

## Immunosuppressants in Composite Tissue Allotransplantation

a report by

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### Introduction

Composite tissue allograft (CTA) such as hand and face transplant has now become a clinical reality. Various treatment protocols have been implemented to prevent rejection. These have been derived from solid organ transplant protocols. However, CTA represents a construct containing tissues derived from all three germ layers: ectoderm, mesoderm, and endoderm. CTAs differ from solid organ transplants not only in their relative antigenicities, but also in their requirements for immunosuppression. Consequently, skin—being highly antigenic—requires maximum immunosuppression.<sup>1</sup> In addition, skin is easily accessible and therefore lends itself to the possibility of topical immunosuppression. The first hand transplantation was performed in Ecuador in 1964 using azathioprine and steroids.<sup>2</sup> This protocol, though successfully used in solid organ transplants, was insufficient for CTA and the hand allograft was rejected after 14 days. This demonstrates the complexity of designing immunosuppressive protocols for CTA.

Over the past century, the concept of interfering with the immune

response at various sites by blocking the formation, stimulation, proliferation, and differentiation of lymphocytes has led to the relentless development of new immunosuppressive drugs. These agents are associated with reduced risk of short- and long-term toxicity and have dramatically improved allograft and patient survival. This brief review focuses on agents currently used in clinical CTA.

### Rationale for Immunotherapy in CTA

The host response to an allograft determines its overall survival. Recognition of non-self minor histocompatibility (MHC) antigens triggers the immune system to reject the allograft. Therefore, allogenic transplantation will not succeed unless the recipient immune system is downregulated. Furthermore, downregulation through immunomodulation must be maintained on a lifelong basis, as the antigen (the allograft) is not self-limiting; it is always present. There are two main strategies for achieving this objective: immunosuppressive therapy (immunotherapy) and tolerance induction. Immunotherapy has been a feature of the transplant arena since the 1950s, while tolerance induction is currently under intensive investigation.

The rationale supporting immunomodulation in transplantation is simple—downregulate the recipient immune response against the allograft in such a way that all other immune responses remain intact. Thus, the following must be considered when designing an immunosuppressive regimen:

- Sufficient immunosuppression must be administered to circumvent short- and long-term damage to the transplanted CTA by allogenic response.
- The total immunosuppressive load should be low enough to allow the recipient's immune system to respond to infectious organisms and carry out surveillance for tumor cells.
- Since CTA is usually performed as a 'non-lifesaving' procedure on relatively healthy individuals, it is imperative that immunosuppressive agents with complementary mechanisms of action be combined to optimize the efficacy of individual agents and to reduce the risk of toxicity associated with them.

### Immunosuppressive Drugs

There are three distinctive eras of immunosuppressive drugs.<sup>3</sup> The first era extended from 1956 to 1984, starting with the use of total body irradiation, steroids, and then azathioprine, as well as initial use of polyclonal anti-lymphocyte antibodies. With the introduction of cyclosporine (CsA) in 1984, the transplantation field took a giant leap forward and was associated with the initial use of monoclonal antibodies, particularly OKT3. From 1994, there was a widespread proliferation of newer immunosuppressive drugs, particularly monoclonal

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antibodies directed against the IL-2 receptor and various T-cell receptors. In addition, newer synergistic drugs targeting the signaling between cells—e.g. tacrolimus and sirolimus—and anti-proliferative drugs—e.g. mycophenolate—were increasingly being used. All agents currently used in the mainstay of immunosuppressive regimens to prevent rejections (except corticosteroids) interfere with discrete sites in the T- and B-cell activation and migration cascade.

Immunosuppressive drugs may be classified depending on their mechanism of action or according to how they are used in immunosuppression—induction versus maintenance. Furthermore, the same drug—depending on its dose—can be used for induction, maintenance, or treatment of acute rejection.

### Induction Therapy

The goal of induction therapy is to turn off the recipient immune system in anticipation of the allograft. These drugs are very potent and induce profound T-cell depletion before allotransplantation, with gradual post-transplant T-cell repopulation. The mechanism of allo-engraftment is donor leukocyte-driven exhaustion and deletion of the anti-donor immune response.<sup>4</sup> These drugs reduce the risk of acute rejection, help to decrease the dose of maintenance immunosuppressive drugs, and facilitate delayed introduction of calcineurin inhibitors.<sup>5,6</sup>

