

Alcohol Ingestion by Donors Amplifies Experimental Airway Disease after Heterotopic Transplantation

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Rationale: Obliterative bronchiolitis (OB) after lung transplantation is triggered by alloimmunity, but is ultimately mediated by transforming growth factor (TGF)- β_1 -dependent airway fibrosis.

Objectives: Chronic alcohol use increases TGF- β_1 expression and renders the lung susceptible to injury. Therefore, we hypothesized that donor alcohol abuse could prime the lung allograft for OB, as many organ donors have a history of alcohol abuse.

Methods: Tracheas from control and alcohol-fed rats (8 wk) were heterotopically transplanted into recipients with varying degrees of alloimmune mismatch and analyzed for obliterative airway disease severity on Postoperative Day 21.

Measurements and Main Results: Although donor alcohol ingestion did not increase the number of antigen-presenting cells or infiltrating lymphocytes, it nevertheless increased allograft luminal collagen content fourfold compared with allografts from control donors. In parallel, alcohol increased TGF- β_1 and α -smooth muscle actin expression in allografts. Alcohol amplified airway disease even in iso-grafts with minor alloimmune mismatches. In contrast, it did not cause any airway disease in isografts in a pure isogenic background, suggesting that a minimal alloimmune response is necessary to trigger alcohol-induced airway fibrosis.

Conclusions: Although alloimmune inflammation is required to initiate airway disease, alcohol primes the allograft for greater TGF- β_1 expression, myofibroblast transdifferentiation, and fibrosis than by alloimmune inflammation alone. This has serious clinical implications, as many lung donors have underlying alcohol abuse that may prime the allograft recipient for subsequent OB.

Keywords: fibrosis; lung transplant; obliterative bronchiolitis; transforming growth factor- β_1

Obliterative bronchiolitis (OB) is a histological diagnosis of small airway fibrosis and occlusion for which no effective treatment exists. OB is the major long-term cause of allograft dysfunction after lung transplantation (1, 2). OB is believed to result from immunologically mediated airway epithelial destruction and subsequent luminal fibrosis. Although a variety of risk factors have been implicated in the development of OB (3), predicting which transplant recipients develop OB has been as yet unreliable. Several studies have demonstrated a correlation between the presence of a variety of biomarkers and the development of OB (4–10). Studies have shown that acute rejection is a risk factor for long-term graft dysfunction (3, 11, 12); however, others have failed to establish this correlation (13, 14).

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AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Obliterative bronchiolitis (OB) is the main long-term cause of graft dysfunction after lung transplantation. Although some risk factors have been implicated in the development of OB, predicting which graft recipients develop OB has been as yet unreliable.

What This Study Adds to the Field

Donor alcohol abuse amplifies the severity of OB in the recipient.

Using heterotopic tracheal transplantation (HTT) in animals to induce obliterative airway disease (OAD) as an experimental model of OB, recent studies have demonstrated that alloimmunity represents but one early component of OAD pathophysiology (15, 16). Rather, OAD development appears to be mediated via two mechanisms. First, an alloimmune trigger leads to inflammation and subsequent destruction of the airway epithelium. Second, the alloimmune response wanes, and is superseded by tissue remodeling pathways. Taken together, the majority of experimental evidence suggests that therapies designed to prevent or treat OB by attenuating the alloimmune response may not be clinically viable. This contention is supported by the fact that progression of OB is refractory to augmented immunosuppressive therapy (17).

Using animal models of alcoholism, studies have shown that alcohol ingestion primes the lung for injury (18–20) and increases transforming growth factor (TGF)- β_1 in the lung (21, 22). Clinically, subjects with alcoholism increased risk of acute respiratory distress syndrome after stresses, such as sepsis or trauma (23, 24). Young and otherwise healthy subjects with alcoholism have impaired alveolar epithelial barrier function despite having normal airway physiology (25). According to the United Network for Organ Sharing, 19% of deceased organ donors have a history of alcohol abuse. In addition, donor alcohol abuse increases the risk of graft dysfunction after heart transplantation (26, 27). Overall, these studies suggest that clinically silent, alcohol-mediated lung dysfunction in the allograft donor could promote development of OB.

In this study, we used the HTT model to test the hypothesis that alcohol ingestion by donors amplifies development of OAD in the recipient. To test this hypothesis, we transplanted airways from control or ethanol-fed rats into recipients with varying degrees of alloimmune mismatch. The data reported in this study support the contention that alcohol potentiates the development of OAD. In addition, we show that alcohol mediates its effects only when a sufficient alloimmune trigger is present. The data presented here are relevant in the context of lung transplantation, as many donors are otherwise young, healthy individuals

who die in alcohol-related accidents. Some of the results of this study have been previously published in the form of abstracts (28, 29).

