Immune response to perforated and partially demineralized bone allografts

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Abstract Immune responses have been shown to be involved in the pathogenesis of clinical complications of cortical bone allografts. In an attempt to reduce the immunogenicity of these allografts, we evaluated cortical bone allografts modified by laser perforation and partial demineralization transplanted orthotopically into sheep tibiae. The recipient animals were divided into three groups, of eight animals each, according to the type of cortical allograft that was transplanted: group 1, no treatment (control); group 2, demineralization only; and group 3, laser perforation and partial demineralization. All animals were tissue-typed by biochemical definition of MHC class I molecules, using unidimensional isoelectric focusing and Western blotting. Mismatches of donors and recipients were assessed by testing samples of each donor and recipient pair in parallel and by comparing their individual bands. Donor-specific alloantibodies were detected by a similar technique, using an enzyme-linked immunosorbent assay (ELISA) format. Negative controls were included in all tests. All grafts were poorly immunogenic, whether they were untreated, processed by partial demineralization, or processed by both laser perforation and partial demineralization. Only two recipient animals showed a transient, antibody-mediated donor-specific immune response. One of these animals had received a control allograft, whereas the other animal had received a laser-perforated and partially demineralized bone allograft. All of the grafts in this study, including control grafts, were stripped of soft tissues and their bone marrow was removed; cellular sources of alloantibody stimulation may have been eliminated by these processes. The results of this study suggest that immune responses to bone allografts may be reduced by removing the bone marrow and adjacent soft tissues. The processing of cortical bone allografts by laser perforation and partial demineralization appeared to have little effect on immune responses.

Key words Immune response · Cortical allograft · Demineralization · Porosity

Introduction

The major complications of massive cortical bone allografts include fractures, nonunion, and infection. The causes of these complications are still poorly understood. However, many investigators have hypothesized that at least some of these complications are immunologically mediated.

Immune-competent cells secrete cytokines such as interleukin 1 and tumor necrosis factor alpha and beta. These cytokines are also potent activators of the osteoclastic cell lineage and, therefore, may stimulate accelerated bone resorption. Because bone resorption must occur before allograft bone can be replaced by newly formed bone, a delicate balance between the two concurrent processes must be maintained for the graft to be revascularized and substituted by host bone without appreciable loss of strength.

In an attempt to enhance the incorporation of cortical bone allografts into host bone, we have developed a new processing method that consists of laser drilling followed by partial demineralization. Previous studies have attempted to improve host incorporation by altering the geometrical surface configuration of cortical bone. The mechanism by which the presence of laser holes may promote osteogenesis and incorporation in partially demineralized grafts is thought to be related to either the greater surface area of the partially demineralized bone or to increased access to vascular tissue, or to a combination of these two factors.

Hydrochloric acid demineralization of the grafts was chosen for two reasons. First, it is known to result in the exposure of osteoinductive noncollagenous bone matrix growth factors, such as transforming growth factor...
(TGF)-β. These factors contribute to the transformation of mesenchymal cells into osteogenic and chondrogenic cells required for the induction of bone resorption and the formation of new bone.

Second, acid demineralization may lead to the depletion of cellular components within the graft that express transplantation antigens, which would reduce immune responses to the graft. Both antibody-mediated and cellular-mediated immune responses to bone allografts have been shown through in-vivo and in-vitro experiments. These studies have indicated that the primary response of the host to the bone allograft is predominately a cellular-mediated response to the MHC-encoded cell surface antigens which are carried by cells within the allograft and recognized by responding T lymphocytes in the host. In clinical studies, this response has been observed to develop as early as 1 month after surgery.

Materials and methods

Preparation of grafts and animal model

Mature outbred mismatched black mutton sheep (Westfälischer Schwarzkopf) were used as both donor and recipient animals. A total of 24 grafts were procured from both hind legs of 12 donor animals. Before transplantation, the grafts were stripped of soft tissues and the bone marrow was removed (Fig. 1). The grafts were 30-mm long and were used to replace a defect of similar size created in the mid-shaft tibia of the left hind leg. Fixation was achieved with an intramedullary nail and with two proximal and two distal locking screws. Three different types of bone transplants were used in each of the three recipient groups, of 8 animals each: group 1, fresh frozen control allografts; group 2, partially demineralized bone allografts; and group 3, laser-perforated and partially demineralized bone allografts with the extent of demineralization being the same as that in group 2.

An Er:YAG laser (Schwartz Electro-Optics, Concord, MA, USA), operating at a wavelength of 2940 nm, was used to drill holes of 300-µm diameter through the entire thickness of the diaphyseal cortex, producing a hole that was, typically, 3-mm-deep. The laser was connected optically to an OPMI 1 F/C operating microscope (Zeiss, Oberkochen, Germany) by an articulated arm. A 100- to 200-µs pulse train, consisting of 1-µs-long pulses was used for drilling. The energy delivered per pulse was measured using a pyroelectric joulemeter (ED-200; Gentec, Ste. Foy, Quebec, Canada) and an oscilloscope. A collinear helium-neon laser with a visible beam was used to aim the Er:YAG laser. The beam diameter at the tissue surface was 330 µm, as defined by the e^-2 intensity points. The lasers were aimed by means of a micro manipulator (Laser Mechanisms, Southfield, MI, USA). Typically, drilling was performed using a 53-mJ pulse, with 25 to 30 pulses required to drill each hole. The fluence per pulse was typically 60 J/cm². Laser holes were drilled at 2.5-mm intervals. Typically, an average