Therapeutic approach to organ transplantation

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Introduction

Renal transplantation is firmly established as the preferred treatment for many patients suffering from end-stage renal disease. Nonetheless no absolute consensus has developed on how to achieve optimal immunosuppression, and many individual centres employing somewhat different protocols report excellent graft and patient survival. Immunological considerations, including antirejection therapy, are organized around a few general principles.

The first consideration is careful patient preparation and, in the circumstance of living donor renal transplantation, selection of the best available ABO compatible, human leukocyte antigen (HLA) match in the event that several potential living related donors are available for organ donation. Second is a multitiered approach to immunosuppressive therapy similar in principle to that used in chemotherapy; several agents are used simultaneously, each of which is directed at a different molecular target within the allograft response (Figure 1, Table 1). Additive/synergistic effects are achieved through application of each agent at a relatively low dose, thereby limiting the toxicity of each individual agent while increasing the total immunosuppressive effect. Third is the principle that higher immunosuppressive drug doses and/or more individual immunosuppressive drugs are required to gain early engraftment and to treat established rejection than are needed to maintain immunosuppression in the long-term. Hence intensive induction and lower dose maintenance drug protocols are used. Fourth is careful investigation of each episode of post-transplant graft dysfunction, with the realization that most of the common causes of graft dysfunction, including rejection, can (and frequently do) coexist. Successful therapy therefore often involves several simultaneous therapeutic manoeuvres. Fifth, the appropriate reduction or withdrawal of an immunosuppressive drug when that drug's toxicity exceeds its therapeutic benefit.

Pretransplant transfusions

Although pretransplant random whole blood transfusion was a powerful adjunct to transplant therapy when cyclosporin (CsA) was not available, the short-term benefits of random transfusion have recently been more difficult to demonstrate in the CsA era. There is no agreement concerning the role of donor-specific transfusions (DST) for recipients of HLA mismatched living related donor renal transplants (LDR). Occasionally DST produces adverse presensitization to the donor. Because these sensitized patients cannot be transplanted with tissues procured from the transfusion donor, many units do not employ routine DST. Owing to the powerful tolerizing effects of DST in experimental models, there are several active clinical trials evaluating various forms of pre/perioperative donor blood element infusions into graft recipients as a therapeutic modality.

Table 1. Mechanism of action of immunosuppressants

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mode of action</th>
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<tbody>
<tr>
<td>CsA/FK506</td>
<td>Blocks Ca^{2+}-dependent T-cell activation pathway via binding to calcineurin</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>Blocks cytokine gene expression</td>
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<tr>
<td>Azathioprine</td>
<td>Inhibits purine synthesis</td>
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<tr>
<td>Mycophenolate</td>
<td>Inhibits a lymphocyte specific guanosine synthesis pathway</td>
</tr>
<tr>
<td>mofetil</td>
<td>Inhibits the response of antigen activated lymphocytes to growth factors</td>
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<td>Rapamycin</td>
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Immunopharmacology of allograft rejection

**CsA and tacrolimus (FK506)**

CsA, a small neutral hydrophobic cyclic peptide of fungal origin, and tacrolimus (FK506), a water-soluble macrolide lactone produced by *Streptomyces tsukubaensis*, block the Ca\(^{2+}\) dependent T cell activation pathway [6–9]. Oral doses of both agents are erratically absorbed. The immunosuppressive effects of CsA and FK506 are dependent upon the formation of a heterodimeric complexes that consist of the native compound (CsA or FK506) and its respective cytoplasmic 'immunophilin' receptor protein, cyclophilin [10] or FK binding protein (FKBP) [11,12]. Both CsA:cyclophilin and FK506:FKBP complexes bind calcineurin, a calcium- and calmodulin-sensitive phosphatase, and inhibit its enzymatic activity (Figure 1; Table 1) [13–15]. CsA/FK506-mediated inhibition of calcineurin's phosphatase activity prevents the dephosphorylation of cytoplasmic NF-AT and thereby impedes subsequent import of this DNA binding protein into
the nucleus [7,8]. CsA/FK506 inhibits the expression of not only NF-AT [16,17] but also the activities of other DNA-binding proteins such as NF-kB and AP-1 factors [18–20]. The phosphorylation status of transcription factors affect not only their nuclear import but also their DNA binding ability and interaction with the cellular transcriptional machinery, e.g., c-jun [21].

