Cytotoxic T Lymphocyte–associated Protein 4 Acts on Local Draining Lymph Nodes in the Allogeneic Abdominal Skin Flap of a Rat Model to Extend Its Survival

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ABSTRACT

Background. Early acute allograft rejection is the result of immune cells mainly composed of thymocyte (T) cells, which are activated, mature, and differentiate in lymphoid tissue.

Methods. Cytotoxic T lymphocyte–associated protein 4 immunoglobulin (CTLA4-Ig) competitively suppresses the cluster of differentiation 28/CTLA4-B7 costimulatory signaling pathway and alters the ratio of T helper 1 to T helper 2, leading to the inhibition of T cell activation.

Results. We used lentivirus-packed CTLA4-Ig to infect the local draining lymph nodes of the allograft skin flap in a rat model, which effectively extended the survival of the allograft. Meanwhile, it also reduced the dose of immunosuppressant necessary in the early stages of transplantation, which lowered the toxicity and side effects of the drug.

Conclusions. Our findings provide new insights into transplantation immunology, facilitate the exploration of a safe and effective immunosuppressive therapy, and promote the wide clinical application of composite tissue allotransplantation.

COMPOSITE tissue allotransplantation (CTA)–induced early acute rejection is regulated primarily by immune cells composed mainly of thymocyte (T) cells, which, together with an immune response, are localized mainly in lymphoid organs and tissues. Local draining lymph nodes are secondary lymphoid tissues that play an important role in naïve T cell activation, maturation, and differentiation. In the microenvironment, adaptive immune response [1,2] induced by contact with exogenous antigens differentiates T cells into both effector and memory T cells that protect the body by eliminating non-self antigens in the peripheral tissues [3,4]. Complete activation of T cells requires not only the primary signals but also the secondary signals (costimulatory signaling pathway). There are many types of costimulatory signals and each possesses distinct functions. For example, the cluster of differentiation 28 (CD28)/cytotoxic T lymphocyte–associated antigen 4 (CTLA4)–B7 costimulatory pathway is a critical secondary signal involved in T cell proliferation and activation [5].

CTLA4 shares homology in structure with CD28; however, CTLA4 negatively regulates T cells. Upregulation of CTLA4 not only suppresses T cell activation by competitively binding to its ligand CD80/CD86, but also changes the immune tolerance of the body to an allograft by regulating the ratio of T helper 1 to T helper 2 (Th1/Th2); therefore, the CD28/CTLA4-B7 signaling pathway is a key target in the research and treatment of immune rejection and tolerance. In this study, we used lentivirus-packed CTLA4-immunoglobulin (Ig) to infect the draining lymph nodes in the receptor graft, which blocked a critical pathway