### Polyclonal Antibodies

Anti-thymocyte globulin (ATG) is a polyclonal antibody preparation of purified immunoglobulin from animals—rabbits or horses—after immunization with human lymphocytes.<sup>7</sup> These polyclonal antibodies opsonize and deplete T-cells by causing complement-mediated lysis or reticuloendothelial cell-mediated phagocytosis.<sup>8</sup> Use of ATG as an induction agent along with other immunosuppressive drugs has been shown to result in a significant reduction in acute rejection rates for various solid organs.<sup>9-11</sup> ATG, along with tacrolimus, steroids, and mycophenolate, has been used for induction therapy in hand transplant patients at various centers.<sup>12-14</sup> In addition, polyclonal antibodies (thymoglobulin) have been used with other immunosuppressants for induction therapy in the first human face transplant.<sup>15</sup> Although polyclonal antibodies are inexpensive to produce, they have a low degree of specificity to the immunizing antigen. Their side effects include formation of anti-antibodies, serum sickness, dose-limiting leucopenia, and infusion-related reactions such as fever, chills, and other systemic effects.<sup>16</sup> These side effects can be partially circumvented by using monoclonal antibodies.

### Monoclonal Antibodies

#### *Muromonab-CD3 (OKT3)*

Muromonab-CD3 (OKT3) is a murine monoclonal antibody directed against a molecular subunit of the T-cell antigen receptor. Although it has been used in solid organ transplantation,<sup>17</sup> side effects have prevented its use for CTA. More recently, a humanized anti-CD3 antibody has been developed to reduce the side effects of the murine counterpart and is currently undergoing clinical trials.<sup>18</sup>

#### *Anti-interleukin-2 Receptor (IL-2R) Antibodies*

Daclizumab and Basiliximab are humanized and chimeric immunoglobulin G (IgG) monoclonal antibodies with a high affinity for the alpha subunit of the

IL-2R (CD25). These agents inhibit the binding of IL-2 to IL-2R on T-cells, thereby competitively inhibiting the activation and proliferation of T-cells.<sup>19,20</sup> They have been used as induction agents in various protocols for solid organ transplantation.<sup>19,20</sup> Basiliximab has been used with tacrolimus in induction therapy for hand transplantation.<sup>21</sup>

#### *Anti-CD52 Antibody*

Alemtuzumab (campath-1H) is a humanized monoclonal antibody against membrane glycoprotein CD52 that is present on T-cells, B-cells, monocytes, macrophages, natural killer cells, and granulocytes.<sup>22</sup> Alemtuzumab rapidly depletes CD52-expressing cells both centrally and peripherally.<sup>23</sup> It has been shown to allow a reduction in maintenance therapy without an increase in infectious or malignant complications in solid organ transplantation when compared with historical controls.<sup>23-26</sup> Alemtuzumab is a potent cellular-depleting agent. It has been used as an induction immunosuppressant for human abdominal wall transplantation<sup>27</sup> and hand transplantation (personal communication). In addition, acute rejection that has been resistant to steroid and ATG reversal following hand transplantation has been shown to respond to alemtuzumab therapy.<sup>28</sup>

### Maintenance Therapy

The highest risk of rejection occurs during the first few months post-transplant, necessitating potent immunosuppressants as an induction. The goal of maintenance therapy is to use minimal immunosuppression—just enough to prevent rejection while keeping the host immunocompetent to infectious diseases and tumor surveillance. Multiple immunosuppressants targeting the immune system at different levels are used at a low dose to produce a synergistic effect with minimal toxicity.

### Corticosteroids

Corticosteroids have been the mainstay of immunosuppression since the 1920s. They interrupt the immune system at various stages due to universal expression of glucocorticoid receptor. They have a negative effect on peripheral blood lymphocyte and monocyte count.<sup>29,30</sup> In addition, they inhibit T-cell activation, pro-inflammatory cytokine production, and prostaglandin production.<sup>31</sup>

Prednisone has been used as an integral part of maintenance therapy for most solid organ and CTA transplantation. High doses of methylprednisone have been used successfully to treat acute rejections following transplantation. However, long-term complications such as delayed wound healing, opportunistic infections, metabolic derangements, and avascular necrosis of the hip warrant judicious use of steroids as maintenance therapy. Newer steroid-free protocols are being implemented to avoid these complications.<sup>32,33</sup>

### Calcineurin Inhibitors

Calcineurin is an important protein in the intracellular signaling cascade for cytokine production. Calcineurin inhibitors downregulate cytokine production without turning it off completely.

### Cyclosporine (CsA)

With the introduction of CsA in the 1980s, transplantation became the preferred treatment for end-stage organ disease. It has been used as an integral part of immunosuppressive regimens around the world. In combination with steroids, CsA dramatically reduces acute rejection rates post-transplant. However, CsA causes dose-related nephrotoxicity. In addition, hepatotoxicity, neurotoxicity, hypertension, and gingival

hyperplasia have limited the use of CsA in CTA.

