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Abscence of Skin Graft Rejection in Cross-Strain Transplantation between BALB/C and C3H Mice Strain

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Abstract

Skin transplantation is a medical or experimental procedure in which the skin from one individual (donor) is transferred to another individual (recipient) to cover wounds, skin damage, or for research purposes. In clinical contexts, skin transplantation is used to treat burns, chronic ulcers, or other skin injuries. In research, skin transplantation is often utilized to study immunological mechanisms, including transplant tolerance and rejection. Skin transplant rejection in genetically different individuals generally occurs due to differences in immunogenic antigens, such as the Major Histocompatibility Complex (MHC), which are recognized by the recipient's immune system as foreign. This rejection is the result of the activation of the adaptive immune response, primarily mediated by T cells. In this experiment, skin transplantation was performed on BALB/c and C3H mouse strains. The experimental results showed that skin transplantation between mice of different strains did not trigger rejection reactions, which are typically characterized by inflammation, erythrocyte infiltration, or tissue necrosis.

Introduction

The history of skin grafting dates back over 3000 years ago, to the practices of the Hindu Tilemaker Caste. In 1804, G. Baronio performed skin transplantation on sheep tails, while Sir Astley Paston Cooper covered a stump defect in 1817. In the 19th century, Reverdin and Thiersch used pinch graft for wound healing. Split-thickness grafts were documented in the late 19th century by Ollier and Thiersch. Full-thickness grafting was first described by Wolfe in 1875 and later refined by Krause in 1893. The development of intermediate split-thickness grafts was pioneered by Blair and Brown, as outlined in their seminal 1929 textbook. Padgett in 1946 developed "thick skin grafting" for burn patients and developed the first mechanical dermatome. Skin grafting is now widely used in dermatology and plastic surgery [1].

Skin substitutes, including acellular biomaterials and composite cultured skin analogs, have been developed to treat various injuries, including burn and traumatic wounds. These substitutes play a critical role in wound protection, infection control, and restoration of skin functionality. Nonetheless, despite significant advancement in biomaterial, no existing dermal substitute can fully replicate the complex physiological dan

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mechanical properties of human skin. Skin allografts are costly, resulting in heightened dependence on xenografts and alternative dermal substitutes [1].

Organ transplantation remains the preferred treatment for patient with end-stage organ failure, with significantly improvements in patient outcomes achieved through advancements in surgical techniques and immunosuppression protocols [2]. Progress in transplantation medicine has been driven by interdisciplinary collaboration. In the last century, researchers have executed more intricate transplant procedures, and begun implementing immune tolerance induction therapies in clinical practice [3].

Animal models have been extensively utilized in transplantation research to investigate mechanisms of rejection and strategies for immunosuppression. Among these models, mice are commonly utilized due to their well-characterized genetic strains and the availability of a broad range of diagnostic and therapeutic antibodies. Skin transplantation in mice offers a practical and efficient methods for studying alloimmune responses, as it requires minimal surgery expertise and allows for straightforward post-operative monitoring. Mice skin transplantation serves as an effective model for investigating alloimmune responses, encompassing antigen delivery, cellular trafficking, and tissue destruction during graft rejection. This study examines the crucial function of animal models in the advancement of transplant immunology.

Methods

Animal model

In this study used three mice, two BALB/c strain and one C3H. Experimental design is showed in Figure 1.

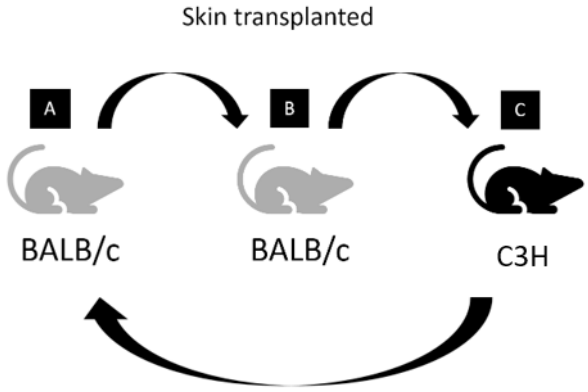

Figure 1: The experimental design of skin transplantation. The skin of 1st BALB/c mice **(A)** transplanted to the 2nd BALB/c mice **(B)**; the skin of 2nd BALB/c mice transplanted to the C3H mice; and the skin of C3H mice transplanted to the 1st BALB/c mice.