METHODS

HTT of Tracheas in Rats

All procedures involving animals were performed with approval by the Veterans Affairs Medical Center institutional animal care and use committee, and in accordance with National Institutes of Health guidelines, the Animal Welfare Act, and applicable Veterans Affairs Medical Center policies. Male Sprague Dawley (SD; Charles River Laboratories, Wilmington, MA) or Fischer 344 (F344; Harlan, Indianapolis, IN) donor rats (age, 3–5 mo) were fed the Lieber-DeCarli isocaloric liquid diet, containing either ethanol (36% total calories) or Maltin-Dextrin as substitute (control diet) for 8 weeks. HTT was performed as described previously (16). Briefly, isografts or allografts were obtained by transplanting tracheas from control or alcohol-fed donor rats subcutaneously into recipient rats. Tracheas were harvested before transplant or at 3, 7, 14, or 21 days after transplant, and either cut into 6- μ m-thick sections for histological and immunohistological analyses, or homogenized for hydroxyproline assays. We used three transplant paradigms to determine alloimmune involvement in the development of OAD. First, to minimize alloimmunity, isogenic transplants were performed between inbred F344 rats. Second, to elicit a mild alloimmune response, isogenic transplants were performed using outbred SD rats. The RT1 haplotype of SD rats is not monitored; however, SD rats would be expected to be heterozygous between each animal across the genome. Third, to model clinically relevant transplantation whereby a strong alloimmune response follows transplantation, we performed allogeneic transplants between SD or F344 rats as donors and F344 or SD rats as recipients, respectively. All recipients were fed normal rodent chow and water.

Hydroxyproline Assay

Collagen content was assayed biochemically by determining hydroxyproline content according to Woessner (30). Additional details on hydroxyproline determination are provided in the online supplement.

Immunohistochemistry

Anti-TGF- β_1 and anti- α -smooth muscle actin (SMA) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and LabVision Corp. (Fremont, CA), respectively. All other primary antibodies were obtained from BD Pharmingen (San Jose, CA), including OX-6 (major histocompatibility complex [MHC] II), anti-CD54 (intercellular adhesion molecule [ICAM]-1), OX-38 (CD4), and OX-8 (CD8a). Biotinylated donkey anti-mouse and anti-rabbit antibodies were obtained from Jackson ImmunoResearch (West Grove, PA). Immunohistochemical staining was performed as described previously (31). Additional details on immunohistochemical methods are provided in the online supplement.

Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick-End Labeling

Tracheal sections from control and ethanol-fed SD donor rats were evaluated pretransplant (1 and 8 wk of ethanol) using an *in situ* cell death detection kit (POD; Roche Diagnostics, Indianapolis, IN) to assess tracheal epithelial apoptosis. Additional details on terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining are provided in the online supplement.

Histochemical Evaluation of OAD

Allograft injury, as determined by epithelial loss, cellular infiltration, and collagen deposition within the lumen of the airway, was assessed at 3, 7, 14, and 21 days after transplantation, as described previously (16). Image analyses were performed as described by Schacker and colleagues (32). Additional details on histological evaluation of OAD are provided in the online supplement.

Statistical Analyses

To determine significance between two groups, comparisons were made using Student's *t* test. Analyses of multiple groups were performed using one-way analysis of variance with Bonferroni's posttest using GraphPad Prism version 4.0a (GraphPad Software, San Diego, CA). For all statistical tests, *P* less than 0.05 was accepted for statistical significance. All graphical data are displayed as means (\pm SEM).

RESULTS

Donor Alcohol Ingestion Exacerbates OAD Depending on the Degree of Alloimmune Mismatch

To determine the effect of donor alcohol ingestion on OAD pathology, allogeneic transplants using control or alcohol-fed SD donors and F344 recipients were performed (SD-F344). Allografts were analyzed histochemically and biochemically at 21 days after transplant. Quantification of trichrome-stained sections revealed significantly increased collagen deposition (fourfold) within the airway lumen in allografts from alcohol-fed donors compared with control donors (Figures 1A and 1B, *bottom panels*). All allografts (from control and alcohol-fed donors) displayed complete obliteration at 21 days after transplantation. To rule out the possibility that strain differences contributed to the amplified pathology, F344-SD allogeneic transplants were also performed. This transplant paradigm similarly resulted in a fourfold (3.911 ± 0.7268) increase in luminal collagen in allografts originating from alcohol-fed donors. Determination of hydroxyproline content in allografts also revealed a significant increase in collagen content in allografts from alcohol-fed donors (Figure 1C). These results indicated that OAD in the recipient was amplified by donor alcohol ingestion, and that these effects were independent of which strain was used as donors versus recipients.

Because OB pathology has been attributed to alloimmune-dependent and alloimmune-independent mechanisms, we sought to determine the contribution of the alloimmune response to alcohol-mediated exacerbation of OAD. To assess alloimmune involvement, we performed two types of syngeneic transplants: F344-F344 and SD-SD, using control and alcohol-fed donor rats. When alcohol-fed donors were used for F344-F344 transplants, we did not observe increased OAD pathology compared with isografts originating from control donors (Figures 1A and 1B; *top panels*). All F344-F344 isografts were free from OAD pathology. However, when SD-SD transplants were performed, isografts from alcohol-fed donors displayed significant OAD pathology (as assessed by trichrome staining and hydroxyproline content) and had no airway epithelium present (Figures 1A and 1B, *middle panels*; and Figure 1C). As expected, the majority of isografts from control donors did not exhibit OAD, and had a fully regenerated and intact airway epithelium. Of seven SD-SD control animals, only one isograft had OAD pathology, whereas all eight SD-SD alcohol isografts displayed OAD pathology. These data support the contention that prior alcohol ingestion by donors exacerbates OAD pathology in the recipient via a process that requires a threshold alloimmune trigger.

