Protein therapies and antiproliferatives: a new paradigm in immunosuppression

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Abstract

The development of immunosuppressive therapies has focused on inhibiting effects of the activated T cell. The introduction of powerful immunosuppressive agents that interrupt the effects of T-cell activation, such as the calcineurin inhibitors (CNIs), has revolutionized solid organ transplantation. However, the ubiquitous location of their targets causes a number of side effects, which can compromise recipient health and long-term allograft survival. Therefore, a common goal in the development of emerging immunosuppressive strategies is to maintain efficacy and minimize toxicities related to these immunosuppressant compounds. The rationale for CNI-free regimens that exploit combinations of antiproliferative and protein therapeutic agents is attractive. Recently, studies employing these agents in CNI-free regimens have begun to offer additional insight into both the potential benefits and limitations of currently available strategies. The currently available biologic agents provide either too potent immunosuppression (eg, T-cell depletion) or inhibit an aspect of T-cell activation too limited to provide adequate rejection prophylaxis (eg, interleukin 2 receptor [IL-2R] blockade). Growing evidence suggests that costimulation blockade, particularly those protein therapeutics targeting CD28 and CD40, provides the correct balance between immunosuppressive and low toxicity, with a more specific, nondepleting, and timely targeting of the immune response. Already, results from a phase 2 trial suggests that combination with a costimulation blockade using belatacept with mycophenolate mofetil as a maintenance therapy after induction with an IL-2R blocker is closest to fulfill this promise. Belatacept represents an emerging immunosuppression paradigm with maintenance protein therapy that fulfills the need of more selective immunosuppression with reduced toxicities, which offers the potential of improving long-term outcomes in renal transplant.

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1. Introduction

The goal of immunosuppressive therapy in transplantation is to facilitate the adaptive mechanisms that lead to the acceptance of the allograft by the recipient. The goal of successful pharmacologic management is to accomplish this outcome without inducing an immunodeficient state in the recipient and with the minimum nonimmunologic side effects. With the introduction of the calcineurin inhibitors (CNIs) cyclosporine (CsA) (1983) and tacrolimus (1995), dramatic improvements in short-term graft survival rates [1] of renal transplants have been achieved. Between 1988 and 1999, the 1-year living-donor transplant recipient survival rate improved from 89.7% (1988) to 94.3% (1999), whereas the 1-year deceased-donor transplant recipient survival rate improved from 76.8% to 89.3% over the same period [2].

However, equivalent improvements have not been realized for the long-term survival of renal allografts [3]. In fact, the introduction of more effective immunosuppressive regimens in the late 1990s produced dramatic reduction in acute rejection rates that were not associated with concomitant improvement in long-term graft survival. The use of CNIs not only was an important catalyst for the reduction of acute rejection rates and improvement in short-term graft survival, but also highlighted a number of new concerns. Calcineurin inhibitors and corticosteroids contribute to the high incidence of cardiovascular and metabolic disorders after transplantation. These disorders account for most of the patients who die with a functioning graft [4,5]. Furthermore, the nephrotoxicity associated with the use of CNIs may limit long-term graft survival [6,7].

In the current era of low acute rejection rates, advancing immunosuppressive therapy is now focused on better graft
2. Lessons learned with current immunosuppressive therapy

The CNIs CsA and tacrolimus are well-established cornerstones of immunosuppressive maintenance therapy and proven to significantly reduce acute rejection [1]. However, the lack of comparable improvement in long-term outcomes is, in part, due to the toxicities associated with CNIs. These toxicities are due to action on ubiquitously expressed pathways [9,10] and result in nephrotoxicity, neurotoxicity, hypertension, diabetes, and hyperlipidemia, as well as quality of life issues (ie, cosmetic side effects) [11,12].

Several studies suggest that one of the limiting factors to achieving improved long-term graft survival rates is chronic allograft nephropathy, a multifactorial process characterized by tubular atrophy, interstitial fibrosis, increasing arteriolar hyalinosis, and progressive ischemic glomerulosclerosis [6,7,11]. Several studies have established an association between the use of CNIs and nephrotoxicity [11,13,14]. The resulting benefit seen in the short term, namely, prevention of acute rejection, is limited by the nephrotoxicity of these agents in the long term [15,16]. A recent study by Nankivell et al [11,17] evaluated kidney transplant biopsies obtained regularly for 10 years from the time of transplantation in patients with type 1 diabetes who received kidney-pancreas transplants. By 10 years after transplantation, histologic evidence of irreversible CNI-induced nephrotoxicity was universally found, despite reductions in CsA dose, and demonstrated an increase in prevalence with prolonged time after transplant [11,17] (Fig. 1).

