Journal of Surgical Research **153,** 268–273 (2009)
doi:10.1016/j.jss.2008.03.050

Swine Hemi-Facial Composite Tissue Allotransplantation: A Model to Study Immune Rejection[1]

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Submitted for publication January 26, 2008

Objective. **Partial face composite tissue allotransplantation was recently achieved in a human subject. However, the side effects of long-term immunosuppression and chronic rejection area still need concerning. This preliminary study investigated the reproducibility of swine hemi-facial transplantation for preclinical studies.**

Materials and methods. **Eleven out-bred miniature swine underwent hemi-facial transplant. The hemi-facial orthotopic transplant consisted of ear cartilage, auricular nerve, parotid gland and lymphoid tissue, muscle with surrounding hemi-facial skin paddle supplied by the carotid artery, and external jugular vein transplanted to recipient swine. Three different experimental designs were studied, as follows: group I (n = 4): autologous hemi-facial transplantation as a normal control; group II (n = 4): hemi-facial allotransplantation without treatment; group III (n = 3): hemi-facial allotransplantation with cyclosporine-A treatment for 4 wk. The transplanted face was observed daily for signs of rejection. Biopsy of donor skin, gland lymphoid tissue, and cartilage were obtained at specified predetermined time (d 7, 14, 28), or at the time of clinically evident rejection.**

Results. **The results indicated the survival of group I following autologous hemi-facial transplant was 100% and indefinite until sacrifice. Group II without treatment as the controls revealed allograft rejection by d 7 to 28. The allograft with short-term cyclosporine-A treatment revealed delayed rejection by d 38 to 49 postoperatively. The histological examination in group I revealed abundant lymphocyte infiltration, especially in lymphoid gland and alloskin at 1 wk and sacrifice. In contrast, the cyclosporine treatment group showed no significant rejection signs in 4 wk posttransplants. These results demonstrated that lymphoid tissue and alloskin are both susceptible to early rejection.**

Conclusion. **The experimental results revealed this model is suitable to investigate the new strategies for preclinical facial allotransplantation studies. Monitoring and modulation of early rejection in alloskin and gland lymphoid tissue is a useful strategy to evaluate composite tissue allotransplantation survival.**

Key Words: **hemi-facial transplantation; composite tissue allotransplantation; swine; immune rejection.**

INTRODUCTION

Composite tissue allotransplantation (CTA) has many applications in reconstructive microsurgery [1]. Advances in reconstructive microsurgery, increased experience with organ transplantation, and recent developments in immunosuppressive therapy have increased interest in CTA research and its clinical application [2]. The CTA presents an alternative to conventional reconstructive methods for repairing tissue damage caused by trauma, burn injury, cancer ablation, and congenital defects. In patients lacking their own "autologous" tissue for reconstruction, this surgical procedure enables reconstruction with tissue structurally similar to their own.

[1] Presented at the 23th Annual Meeting of American Society for Reconstructive Microsurgery, Jan. 12–15, 2008, Los Angeles, CA.

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0022-4804/09 $36.00

The first human hand transplantation was performed in 1998 in Lyon, France [3]. Since that time numerous hand transplantations have been performed with varying reports of success and failure [1]. The first partial face allotransplantation performed in a human subject in November, 2005, demonstrated the technical feasibility of this procedure [4]. Although not quite routine yet, the practice of CTA is not rare. Among many others such as donor source, ethics, psychology of recipient, and so on, immune rejection and its treatment are continuously one of many big issues. As a matter of fact, application of immune suppress therapy is required. Despite its promising applications, the side effects of long-term immunosuppressive therapy and chronic rejection are still concerning [3, 5, 6]. Unlike many lifespan-prolonging solid organ transplants, CTA is an elective procedure for improving quality of life. Therefore, preclinical trials are needed to evaluate the long-term efficacy of new immunosuppressive strategies.