Airway Epithelial Viability Pretransplant Is Not Compromised in Alcohol-Fed Donors

Using the HTT model, we have shown that alcohol ingestion by donors increases OAD pathology in recipients depending on the degree of alloimmune mismatch. To investigate mechanisms by which alcohol ingestion by donors increased OAD, we first examined the possibility that alcohol ingestion by donors exacerbates OAD in allografts by decreasing the viability of the

tracheal epithelium. To test this hypothesis, we analyzed pre-transplant airway from control or alcohol-fed SD donors at 1 and 8 weeks of control/alcohol diet for the presence of TUNEL-positive cells. We did not observe an increase in the percentage of apoptotic epithelial or submucosal cells between control and

alcohol-fed donors at either time point (Figures 2A and 2B). In fact, TUNEL-positive cells were not observed in the airway epithelium in sections from any experimental group. These results suggest that alcohol does not mediate its effects via heightened airway epithelium injury due to increased apoptosis in the tracheal epithelium.

Pretransplant Airways from Alcohol-Fed Donors Do Not Appear to Be Primed for Heightened Alloimmune Injury

As another putative mechanism by which donor alcohol ingestion exacerbates OAD, we sought to determine if alcohol primes the transplanted tissue for heightened alloimmune activity by increasing numbers of antigen-presenting cells. Alternatively, alcohol may cause lymphocytic infiltration in untransplanted tracheal tissue. To characterize the immunological state of the alcohol-fed tissue before transplantation, tracheae from control and alcohol-fed SD donors (8 wk of dietary alcohol) were analyzed for the presence of antigen-presenting cells and T lymphocytes. Sections from pretransplant tracheae were immunostained for the presence of MHC II-, ICAM-1-, CD4-, and CD8a-positive cells. Tracheae from 8-week alcohol-fed rats show a significant decrease in the prevalence of MHC II-positive cells; however, no significant differences were noted for ICAM-1, CD4, or CD8a expression (Figures 3A and 3B). Thus, tracheae from alcohol-fed donor rats do not appear to be primed to induce a heightened alloimmune response.

Donor Alcohol Ingestion Exacerbates Epithelial Injury after Transplantation

Destruction of the epithelium appears to be a critical step in the development of OAD (15, 33). To investigate the possibility that donor alcohol ingestion renders the airway epithelium more susceptible to injury, we analyzed the degree of epithelialization in SD-F344 allografts from control and alcohol-fed donors at 3, 7, 14, and 21 days after transplantation. In allografts from control donors, considerable epithelial loss occurred by Day 3, followed by regeneration by Day 7, and permanent loss by Day 14 (Figures 4A and 4B). In contrast, allografts from alcohol-fed donors displayed significantly reduced epithelialization at Day 7 (Figures 4A and 4B), suggesting either that regeneration of the epithelium is inhibited, or that permanent loss of the epithelium occurs earlier.

Lymphocytic Infiltration Is Not Increased in Allografts Originating from Alcohol-Fed Donors

Because infiltrating T lymphocytes are indicative of increased alloimmune activity, we determined if allografts from alcohol-fed donors displayed augmented alloimmune infiltration relative