Thus, despite the benefits of CNIs in the prophylaxis of early acute rejection, it is now clear that these agents are associated with long-term renal allograft injury. Therefore, the focus of transplant immunology has moved to identify-

3. Protein therapeutics and antiproliferative combinations—addressing the deficiencies in immunosuppressive therapy

The rationale for CNI-free regimens that exploit combinations of protein therapeutics and antiproliferative agents is attractive. Technological advances in protein therapeutics have enabled the construction of molecules that exert more specific mechanisms of action and, therefore, greater selectivity in terms of immunosuppression. Combining these protein therapeutics with antiproliferative agents may enable more selective targeting and inhibition of immune cells.

Current antiproliferative agents include the mycophenolate mofetil (MMF) and the mammalian target of rapamycin (mTOR) inhibitors sirolimus and everolimus. These agents have been combined with protein therapeutics, including IL-2R antagonists, polyclonal rabbit antithymocyte (rATG) preparations, and alemtuzumab, a humanized anti-CD52 monoclonal antibody. These combinations have allowed new opportunities to explore potentially less toxic CNI-free maintenance regimens. These strategies currently used are being investigated to achieve either CNI- or steroid-free regimens.

3.1. Interleukin 2 receptor blockade and mycophenolate mofetil therapy

One approach is to prevent immune engagement and early T-cell activation and, thus, induce a quiescent state during the early influx of cells, responding to tissue injury from preservation, reperfusion, and alloresponse. The introduction of nondepleting monoclonal antibodies, the IL-2R blockers basiliximab and daclizumab [18], which inhibit IL-2 signaling and thus inhibit growth and proliferation (ie, amplification) of activated T-cells, provided an
opportunity to spare CNIs. Their specificity for T cells means they have the potential to exert a lower incidence of nonimmune side effects. Mycophenolate mofetil is used as maintenance immunosuppression to decrease the proliferative response of T and B cells by inhibiting the activity of inosine 5’-monophosphate dehydrogenase, a key enzyme in the de novo pathway of guanosine nucleotide synthesis [19]. Combining a highly specific biologic (the IL-2R monoclonal antibody) with a nonnephrotoxic agent (MMF) offered a regimen with minimal toxicity [20].

A multicenter trial used daclizumab in combination with MMF and conventional corticosteroid therapy as part of a CNI-avoidance regimen. In total, 48% of patients had biopsy-proven rejection during the first 6 months after transplant; the median time to the initial biopsy-proven rejection in these same patients was 39 days. At 1 year, patient and graft survivals were 97% and 96%, respectively. The mechanism of rejection was postulated to be caused by other pathways, such as effects mediated by IL-15, because circulating and intragraft lymphocytes during rejection had saturated IL-2Rs [20].

In another study, patients (n = 45) who had received renal allografts were treated with daclizumab, MMF, and steroids, without CNIs. Cyclosporine A was added to the regimen if patients developed acute rejection episodes or adverse reactions to steroids or MMF. In total, 51% of patients required CsA therapy [21].

These studies demonstrated that a regimen combining IL-2R blockade with an antiproliferative, although less toxic, is not sufficiently effective to be used in the long term as maintenance therapy. This may be because of the fact that other pathways that perpetuate the immune response, such as those leading to T-cell activation, can still exist. The use of anti–IL-2 antibodies in combination with 2 antiproliferatives (MMF and sirolimus) appears to be more effective but may be less well tolerated [22,23]. Rigorous studies to test the safety and effectiveness of the combination of MMF and sirolimus are underway.

3.2. rATG/alemtuzumab and sirolimus

Another approach to preventing the initial immune engagement is to delete immune effector cells completely to prevent the early steps in immune engagement and activation. Depleting protein therapeutics specifically target extracellular T-cell receptors and decrease T-cell activities by removing lymphocytes. These depleting biologics have been utilized in conjunction with mTOR inhibitors. The mTOR inhibitors sirolimus and everolimus inhibit pathways leading to cell cycling and, hence, limit T-cell clonal expansion [24].

Alemtuzumab (Campath) is a humanized monoclonal antibody against CD52, which is expressed on lymphocytes, monocytes, and macrophages. Alemtuzumab causes profound and prolonged T-cell depletion via complement-dependent lysis [25]. A recent study in recipients of living-donor kidneys who received alemtuzumab perioperatively as monotherapy with no maintenance immunosuppression exhibited profound lymphocyte depletion in the periphery and secondary lymphoid tissue, but all patients (n = 7) developed rejection episodes within the first month, characterized by monocytic infiltration, which responded to steroid and/or sirolimus treatment [26].