CsA/FK506 inhibits activation of several cytokine genes, including the IL-2, IL-4, and gamma interferon (IFNγ) genes; however, this activity does not totally account for the antiproliferative effect of CsA/FK506 upon activated T-cells. Inhibition of expression of protooncogenes (e.g. H-ras, c-myec) as well as prevention of expression of receptors for cytokines (e.g. the IL-2 alpha chain receptor) might also be quite important in this regard [22,23].

It is also significant that CsA, in striking contrast to its inhibitory activity on the induced expression of IL-2, enhances the expression of transforming growth factor-β (TGF-β) [24]. As TGF-β is a potent inhibitor of IL-2 stimulated T-cell proliferation and generation of antigen specific CTL, enhanced expression of TGF-β is likely to contribute to the immunosuppressive activity of CsA. This novel effect of CsA suggests also a mechanism for some of the complications (e.g., renal fibrosis) of CsA therapy since TGF-β is a fibroblast growth factor [25].

Corticosteroids

Corticosteroids were first used in clinical transplantation to reverse acute rejection reactions in patients treated with maintenance doses of azathioprine. It is now customary to use modest doses of a corticosteroid in maintenance protocols that also utilize CsA or tacrolimus ± azathioprine. A short course of high doses of corticosteroids is often used to treat acute rejection episodes. Corticosteroids inhibit T-cell proliferation, T-cell-dependent immunity, and cytokine gene transcription (including IL-1, IL-2, IL-6, IFN-γ and tumour necrosis factor-alpha (TNF-α) genes) (Figure 1, Table 1) [26–28]. While no individual cytokine can totally reverse the inhibitory effects of corticosteroids upon mitogen-stimulated T-cell proliferation, a combination of cytokines is effective [29].

Some cytokine genes possess a glucocorticosteroid response element in the 5' regulatory region that serves as a target for the heterodimeric complex formed by the association of corticosteroids with the intracellular glucocorticosteroid receptor protein. Binding of this complex to the glucocorticosteroid response element can, in theory, block gene expression. Blockade of IL-2 gene transcription, however, involves impairment of the cooperative effect of several DNA binding proteins [30], although the IL-2 gene does not possess a glucocorticosteroid response element.

There are several additional mechanisms by which glucocorticoids might block T-cell activation. Glucocorticoids can block expression of numerous genes through the non-covalent association of the interaction of the activated hormone-receptor complex with the c-Jun/c-fos heterodimer (activation protein-1, AP-1) [31], c-Jun and c-fos heterodimers bind to the AP-1 site of the promoter of many cytokine genes. In keeping with this observation, glucocorticoids interfere with IL-2 gene expression through prevention of nuclear transcription factors binding to the AP-1 and NF-AT sites [32]. Glucocorticoids also inhibit the pretranscriptional calcineurin-dependent pathways for T-cell activation [33]. Inhibition by corticosteroids of cytokine production represents an important rationale for its usage in the control of the anti-allograft response (Figure 1, Table 1).

Azathioprine

Azathioprine is the 1-methyl-4-nitro-5-imidazolyl derivative of 6-mercaptopurine [34,35]. This purine analogue functions as a purine antagonist and inhibits cellular proliferation (Figure 1, Table 1). Allopurinol blocks the catabolism of azathioprine, causing a dramatic increase in bone marrow suppression. Azathioprine is often used in conjunction with CsA or tacrolimus and corticosteroids in maintenance protocols. Although application of azathioprine diminishes the incidence and intensity of rejection episodes, it is not valuable in the therapy of ongoing rejection. An agent, mycophenolate mofetil, blocks purine metabolism through its inhibitory effect upon inosine monophosphate dehydrogenase, an enzyme in the de-novo purine biosynthetic pathway [36,37]. The effects of mycophenolate mofetil upon purine metabolism are rather selective for activated lymphocytes [38]. As a consequence it is possible that mycophenolate mofetil has replaced azathioprine in many drug regimens [39].

OKT3 monoclonal antibody (mAb)

The multimeric CD3 complex protein is non-covalently associated to the α and β chains of the T cell receptor for antigen. This complex is expressed on the surface of all functionally competent T lymphocytes. OKT3 binds to the Σ-chain of the CD3 complex; OKT3 binding to T-cells leads to modulation of all components of the TCR/CD3 complex from the T cell surface, either by shedding or internalization [40]. Moreover T cells virtually disappear from the peripheral blood following the administration of OKT3 mAb.