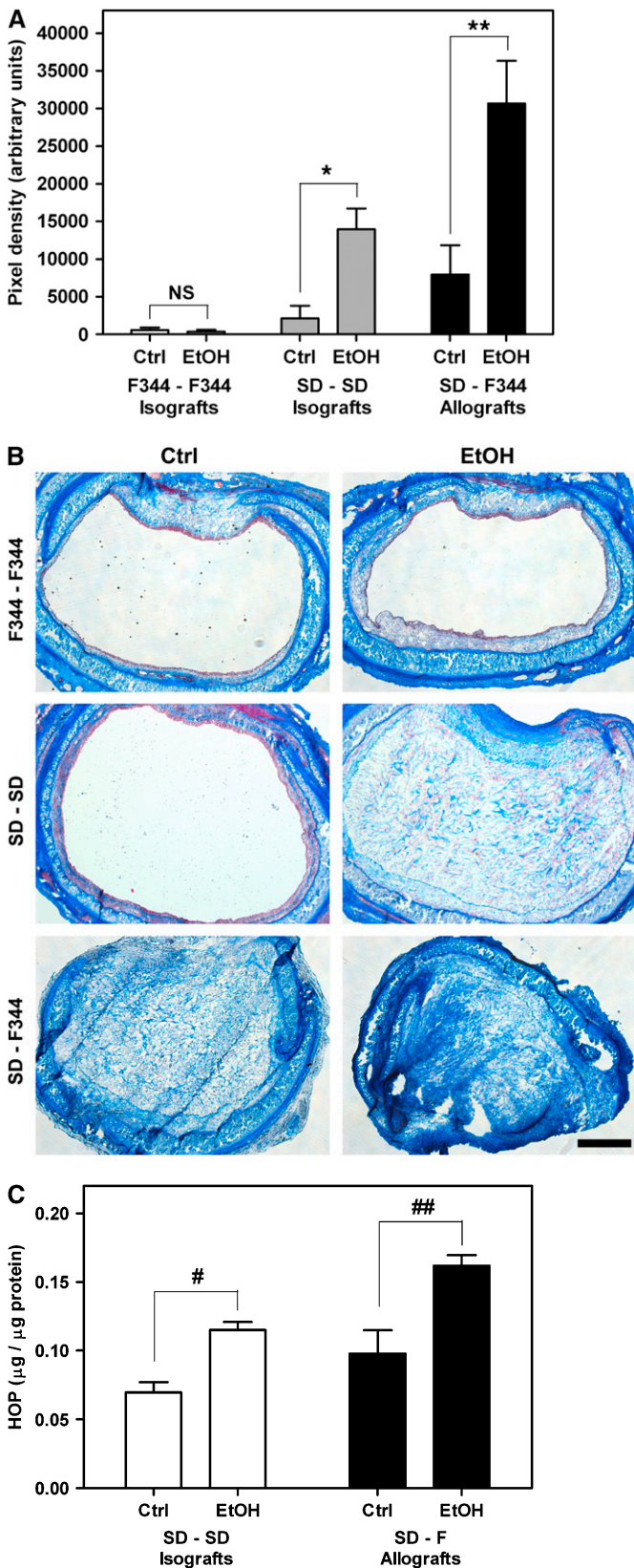


Figure 1. Donor alcohol ingestion exacerbates obliterative airway disease, depending on the degree of alloimmune mismatch. (A) Quantification of Masson's trichrome blue staining of 21-day isografts and allografts from control (Ctrl) and alcohol-fed (EtOH) donor rats. No significant difference was observed between control (n = 4) and alcohol (n = 4) groups for Fischer 344 (F344)-F344 isografts. Statistical significance was observed between control (n = 8) and ethanol (n = 7) groups for Sprague Dawley (SD)-SD isografts (*P < 0.05), and control (n = 10) and ethanol (n = 12) SD-F344 allografts (**P < 0.05). (B) Masson's Trichrome Blue histology staining of F344-F344 isografts (top panels), SD-SD isografts (middle panels), and SD-F344 allografts (bottom panels). Scale bar = 500 μm. (C) Hydroxyproline (HOP) content (indicative of collagen content) is significantly increased in SD-SD isografts (#P < 0.05) and SD-F344 allografts (##P < 0.05) from ethanol-fed donors.

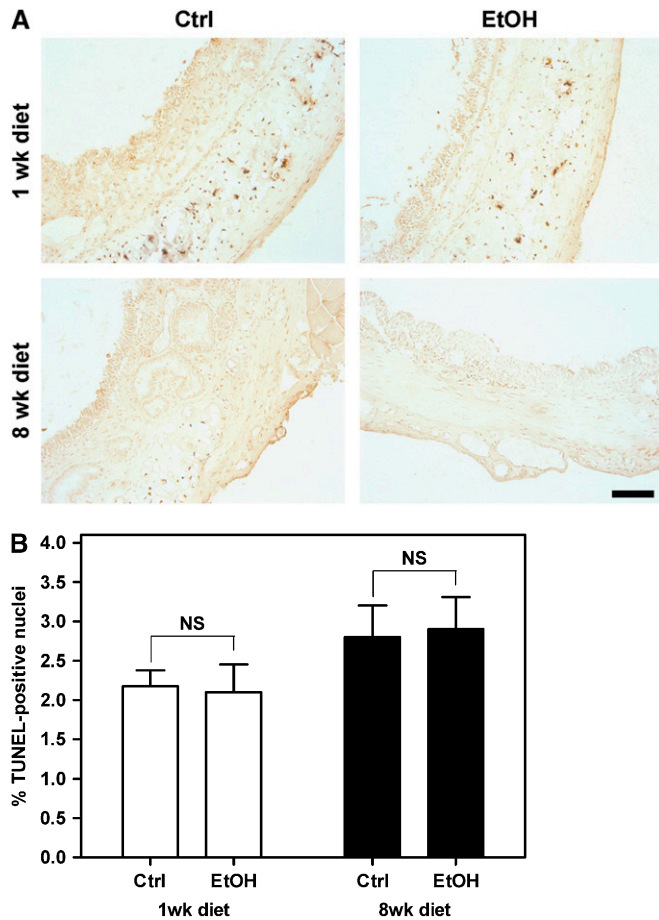


Figure 2. Pretransplant airway epithelium viability is not compromised in alcohol-fed SD donors. (A) Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining of pretransplant tracheal sections at 1-week ethanol diet (*top panels*) and 8-week ethanol diet (*bottom panels*). TUNEL-positive nuclei were not observed in the airway epithelium of any groups. Scale bar = 100 μ m. (B) Quantification of TUNEL staining in the airway subepithelial layers. No significant differences were observed in the prevalence of TUNEL-positive cells between control ($n = 4$) and alcohol-fed ($n = 4$) donors at either 1 or 8 weeks of dietary ethanol ($P > 0.05$). NS = not significant.

to control animals at 3, 7, 14, and 21 days after transplantation. Allografts were analyzed for the presence of infiltrating CD4- and CD8a-positive T lymphocytes in the subepithelial layers between the cartilage and airway epithelium/epithelial remnants. Representative images are of CD8a-positive lymphocytes, as this subset of cells was significantly more prevalent (Figure 5A). No significant differences were observed in the total number of CD4- and CD8a-positive cells between allografts originating from control or alcohol-fed SD donors at the time points analyzed (Figure 5B). These data suggest that alcohol does not mediate its deleterious pathological effects via increased infiltration of CD4- and CD8a-positive cells.