Preclinical animal models are essential for advancing CTA to clinical application. Investigations involving small animal models have comprehensively evaluated CTA rejection [7, 8]. Although rat models have shown predictable patterns of rejection, there exist fundamental differences between the human and rat immune systems [9, 10]. Therefore, rodent models may not be applicable in humans. The experimental findings are an important step toward assessing in humans. Large animal models, especially swine and primate, offer better characterization of the major histocompatibility complex (MHC), which is similar to that seen in humans, as compared to rodents [11, 12]. Although large animal models are still different than humans, however, a large animal is necessary to be applied toward human clinical trial for surgeon's training and new immunosuppression protocol. Facial CTA, including total and hemi-facial, has been performed previously in rodent models [13, 14]. However, rare facial allotransplantation has not been reported in a preclinical large animal study [15, 16]. Therefore, this study investigated the reproducibility of swine hemi-facial transplantation for preclinical immune rejection studies.

MATERIALS AND METHODS

Animals

Eleven out-bred domestic miniature swine (Lan-Yu strain and Hwa-Ban strain; age, 3 mo; size, 12-20 kg) were studied. The study was conducted in accordance with *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (Bethesda, MD). Animals were obtained from Tai-Tung Veterinary Research Institute, Taiwan. Experiments were conducted under the Institutional Animal Care and Use Committee protocol approved by the Chang Gung Memorial Hospital in Kaohsiung, Taiwan. The miniature swine were divided into 2 experimental groups. Group I (n = 4) received autologous hemi-facial transplantation (Lan-Yu strain to Lan-Yu strain) as a normal control. Group II (n = 4) received hemi-facial allotransplantation (Hwa-Ban strain to Lan-Yu strain) without treatment. Group III (n = 3) received cyclosporine-A (CsA; d 0 to +28; 10 mg/kg for 2 wk then 5 mg/kg for 2 wk). Preliminary studies had actually been performed before we applied this model to immunological studies. First, anatomical dissection of swine face and clarification of the blood supply by angiography were done. After that, more than 10 cases have been performed to test whether or not this model is reproducible. During these practices, no graft loss due to vessel-compromised problems was found.

Surgical Anatomical Dissection for Harvesting Hemi-Facial Flap

The animals were premedicated with ketamine (10 mg/kg) and xylazine (1.5 mg/kg) intramuscular injection and then placed in a supine position on the operating table and intubated. Anesthesia was maintained with pentobarbital (50 mg/kg) and oxygen inhalation. The head and neck were shaved and painted with antiseptic iodine solution. The hemi-facial flap was schematically marked on each animal (Fig. 1A). Upper and lower eyelids were not included in the flap. To design the hemi-facial composite flap containing skin, muscle, ear cartilage, nerve, parotid gland, and surrounding tissue,

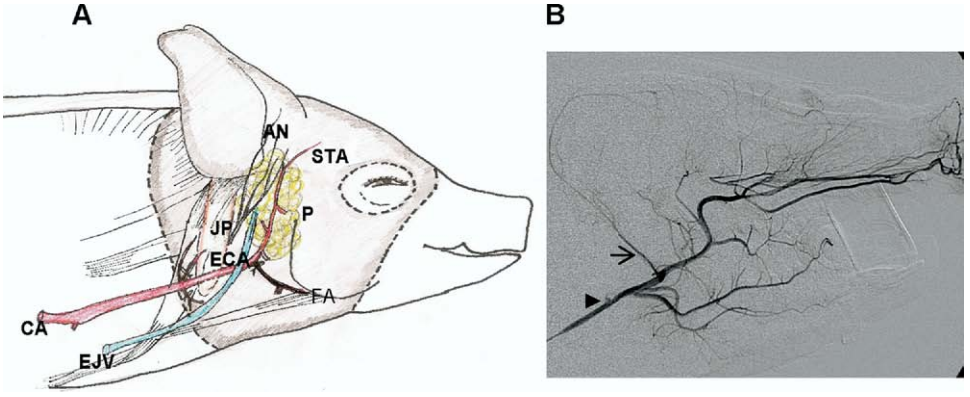

FIG. 1. (A) Schematic diagram of the orthotopic hemi-facial composite tissue transplant model. The hemi-facial flap contained vascularized skin, lymphoid parotid gland tissue, ear cartilage, part of muscle, auricular nerve, and surrounding soft tissue. (B) Angiography revealed vascular distribution of the hemi-facial composite flap supplied by the superficial temporal artery (arrow) and its branches originating from external carotid artery (arrowhead). CA = common carotid artery; EJV = external jugular vein; JP = jugular process; AN = auricular nerve; FA = facial artery; P = parotid gland. (Color version of figure is available online.)