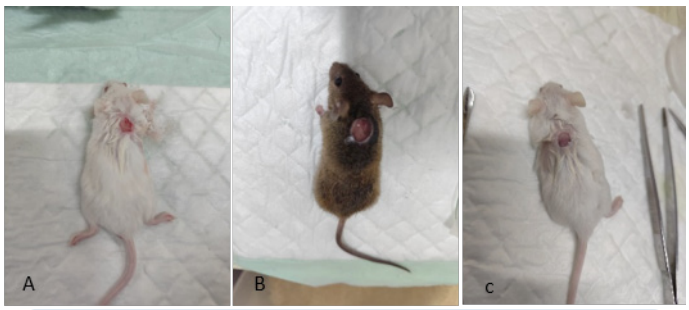

Figure 2: Dimension of skin dissection was width 1 cm in dorsal.

Transplantation

Mice were anesthetized with ketamin-xylazyne. Skin biopsies was harvested from an upper dorsal area with width 1 cm. The dissection was done by sterile procedure. Skin biopsies was placed in NaCl solution. Then, skin from one mice transplanted on the othe mice corresponding to experimental design. The wound healing progress was observed every 2-3 days during 2 weeks (Figures 2 & 3).

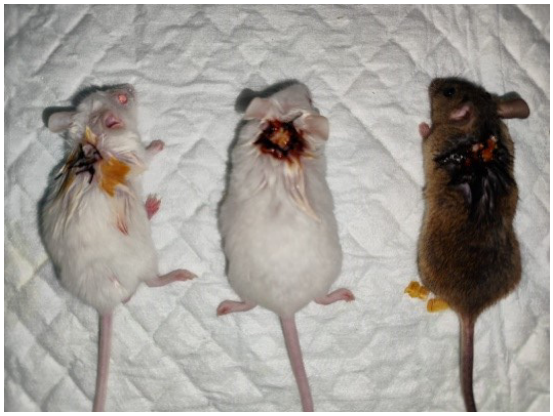

Figure 3: After cross-transplanted skin.