Allografts from Alcohol-Fed Donors Display Increased TGF- β_1 and α -SMA Expression

Because the alloimmune-independent component of OAD pathology is dependent on increased fibrogenic activity, we investigated the role of TGF- β_1 and α -SMA in alcohol-mediated

exacerbation of OAD. Previous studies have demonstrated that OAD pathology in allografts is TGF- β_1 -dependent (16, 34, 35). To determine if components of the TGF- β_1 fibrogenic pathway are increased in alcohol-mediated exacerbation of OAD, 21-day allografts from control and alcohol-fed SD donors were immunostained for TGF- β_1 . TGF- β_1 expression is significantly increased in allograft sections from alcohol-fed donors (Figure 6A, top panels; and Figure 6B).

Fibrosis, one of the hallmarks of OAD, is characterized by excessive synthesis of collagen and accumulation of myofibroblasts. TGF- β_1 , a potent inducer of collagen synthesis, and myofibroblast transdifferentiation, are implicated in fibrosis (for review, see Reference 36). First, pretransplant tracheae from control and alcohol-fed rats were examined for the presence of α -SMA-positive cells. No differences in TGF- β_1 and α -SMA expression were observed between pretransplant control and alcohol tracheae (data not shown). Allografts from control and alcohol-fed SD donors were assayed immunohistochemically at Day 21 after transplant for the presence of myofibroblast transdifferentiation. α -SMA-positive cells, indicative of increased myofibroblast transdifferentiation, were significantly increased in the allograft lumen from alcohol-fed donors (Figure 6A, bottom panels; and Figure 6B). These data show that alcohol-mediated amplification of OAD is associated with increased TGF- β_1 and α -SMA expression, and suggest that alcohol mediates its effects via increased fibrogenesis.

DISCUSSION

In the current study, we determined that donor alcohol ingestion amplifies OAD in the recipient. Using a variety of different transplant paradigms to elicit varying degrees of alloimmune mismatch, we showed that alcohol mediates its effects only when a threshold alloimmune trigger is present. Specifically, we demonstrated that a slight alloimmune trigger (HTT between outbred rats) is sufficient to elicit amplified OAD pathology if the airway is exposed to alcohol before transplantation. In the more clinically relevant transplant paradigm (HTT between different strains of rats), donor alcohol ingestion amplifies OAD significantly. In experiments designed to test the mechanism by which prior alcohol ingestion amplifies OAD pathology, we determined that alcohol ingestion does not decrease airway epithelium viability before transplant. Moreover, alcohol did not appear to prime the alloimmune trigger in grafted tissue, as no increase in the number of antigen-presenting cells was observed. In addition, allografts from alcohol-fed donors did not display increased lymphocytic infiltration. However, epithelial integrity at Post-Transplantation Day 7 was decreased in allografts originating from alcohol-fed donors. Finally, allografts from alcohol-fed donors had increased numbers of luminal TGF- β_1 - and α -SMA-positive cells, indicating that tissue remodeling/fibrotic pathways are amplified.

Development of OAD pathophysiology after transplantation occurs via two distinct yet codependent mechanisms. First, an alloimmune trigger occurs in the grafted tissue, leading to oxidative stress and up-regulation of proinflammatory mediators. Subsequent injury to the airway epithelium likely represents one of the critical steps in OAD pathophysiology (15, 37). In support of this concept, denuded allografts develop severe OAD pathology, and replacement of epithelium in denuded allografts can prevent this (33).

Damage to the airway epithelium exposes subepithelial layers of cells, which further amplifies the alloimmune response, as well as activating profibrotic pathways in the underlying connective tissue. At some stage of OAD pathogenesis, alloimmune