the vascular territories of the composite flap supplied by the superficial temporal artery and its branches originating from the carotid artery were defined by angiography in preliminary anatomical studies (Fig. 1B). The skin was incised to the depth of the brachiocephalicus muscle in the anterior and posterior neck, to the depth of facial muscles in the facial region, and above the periosteal plane in the nasal and fronto-parietal region. In the neck, dissection was continued superiorly above the sternomastoideus muscle to the level of angle of mandible, preserving the external jugular vein. The submandibular gland was excised after ligation of the glandular branches of facial artery and vein. Facial artery and facial nerve were identified and excluded from the flap. Dissection was performed above the masseter muscle toward the ear. To preserve pre-auricular vascular structures, the parotid gland was included in the flap. In the retro-auricular region, the internal maxillary vein and the main trunk draining the pterygoid plexus were ligated and transected. The auricular nerve was preserved and included in the flap. At the back of the neck, after transaction of platysma and levator auris longus muscles, the flap was elevated above the trapezius up to the posterior wall of the cartilaginous area of the external ear canal. The sternomastoideus muscle was detached, and bony ostectomy of jugular process in cervical spine was performed to expose the common carotid artery and its main branches, the external and internal carotid arteries. The internal carotid artery, cranial thyroid artery, ascending pharyngeal artery, and lingual artery were ligated and transected. The external ear canal was detached at the osteocartilaginous junction, and the external ear was kept within the flap. The common carotid artery and external jugular vein were dissected as the vascular pedicle of the flap. The flap revealed good circulation after harvest (Fig. 2A).

Preparation of the Donor Hemi-Facial Flap

After standard sterile preparation of the donor swine, the hemi-facial composite flap was harvested as described above. The common carotid artery and external jugular vein were divided to create the vascular pedicle of the flap. After dividing the vascular pedicle, heparinized normal saline solution was flushed into the allograft through the carotid artery until the venous outflow was clear. The donor animal was euthanized with an overdose of pentobarbital upon completion of the allograft harvest.

Preparation of the Recipient

The recipient animal was prepared in a similar fashion. Intravenous catheter was placed for intraoperative fluid management. This catheter was subsequently used for drawing blood samples and administering medicine postoperatively. A single lumen Hickman catheter was inserted on the contralateral side of the external jugular vein under direct vision and tunneled in a posterior direction to exit high on the dorsal neck. The incisions were closed in layers using absorbable and nonabsorbable sutures.

On the ipsilateral side of the recipient the full thickness of skin and subcutaneous tissue was removed, sparing the peri-orbital and peri-oral skin to avoid disturbing vital functions of the recipient after transplantation. The external jugular vein was isolated anteriorly to the sternomastoideus muscle and prepared for venous anastomosis. Next, the sternomastoideus muscle was freed to expose the common carotid artery. Special attention was paid to keep vagus and phrenic nerves intact. The inferior half of the sternomastoideus muscle was resected to facilitate arterial end-to-side anastomosis and to prevent exertion of pressure to the anastomotic site after surgery. After preparation, the hemi-facial flap was secured and sutured in the recipient. Next, venous anastomosis was performed using standard end-to-end microsurgical technique between the external jugular vein of the donor and recipient. Next, end-to-side anastomosis between common carotid artery of the recipient and donor was performed under operating microscope magnification using 9-O nylon sutures. The external ear canal and flap skin was closed using 3-O Vicryl and 3-O nylon (Fig. 2B).

Postoperative Care

The experimental animal recovered fully with uneventful postoperative course. After the animal revived and was comfortably breathing, it was returned to its pen. No anticoagulant drug was given postoperatively. Intravenous systemic antibiotics (ampicillin) was given for 5 d. The intravenous catheter was flushed by heparin (2000 units heparin in 1000 mL 0.9% normal saline) once a day. The transplant was monitored on a daily basis for signs of rejection. The animal was also monitored for signs of distress, sepsis, or wound complications.

Histological Evaluation of Graft Rejection

The transplanted face was observed daily for signs of rejection occurring in a reproducible sequence of epidermilysis, desquamation, eschar formation, and necrosis. Biopsy of donor skin, gland lymphoid tissue, and cartilage were obtained at specified predetermined times (d 7, 14, 28), or at the time of clinically evident rejection. Tissues were harvested, fixed in 10% neutral buffered formalin, sectioned, and stained with hematoxylin and eosin. At the clinically defined endpoint, animals were sacrificed.