## Tacrolimus (Tac)

Tac is a macrolide antibiotic produced by *Streptococcus tsukubaensis*. It is 10–200 times more potent than CsA.<sup>34</sup> It binds to the intracellular FK506 binding protein (FKBP 12) and blocks calcineurin-mediated cytokine production. It has become a standard immunosuppressant for organ and CTA transplantation. Due to its potency, Tac has been used as monotherapy for kidney transplantation.<sup>35,36</sup> In addition, Tac has a beneficial effect on nerve growth—probably through FKBP-52, hsp-90, and p23<sup>37</sup>—and has been used for human nerve allotransplantation. It has been used systemically as well as topically for hand and face allotransplantation for maintenance and treating acute skin rejection.<sup>33</sup> However, nephrotoxicity, neurotoxicity, and inability to prevent chronic rejection have led to a search for newer immunosuppressants.

## Mammalian Target of Rapamycin (mTOR) Inhibitors

Sirolimus (rapamycin) is an antibiotic isolated from *Streptococcus hygroscopicus*. It binds to FKBP-12 and inhibits serine-threonine protein kinase (mTOR), thereby preventing cell cycle progression from the G1 to the S phase. It has been shown to induce FoxP3 T regulatory cells.<sup>38</sup> In addition, it inhibits fibroblasts,<sup>39</sup> platelet-derived growth factor, vascular smooth muscle proliferation,<sup>40</sup> and endothelial and intimal thickening.<sup>41</sup> Sirolimus has beneficial effects in preventing post-transplant malignancies.<sup>42,43</sup> This profile has prompted many transplant centers to include sirolimus in maintenance therapy. There is only one animal study using sirolimus in CTA; however, its impressive ‘anti-chronic rejection’ profile has prompted its use with mycophenolate in a human hand transplant patient.<sup>33</sup> Its major adverse effects include delayed wound healing, hyperlipidemia, anemia, and thrombocytopenia.

## Anti-proliferative Agents

Due to its systemic toxicity, most transplant centers have stopped using azathioprine. Mycophenolate mofetil (MMF) is a reversible, non-competitive inhibitor of inosine monophosphate dehydrogenase-2, which is required for DNA synthesis in proliferating T- and B-cells. It thus blocks proliferation of T-cells and suppresses B-cell antibody production without affecting bone marrow and parenchymal cells.<sup>44</sup> Its efficacy has been shown in various animal models of CTA transplantation<sup>45,46</sup> and has been shown to delay the onset of chronic rejection.<sup>47</sup> MMF in combination with Tac and steroids has been the mainstay of maintenance immunosuppression in current CTA transplants. In combination with sirolimus, it has been used in a hand

transplant patient to prevent anticipated chronic rejection.<sup>33</sup> Its side effects are mild and include leucopenia, gastritis, and opportunistic infections with cytomegalovirus.

## Treatment of Acute Rejections

Due to the heightened antigenicity of CTA, in spite of induction therapy and maintenance therapy, all CTA patients have had breakthrough acute rejection episodes. These vary in intensity and frequency, and also vary with the immunosuppressive protocol used, donor-recipient human leukocyte antigen (HLA) matching, and the time interval post-transplant. In milder cases, a transient increase in the dose of maintenance drugs can reverse the episode. Steroids in particular have been the mainstay in this regard. Topical Tac or steroids have been used for clinical skin rejection.<sup>48</sup> In one hand transplant patient, alemtuzumab was used successfully to rescue steroid and ATG-resistant acute rejection in an otherwise lost allograft. This underlines the complex requirements of CTA for immunosuppression.

## Newer Immunosuppressive Agents

Advances in transplantation pharmacology have led to an explosion in immunosuppressant drug design, with newer drugs specifically designed to inhibit selective aspects of the immune system while at the same time minimizing toxicity. Everolimus is a macrolide antibiotic similar to sirolimus, but with a better oral bioavailability profile and efficacy. It has been used in solid organ transplantation with success. FTY720 is a sphingosine 1 phosphate receptor agonist that prevents egression of lymphocytes from secondary lymphoid tissue and prevents CD4, CD8, and memory T-cells from accessing inflammation sites.<sup>49</sup> Co-stimulation blockade has been trialed successfully in animal models of organ transplantation, but has not replicated these results in human studies. Belatacept in particular has shown promise and is currently undergoing phase III trials.<sup>50</sup> LF15-0195 is a new analog of 15-deoxypergualin that decreases macrophage and T-cell infiltration into the allograft.<sup>51</sup>

## Conclusion

The role of safe immunosuppression is crucial for patient and graft survival. As CTA is a ‘non-life saving’ transplant, it warrants careful consideration in immunosuppression. The key is to cause initial immune switch-off using potent induction agents, then use drug therapy to minimize individual toxicity while at the same time maximizing clinical benefit. New immunosuppressants need to be thoroughly investigated in animal models before their use in composite tissue allotransplantation. ■

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