MANAGEMENT OF IMMUNOSUPPRESSION IN KIDNEY POST TRANSPLANTATION

J. Karamehic¹, M. Lorber², F. Gavrankapetanovic¹, B. Heljic³, R. Formica², D. Subasic¹

¹The Institute of Clinical Immunology, University Clinical Centre, Sarajevo, Bosnia and Herzegovina, ²The Yale University School of Medicine, Department of Organ Transplantation, New Haven, USA, ³Department of Endocrinology, University Clinical Centre of Sarajevo, Bosnia and Herzegovina.

ABSTRACT

When histocompatibility differences exist between donor and recipient, it is necessary to modify or suppress the immune response in order to enable the recipient to accept a graft. Immunosuppressive therapy, in general, suppresses all immune responses, including those to bacteria, fungi and even malignant tumors. In the 1950s when clinical renal transplantation began, sublethal total body irradiation was employed. Currently immunosuppression is more safely induced pharmacologically. Agents used in humans to suppress the immune response are discussed in our paper.

Key words: immunosuppression medications, protocols, renal transplantation

INTRODUCTION

The 1990 Nobel Prize was awarded to Dr. Joseph Murray for his discovery that renal transplantation between identical twins could reverse renal failure in humans (1). The subsequent discovery by Schwartz and Dameshek that 6-mercaptopurine could suppress the immune response of the rabbit to human serum albumin and to rabbit skin allografts, led sequentially to large animal experiments, then clinical work during the 1960s confirming concept that human allotransplantation could be applied to reverse renal failure (2). However, it was not until the discovery of cyclosporine in the middle 1970s, followed by widespread application to clinical transplant practice during the 1980s that transformed clinical transplantation into preferred treatment for many causes of organ failure. In this paper we will review the growing list of immunosuppressive agents available for clinical use, outline prospects for newer agents presently under investigation, and suggest a possible clinical strategy for immunosuppression after renal transplantation.

INDUCTION OF IMMUNOSUPPRESSION

Induction for the purpose of transplant immunosuppression refers to a general strategy designed to provide a more intense regimen during the immediate peri- and post-transplant period seeking to avert or delay the onset of acute rejection. The rationale for this approach was largely based upon one observation that early acute rejection episodes have been associated with deleterious long-term effects on transplanted kidneys, and most allograft failure due to acute rejection occurs during the initial 3 post transplant months. Unfortunately, despite the underlying logic of this approach, it has been difficult in clinical practice to demonstrate clear benefit (3).

The clinical application of strategies designed to reduce alloimmune responses to transplanted organs has continued to evolve. A list of available agents and approaches for immunosuppressive therapy used currently, or in the past, has been classified according to their general mechanism of action (Table 1).
Table 1 Immunosuppressive approaches with a potential for clinical practice

**Calcineurin inhibitors**
- Cyclosporine
- Tacrolimus

**Proliferation signal inhibitors**
- Sirolimus
- Everolimus

**Non-specific inhibitors of cell division/ nucleotide metabolism**
- Azathioprine
- Cyclophosphamide

**Lymphocyte selective inhibitors of cell division/ nucleotide metabolism**
- Mycophenolate mofetil
- Mycophenolic acid

**Agents affecting antigen presentation**
- Corticosteroids

**Biologic agents/ approaches**
- Polyclonal anti-lymphocyte antibodies
- Anti-lymphocyte globulins
- Anti-thymocyte globulins
- Campath –1H
- Murine monoclonal anti-lymphocyte antibodies
- OKT-3
- Humanized monoclonals
- Basiliximab (anti-CD25 – Chimerized)
- Dacluzimab (anti-CD25 – Humanized)
- Plasmapheresis +/- intravenous immunoglobulin
- Irradiation (ultraviolet; x-ray)

Initial approaches to induction used various polyclonal anti-lymphocyte antibody preparations, then beginning in the late 1980s, the murine monoclonal anti-CD3 preparation, OKT3 was placed into widespread use. However, despite anecdotal reports suggesting benefit, it was very difficult to demonstrate a benefit when such preparations were used in randomized trials (4). A convincing argument supporting the application of anti-lymphocyte antibody induction strategies was reported as a meta-analysis by Szczech et al. in 1997 (5). This analysis included results from 7 randomized, controlled trials using anti-lymphocyte induction in renal transplantation where the authors felt outcomes could be reliably compared. The findings demonstrated a statistically sound argument supporting induction therapy with improved graft survival among patients receiving anti-lymphocyte antibody therapy (85.6% vs. 79.6%) More recent results using the rabbit anti-thymocyte preparation, Thymoglobulin, have similarly suggested efficacy when used for induction of immunosuppression.

