to be secondary to the progressive arteriolar vasculopathy leading to the glomerular ischaemia and tubular atrophy. This pattern of loss initially results in a characteristic appearance of striped tubular atrophy and fibrosis which begins in the medulla and progresses to the medullary rays of the cortex. In a recent study of renal pathology in 22 heart- and lung-transplant recipients, we have emphasized the presence of changes affecting both
endothelial and vascular smooth muscle cells. All patients had creatinine clearance estimated as less than 50 ml/min, and conspicuous vasculopathy was always seen. Glomerular pathology ranged from typical features of focal and segmental sclerosis that one associates with other situations in which glomerular hyperfiltration occur, to segmental pathology and mesangiolysis that appeared to be the consequence of capillary thrombotic microangiopathy [11].

Dissociation between structure and function in cyclosporin nephropathy

Short-term follow-up studies of renal function after the first year of cardiac transplantation may be misleadingly reassuring. Renal function can remain stable [2,7,12], but increasing proteinuria may develop [2,12], and follow up biopsies have shown progressive injury even after cyclosporin has been withdrawn [2]. The NIH uveitis study [13] and careful follow up after pancreatic transplantation [14] provide further evidence of dissociation between progressive tubulointerstitial and arteriolar disease and renal function. This is supported by experimental studies of cyclosporin nephropathy in which GFR may return to normal after cessation of therapy whereas tubulointerstitial pathology persists [15]. This dissociation appears to reflect pathogenesis, since Kon and her colleagues [16] have shown that haemodynamic changes can be inhibited by endothelin antagonists whereas ACE inhibitors reduce fibrosis.

Recent advances

The paradox that chronic models of experimental cyclosporin nephrotoxicity did not produce the vascular pathology and progressive tubulointerstitial fibrosis seen in man has now been resolved. If rats are given cyclosporin and simultaneously volume contracted by salt depletion, then the full gamut of pathology is seen [15,17]. Withdrawal of cyclosporin allows GFR to return to normal, while the morphological features remain [15]. Blockade of angiotensin II, by either ACE inhibitors or receptor blockers, strikingly protects against the morphological injury without preventing the fall in GFR [18,19]. Conversely, endothelin blockade prevents the fall in GFR without protecting against morphological injury [16]. These data suggest that angiotensin II, but not endothelin, may participate in cyclosporin induced fibrosis. Cyclosporin may also augment fibrogenesis by stimulation of collagen synthesis. Both in vivo and in vitro [20] cyclosporin enhances the expression of transforming growth factor-β [21] and in chronic toxicity models there is an early macrophage infiltration that is associated with upregulation of chemokines [22].

Summary

The greatest change in GFR in response to treatment with cyclosporin occurs in the first 3–6 months and the magnitude of the decrement in the first year (or perhaps the first few months) appears to be a vital indicator of future problems. However, the apparent stabilization of renal function, particularly when monitored only by plasma creatinine, can conceal progressive tubulointerstitial injury, and increasing proteinuria is an ominous sign.

Although lower doses of cyclosporin and careful monitoring of renal function may be helpful, there is at present no pharmacological intervention to protect or reverse the reduction in GFR that occurs. We believe that the vascular lesion induced by cyclosporin is fundamental, with early and initially reversible cyclosporin-induced vasospasm leading to progressive vascular damage with activation of endothelial cells and increased platelet interactions. Amongst other determinants, the renal response to this vasculopathy will depend on the balance between the presence of vasoactive factors with the vasoconstrictors promoting interstitial fibrosis and the vasodilators inhibiting proliferation [23]. It is likely that the kidneys of heart-transplant recipients are chronically ischaemic and as a consequence their renin–angiotensin systems massively activated, which may further sensitize their kidneys to cyclosporin. Overproduction of angiotensin II, associated with the DD ACE genotype, has already been associated with poor prognosis in diabetic [24] and IgA [25] nephropathy. It is interesting to speculate that this ACE genotype, which is associated with a poor outcome in non-ischaemic heart disease [26] can influence renal sensitivity to cyclosporin and predict the development of morphological injury.

Extension of these experimental findings into the clinical arena with a placebo-controlled trial of early introduction of ACE inhibitor therapy in recipients of cardiac transplants would be timely.

References

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