Experimental Models of Composite Tissue Allograft Transplants

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ABSTRACT

Composite tissue allotransplantation has been recently introduced as a potential clinical treatment for complex reconstructive procedures including traumatic injuries, cancer ablative surgeries, or extensive tissue loss secondary to burns. Composite tissue allografts (CTAs) consist of heterogeneous tissues including skin, fat, muscle, nerves, lymph nodes, bone, cartilage, ligaments, and bone marrow with different antigenicities. Thus, composite tissue structure is considered to be more immunogenic than solid organ transplants. In this article, we present the experimental applications of CTA transplantation. To study the mechanisms of CTA acceptance and rejection, different experimental models, strategies, and different immunosuppressive protocols were used.

KEYWORDS: Composite tissue allotransplantation, experimental models, limb, face, vascularized bone

Composite tissue allotransplantation represents the newest transplant area, and it has considerable potential for reconstructive plastic surgery. However, the immunology of composite tissue allografts (CTAs) is complex because they are composed of a variety of discrete tissues such as skin, fat, muscle, nerves, lymph nodes, bone, cartilage, ligaments, and bone marrow with different antigenicities. Each of these components elicits a different immune reaction; consequently, tolerance is more difficult to achieve here than is the case with solid-organ allografts. At the same time, although these tissues are structurally, functionally, and aesthetically important to patients who need functional restoration of musculoskeletal defects, they are nevertheless not vitally important to life. Moreover, even when the autologous tissue is sufficient for the reconstruction of large defects, functional and aesthetic recovery is not always ideal. Besides morbidity problems associated with the donor

site, the recipient may require multiple surgeries. Nevertheless, despite these complexities, CTA retains great potential for the reconstruction of various parts of the body.^{1,2}

EXPERIMENTAL MODELS OF CTA TRANSPLANTS

We have designed and developed different CTA models testing different immunosuppressive protocols of tolerance induction.

1. Hind-Limb Transplant Models

We have performed more than 1000 hind-limb transplants across major histocompatibility complex (MHC) barriers between fully allogeneic BN (RT-1ⁿ) and semi-allogeneic LBN (RT1¹⁺ⁿ) donors and Lewis (RT11)

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recipients. Different immunosuppressive protocols of cyclosporin A (CsA) monotherapy, combined CsA with topical fluocinolone acetonide, tacrolimus (FK506) monotherapy, combined CsA/ $\alpha\beta$ -T cell receptor monoclonal antibody ($\alpha\beta$ -TCR mAb) and CsA/Antilymphocyte Serum (ALS) were used for different dose and time regimens. Flow cytometry was used to assess the efficacy of immunosuppressive protocols and donor-specific chimerism. Clinical tolerance and immunocompetence were confirmed by skin grafting in vivo and by mixed lymphocyte reaction in vitro. Combined protocols of CsA/ $\alpha\beta$ -TCR mAb and CsA/ALS resulted in long-term survival and donor-specific tolerance in the hind-limb allografts³⁻⁷ (Fig. 1).

We also evaluated the role of host thymus in tolerance induction in CTAs across MHC barriers during a 7-day $\alpha\beta$ -TCR/CsA protocol.⁸ Treatment with $\alpha\beta$ -TCR/CsA resulted in indefinite allograft survival (Mean Survival Time (MST) = 370 days). We found that the presence of the thymus was essential in inducing donor-specific tolerance in rat hind-limb CTAs using an $\alpha\beta$ -TCR/CsA protocol.⁸

2. Cremaster Muscle Composite Tissue Allograft Transplantation Model

We developed a new CTA transplantation model to study the microcirculatory changes during acute allograft rejection and ischemia/reperfusion (I/R) injury. The donor cremaster muscle allografts were prepared as a tube flap, harvested on the common iliac vessels, transplanted to the neck region of the recipient, and anastomosed to the recipient's ipsilateral carotid artery and external jugular vein using the standard end-to-end microsurgical technique. In group 1 ($n=6$), the hemodynamics of cremasteric muscle microcirculation was measured in C57BL/6N mice without transplantation for baseline data. In group 2 ($n=6$), isograft trans-

