Alcohol ingestion by donors amplifies experimental airway disease after heterotopic transplantation

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Online Data Supplement

Supplementary Material:

MATERIALS AND METHODS

Hydroxyproline assay:

Isografts and allografts were homogenized and digested overnight at 110^{o}C in 6N HCl. Allograft and isografts homogenates were incubated for 20 minutes with Chloramine T solution, and then incubated with Ehrlich's solution for 15 minutes at 65^{o}C. Hydroxyproline content was assayed at 550 nm using hydroxyproline standards (Sigma). Hydroxyproline content for each sample was normalized to protein content and expressed as micrograms hydroxyproline per microgram protein.

Immunohistochemistry:

Briefly, untransplanted (from SD rats), isograft, and allograft sections (6 μm) were fixed in 4% formaldehyde (10 minutes; for α-SMA and TGFβ$_1$) or cold acetone (2 minutes; for OX-6, CD54, CD4, and CD8a). Endogenous peroxidase activity was quenched with H_2O_2. Sections were permeabilized with 0.05% Tween-20 (PBS-T), blocked with 5% donkey serum, incubated overnight at 4^{o}C with primary antibodies. Sections were incubated with biotinylated secondary antibodies followed by horseradish peroxidase-streptavidin (Vectastain kit; Vector Laboratories). Color was developed with 3,3'-diaminobenzidine tetrahydro-chloride substrate (Vector), counterstained with hematoxylin, and coverslipped. Appropriate isotype IgGs were used to control for nonspecific antibody binding. Multiple high-power photomicrographs were obtained using a Leica DM4000B microscope. To quantify the amount of immunohistochemical staining

in sections, the number of immuno-positive cells was expressed as a percentage of the total number of nuclei (determined by counting the number of hematoxylin-positive nuclei) per field. In some experiments, the number of immuno-positive cells was determined by counting positive cells per high power microscopic field (20x magnification).

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL):

Sections (6μm) were prepared, and stained by the TUNEL method according to the manufacturer's instructions. Sections were counterstained with hematoxylin and coverslipped. Each stained section was examined at high power fields for the presence of TUNEL-positive cells in the tracheal epithelial and submucosal layers. To quantify the extent of apoptosis in tracheal sections, the number of TUNEL-positive nuclei was expressed as a percentage of the total number of nuclei per field.

Histochemical evaluation of OAD:

First, H&E-stained sections of grafts were analyzed for epithelial damage and cellular infiltration. The extent of epithelialization was quantitated by importing photo-micrographs into Scion Image Beta 4.02 (Scion Corporation, Frederick, MD) and determining the circumference of the entire lumen and epithelialized lumen. Percent epithelialization was calculated by dividing values for 'epithelialized lumen' by values for 'entire lumen'. Second, relative collagen deposition within the airway lumen between experimental groups was determined by quantifying Trichrome staining of multiple sections throughout the tissue. Briefly, multiple photo-micrographs were imported into Photoshop 7.0 (Adobe Systems Inc.). A color sampler tool was used to gate representative shades of blue Trichrome staining. To determine the stain density of

each field, the area of the field containing the selected color stain was selected, the remaining background was removed, and the resulting image was imported into Scion Image. The density of staining was averaged and calculated for each section.