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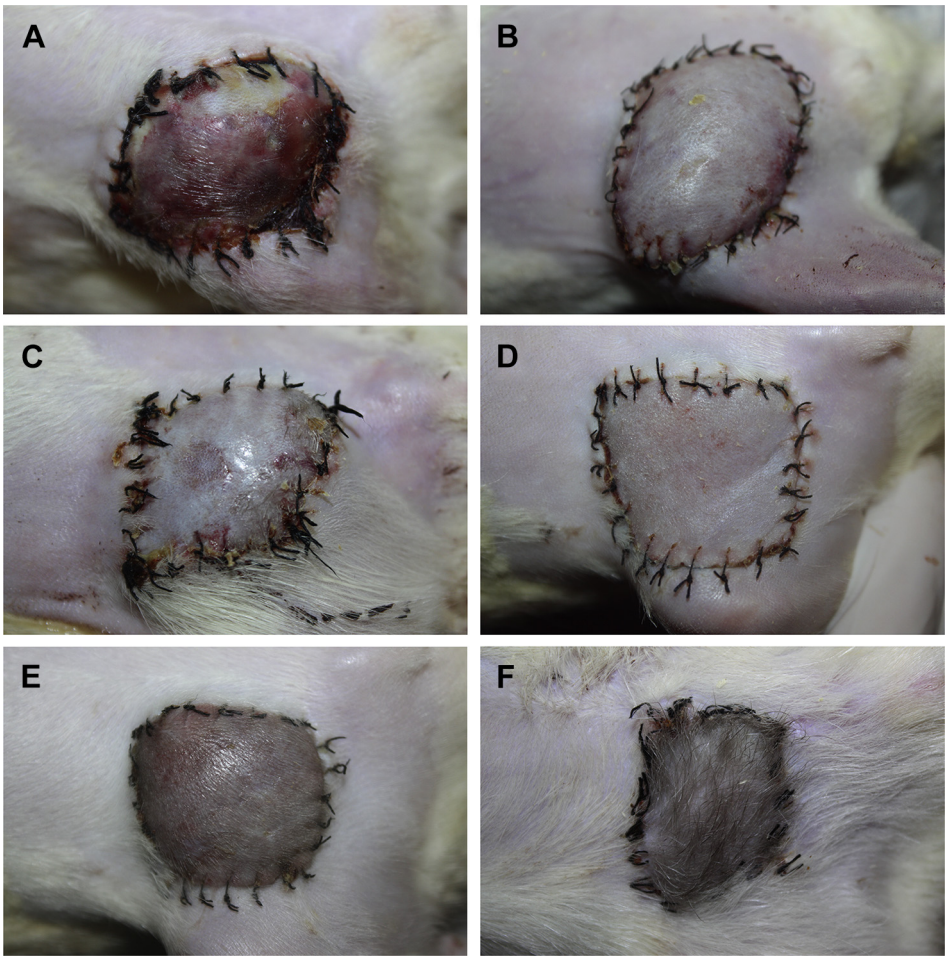

Fig 1. The condition of the skin flap after surgery. (**A**) Seven days after surgery, the skin flap of group A showed partial necrosis and hardening, which met the criteria of grade-III skin rejection. (**B**) Seven days after surgery, the skin flap of group B showed edema and bleeding spots. (**C**) Seven days after surgery, quitting injection of rapamycin, there were edema and bleeding spots occurred in the flaps. (**D**) Seven days after surgery, the skin flap of group D was in good condition with no edema or bleeding spots and a normal color. (**E**) Group D on day 12, edema and bleeding spots appeared and the color became darker. (**F**) The skin flaps were in good condition until 3 weeks after surgery of group E. Skin color was normal and the incision recovered well.

for T cell activation from the origin and altered T cell activation and differentiation. Our findings confirmed the crucial role of draining lymph nodes in allotransplantation and provided a new strategy for transplantation immunology.

MATERIAL AND METHODS

Experimental Animals

Male Lewis rats ($RT1^1$) and brown Norway rats (BN, $RT1^n$) approximately 8 weeks to 10 weeks old were purchased from Vital River, Inc. (Beijing, China) and maintained in clean rooms. The rats were fed a regular diet and provided access to water at all times. BN rats were the donors and Lewis rats were the receptors of the allograft. The animals were maintained in the Department of Experimental Surgery at Xijing Hospital in strict accordance with standard operating practices (SOPs) for the maintenance and management of specific pathogen-free–grade animals. We began the experiment 1.0 week after the purchase when the rats were accustomed to the environment. The bodies were processed according to SOPs of experimental animals and wastes by the Department of Experimental Surgery to avoid environmental contamination. The experimental animals and their environment followed the laboratory animal regulations published by the National Technology Committee. The experimental protocol was approved by the ethics committee of Fourth Military Medical University, China.

Procedures

The transplantation was performed using the abdominal free flap. The rats were anesthetized intraperitoneally with 1.0% pentobarbital. A 3.0 × 3.0–cm abdominal skin flap was transplanted from a BN to a Lewis rat by first determining the positions of the draining lymph nodes in the receptor transplant area to ensure that they would not be destroyed during transplantation and then ensuring that the donor skin flap did not contain draining lymph nodes. The