Figure 1 Appearance of the transplanted hind limb (LBN to LEW) at day 65 under combined CsA/ $\alpha\beta$ -TCR mAb therapy showing no signs of rejection.

plantations were performed between C57BL/6N mice. In group 3 ($n=5$), allograft transplantations were performed across a high histocompatibility barrier between C3H and C57BL/6N mice. Hemodynamic parameters of microcirculation did not differ significantly among the three groups; however, the number of rolling, adhering, and transmigrating polymorphonuclear leukocytes and lymphocytes showed significant increase in the allograft group ($p < 0.001$) as early as 2 hours after transplantation. We found that cremaster muscle transplantation in mice is a reliable and reproducible model with a 95% immediate success rate. The model offers the unique possibility of studying leukocyte-endothelial interaction during acute allograft rejection and I/R injury in mouse.⁹

3. Hind-Limb Cremaster Transplantation Model

To determine the potential use of antibodies to adhesion molecules in CTAs, we investigated the effects of anti-Intracellular Adhesion Molecule (ICAM)-1 and anti-Lymphocyte Function Associated Antigen (LFA)-1 monoclonal antibody treatment in a rat hind-limb cremaster transplantation model. This model allows monitoring directly in vivo shifts and trafficking of the population of rolling, adhering, and transmigrating neutrophils and lymphocytes in response to antibody therapy. This unique approach provides important understanding of the hemodynamics of rejecting composite tissue allografts. Twenty transplantations were performed across a major histocompatibility barrier between Lewis-Brown-Norway (LBN, RT11 + n) and Lewis (LEW, RT11) rats in four experimental groups of five animals each. Group 1 animals received only vehicle solution; groups 2 and 3 received monoclonal antibodies against ICAM-1 and LFA-1, respectively; group 4 received a combination dose. Treatments were continued for 7 days. Clinical signs of rejection were noted daily and correlated with in vivo microcirculatory measurements. Monoclonal antibodies against LFA-1 or ICAM-1 alone inhibit the activation of leukocytes at the microcirculatory level but do not prolong graft survival; however, the combination of anti-ICAM-1 and anti-LFA-1 monoclonal antibodies significantly prolonged allograft survival in this composite tissue transplantation model.¹⁰

4. Composite Vascularized Skin and Femoral Bone Transplant Models

We have designed a new model of combined vascularized groin skin and bone marrow transplantation. The rat femur is transplanted with the groin cutaneous skin flap. Transplants were performed between genetically identical Lewis (LEW, RT11) rats. Combined groin skin and femoral bone flaps were transplanted based on the femoral vessels. Five different experimental designs

in five groups of five animals each were studied: group I, bilateral vascularized skin (VS) transplantation; group II, bilateral vascularized skin/bone transplantation; group III, vascularized skin transplantation on one side and vascularized skin/bone transplantation on the contralateral side; group IV, vascularized bone transplantation on one side and vascularized skin/bone transplantation on the contralateral side; group V, vascularized bone transplantation on one side and vascularized skin transplantation on the contralateral side. All flaps survived more than 100 days posttransplant. Histologic examination of the femoral bone revealed active hematopoiesis with viable compact and cancellous bone components at day 100 posttransplant. These models can be applied directly to a tolerance induction study across MHC barriers, where bone will serve as source of delivery of donor stem and progenitor cells, and the skin component will serve as a monitor of graft rejection^{11,12} (Fig. 2).

5. Bone Marrow Transplantation Models

We investigated the effect of crude bone marrow cotransplantation on vascularized skin allograft survival. Thus, we have cotransplanted crude bone marrow without marrow processing or recipient conditioning. Skin graft transplants were performed between semi-allogeneic LBN (RT11 + n) donors and Lewis (RT11) recipients under CsA or $\alpha\beta$ -TCR mAb alone, or combined CsA and $\alpha\beta$ -TCR mAb for 35 days. The use of combined CsA and $\alpha\beta$ -TCR mAb therapy with crude bone marrow transplantation extended skin allograft survival up to 65 days in this vascularized skin allograft model.¹³