However, the disadvantages of lymphocyte depleting antibody therapy including side effects such as the cytokine release syndrome, and possible consequences of excessive immunosuppression encouraged investigation into potential alternative induction strategies (6). One approach used murine monoclonal antibodies directed toward the IL-2 receptor (CD25), reasoning that this approach would target only activated T-lymphocytes. Initial efforts were disappointing with unacceptable rates of associated acute rejection. However, monoclonal anti-CD25 preparations were subjected to molecular engineering to create a chimerized preparation, basiliximab, (Simulect®; Novartis Pharmaceuticals), as well as a humanized preparation daclizumab (Zenapax®; Hoffman-LaRoche) (7). Each agent was applied for induction of immunosuppression in combination with cyclosporine and corticosteroids in large scale international, randomized and blinded clinical trials. Each trial resulted in a clear statistical benefit favoring anti-CD25 antibody induction with and excellent safety profile and no associated cytokine release syndrome so common with other anti-lymphocyte antibody preparations (8).

**Acute rejection prophylaxis and maintenance of immunosuppression**

Maintenance of immunosuppression defines the agents used over time to prevent acute rejection. The specific agents have changed over the years, but the maintenance regimen represents the cornerstone of long-term anti-rejection therapy. Most strategies used over the years have included two or three agents, usually with one of those being dominant and the others adjunctive, either to enhance the overall level of immunosuppression or to allow reduced dosing of the dominant agent (usually to minimize toxicities). Most patients tend to require lower doses of immunosuppressants as the time after transplant passes. This practice has long been based on the theoretic construct of “graft accommodation”. Although formal proof of this concept has not been established, the phenomenon has usually been attributed to findings of reduced alloimmune activity and reduced elaboration...
of pro-inflammatory cytokines from the host over time (9). The various clinically relevant agents will now be described, according to their mechanism of action, and where possible to the time line of their introduction into clinical transplant practice.

**Inhibitors of nucleotide biosynthesis**

The first agent widely applied for immunosuppression after renal transplantation was the purine anti-metabolite azathioprine (AZA). After initial success in canine models, successful human transplantation under AZA immunosuppression was first reported in 1963, and in combination with prednisone in 1964 (10). The two drug regimen of AZA and prednisone then became the standard approach to immunosuppression after renal transplantation until 1983. Unfortunately, although this approach resulted in some clinical success, acute rejection episodes were observed among more than 80% of patients, and one year renal graft survival was only approximately 50%.

After absorption, AZA is rapidly metabolized into 6-mercaptopurine, which in turn is metabolized into thiosinic acid, the active metabolite which is inserted into developing strands of DNA, thereby interfering with DNA synthesis. This inhibition of DNA synthesis, in turn results in suppression of T and B lymphocyte proliferation resulting in suppression of immune responses. The rate limiting toxicity of AZA has generally been reversible (with dose reduction or discontinuation) suppression of the bone marrow with leukopenia, thrombocytopenia and anemia (11). Additionally described albeit to a lesser degree are hepatotoxicity and reversible hair loss. Therefore, AZA exhibits a clinically important interaction with allopurinol, a xanthine oxidase inhibitor, used widely for therapy of gout (12). When allopurinol is administered to patients receiving AZA, metabolism is diminished. This results in accumulation of intact AZA and AZA metabolites, potentially leading to severe and prolonged neutropenia with associated sepsis. Mycophenolate mofetil (MMF) was evaluated in large international randomized, blinded, multicenter clinical trials during the middle and late 1990s. Compared to AZA or placebo, each in combination with cyclosporine and corticosteroids, results demonstrated significant reduction in the incidence of acute renal allograft rejection with a very favorable safety profile among patients receiving MMF (13). MMF is metabolized to the active metabolite, mycophenolic acid. It selectively inhibits lymphocyte proliferation by reversible inhibition of inosine monophosphate dehydrogenase (IMPDH), a key enzyme in the de novo purine synthetic pathway. Unlike other cells, lymphocytes are particularly sensitive to mycophenolic acid (MPA), because of their relative dependence upon the de novo compared to the salvage purine pathway (14). Experimentally, MPA has been found to suppress antibody formation, inhibit generation of cytolytic T cells, and reduce expression of adhesion molecules on lymphocytes. It is usually administered at a fixed dose ranging between 1.5 mg to 3 mg daily, generally in two doses, twelve hours apart (15). Clinical side effects are usually gastrointestinal, likely owing to the observation that GI tract epithelial cells are also IMPDH dependent (16). MMF was initially used without drug level monitoring with dosing based upon the presence or absence of gastrointestinal side effects. However, recently many researchers have begun to use circulating MPA levels to guide dosing strategy.

