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Survival of human cadaver skin on SCID pigs: proof of concept

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Abstract

Transplantation of human xenografts onto immunocompromised mice is a powerful research tool for studying wound healing. However, differences in healing between humans and mice and their small size limits this model. We determined whether human cadaver skin xenografts transplanted onto pigs with severe combined immune deficiency (SCID) would survive and not be rejected. Meshed (1:1.5), cryopreserved human cadaver skin was transplanted onto 10 partial thickness dermatome wounds in each of two normal domestic pigs and two SCID pigs. Autografts (n=2/animal) from the 4 animals were used as controls. In normal pigs, all autografts were engrafted and healed with minimal if any inflammation and scarring. All human xenografts were rejected by the normal pigs within 5-11 days and associated with an intense T-cell inflammatory response. In contrast, both autografts and xenografts were engrafted and survived the 28-day study in the SCID pigs with minimal inflammation and no gross scarring.

Hypertrophic scars (HS) and keloids are excessive forms of pathological scarring, which can result in significant patient disfigurement and dysfunction.¹ Greater understanding of the molecular and cellular mechanisms leading to excessive scarring has been limited by the paucity of animal models that mimic wound healing in humans.² Transplantation of human skin onto nude mice was first described in the early 1970s.^{3–5} However, until recently, there have been few if any good models of immune deficient pigs. Researchers at Iowa State have serendipitously discovered an immune deficient pig suffering from a condition similar to severe combined immune deficiency (SCID), in which the animals lack T and B cells, but not natural killer (NK) cells.^{6, 7} Prior to transplanting human keloids and hypertrophic scars onto SCID pigs, we hypothesized that human cadaver skin xenografts would be rejected by normal but not immune deficient pigs. We further hypothesized that rejection of the xenografts in normal pigs would be characterized by T-cell infiltration and that human xenografts would heal similarly to autografted skin from SCID pigs.

METHODS

Human cadaver skin

Pathogen-free, freshly donated, glycerin-cryopreserved, partial thickness human cadaver skin was procured from the NYC skin bank at Cornell Burn Center. The skin grafts were

kept at -80°C until immediately prior to experimentation and thawed in room temperature normal saline.

SCID pig breeding and handling

The SCID pig discovered at Iowa State is the result of two separate point mutations in the Artemis gene located on chromosome 10.⁸ Mutations in this gene are also associated with SCID in humans.⁹ A colony of homozygous SCID boars and specific pathogen free (SPF) carrier sows has been created and maintained through selective breeding and bone marrow transplants on the male side. SPF SCID pigs are kept in a special 'barrier facility' designed to minimize human-to-pig pathogen transfer.¹⁰

Transplantation of human skin grafts onto pigs

All studies were approved by the institutional Animal Care and Use Committees and were conducted in accordance with the recommendations provided in the Guide for the Care and Use of Laboratory Animals, 8th edition. We used two 25–30-kilogram domestic Yorkshire (Archer Farms, PA) and two 32–46 kg SCID pigs bred at Iowa State for the experiments. After allowing a one-week acclimation period, pigs were fasted overnight and sedated with an intramuscular combination of ketamine 20 mg/kg, xylazine 2 mg/kg, acepromazine 0.1 mg/kg and atropine 0.02 mg/kg. After endotracheal intubation the animals were anesthetized with inhalational isoflurane (1–5%) and placed in the prone position and the hair was removed with electric clippers. The skin was washed with soap and water and scrubbed with isopropyl alcohol 70%. We created 12 partial thickness dermatome wounds measuring 2.5 by 2.5 cm on each of the four pigs using an electric dermatome set at 0.75 mm depth, evenly distributed between the two paravertebral sides of the animal. In each pig, 10 wounds were closed with 1:1.5 meshed split thickness human cadaver skin xenografts while the remaining two wounds were closed with an autograft obtained from the same pigs' hindlegs. The grafts were secured with staples and covered with a triple antibiotic ointment and petrolatum impregnated gauze. Periodic digital images and full thickness skin biopsies were obtained and the animals were euthanized at the end of the 28-day study period.

Processing and analysis of skin biopsies

The punch biopsies were bisected and fixed in 2% formaldehyde. Five-micron sections were stained with Haematoxylin and Eosin (H&E) and imaged using an EVOS microscope (ThermoFisher Scientific) with an internal camera. The presence or absence of the skin graft and the degree of inflammation was noted. Inflammatory cells were recognized by their typical morphology on H&E staining. The relative thickness of the epidermis and presence of rete pegs was noted as well as whether there was scarring typified by thin bundles of collagen mostly oriented in a single horizontal plane.

Immunohistochemical analysis

Immunohistochemistry for CD3 (a marker of T-cells) and CD56 (a marker of natural killer [NK] cells) was performed on paraffin embedded tissue sections following the manufacturer's instructions (Millipore, Tamecula, CA). Survival of transplanted human

cadaver xenografts was determined using human-specific anti-HLA Class 1 ABC (Abcam, Cambridge, MA) antibodies following the manufacturer's instructions.

RESULTS

A total of 4 pigs and 48 grafted partial thickness wounds were included in the study analysis. All of the animals survived and there were no obvious wound infections noted in any of the wounds from all four animals.