Maintenance immunosuppressive regimens

The basic immunosuppressive protocol used in most transplant centers involves the use of multiple drugs, (usually CsA or FK506 + corticosteroids ± a purine antagonist) each directed at a discrete site in the T-cell activation cascade (Figure 1, Table 1) and each with distinct side-effects [35]. CsA, FK506, azathioprine, mycophenolate mofetil and corticosteroids are already approved by the FDA while the clinical efficacy of rapamycin (an agent that inhibits the prolif-
Corticosteroids act to reduce the intensity of leukocytic infiltration in a rejecting allograft has not been fully elucidated; however, release of numerous cytokines is blocked by high-dose steroids, and T-cell trafficking patterns are altered. OKT3-treated T cells lose their antigen receptor proteins and become literally blinded to the presence of the allograft; thus, rejection abates. OKT3 is superior to standard high-dose corticosteroid therapy for reversing allograft rejection (90% vs 70% success [40]. More than 90% of first rejections and a high percentage of second rejection episodes respond to OKT3 therapy. Nonetheless, OKT3 is often reserved as treatment for corticosteroid-resistant rejection episodes. As antirejection treatment, OKT3 is given as a daily 5-mg i.v. bolus for 5–10 consecutive days.

While prophylactic administration of OKT3 to patients in the immediate post-transplant period is well tolerated, administration of the first and occasionally second dose of OKT3 to patients treated for ongoing rejection often causes a 'capillary leak' syndrome that can lead to severe ARDS-type pulmonary oedema, hypotension, and/or aseptic meningitis [1,2,44]. This syndrome is caused by the release of lymphokines from the OKT3-targeted activated T cells. Because of these troublesome symptoms as well as additional expense, we reserve OKT3 therapy for steroid-insensitive rejection episodes. Subsequent doses are well tolerated. Approximately 75% of patients develop IgG or IgM anti-idiotype or antiisotype antibodies against OKT3. Azathioprine at doses of 1–2 mg/kg/day and prednisone at 30 mg/day are employed to limit the frequency and delay the onset of occurrence of host anti-OKT3 antibodies. OKT3 is not efficacious in patients who have developed high titre anti-idiotypic antibodies against OKT3. Anti-isotypic antibodies do not neutralize the immunosuppressive properties of OKT3.

Polyclonal or antilymphocyte antibody preparations are derived from animals immunized with human lymphocytes. The antibodies are directed against both lymphocyte-specific and more broadly expressed antigens. More than 80% of steroid-resistant first rejection episodes will respond to these polyclonal antibodies. Patients are skin tested with 0.1 mg of a 1:1,000 dilution of polyclonal antilymphocyte antibodies prior to administration of the first dose and pretreated before each dose with diphenhydramine and steroids. Antilymphocyte antibodies, often at a dosage of 10–15 mg/kg, are administered daily by slow i.v. infusion over 4–8 h for 10–14 days. Adverse reactions include anaphylaxis, haemolysis, thrombocytopenia, neutropenia, dyspnoea, chills, fever, hypotension, chemical phlebitis, pruritus, serum, sickness, and chest, flank, and back pain. Unlike the first-dose complications noted with OKT3, the severity of anaphylactoid side-effects to these polyclonal antilymphocyte preparations can increase with subsequent doses. Frank anaphylaxis can occur at any time during treatment. The use of polyclonal antilymphocyte antibodies has decreased because OKT3 is less toxic and comparably effective in reversing rejection.

We rarely treat a kidney transplant recipient for...
more than three rejection episodes in the early post-transplant period, because third and fourth rejections tend to be vasculitic forms that are therapeutically resistant, and the risks to the patient from zealous immunosuppression are unacceptably high by that point. In contrast, patients with cardiac allografts who will die with the cessation of cardiac function are treated more vigorously because complete rejection, in the absence of retransplantation, is fatal.

While current drug protocols are far superior to those employed a decade ago, the situation is far from ideal. Most allografts eventually succumb to chronic rejection. Long-term therapy is mandatory. We anticipate clinical application in the near future of more refined immunosuppressive regimens: new drugs, humanized mAbs, and fusion proteins that target discrete steps in antigen recognition, signal transduction, and effector immunity. We are also optimistic regarding the inducibility of antigen specific tolerance in the clinical setting, but a delivery date cannot yet be guaranteed.

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