We have also investigated the effect of different routes and dosages of donor-derived bone marrow cell transplantation on donor-specific tolerance induction and chimerism across MHC barriers under a short 7-day protocol of CsA monotherapy and combined CsA/ $\alpha\beta$ -TCR therapy. Intraosseous and intravenous

bone marrow cells were transplanted between BN (RT1ⁿ) donors and Lewis (RT11) recipients. Flow cytometry assessed immunodepletion and donor-specific chimerism. All animals survived without graft-versus-host disease. Intraosseous transplantation of donor-specific bone marrow cell was 75% more efficient in induction of donor-specific chimerism compared with intravenous transplantation, and the level was 50% higher in animals that received 70×10^6 bone marrow cells (9.9%) when compared with animals that received 35×10^6 bone marrow cells (4.9%). These studies confirmed the tolerogenic properties of donor bone marrow transplantation directly into the bone, representing a natural microenvironment for bone marrow seeding and repopulation.^{14,15}

6. Vascularized Skin (Groin) Allograft Transplant Model

A vascularized skin allograft transplant model was used to evaluate the potential for tolerance induction in highly immunogenic tissue grafts (e.g., skin). We tested the efficacy of different immunosuppressive protocols and their potential to extend survival and to induce chimerism in vascularized skin allografts. Vascularized skin allograft transplants across strong MHC barriers were performed between fully allogeneic ACI (RT1^a) donors and LEW (RT11) recipients. Skin flaps (3×4 cm), based on the femoral artery and vein, were harvested from the donor groin and included the underlying panniculus carnosus and inguinal fat tissue. In the recipient animal, a defect of the same size was created on the right side, and the artery and vein were anastomosed end-to-end with the recipient's femoral artery and vein. Animals received $\alpha\beta$ -TCR mAb, CsA, or FK506 therapy; or immunosuppressive therapy combining $\alpha\beta$ -TCR + T cell receptor mAb/CsA and $\alpha\beta$ -TCR mAb/FK506, given for only 7 days to test the potential for chimerism induction and to extend graft survival.

Figure 2 Composite vascularized skin/bone graft model. (A) Anatomy of harvested composite vascularized skin/bone graft. (B) Composite vascularized skin/bone graft at day 60 posttransplant revealed complete survival.

Figure 3 Vascularized skin (groin) allograft transplant model. (A) Appearance of the harvested vascularized skin allograft from semi-allogeneic LBN donors. (B) Vascularized groin skin transplants based on the femoral vessels were performed between semi-allogeneic LBN donors and Lewis recipients (early posttransplant view). (C) The recipient of skin (groin) allograft 150 days after transplant shows no sign of rejection under low-dose cyclosporin A monotherapy.

The combined $\alpha\beta$ -TCR mAb/CsA and $\alpha\beta$ -TCR mAb/FK506 protocols were effective in inducing and maintaining chimerism, and they substantially extended the survival of the vascularized skin allograft transplants across MHC barriers^{16,17} (Fig. 3).

7. Vascularized Bone Marrow Transplantation Models

Different models of vascularized bone marrow transplantation (VBMT) have been introduced. Limb transplantation was the first vascularized bone marrow model used for this purpose. Hind-limb transplants were performed between Lewis-Brown-Norway (LBN, RT11 + n) donors and Lewis (LEW, RT11) recipients to test the effect of 21-, 7-, and 5-day protocols of combined $\alpha\beta$ -TCR mAb/CsA treatment on tolerance induction.⁵ All transplants under combined $\alpha\beta$ -TCR/CsA therapy survived more than 350 days. Clinical tolerance and immunocompetence were confirmed by skin grafting in vivo and mixed lymphocyte reaction (MLR) in vitro. All recipients at day 100 posttransplant uniformly accepted skin allografts from the donor (LBN) and the recipient (LEW) but rejected third-

party (ACI) grafts. We confirmed that the 5-day protocol was long enough to maintain immunologic unresponsiveness of a new repertoire of recipient T cells, which led to the engraftment of donor hematopoietic stem cells. This was confirmed by donor-specific chimerism of 10 to 12% for double-positive CD4 cells and 6 to 9% for double-positive CD8 T cell subpopulations on day 120 posttransplant in all combined treatment groups.