Survival of grafts in normal pigs

For the first 3 days after grafting all grafts appeared to survive and it was difficult to distinguish between autografts and xenografts. All of the autografts were successfully engrafted and remained intact throughout the study duration (Fig. 1). In contrast, none of the human cadaver xenografts survived, all being rejected within 5–11 days following their transplantation. Histomorphological analysis demonstrated little if any inflammation in wounds that were grafted with autografts. In contrast, an intense inflammatory response was noted in all xenograft treated wounds (Fig. 1). The inflammatory cells included predominantly mononuclear cells and a moderate amount of eosinophils. Immunostaining demonstrated that most inflammatory cells stained positive with the T cell specific anti-CD3 antibody but not with the NK cell specific CD56 antibody (not shown).

Survival of grafts in SCID pigs

All of the autografts became engrafted and survived throughout the study duration (Fig. 2). In addition, all but one human cadaver xenografts (which sloughed off on day 3 due to mechanical reasons) were successfully engrafted and remained viable throughout the entire 28-day study. Minimal if any inflammatory response was noted in all autografts and in 19/20 human cadaver xenografts. In comparison to the engrafted autografts, the epidermis of the xenografts was hypercellular and lacked the typical rete pegs that were present in the autografts. Immunostaining for human specific HLA antibodies demonstrated a net-like pattern surrounding the epidermal cells in the transplanted epidermis, but not seen in pig autografts, confirming the human origin of the newly formed epidermis in the SCID pigs (Fig. 2).

DISCUSSION

Our study serves as a proof-of-concept that human cadaver skin is rejected when transplanted onto normal immunocompetent pigs and that the rejection is mostly by T-lymphocytes and not by NK cells. Of note, eosinophils were also a prominent part of the inflammatory response. The presence of eosinophils is typical of a response to a foreign body. In contrast, human cadaver skin xenografts were successfully engrafted when transplanted onto SCID pigs with minimal if any inflammation, thus supporting our study hypothesis. The persistence of human derived keratinocytes in some of the transplanted xenografts is not surprising as prior studies have demonstrated up to 50% viability of cryopreserved skin samples.¹¹ The ability of xenografts to survive on SCID pigs is further

supported by the remarkable resemblance of transplanted xenografts and autografts in SCID pigs both grossly and histomorphologically.

Our findings have several potential implications. First, it demonstrates for the first time that xenografts of human skin can survive on SCID pigs. Second, it opens the opportunity to transplant diseased human skin onto SCID pigs such as hypertrophic scars, keloids, and various cutaneous inflammatory or malignant skin conditions. This may allow further investigation of the pathobiology of cutaneous injuries and illnesses as well as the exploration of novel therapies in this model. The pig is a particularly good model for these types of studies given the similarity in immune gene sequences, anatomy, physiology, and pharmacokinetics. The large size of the pigs, which allows transplantation of multiple large pieces of human skin, is another advantage over mice.

Transplantation of skin onto immune deficient animals other than pigs is not new. In 1987, Robb et al. developed a new model for studying the development of human hypertrophic burn scar formation.¹² In their study, normal and hypertrophic skin from humans was transplanted onto athymic mice. The normal human skin adhered to the murine wound bed and revascularized in a manner similar to a normal human recipient bed, remaining viable for the duration of the study (6 months). Transplanted human skin that was subsequently burned and allowed to heal demonstrated increased scarring and collagen content. Hypertrophic scars transplanted onto the nude mice also were able to vascularize and retained their original gross and microscopic appearance. Yang,¹³ as well as Tredget et al. have also established a hypertrophic scar model by transplanting full-thickness human skin grafts onto the backs of nude mice.^{14, 15}

Our study is preliminary and has notable limitations. First, the sample size was small and our results need to be validated in a larger number of wounds and animals. Second, pigs were studied only for 28 days. Thus, we cannot comment on the longer-term results. Finally, we did not determine the viability of the skin graft prior to transplantation.

CONCLUSIONS

We have proven that human derived skin can be successfully transplanted on immune deficient pigs, surviving for at least 28 days without rejection. Further studies will determine whether human keloids and hypertrophic scars can be successfully transplanted on SCID pigs to be used as a novel method for studying excessive scarring in swine.

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Figure 1.

Image from normal pig, 5 days after grafting demonstrating complete rejection of xenograft (A) and survival of autograft (B). Representative H&E stained micrographs from normal pig wounds treated with xenograft (C) and autograft (D). Image C demonstrates the outer layer of the rejected xenograft with healing underneath. Image D demonstrates complete engraftment of the autograft. High power H&E stained images from normal pig demonstrating an intense inflammatory response with occasional eosinophils with xenograft (E) but not autograft (F). The inflammatory response in the xenografts is characterized mostly by CD3 red-stained T-cells (G) that are less prominent in autografts (H).

Figure 2.

The upper gross images from SCID pigs 28 days after injury demonstrate survival of xenograft (A) and autograft (B). The middle H&E stained micrographs demonstrate survival of the xenograft (C) and autograft (D) with minimal if any inflammation. The bottom images stained with anti-human leukocyte antigen-ABC confirm survival of transplanted human skin. The red, net-like staining pattern in image E is from a SCID pig transplanted with the human cadaver skin. Image F is from normal uninjured pig skin with no visible staining.