Statistical Analysis

Graft survival between groups or transplanted animals was compared by Kaplan–Meier analysis and log-rank test. P value of <0.05 was considered to be statistically significant.

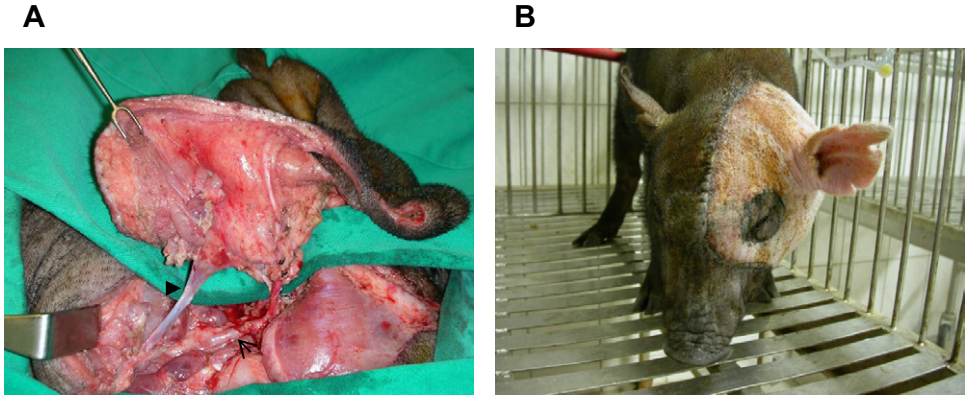

FIG. 2. (A) Intraoperative photo of hemi-facial flap harvesting. The common carotid artery (arrow) and external jugular vein (arrowhead) were used as the vascular pedicle of the flap. (B) Postoperative photograph of hemi-facial transplants. (Color version of figure is available online.)

RESULTS

Short-Term Immunosuppressant Prolonged Allograft Survival

The animal was monitored immediately after surgery in the recovery cage. No special care was required. Following recovery from surgery, the animal ambulated freely in its cage with no difficulty. The mean time to complete the hemi-facial transplant procedure was 8 h, and mean time of warm ischemia was 105 min.

All hemi-facial flaps succeed reperfusion without pedicle compromise complications immediately. However, all hemi-facial flaps remained swollen for 2 wk due to postoperative saliva gland hypersecretion. The autologous hemi-facial transplant achieved 100% survival indefinitely until sacrifice. In the control group, the results showed a progressive rejection of the allograft by d 7 to 28. The allograft with short-term CsA treatment revealed delayed rejection between d 38 and d 49 postoperatively. This demonstrated that short-term immunosuppressant with CsA treatment could significantly prolong allograft survival as compared to the controls (Fig. 3).

Histological Analysis of Allograft Rejection

In the control group, the histological examination revealed severe rejection signs and abundant mononuclear infiltrations in lymphoid gland tissue and alloskin, especially lymphoid tissue at 1 wk and sacrifice as compared to that in normal autologous transplant lymphoid tissue and skin. In contrast, the cyclosporine treatment group showed mild lymphocyte infiltration without significant rejection signs in 2 wk and 4 wk posttransplants (Fig. 4). However, there were no apparent differences in allo-cartilage between the control and CsA treatment group. These analytical findings indicated different antigenicities of the composite allografts tissues. Lymphoid gland tissue and alloskin are both susceptible to early rejection.