We introduced a new model of combined vascularized groin skin and femoral bone marrow transplantation based on femoral vessels. Our research on the VBMT by using rat epigastric free flap alone or in combination with vascularized femur allografts under treatment of $\alpha\beta$ – TCR/CsA for 7 days revealed that when the epigastric free flaps were used alone, the survival rate of the flap was 25 days; however, when the skin flap transplantation was combined with vascularized femoral allografts, survival greater than 60 days was achieved with no signs of rejection or graft-versus-host disease.¹⁸

We also harvested vascularized femoral bone allograft and transplanted into the recipient's inguinal

region. Briefly, after wide exploration of the right inguinal region and the leg, the superficial epigastric and saphenous vessels were ligated and divided. The popliteal space was explored, and the muscular branches and the femoral vessel were ligated and transected distally. Both the lateral femoral circumflex and superficial circumflex iliac arteries were preserved, for they are the nutrient and periosteal arteries to the femoral bone. The femur bone was disarticulated both proximally and distally. The femoral vessels were then dissected proximally, and the flap was harvested. In the recipient, the vascular anastomoses were performed to the femoral vessels end-to-end. Flap inseting was completed in the inguinal region of the recipient. After approximation of the inguinal fat pad over the flap with three interrupted absorbable sutures, the skin was closed using 4-0 chromic catgut sutures. More than 50 transplants were performed, and the patency of the anastomosis was confirmed in all animals. Placement of the vascularized bone graft in the inguinal region did not interfere with the mobility of the recipients.¹⁹

8. Bilateral Vascularized Femoral Bone Marrow Transplant Model

Encouraged by tolerogenic properties of vascularized bone marrow transplant, we have introduced a new model of VBMT: the bilateral vascularized femoral bone (BVFB) isograft and allograft transplant based on abdominal aorta and inferior vena cava. Transplants were performed between Lewis (RT11) rats. In the donor, both femoral bones were harvested based on the abdominal aorta and inferior vena cava. In the recipient, the harvested isograft transplants were transferred into the abdominal cavity. The vascular pedicles of transplants were patent, and the bones were viable during the follow-up period of 63 days posttransplant. We have confirmed the feasibility of BVFM transplantation based on abdominal aorta and inferior vena cava.²⁰

As a continuation of the previous study, we have tested the efficacy of the BVFB model in induction of chimerism across MHC barriers under a combined CsA/ $\alpha\beta$ -TCR mAb protocol given for 7 days. Transplants were performed between BN (RT1ⁿ) donors and Lewis (RT1¹) recipients. At day 21, a peak level of donor-specific chimerism of 24.2% was confirmed in the peripheral blood of BVFB recipients. At day 63, the level of chimerism had declined to 1.5% and was maintained at this level thereafter. Histologic examination revealed viable bone marrow cells up to 35 days posttransplant. Thus, the bilateral vascularized femoral bone transplant model can be used for tolerance induction protocols.²¹

9. Combined Semimembranosus Muscle and Epigastric Skin Flap Model

We have developed a new model of combined semimembranosus muscle and epigastric skin free flap based on a single pedicle consisting of the muscular branch of semimembranosus muscle and superficial epigastric vessels in continuity with femoral vessels. To the neck (four flaps) and contralateral groin (four flaps) recipient sites, we transferred eight combined semimembranosus muscle and epigastric autogenous skin flaps based on the muscular branches and superficial epigastric vessels in continuity with femoral vessels. All animals survived after surgery, and all flaps were viable as checked by direct observation of color and temperature. All flaps, including the muscle and skin components, were viable at postoperative day 7. The vascular patency of the pedicles was confirmed under an operating microscope. The success rate for the flap transfer was 100%. This model of combined muscle and skin flap has wide applicability, for example to microcirculatory, pharmacologic, physiologic, biochemical, and immunologic studies.²²

10. Vascularized Laryngeal Allograft Transplantation Model

In 1992, Strome et al developed a vascularized laryngeal allograft transplantation model to reexamine the potential for laryngeal transplantation. This model contributed to the first successful human larynx transplant in 1998. In the 1992 model, the allografts were sited in tandem with the intact recipient larynges and were not innervated. A total of 16 animals were studied, and 14 rats had a 64% arterial patency at intervals of 1 to 14 days. More than 1500 rat transplants later, numerous modifications have improved the applicability of this model to the CTA transplantation field.^{23,24}

