Introduction

Composite tissue allotransplantation has been recently introduced as a potential clinical treatment for complex reconstructive procedures, include traumatic injuries, cancer ablative surgeries, or extensive tissue loss secondary to burns. Composite tissue allografts (CTAs) consist of heterogeneous tissues derived from ectoderm and mesoderm, including skin, fat, muscle, nerves, lymph nodes, bone, cartilage, ligaments, and bone marrow, with different antigenicity. Thus, composite tissue structure is considered to be more immunogenic than solid organ transplants. While cartilage, ligaments, and fat present low antigenicity, bone, muscles, nerves, and vessels present moderate antigenicity, and skin is the component that develops the most severe rejection because of the abundance of dendritic cells within the epidermis and dermis. To study the mechanisms of CTA acceptance and rejection, different experimental models, strategies and different immunosuppressive protocols have used [1, 2].

Composite Tissue Allograft Transplantation Studies Using Immunosuppressive Protocols

In 1971, before the introduction of the cyclosporin A (CsA) to the CTA transplantation area, Lance et al. developed a canine hind-limb allotransplantation model and undertook painstaking comparison of immunomodulatory regimens that included combinations of azathioprine, hydrocortisone, and antilymphocyte serum. The researchers reported in excess of 300 days' graft survival rate in one animal. For the first time in the literature, this study revealed that immunologic barriers in CTA could be overcome and that long-term successful CTA transplantation was possible [3, 4]. In 1978, developments in the technical expertise of microvascular repair of rat hind limbs revealed alloantigenic systems which established the rat hind limb as the prototype model in CTA transplantation in [5–7]. In 1979, Doi published a study of rat limb allotransplantation across known histocompatibility barriers utilizing three nonspecific immunosuppressive agents (azathioprine, 6-mercaptopurine, and prednisolone) in different doses and combinations [8]. In this study, graft survival was shown to be extended in the treatment groups, especially in those treated with a combination of azathioprine and prednisolone. However, the most significant feature of this study was that 100% of the treated rats died from side-effects from the immunosuppressive regimen. For this reason, graft survival in these groups could not be ascertained because of early animal death (9–24 days posttransplant); histologic evidence of rejection was not apparent. All untreated allografts rejected between 10 and 15 days, with histologic confirmation. The researcher concluded that immunosuppressive therapy would require dramatic improvement
before CTA could be realized in clinical application [4].

The first report of CTA using CsA appeared in 1982. Black et al. [9] performed rat hind-limb transplantation in inbred strains of rats, with Lewis (LEW) (RT11) rats serving as recipients and Lewis Brown Norway (LBN) (RT11+n) F1 animals serving as donors. They reported extension of rat hind-limb survival from 18±5 days in untreated grafts to 101±13 days in animals receiving a 20-day perioperative course of 25 mg/kg per day dosage of CsA. This marked a significant breakthrough in CTA, making CsA the mainstay of immunomodulatory therapy in subsequent investigations for many years [4].

Most experimental studies on CTAs have been performed on rodents by using a hind-limb transplantation model. Monotherapies using calcineurin inhibitors [CsA, tacrolimus (FK-506)] are known to prolong allograft survival only if they are given in high doses and throughout the recipient’s life. Only a few studies reported survival longer than 1 year by using CsA on major histocompatibility complex (MHC)-mismatched animals [10, 11]. The combined use of CsA with prednisone reduced the amount of CsA dose and prolonged graft survival up to 210 days, but again, infection and rejection rates were high [12]. We found that CsA combined with topical fluocinolone acetonide to prevent skin rejection extended allograft survival and allowed for the use of a reduced dose of CsA to achieve long-term survival [13]. The use of FK-506 as a singledose (10 mg/kg) protocol on the day of transplant followed by single weekly injections (3 mg/kg) produced complete graft survival over 200 days [14]. FK-506 was also found to be more potent than CsA in a study using 114 hind-limb transplants across an MHC mismatch [15]. It is clearly understood that combination therapies are more successful than monotherapy protocols. Low doses of CsA with mycophenolate mofetil (MMF) showed long-term survival over 231 days in 89% of recipients, with a return of full sensory and partial motor function [16]. Combining FK-506 and 15-deoxyspergualin showed a 120-day rejection-free survival when both drugs were given for 30 days after transplantation [17]. Experimental rodent data reveal that effective immunosuppression and graft survival in CTAs can be achieved by using chronic administration of combination therapies, with substantial morbidity and mortality.

Experimental data on CTAs from large animals are limited. Ustuner and colleagues employed the swine as a large animal model for CTA research. Transplantation of a radial forelimb osteomyocutaneous flap between outbred swine using the combined treatment of CsA, MMF, and prednisone was reported. In this model, the graft consisted of a segment of the forelimb that included a portion of radius, the flexor carpi radialis, and overlying skin. The vascular supply was through the brachial artery and cephalic veins, and a segment of median nerve was included in to the CTA model [18]. In this study, three of eight allografts were found to be rejection free at 90 days after transplantation. Antirejection effect and systemic side-effects of combined FK-506 and MMF were assessed in a radial forelimb osteomyocutaneous flap model in outbred pigs. Five of nine animals survived for 90 days without any signs of rejection. It was found that this combination provided superior antirejection effect when compared with CsA/MMF regimen but showed more toxicity [19].

Lee et al. recently reported an inbred swine model of heterotopic partial limb allotransplantation [20]. The graft consisted of donor tibia, fibula, knee joint, distal femur, and associated musculature on a femoral arteriovenous pedicle; no skin was included. The vessels were anastomosed in an end-to-side fashion to recipient vessels and the graft inserted into a subcutaneous pocket on the recipient’s abdomen. This is the only large animal model of CTA with a genetically defined histocompatibility barrier enabling the study of specific transplantation barriers between the donor and the recipient. CsA at 10mg/kg per day was administered intravenously to the recipient pig for 12 days, and the dose was adjusted based on serum levels. In this study, allografts from MHC-mismatched donors treated with CsA showed signs of rejection in less than 6 weeks, but in similarly matched donors, 178- to 280-day allograft survival was accomplished. Allografts in similarly matched group were harvested between 178 and 280 days after transplant. All grafts demonstrated patent vessels, bleeding from marrow cavities, and