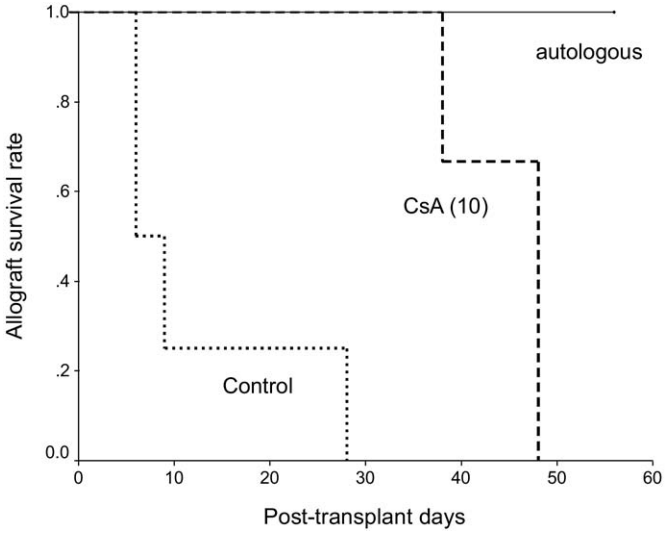

FIG. 3. Short-term immunosuppressant prolonged allograft survival. The autologous transplant revealed 100% survival indefinitely 8 wk postoperatively. The alloskin paddle of allograft in control group revealed progressive rejection of the allograft by d 7 to 28. The short-term CsA treatment group showed only early mild rejection sign from d 38 to 49 postoperatively. This demonstrated that short-term CsA treatment significantly prolonged allograft survival as compared to the controls ($P = 0.0158$).

DISCUSSION

CTA offers many advantages over autologous tissue reconstructive procedures including superior functional and esthetic outcome, no donor site morbidity, and reduced necessity for subsequent surgical revision [17]. CTA could provide an attractive strategy for reconstituting facial defects including skin, muscle, bone, or even the peripheral nervous system [18, 19].

Facial allotransplantation in experimental rodents have been reported previously [13, 14]. However, these models almost all involved small animals not applicable to humans. Clinical evidence indicates that small animal (rodent) model immunosuppression protocols are not consistently applicable because rodents tend to be more tolerant than humans to allograft transplantation [9]. To assess new immunosuppressive protocols and the possibility of tolerance induction, further large animal model studies are needed prior to initiation of human clinical trials.

Large animal studies for CTA transplantation are superior to small animal models for many reasons. From an immunological viewpoint, large animal models offer better characterization of the MHC complex, especially miniature swine and primates [10–12]. Predictable rejection processes in solid organ studies incorporating animals with MHC disparities resemble those of humans. The immunological system of swine also resembles that of humans and has been used extensively for transplantation studies [20, 21].

It is not trivial to establish a model for scientific research. As a scientifically justified surgical model, it has to be reproducible with a high success rate. It is always expensive and a lot of work to use big animals such as swine and primate as a model. Silverman and colleagues recently developed a heterotopic nonhuman primate facial CTA model including skin, masseter and a portion of pterygoid muscle, and mandible bone [22]. The results indicated this primate allograft model showed a big variation of allograft survival. In this study, a swine hemi-facial allotransplantation model including the skin, lymphoid gland tissue, parts of the sternomastoideus and trapezius muscles, ear cartilage, and sensory nerve was successfully developed. No graft loss due to vessel-compromised problem was found perioperatively. This demonstrated that this operative technique is feasible. Although the graft survival of our preliminary trials was 100%, the surgical procedure is

