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Antibody-functionalized peptidic membranes for neutralization of allogeneic skin antigen-presenting cells

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ABSTRACT

We report herein application of an in situ material strategy to attenuate allograft T cell responses in a skin transplant mouse model. Functionalized peptidic membranes were used to impede trafficking of donor antigen-presenting cells (dAPCs) from skin allografts in recipient mice. Membranes formed by self-assembling peptides (SAPs) presenting antibodies were found to remain underneath grafted skins for up to 6 days. At the host–graft interface, dAPCs were targeted by using a monoclonal antibody that binds to a class II major histocompatibility complex (MHC) molecule (I-A^d) expressed exclusively by donor cells. Using a novel cell labeling near-infrared nanoemulsion, we found more dAPCs remained in allografts treated with membranes loaded with anti-I-A^d antibodies than without. In vitro, dAPCs released from skin explants were found adsorbed preferentially on anti-I-A^d antibody-loaded membranes. Recipient T cells from these mice produced lower concentrations of interferon-gamma cultured ex vivo with donor cells. Taken together, the data indicate that the strategy has the potential to alter the natural course of rejection immune mechanisms in allogeneic transplant models.

1. Introduction

The performance of an in situ forming fibrillar membrane in attenuating T cell responses toward allogeneic skin grafts was investigated. Human skin allografts are important biological dressings for temporary wound closure [1]. Patients with partial and full-thickness burns benefit from intact epidermis and dermis; together, these structures serve as a protective barrier to minimize desiccation of the underlying exposed tissues, limit water evaporation, reduce bacterial contamination, relieve pain and promote wound healing by accelerating re-epithelialization [1]. However, the heightened antigenicity of skin allografts drives powerful allospecific T cell responses in recipients [2].

Calcineurin inhibitors are the mainstays in managing allograft rejection [3–5]. These agents exert their immunosuppressive effects mainly by dampening the activation, proliferation and survival of all T cells through down-regulation of interleukin-2. Patients exposed to these drugs have increased risk of developing opportunistic infections because skin flora may contain antibiotic-resistant *Staphylococcus aureus* [6,7]. Herein we propose to generate selective immuosuppression by exploiting a fundamental molecular difference between donor and recipient cells: class II MHC (MHC-II) molecules expressed by donor antigen-presenting cells (dAPCs).

Acute rejection is driven by mismatched class I and II MHC molecules expressed by donors and recipients whereby the latter mount potent T cell responses against skin allografts [8]. Allograft survival correlates with the density of resident dAPCs. Within hours following allogeneic skin transplantation, dAPCs residing within allografts begin migrating to recipient draining lymph nodes [9–11]. More than three-quarters of the resident dAPCs

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would egress from skins within 3 days [12]. Once in lymph nodes, dAPCs activate allospecific T cells via MHC and costimulatory molecules [2,8]. Inside lymph nodes, host CD4 helper T cells recognizing mismatched MHC-II molecules are activated to drive CD8 T cell differentiation into cytotoxic T cells (CTLs) (Fig. 1). Presentation of MHC-II antigens is critical because the generation of CTLs is compromised without CD4 T cell participation. With visceral allografts, activation of CD4 and CD8 T cells is correlated with the frequency of dAPCs in lymph nodes [13–15]. Activated allospecific CD8 CTLs in turn migrate to the transplant site and damage graft parenchyma via recognition of MHC class I molecules [8]. The magnitude of the rejection depends on the qualitative and quantitative encounter between dAPCs and recipient T cells shortly after grafting [12].

Because acute rejection is a function of dAPCs accumulating in recipient lymph nodes, preclinical modalities have been devised to deplete dAPCs prior to transplantation. Typically the methods require systemic infusion of anti-leukocyte antibodies into donor animals before organ harvest [16–18]. While such pre-emptive strategies can be effective in delaying rejection of allografts in animal models, translation to humans is complicated by the potential harm that can be done to the donors. Recognizing the unmet need, we envisaged a new strategy by which dAPCs trafficking can be impeded selectively after transplantation.

Previously we have reported an injectable platform by which retention of IgG molecules in local tissues can be enhanced using EAK16-II, a self-assembling peptide (SAP) with the sequence AEAEAKAKAEAEAKAK [19]. This and related SAPs are primarily utilized for their environmental responsiveness [20,21]. These peptides undergo sol–gel phase transition at high ionic strengths (>20 mM NaCl); in deionized water, EAK16-II can be injected into physiological environment to establish gels in situ. A system containing EAK16-II, its histidinylated analogue EAKIIH6 and protein linkers (αH6-IgG and pAG) can localize model therapeutic IgG molecules in vivo through directional binding [19,22]. Membranes loaded with antibodies can be established in vivo by subcutaneous injection. Such immobilization partially overcomes antibody clearance mechanisms in tumors, as evidenced by prolonged retention of IgG in mouse mammary and melanoma lesions [22]. In both tumor types, localized IgG remained in tumors significantly longer than free IgG.