A better example of the concept of proteins and antiproliferatives is the trial reported by Knechtle et al [27]. Twenty-nine primary renal transplant patients were treated with 2 doses of alemtuzumab (20 mg at days 0 and 1 or at days –1 and 0), 1 dose of solumedrol, 500 mg, steroids for 14 days and maintenance sirolimus therapy. However, 12 of 29 patients developed acute rejection and half of the rejections were vascular. These findings demonstrate an unacceptably high incidence of early aggressive rejection [28]. Thus, alemtuzumab and antiproliferatives without CNI do not provide adequate safety.

The findings from studies with depleting protein therapeutics such as alemtuzumab suggest that, although promising, the immunosuppression provided is probably insufficient to dramatically improve long-term outcomes. The results suggest that T-cell depletion alone does not induce tolerance, can dysregulate B-cells (thus, the high incidence of humoral rejection), and demonstrate a key role for monocytes in the early response to the allograft [26]. Indeed, monocytes are still able to trigger an immune response [28,29]. Additionally, treatment with these agents does not address the development of a preferential memory T-cell proliferation as a response to T-cell depletion [30]. Thus, recent trials with alemtuzumab have reincorporated CNIs for the first few months after transplantation.

3.3. Interleukin 2 agonist, IL-15 antagonist, and sirolimus

A series of studies from Terry Strom’s laboratory has demonstrated that the use of 2 proteins, an IL-2 agonist and an IL-15 antagonist (IL-15 mutant/Fcγ2a fusion protein) in combination with sirolimus, can result in prolonged tolerance in rodent models of transplantation [31]. The combined protein therapies accelerate activation-induced apoptosis, limit T-cell clonal expansion, and preserve the presence of T regulatory cells. Whether this successful approach can be duplicated in nonhuman primates remains to be determined. It is clear that human trials with this combination will be more challenging in organ transplantation than autoimmunity. In contrast, the addition of an IL-15 antagonist to a combination of anti–IL-2 antibodies and an antiproliferative may represent an intriguing immunosuppression regimen.

4. Selective costimulation blockade—a new paradigm in maintenance therapy

T-cells require 2 signals for their full activation, a phenomenon first described by Bretscher and Cohn in 1970 [32]: signal 1 is T-cell/antigen interaction; signal 2 is a costimulatory signal caused by ligation of a distinct receptor
on the T cell (Fig. 2). In the absence of the costimulatory signal, T cells fail to proliferate, do not secrete proinflammatory cytokines, and can become anergic or apoptotic [33]. Thus, inhibiting this second signal has become the focus of new agents for maintenance immunosuppressants.

There are several costimulatory pathways, which can either up-regulate or down-regulate T-cell activation. One of the best-characterized costimulatory pathways is interaction or binding of the T-cell surface receptor CD28 with CD80 (B7-1) and CD86 (B7-2) on the antigen-presenting cell [34]. The homologue to CD28, CTLA4, is expressed transiently after T-cell activation and is a negative regulator of T-cell activation [35]. CTLA4 competitively inhibits the binding of CD28 to CD80 and CD86 as it binds with greater affinity compared with CD28 [36,37].

Another important costimulatory pathway involves the interaction between CD40 on T cells and its ligand CD154. CD40-CD154 interactions have been demonstrated to be critical in the activation of B cells [38]. It has also been demonstrated that the interaction of CD40 with CD154 plays an important role in T-cell activation, in part, via up-regulation of CD80/86 [39,40].

4.1. Early work using selective costimulation blockade in transplantation

Agents that block costimulation are being tested in the clinic. One of the earliest agents to be tested targeted the CD40-CD154 costimulatory pathway. CD154 is expressed on vascular endothelial cells, smooth muscle cells, platelets, and macrophages, suggesting a possible role for CD40-CD154 during transplant rejection. The CD40/CD154 interaction has been identified as playing a role in chronic rejection [41]. In the rhesus monkey, treatment with humanized anti-CD154 enabled allogeneic islet engraftment and long-term insulin independence after transplantation of intrahepatic islet allografts [42]. Humanized anti-CD154 therapy in nonhuman primates induced prolonged renal allograft survival in a CNI- and steroid-free regimen [43]. The ability of anti-CD154 to achieve prolonged graft survival in nonhuman primates led to a pilot study in humans using hu5C8, a humanized anti-CD154 with MMF (with only 2 weeks of steroid therapy). Treatment with hu5C8 was associated with thromboembolic complications, possibly owing to expression of CD154 on platelets and its role in stabilizing arterial thrombi [44,45]. The trial was halted. Furthermore, in this trial, treatment with anti-CD154 was associated with a high acute rejection rate. The combination of thromboembolic complications and questionable efficacy has halted clinical development of all anti-CD154 therapies [46]. A potential alternative is the targeting of CD40 with monoclonal antibodies (already in clinical development).