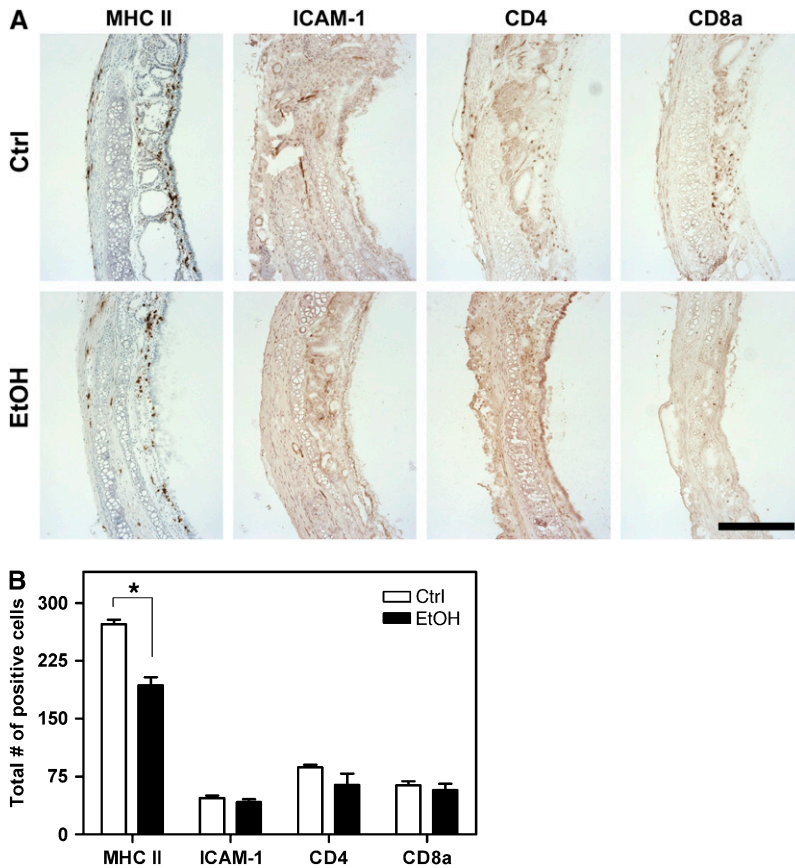


Figure 3. Pretransplant airways from alcohol-fed SD donors do not appear to be primed to heightened alloimmune injury. (A) Untransplanted tracheal sections (8-wk dietary ethanol) were analyzed by immunohistochemical staining for major histocompatibility complex (MHC) II, intercellular adhesion molecule (ICAM)-1, CD4, and CD8a expression. Scale bar = 250 μ m. (B) Quantification of MHC II-, ICAM-1-, CD4-, and CD8a-positive cells in untransplanted tracheal sections. MHC II expression is decreased approximately 50% in sections from alcohol-fed donors (* $P < 0.05$); however, no significant differences were noted between control (open bars) and alcohol (closed bars) groups for ICAM-1, CD4, or CD8a (n = 4 for each group).

injury wanes and TGF- β_1 -dependent components of tissue remodeling are activated. Although TGF- β_1 is thought to have desirable antiinflammatory properties, increased levels of TGF- β_1 in patients with OB appear to correlate with the severity of the disease (38, 39). TGF- β_1 is a critical factor known to induce myofibroblast transdifferentiation (40). Activation of this pathway leads to increased numbers of fibrogenic cells, migration of fibrotic cells into the airway lumen, and subsequent transdifferentiation into myofibroblasts. Myofibroblasts are a key source of extracellular matrix protein deposition that occurs in the airway lumen (34, 41). Thus, it is likely that factors that modulate components of the tissue remodeling pathway play a role in OAD pathogenesis.

Using the HTT model in rodents, King and colleagues demonstrated that, when alloimmunity is removed before permanent destruction of the airway epithelium, OAD does not develop (15). In contrast, OAD develops, regardless of whether alloimmunity is absent, if the integrity of the airway epithelium is abrogated. Thus, a "point of no return" exists for OAD development, whereby an alloimmune trigger alone is not sufficient to cause development of OAD. Ramirez and colleagues demonstrated the importance of the TGF- β_1 pathway in OAD pathophysiology by showing that Smad3, an intracellular signal transducer for TGF- β_1 , is required to elicit OAD, despite the presence of a normal alloimmune response (16). In a subsequent study, it was shown that TGF- β_1 stimulates myofibroblast transdifferentiation via Smad3-dependent and Smad3-independent pathways, contributing to the excessive matrix deposition that is characteristic of OAD (34). Taken together, the results from these studies show that alloimmunity represents but one early component of OAD pathophysiology.

The results presented in this study show that alcohol mediates its effects when a threshold alloimmune trigger is present. When transplants were performed between inbred F344 rats, a minimal alloimmune trigger was present. Predictably, isografts from alcohol-fed donors did not develop OAD pathology. These data are not surprising, as the majority of clinical and experimental evidence underscore the necessity for an alloimmune trigger to induce OAD pathogenesis. Transplants between the outbred SD rats provided a mild alloimmune mismatch. However, because a sufficient alloimmune trigger was present, isografts from alcohol-fed donors displayed OAD pathology. In the more clinically relevant transplant paradigm, transplants between different strains (SD-F344 and F344-SD), providing a significant alloimmune mismatch, predictably resulted in OAD pathology in allografts from control donors, but also led to exacerbated OAD pathology in allografts from alcohol-fed donors. By using this combination of syngeneic and allogeneic transplantation paradigms to vary the degree of alloimmune trigger, the experimental data presented here argue against ischemic injury being a possible source of the heightened OAD in grafts from alcohol-fed donors. Importantly, these results also demonstrate that the deleterious effects of alcohol occur independent of the strain of rat used for the donor.