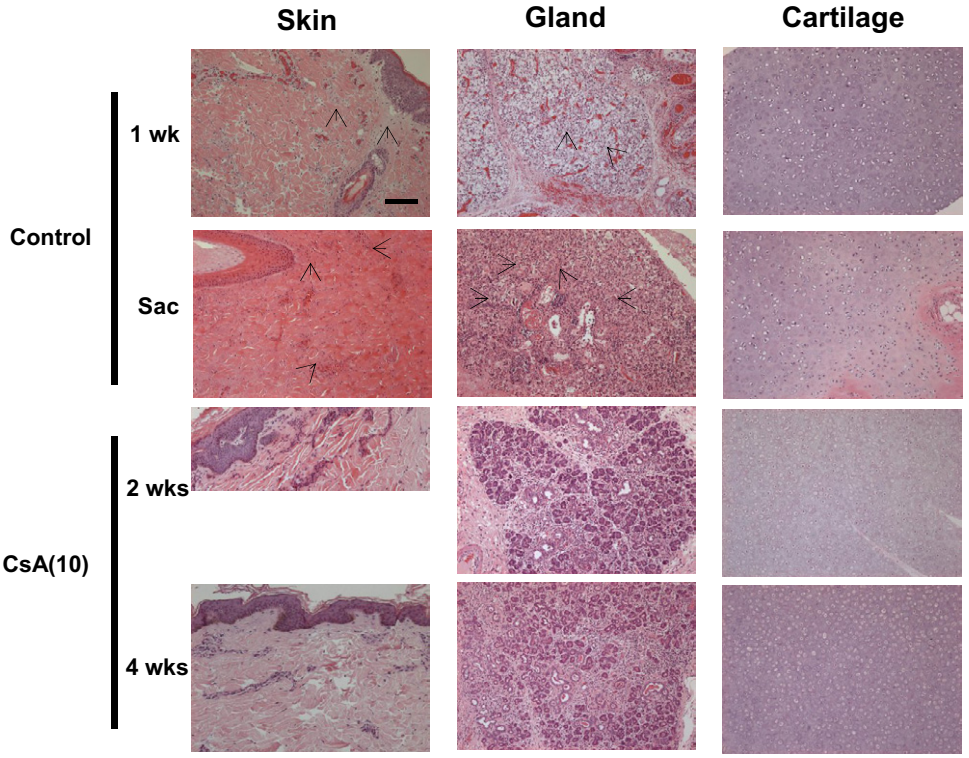

FIG. 4. Histological analysis of allograft tissue by using hematoxylin and eosin staining. In control group, the histological examination revealed severe rejection signs and abundant mononuclear infiltrations in lymphoid gland tissue and alloskin, especially in the lymphoid gland tissue, at 1 wk and sacrifice. In contrast, the CsA treatment group showed only mild lymphocyte infiltration without significant rejection signs in 2 wk and 4 wk posttransplants. However, there were no apparent differences in allo-cartilage between the control and CsA treatment group. Photo magnification, ×100. Scale bar, 100 μm. (Color version of figure is available online.)

not simple and needs experienced surgical teamwork perioperatively. It still took 8 h to complete the allotransplantation model. Nevertheless, if well-trained surgeons realize the anatomical dissection of swine, we believe that it is not difficult for other surgeons to dissect the hemi-facial flap and do microvascular anastomosis in such big recipient vessels (carotid artery and external jugular vein).

In clinical observation, the autologous hemi-facial transplant was 100% survival until sacrifice. However, autografts revealed swelling and saliva accumulation in the first 2 wk postoperatively. The control group revealed progressive rejection by 1 to 4 wk posttransplants. The short-term CsA treatment group showed only early mild rejection sign from 6 to 7 wk postoperatively. This demonstrated that short-term immunosuppressant could significantly prolong allograft survival compared to that in controls.

In marked contrast to the monitoring of solid-organ transplants, measurement of graft function cannot clearly determine allograft rejection. However, since CTA are easily observed, rejection of the graft might be easily detected and monitored by inspection of the skin. In this study, the hemi-facial swine allograft model revealed simple clinical visualization of the CTA skin surface for detecting early rejection and the vascular status of the allograft.

Different antigenicities of the various tissues found within the CTA result in various rejections [23, 24]. In this histopathological analysis, the control group revealed abundant lymphocyte infiltration in lymphoid gland tissue and alloskin at 1 wk and sacrifice. In contrast, the cyclosporine treatment group revealed less lymphocyte infiltration without significant rejection signs in 2–6 wk posttransplants. There were no apparent differences in ear cartilage between the control and CsA treatment group. These results demonstrated that modulation of early rejection in alloskin and gland lymphoid tissue may be a key treatment strategy in CTA survival.

This experimental result warrants further preclinical studies of facial CTA in large animal models. However, some disadvantages were noted in this model. First, parotid saliva-pooling caused transplant swelling and wound infection. The symptoms persisted for up to 2 wk. This complication could be prevented by ligation of salivary gland duct intraoperatively, elongation of i.v. antibiotics, or saliva drainage by untightened suture over wound edge. Another shortcoming of this model is that functional outcome of facial animation could not be evaluated following CTA. However, assessment of innervation and sensation was beyond the scope of this study, and further anatomical feasibility studies are needed. This model included the

greater auricular sensory nerve but not the facial nerve and innervated muscle. An immunological intervention protocol may evaluate functional sensory outcomes by assessing withdrawal from both pain and temperature.

In summary, this hemi-facial transplantation model is reproducible and warrants further preclinical investigation of the new strategies in large animal models. The limited experimental findings of this study are an important step toward assessing the immunological manipulation involved in facial allotransplantation in humans.