5. CTLA4Ig and belatacept

CTLA4Ig, a soluble recombinant immunoglobulin fusion protein, comprised of the extracellular domain of CTLA4 and the Fc portion of IgG1, was developed as a single agent
to competitively inhibit both CD80 and CD86 molecules from interacting with their receptors. Preclinical studies demonstrated CTLA4Ig inhibition of T-cell–dependent antibody responses and prolonged transplanted organ survival [41,47,48]. Abatacept (human CTLA4Ig), a selective costimulation modulator, has shown efficacy in the treatment of human autoimmune disorders, rheumatoid arthritis, and psoriasis [48,49]. CTLA4Ig has also demonstrated an ability to increase cardiac allograft survival in rodents [50].

However, results with CTLA4Ig monotherapy in nonhuman primate transplant models were disappointing [51,52]. This may be because CTLA4Ig binds less strongly to CD86 than CD80 [36]. CD86 is expressed early after signal 1 [53] and is involved in recruiting CD28 to the immunologic synapse, which is crucial for full T-cell activation [54]. As a result of these findings, pursuit of CTLA4Ig as a potential therapy in transplant immunosuppression was discontinued.

5.1. Selective costimulation blockade in transplantation

Given what was learned with CTLA4Ig, it was reasoned that a compound that inhibited CD86 more effectively than CTLA4Ig would offer the efficacy required to prevent allograft rejection. This led to the development of belatacept...
(LEA29Y), a modified version of CTLA4Ig, which was rationally designed to provide more potent immunosuppression, for transplantation (Fig. 3). Belatacept differs by 2 amino acid residues within the region that binds to CD80 and CD86; these amino acid substitutes result in the changed binding profile of belatacept, which possesses an approximate 4-fold greater binding avidity to CD86 and an approximate 2-fold greater binding avidity to CD80 than CTLA4Ig [55]. Belatacept has also demonstrated ~10-fold more potent inhibition of T-cell activation in vitro and increased efficacy at preventing rejection in primate renal transplantation [55]. The clinical development of belatacept offers a new paradigm in immunosuppression: chronic intermittent parenteral protein therapy.

A phase 2 multicenter clinical study was carried out comparing the safety and efficacy of belatacept vs CsA as part of a quadruple immunosuppressive drug regimen in 218 patients who had undergone renal transplant with deceased- or living-donor kidneys [56] (Fig. 4). Patients received a more or less intensive belatacept dosing regimen or CsA, all in combination with MMF, corticosteroids, and basiliximab. At 6 months, there was no significant difference in the incidence of clinically suspected biopsy-proven acute rejection between treatment groups. In addition, the study demonstrated that renal function was significantly better in patients receiving belatacept vs patients receiving CsA. Cardiovascular and metabolic endpoints (hyperlipidemia) were more favorable in patients treated with belatacept [56,57].

Together, these data provide evidence that belatacept has the potential to offer a new treatment paradigm for transplant patients, as evidenced by an improvement in renal function and cardiovascular and metabolic events compared with CsA. Early improvements in renal function have been suggested to correlate with improved long-term outcomes [2]. In addition, improving cardiovascular and metabolic risk factors is likely to have a beneficial effect on patient survival [4]. Thus, these data suggest that, for the first time, a protein therapeutic can be used in place of CNIs as a maintenance immunosuppressant combination.

5.2. Future studies with belatacept

Belatacept is currently in 2 phase 3 trials with protocols similar to the phase 2 (basiliximab induction, MMF, and prednisone). A planned pilot trial at University of California at San Francisco and Emory University, Atlanta, Ga, is being supported by the Immune Tolerance Network to facilitate drug withdrawal. The regimen is free of both steroids and CNIs and utilizes induction with daclizumab and maintenance therapy with sirolimus (Fig. 5). Drug withdrawal will be implemented in selected patients if they can be demonstrated to be tolerant to their renal allograft.

5.3. Future investigation of costimulatory pathways

Combination therapy blocking both the CD28 and CD40 pathways has been demonstrated to effectively prevent T-cell clonal expansion both in vitro and in vivo, promote long-term survival of fully allogeneic skin grafts, and inhibit the development of chronic vascular rejection of primarily vascularized cardiac allografts. These findings suggest that the CD28 and CD40 pathways are critical interdependent regulators of T-cell–dependent immune responses [58]. In a recent study, the effect of belatacept and a chimeric antibody targeting CD40 (Chi220) in a nonhuman primate model of islet transplantation was studied [59]. The study demonstrated that although either agent alone only modestly prolonged islet survival, combination of belatacept and Chi220 significantly increased long-term survival [59]. This provides early evidence of the potential for highly selective therapies using these new costimulation–targeted protein therapeutics as maintenance immunosuppressants to improve long-term outcomes in transplantation.

References


Zheng X, Sánchez-Fueyo A, Sho M, et al. Favorably tipping the balance between cytopathic and regulatory cells to create transplan