However, the experimental evidence shown here suggests that alcohol does not prime the transplanted tissue to elicit an augmented alloimmune response. In this study, histological analyses show that untransplanted tracheal tissue from alcohol-fed donors appears grossly normal, with no obvious signs of immune infiltration. Immunohistochemical analyses of MHC II antigens failed to detect an increase in antigen presentation on the airway epithelium or submucosal layers. In fact, alcohol

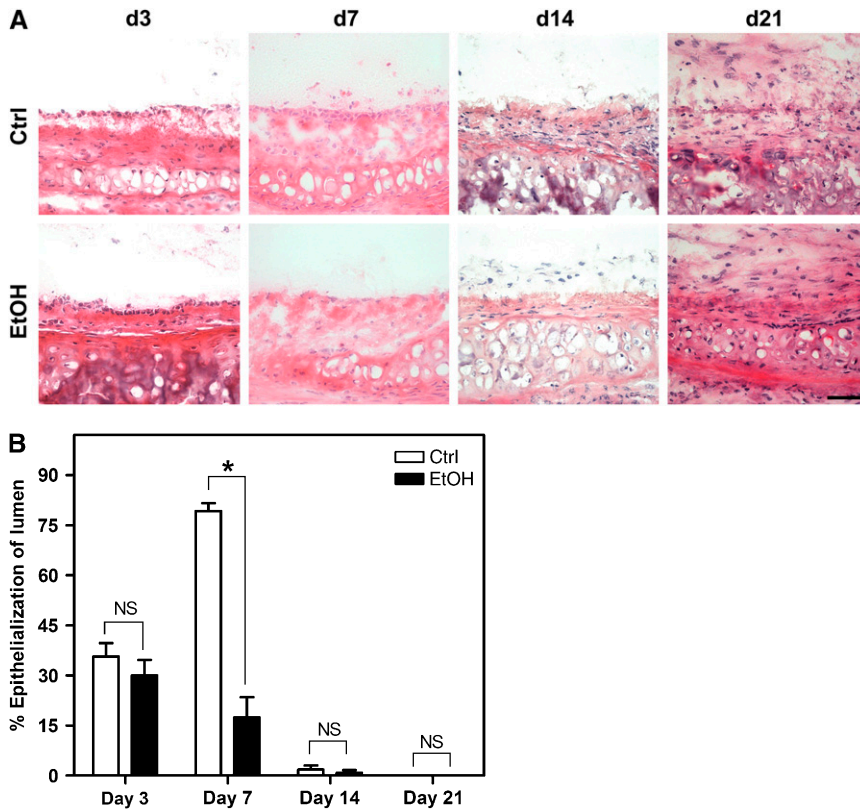


Figure 4. Donor alcohol ingestion exacerbates epithelial injury post-transplantation. (A) Sections from 3-, 7-, 14-, and 21-day allografts originating from control (top panels) or alcohol-fed (bottom panels) donors were stained with H&E. Areas highlighting the epithelium are shown in representative H&E images from each group. Scale bar = 50 μ m. (B) Quantification of the degree of epithelialization of allografts from control (open bars) or alcohol-fed (closed bars) donors at Days 3, 7, 14, and 21 post-transplantation (n = 4–5 for each group; *P < 0.05). NS = not significant.

ingestion decreased the number of MHC II–positive cells in pre-transplanted airway tissue, suggesting that the immunological state of the tissue is compromised. This contention is supported by previous studies showing that alcohol impairs immune func-

tion in the lung (for review, see Reference 42). Furthermore, we show that pretransplanted tracheae from alcohol-fed donors do not have increased expression of the costimulatory ligand, ICAM-1, or CD4/CD8a-positive lymphocytes. In 3-, 7-, 14-, and 21-day

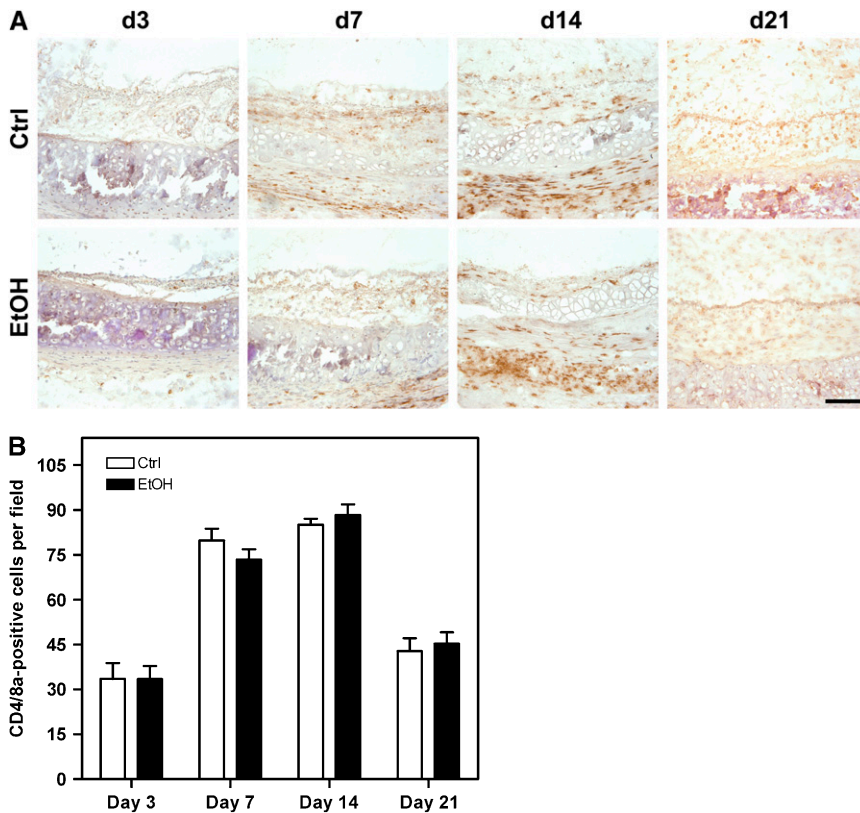


Figure 5. Lymphocytic infiltration is not increased in allografts originating from alcohol-fed donors. (A) Sections from 3-, 7-, 14-, and 21-day allografts originating from control or alcohol-fed donors were immunostained for CD8a. Representative CD8a (brown staining) images from each group show the allograft lumen/epithelial/subepithelial region. Scale bar = 100 μ m. (B) Quantification of CD4- and CD8a-positive cells reveals no difference in the percentage of infiltrating T lymphocytes in allografts from control (open bars) or alcohol-fed (closed bars) donors (n = 4 each group).