We applied the $\alpha\beta$ -TCR mAb protocol along with tacrolimus to the existing rat model of laryngeal transplantation as a tolerance-inducing strategy. Larynges were transplanted from Lewis-Brown-Norway (RT1^{1/n}, F1) donors to Lewis (RT1¹) recipients. Recipients received 7 days of treatment with tacrolimus and mouse anti-rat $\alpha\beta$ -TCR monoclonal antibodies. Histology, MLR, skin grafting, and flow cytometry assessed functional tolerance, efficacy of immunodepletion, and donor-specific chimerism. All 10 recipients survived until sacrifice at 100 days. Histology suggested functional allograft tolerance. In this rat laryngeal-transplantation model, functional tolerance was induced under a combined tacrolimus and $\alpha\beta$ -TCR protocol.²⁵

11. Face Transplantation Models

In preparation for facial allograft transplantation in humans, we have developed full face and hemiface skin

Figure 4 The full face/scalp allograft transplantation model. (A) Full face/scalp flaps including the external ears and scalp, based on the common carotid artery and external jugular vein, were harvested from the donors. (B) Immediate posttransplant view of the full face/scalp allograft recipient. Transplantation was performed between full-allogeneic ACI donors and Lewis recipients. (C) Late posttransplant views of full face/scalp allograft recipient on low dose of cyclosporin A monotherapy at day 200 showing no signs of rejection.

transplant models to test different immunosuppressive protocols and tolerance induction across semi-allogeneic and full allogeneic histocompatibility barriers.^{26–29}

(a) Full Face/Scalp Transplant Model

We performed full face/scalp allograft transplantation across MHC barriers in a rodent model. Transplants were performed between semi-allogenic LBN (RT11 + n) donors and Lewis (RT11) recipients. Postoperatively, the recipient animal received low dose ($2 \text{ mg kg}^{-1} \text{ day}^{-1}$) CsA monotherapy during the follow-up period of more than 200 days^{26,27} (Fig. 4).

(b) Modifications of Full Face/Scalp Transplant Model

To improve the survival of full face/scalp allograft recipients, two different modifications of the arterial anastomoses in the recipients were introduced. The unilateral common carotid artery of the recipient

was used to vascularize the full transplanted facial/scalp flap. These arterial modifications have significantly reduced the complications associated with the bilateral common carotid arteries anastomoses and subsequently the postoperative mortality of the animals.²⁸

(c) Hemiface Transplant Model

We introduced a hemifacial allograft transplant model, which is technically less challenging compared with the full facial/scalp model. The same low-dose CsA monotherapy ($2 \text{ mg kg}^{-1} \text{ day}^{-1}$) immunosuppressive protocol was used during the follow-up period of 330 days in the fully MHC mismatched hemifacial transplant recipients.²⁹ In addition to the hemiface skin model, composite hemiface/calvarium, hemiface/mandible/tongue (Fig. 5), and maxilla models were developed with long-term survival and maintained chimerism in the blood of recipients.^{30–32}

Figure 5 Hemiface/mandible/tongue allotransplantation model. (A) The outer aspects of the flap. Mandibular tooth and tongue are seen. (B) The inner aspect of the flap. Common carotid artery, external jugular vein, tongue, mandibular tooth, and mandibular condyle are seen. (C) Appearance of the hemiface/mandible/tongue allograft transplant at day 150 posttransplant (LBN to LEW).

CONCLUSION

We have presented the experimental applications of CTA transplantation. The functional and aesthetic outcomes after application of conventional reconstructive procedures or prosthetic materials is not satisfactory, especially in patients with severe deformities and disabilities. Since the first successful hand transplantation in France in 1998, composite tissue allograft transplantation has elicited great interest in the field of plastic surgery. Thus far, more than 50 CTA transplants have been reported.

Currently, the main obstacle to CTA transplantation is the necessity of lifelong immunosuppression therapy, which has serious and well-known side effects such as severe infections, organ toxicities, and malignancies. In addition, composite tissue transplantation raises controversial ethical, social, and psychological questions. Future applications of composite tissue transplantation will therefore be determined on the basis of the long-term results of its recent and current applications.

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