**Corticosteroids**

Corticosteroids were introduced very early in the clinical transplant experience, and they have until recently remained a mainstay of the most immunosuppression regimens. They have been well documented to exhibit immunosuppressive as well as anti-inflammatory properties; they suppress macrophage function, prevent lymphocyte proliferation, inhibit production of some cytokines, reduce expression of adhesion molecules, induce lymphocyte apoptosis, may alter lymphocyte trafficking, and under certain conditions effect reduction in expression of major histocompatibility complex molecules (17). The exact mechanism of action is not fully understood, but generally corticosteroids influence the rate of specific gene transcription of important regulatory proteins, including AP-1 and NFκB, and they appear to inhibit certain key cytokines such as Interleukin-1 (IL-1). Induction strategies were applied in conjunction with tacrolimus and MMF (18). Perhaps even more encouraging were the results reported using sirolimus and tacrolimus in a multicenter pilot study of 80 patients where there was an incidence of acute rejection of only 6% with no graft loss or patient deaths. Consequently, a new era of maintaining immunosuppression without the requirement for ongoing corticosteroids may now be on the horizon. A common posttransplant steroid regimen is suggested in Table 2.
Table 2 The sample of post renal transplant corticosteroid maintenance

<table>
<thead>
<tr>
<th>Day</th>
<th>Medication</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraoperative</td>
<td>Methyl-Prednisolone</td>
<td>250 mg (or lesser dose, 4 mg/kg)</td>
</tr>
<tr>
<td>1</td>
<td>Prednisone</td>
<td>30 mg every 6 hours (or lesser of 2 mg/kg/d)</td>
</tr>
<tr>
<td>2</td>
<td>Prednisone</td>
<td>25 mg every 6 hours (or lesser of 1.5 mg/kg/d)</td>
</tr>
<tr>
<td>3</td>
<td>Prednisone</td>
<td>20 mg every 6 hours (or lesser of 1.0 mg/kg/d)</td>
</tr>
<tr>
<td>4</td>
<td>Prednisone</td>
<td>15 mg every 6 hours (or lesser of 0.9 mg/kg/d)</td>
</tr>
<tr>
<td>5</td>
<td>Prednisone</td>
<td>15 mg every 8 hours (or lesser of 0.7 mg/kg/d)</td>
</tr>
<tr>
<td>6</td>
<td>Prednisone</td>
<td>15/10/15 mg every 8 hours (or lesser of 0.6 mg/kg/d)</td>
</tr>
<tr>
<td>7</td>
<td>Prednisone</td>
<td>15/5/15 mg every 8 hours (or lesser of 0.5 mg/kg/d)</td>
</tr>
<tr>
<td>8</td>
<td>Prednisone</td>
<td>15 mg every 12 hours (or lesser of 0.4 mg/kg/d)</td>
</tr>
<tr>
<td>10</td>
<td>Prednisone</td>
<td>25 mg daily (or lesser of 0.35 mg/kg/d)</td>
</tr>
<tr>
<td>14</td>
<td>Prednisone</td>
<td>20 mg daily (or lesser of 0.3 mg/kg/d)</td>
</tr>
<tr>
<td>28</td>
<td>Prednisone</td>
<td>17.5 mg daily (or lesser of 0.25 mg/kg/d)</td>
</tr>
<tr>
<td>35</td>
<td>Prednisone</td>
<td>15 mg daily (or lesser of 0.2 mg/kg/d)</td>
</tr>
<tr>
<td>45</td>
<td>Prednisone</td>
<td>12.5 mg daily</td>
</tr>
<tr>
<td>60</td>
<td>Prednisone</td>
<td>10 mg daily</td>
</tr>
</tbody>
</table>