In the present study, we characterize the system of materials designed to localize anti-MHC-II antibodies specific to dAPCs (Fig. 1). The rationale is that EAK16-II, its histidinylated analogue EAKIIH6, protein linkers and anti-MHC-II antibodies spontaneously complex non-covalently upon mixing in syringes, with the resultant assembly localizing at the graft–host interface. dAPCs migrating toward draining lymph nodes would interact with the antibody-functionalized membranes, resulting in delayed and/or diminished encounter with T cells. The strategy was examined in the current study using an allogeneic mouse skin graft model. All MHC alleles between the donor and recipient are mismatched, rendering this a stringent model. Although delay in rejection was not expected, the work was aimed to characterize membranes presenting anti-I-A^d IgG (referred to hereafter as αI-A^d) in vitro and in vivo to assess feasibility of the strategy. To this end, we investigated retention of IgG co-administered with the self-assembling components under normal and grafted skins. A nanoemulsion encapsulating with a fluorescent tracer was used to image dAPCs in vitro and in vivo. The immune responses were determined by analyzing production of IFN-γ from T cells in recipient mice. The data indicate that the platform has the potential to become a useful clinical tool by which skin graft rejection can be mitigated.

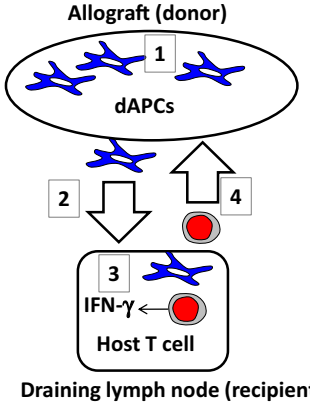

Fig. 1. A generalized depiction of cellular mechanism of acute rejection of allografts. Shortly after the transplantation, dAPCs in the allograft are activated (1) and migrate towards draining lymph nodes (2). Host T cells in the draining lymph nodes are activated via direct allorecognition by dAPCs (3). Activated T cells will migrate to the allograft and mediate graft rejection (4).

2. Methods and materials

2.1. Materials

Peptides were custom synthesized by American Peptide Company (Sunnyvale, CA) at greater than 95% purity with acetylated or amidated termini. Peptides were reconstituted in sterile MilliQ water (18.2 MΩ at 25 °C) at 5 mg ml^{-1} (3 mM, EAK16-II, AcNH-AEAEAKAKAEAEAKAK-$CONH_2$) or 7.5 mg ml^{-1} (3 mM, EAKIIH6, AcNH-AEAEAKAKAEAEAKAKHHHHHH-$CONH_2$). Rabbit anti-His-tag polyclonal antibody (αH6-IgG, 0.2 mg ml^{-1}) was obtained from AnaSpec, Inc. (San Jose, CA). Recombinant protein A/G (pAG) was obtained from Pierce Biotechnology (Rockford, IL) and reconstituted to 0.25 mg ml^{-1} in sterile deionized water. Anti-mouse I-A^d (MHC-II) antibody (αI-A^d, 0.5 mg ml^{-1}; IgG3κ, clone 39-10-8) was purchased from Biolegend (San Diego, CA). Allophycocyanin-conjugated anti-mouse MHC-II I-A^d antibody (0.2 mg ml^{-1}) was obtained from eBioscience (San Diego, CA). DyLight™ 800-conjugated goat anti-chicken IgG (IgG^{800}) was obtained from KPL (Gaithersburg, MD) and reconstituted to 1 mg ml^{-1} in sterile deionized water. RPMI 1640 and fetal bovine serum (FBS) were obtained from HyClone (Logan, Utah). ACK lysing buffer and penicillin/streptomycin solutions were purchased from Lonza (Walkersville, MD). Perfluorocarbon (PFC) and perfluoro-15-crown-5 ether was purchased from Exfluor Research Corporation (Round Rock, TX). Lipophilic carbocyanine DiOC18(7) (DiR) was purchased from Life Technologies and used without further purification.

2.2. Skin transplantation

Six- to eight-week-old female (certified-virus-free) BALB/c and C57BL/6 mice were purchased from Charles River Laboratories (Wilmington, MA) and housed in the Duquesne University Animal Care Facility. The animals were handled in accordance with protocols approved by the Duquesne University Institutional Animal Care & Use Committee. The transplant procedure was modified from a protocol described by Garrod and Cahalan [23]. Briefly, the recipient C57BL/6 mice were anesthetized using isoflurane (induction: 5%; maintenance: 3%) and subsequently administrated with Buprenex at 0.1 mg kg^{-1} via the intraperitoneal route. Ear skin explants (dorsal layer) were prepared from BALB/c mice and transplanted onto graft beds (each ~0.5 cm^2) prepared in the flank of C57BL/6 mice. Upon completion of the surgery, C57BL/6 mice were bandaged and returned to the cage, and their activities monitored regularly.