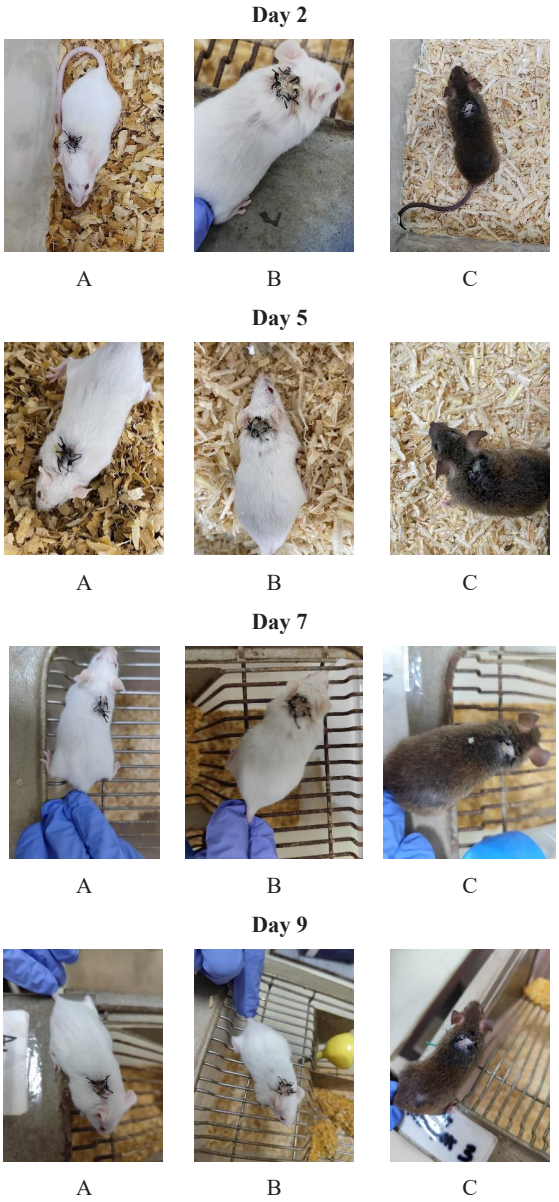

Figure 4: The observation of skin grafts on day 2; day 5; day 7; and day 9 in a mice A, mice B, and mice C.

Skin isolation and hematoxylin-eosin (HE) staining

Skin graft was isolated after 2 weeks. Then, the skin tissues were prepared to HE staining with fixative solution. After HE staining have been done, the skin graft tissue was observed by light microscope to evaluate the wound healing process.

Results

Skin graft observation

Mice was observed in day 2; day 5; day 7; and day 9. The skin graft showed signs of healthy adherence and drying by the fifth day post-transplantation. Signs of inflammation, such as redness and swelling were not obvious in the skin graft area.

Microscopic examination

The skin grafts were isolated by 2 weeks post-transplantation. All skin graft was stained with HE staining.

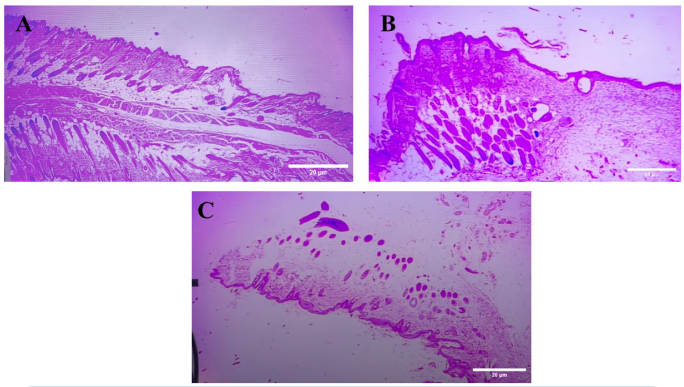

Figure 5: The histological skin graft with HE staining (magnification 400× and scale bar 20 µm). A. Mice A has not showed rejection in second weeks post-transplantation. However, the epidermis structure looks different between mice A, B and C.

In Figure 5, all histological skin graft showed re-epithelization in the epidermal layer with no immune cells infiltration in the dermal layer. No significant difference was observed in the epidermis and dermis across sample. Additionally, all sample exhibited renewed hair follicles in the dermal layer. Compared to all skin graft, mice B demonstrated a better wound healing process than mice A and C. This was indicated by the absence of intermittent gaps between epidermal and dermal layers. Skin graft of mice B also showed fewer adipocyte-like structure compared to mice A and C.

Discussion

There are four types of tissue grafting. They are autograft, isograft, allograft, and xenograft [4]. An autograft is a tissue graft that is removed from one area to another in the same individual. Isograft is a tissue graft performed on individuals with identical genetics. Isograft is difficult to do in humans, but it can be done in animals such as mice with the same strain. Allograft is a tissue graft that is performed on two of the same species, but different strains. A xenograft is a tissue graft on various species. In this study, two types of skin grafting were performed with mice. Skin isograft was performed on two mice with the same strain (BALB/c mice), while skin allograft was performed on mice with different strains (BALB/c and C3H mice). There is no reaction of tissue rejection in autograft and isograft, while allograft and xenograft can cause reaction of tissue rejection in the normal immune system. Reaction of tissue rejection occurs due to genetic differences between donor and recipient tissues, mainly due to differences in MHC genes, so these genetic differences cause an immune response to the graft tissue. Antigens that cause tissue rejection in allografts are called alloantigens, while antigens that cause tissue rejection in xenografts are called xenoantigens. In this study, normal tissue grafts in two mice with different strains will induce an immune response. Two reactions of tissue rejection will occur. They are direct recognition of alloantigens and indirect recognition of alloantigens [5]. Direct recognition of alloantigens occurs when donor dendritic cells found in the graft tissue with different MHC genes from the recipient can directly activate alloreactive T lymphocytes. Indirect recognition of alloantigens occurs when alloantigens found in the graft tissue of the donor are recognized by the recipient's dendritic cells and activate these dendritic cells. Activated dendritic cells will present peptides from alloantigens to naive T lymphocytes and cause activation of alloreactive T lymphocytes. Alloreactive T lymphocytes will go to the graft tissue and cause damage to the graft tissue. Ultimately, graft tissue rejection occurs in the allograft.