ACKNOWLEDGMENTS

The authors thank the Chang Gung Memorial Hospital Research Project, Taiwan for financially/partially supporting this research under Contract No. CMRPG-860451. Dr. Radovan Boca is appreciated for valuable discussions. Dr. Eng-Yen Huang is appreciated for bio-statistical consultation.

REFERENCES

1. Tobin GR, Breidenbach WC 3rd, Pidwell DJ, et al. Transplantation of the hand, face, and composite structures: Evolution and current status. Clin Plast Surg 2007;34:271.
2. Gorantla VS, Barker JH, Jones JW Jr, et al. Immunosuppressive agents in transplantation: Mechanisms of action and current anti-rejection strategies. Microsurgery 2000;20:420.
3. Dubernard JM, Owen E, Herzberg G, et al. Human hand allograft: Report on first 6 months. Lancet 1999;353:1315.
4. Devauchelle B, Badet L, Lengele B, et al. First human face allograft: Early report. Lancet 2006;368:203.
5. Mathes DW, Randolph MA, Lee WP. Strategies for tolerance induction to composite tissue allografts. Microsurgery 2000;20: 448.
6. Hettiaratchy S, Randolph MA, Petit F, et al. Composite tissue allotransplantation—a new in plastic surgery? Br J Plast Surg 2004;57:381.
7. Siemionow M, Oke R, Ozer K, et al. Induction of donor-specific tolerance in rat hind-limb allografts under antilymphocyte serum and cyclosporine A protocol. J Hand Surg Am 2002;27: 1095.
8. Hewitt CW, Black KS, Dowdy SF, et al. Composite tissue (limb) allografts in rats. III. Development of donor-host lymphoid chimeras in long-term survivors. Transplantation 1986;41:39.
9. Gunther E, Walter L. Comparative genomic aspects of rat, mouse and human MHC class I gene regions. Cytogenet Cell Genet 2000;91:107.
10. Yuhki N, Beck T, Stephens RM, et al. Comparative genome organization of human, murine, and feline MHC class II region. Genome Res 2003;13:1169.
11. Chardon P, Renard C, Vaiman M. The major histocompatibility complex in swine. Immunol Rev 1999;167:179.
12. Antczak DF. Structure and function of the major histocompatibility complex in domestic animals. J Am Vet Med Assoc 1982; 181:1030.
13. Demir Y, Ozmen S, Klimczak A, et al. Tolerance induction in composite facial allograft transplantation in the rat model. Plast Reconstr Surg 2004;114:1790.
14. Ulusal BG, Ulusal AE, Ozmen S, et al. A new composite facial and scalp transplantation model in rats. Plast Reconstr Surg 2003;112:1302.
15. Shengwu Z, Qingfeng L, Hao J, et al. Developing a canine model of composite facial/scalp allograft transplantation. Ann Plast Surg 2007;59:185.
16. Yates G, Landon B, Edwards G. Investigation and clinical application of a novel axial pattern flap for nasal and facial reconstruction in the dog. Aust Vet J 2007;85:113.
17. Lee WP. What's new in plastic surgery. J Am Coll Surg 2002; 194:324.
18. Siemionow M, Unal S, Agaoglu G, et al. A cadaver study in preparation for facial allograft transplantation in humans: Part I. What are alternative sources for total facial defect coverage? Plast Reconstr Surg 2006;117:864.
19. Unal S, Agaoglu G, Zins J, et al. New surgical approach in facial transplantation extends survival of allograft recipients. Ann Plast Surg 2005;55:297.
20. Lee WP, Rubin JP, Cober S, et al. Use of swine model in transplantation of vascularized skeletal tissue allografts. Transplant Proc 1998;30:2743.
21. Ustuner ET, Majzoub RK, Ren X, et al. Swine composite tissue allotransplant model for preclinical hand transplant studies. Microsurgery 2000;20:400.
22. Silverman RP, Banks ND, DeTolla LJ, et al. A heterotopic primate model for facial composite tissue transplantation. Ann Plast Surg 2008;60:209.
23. Kuo YR, Sacks JM, Wu WS, et al. Porcine heterotopic composite tissue allograft transplantation as a large animal model for pre-clinical study. Chang Gung Med J 2006;29:268.
24. Lee WP, Yaremchuk MJ, Pan YC, et al. Relative antigenicity of components of a vascularized limb allograft. Plast Reconstr Surg 1991;87:401.