Calcineurin inhibitors

Cyclosporine is a cyclic, 11 amino acid peptide that was originally isolated as a fungal fermentation broth during the early 1970s, and its story in terms of the impact on clinical transplantation is well known. It became the catalyst moving the field of transplantation from an interesting clinical research activity to its present position as the acknowledged treatment of choice for many forms of end stage organ failure, including renal failure (19). Cyclosporine binds to form complexes with a ubiquitous, abundant intracellular protein called cyclophilin (CYP), and the CsA-cyclosporin complex is actually the active immunosuppressive drug. CsA-CYP complexes engage with the calcium dependent serine phosphatase, calcineurin to inhibit enzymatic function. Calcineurin inhibition prevents or reduces activation of a number of important transcription factors, including the nuclear factor of activated T cells (NFAT). Consequently, CsA action prevents the transcription of a many T cell cytokine genes including IL-2, IFN-G, IL-4, TNFα, and GM-CSF. In clinical practice, CsA action results in reduction, but not complete elimination of calcineurin inhibitors CN activity, and the result is immunosuppression leaving sufficient reserve to prevent infection in most circumstances (20).

In summary, although clinical use has at times proved challenging, accumulating evidence suggests that sirolimus represents an important advance in clinical immunosuppression. Sirolimus (SRL) based strategies seem likely to provide important options in selecting individualized, patient specific post transplant maintenance strategies (21).

THERAPY OF ACUTE REJECTION

Percutaneous biopsy of the renal transplant is typically undertaken when clinical evidence is suggestive of renal dysfunction. Histopathologic findings can be classified according to several published schemes with the Banff criteria being the most widely applied (22) (Figures 1-4). Therapy of acute rejection has been associated with at least short term success, defined by improved renal function, with an incidence exceeding 90% (22). However, acute rejection episodes negatively impact long term outcomes when renal function fails to return to pre-rejection values after treatment. Many researchers have interpreted the data to suggest that prognosis is poorer when vascular (arterial) involvement is identified on the allograft biopsy. Therapy of rejection usually falls into one of three strategies (23, 24).

Most clinicians continue to use pulse corticosteroids (usually 3 daily doses of methyl- prednisolone, 3.5-7 mg/kg/dose) as primary treatment of mild to moderate acute rejection, usually without vascular involvement (Banff grade 1) (25, 26). More aggressive or virulent acute rejection, particularly among patients with arteritis and/or more intense interstitial lymphocytic infiltration, or rejection episodes that do not respond to pulse steroids, usually require a more aggressive strategy using anti-lymphocyte antibody preparations (27). The monoclonal anti-CD3, OKT3, was favored for many years, but Thymoglobulin, polyclonal rabbit anti-human Thymocyte globulin, has more recently been favored by many clinicians, largely because it has been associated with excellent outcomes and fewer drug associated adverse side effects (28). Therapy of refractory acute rejection...
rejection has become a subject of significant controversy, especially when a humoral component has been demonstrated. It is this setting where several reports have now suggested efficacy using plasmapheresis and intravenous hyperimmune globulin (29, 30, 31,32).

In conclusion, renal transplantation has evolved dramatically from an experimental curiosity to mainstream therapy for end stage renal disease during the last 25 years, principally resulting from advances in immunosuppression. Beginning in the 1980s with cyclosporine, then the related calcineurin inhibitor, tacrolimus, the outlook has continued to improve. The selective inhibition of cytokine gene activation exhibited by these agents permitted more effective prophylaxis of acute rejection while sparing major other elements of nonspecific host defense. This has allowed more judicious application of more global agents such as azathioprine, anti-T cell antibody preparations, and more recently, mycophenolate mofetil, resulting in overall efficacy and safety. The discovery that sirolimus and more recently everolimus, exert synergistic effects when used in combination with calcineurin inhibitors, cyclosporine and tacrolimus provide opportunities to reduce dosing of each agent. Accordingly, one might anticipate this will yield improved efficacy, while minimizing toxicities associated with each agent when given at higher dose. Similarly now feasible is corticosteroid free immunosuppression, a particularly important goal due to the substantial co-morbidities associated with long term steroid therapy. Newer combination therapies using, for example sirolimus or everolimus with low dose cyclosporine, or tacrolimus, promise to dramatically reduce the incidence of acute rejection. Alternative approaches using calcineurin inhibitors with mycophenolate mofetil, sirolimus with mycophenolate mofetil, may also provide benefit. Together, the available options for post renal transplant immunosuppression will provide increasingly effective, better tolerated approaches, that seem likely to improve the long term outlook for patients after renal transplantation. These approaches will provide the clinician with increasingly more effective therapies, as we eagerly await the future goal of the long term allograft tolerance.

REFERENCES