Skin allografts are one the most immunogenic types of tissue grafts, where it demonstrates higher immunogenicity compared to other tissue allografts, since it contains many antigen-presenting cells, such as langerhans cells, dendritic cells, and macrophages, resulting in greater activation of immune response towards the graft [6-8]. Sign of rejection in skin graft can be observed as early as day 1 after transplantation, to 14 days later [6,9]. The duration post-transplantation significantly influences the sign of rejection in skin grafts [9]. In primary rejection, visible signs of rejection typically appear within 7-14 days if the graft is rejected, while secondary rejection may occure more rapidly [5,10]. These signs include erythema and edema at the graft site as sign of inflammation and tissue damage, graft darkening which indicates ischemia and necrosis, ulceration of the graft, graft peeling as indicates a complete rejection, and loss of hair and hair follicle [4,11,12]. Histological examination of the skin graft tissue using HE staining can reveal certain features that indicates rejection, such as lymphocyte infiltration, endothelial damage, and thrombosis of surrounding blood vessels [7]. Granular and linear patterns of immune deposit can be observed using immunofluorosecence, indicating complement activation and immune complex deposition. on duration post-transplantation [13]. These rejections can be further classified into grades, called Banff grading, with each grade indicating a different severity level of rejection. This grading is widely used in Composite Tissue Allotransplantation (CTA), as an emerging techniques for tissue or limb defects [14].

Absent of rejection observed in the study might be caused by several factors, one of them are genetic factor. BALB/c mice are inbread, leading to reduced genetic variability, while C3H mice exhibit residual heterozygosity due to insufficient inbreeding. This genetic variability in C3H mice can result in unpredictable responses in experiments, as been observed in the study, potentially causing minor mismatches, instead of major mismatches, when compared to the more genetically uniform BALB/c strain [15]. Minor mismatch is a type of graft rejection mediated by minor histocompatibility antigens (mHAgs). This form of rejection typically occurs more slowly and is less severe compared to rejection driven by MHC mismatchs. It is particularly evident in skin grafts between mice of the same strain but different sexes. In such cases, the immune system recognizes sex-differentiated proteins as minor histocompatibility antigens, thereby triggering an immune response and subsequent graft rejection [16,17].

Another factors contributing to the absent of skin graft rejection in the study is the involvement of immunological mechanism that modulates the host's immune response. Antigen uptake by immature dendritic cells in the host's skin tissue will cause immune tolerance towards skin graft, hence the absent of skin graft rejection [18]. An increase in regulatory T cells (Treg) can prolong skin graft survival, leading to graft tolerance in mice of different strain [19]. These Treg cells can be modulated by induction of oral tolerance toward spesific antigen that needed to be tolerated, or can be naturally induced by cryptic infections in mice [20,21]. Parasitic infections, for instance, can create immunosuppressive environment that allows the parasite to persist, partly by increasing Treg population [22]. In the context of skin grafts, this immunosuppressive environment is beneficial as it promotes graft tolerance [21,23].

Conclusion

The condition of C3H mice, which showed no signs of skin transplant rejection from BALB/c mice, typically characterized by inflammation, erythrocyte infiltration, or necrosis, could be influenced by several factors. Based on the conducted experiments, it can be concluded that the occurrence or absence of transplant rejection is influenced by various factors, including the strain, the immune characteristics of the donor and recipient, and the environmental conditions in which the experiment is conducted.

Author Statements

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