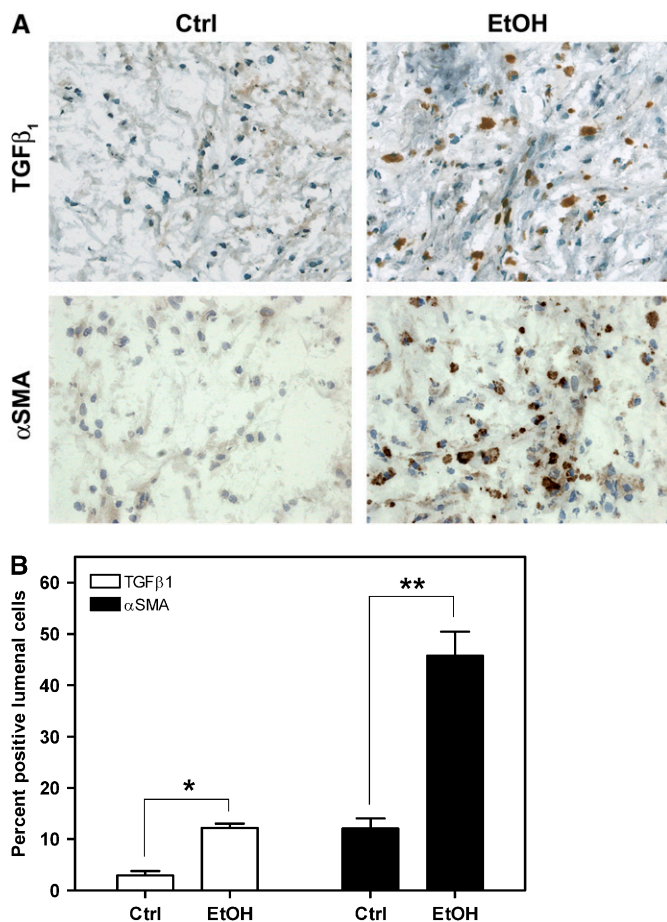


Figure 6. Allografts originating from alcohol-fed donors display increased transforming growth factor (TGF)- β_1 and α -smooth muscle actin (SMA) expression. (A) TGF- β_1 (top panels) and α -SMA (bottom panels) immunohistochemical stain (brown) and hematoxylin counterstaining (blue) of 21-day allografts from control and alcohol-fed rats. Note the increased TGF- β_1 and α -SMA staining in allografts from alcohol-fed versus control rats. Scale bar = 50 μ m. (B) Quantification of TGF- β_1 - and α -SMA-positive cells reveals a significant increase in the percentage of TGF- β_1 - (open bars; * $P < 0.05$) and α -SMA (closed bars; ** $P < 0.05$)-positive cells within the lumen of allografts (fourfold and threefold increase, respectively) ($n = 4$ each group).

allografts, no differences were observed in the prevalence of CD4/CD8a cells in allografts from control or alcohol-fed donors, despite decreased airway epithelial integrity in 7-day allografts from alcohol-fed donors.

Thus, alcohol appears to amplify the tissue remodeling response that is considered to be a later event in the development of OAD. Specifically, alcohol ingestion by donors increases the number of TGF- β_1 - and α -SMA-positive cells in the airway, indicating that components of tissue remodeling are amplified in response to alcohol. These data suggest that prior alcohol ingestion results in increased numbers of luminal myofibroblasts, and provides a possible explanation for the increased collagen deposition observed in allografts from alcohol-fed donors. In agreement with previous studies (15, 16), this work underscores the notion that modulation of fibrotic mechanisms is equally important as the initial alloimmune involvement in development of OAD pathophysiology. These data, taken together, suggest that prior alcohol ingestion by donors does not initiate the alloimmune trigger, but instead primes the grafted tissue for injury and subsequent development of OAD.

Previous studies have shown that, in the alcoholic lung, macrophage function is decreased (43) and other innate and adaptive immune functions are impaired (for review, see Reference 42). Expression of TGF- β_1 is increased in the lungs of alcohol-fed rats (21). Alcohol ingestion also activates the renin-angiotensin system, and angiotensin II is capable of inducing oxidative stress by depleting pulmonary glutathione levels and increasing TGF- β_1 expression (22). However, it is possible that other components of the alloimmune response may be increased in response to alcohol ingestion.

Despite the fact that several post-transplant risk factors for the development of OB have been identified (for review, see Reference 3), no effective therapies exist to prevent or attenuate the development of OB because of its progressive and irreversible nature. Comprehensive studies on how donor characteristics affect the success of lung transplantation in the recipient are lacking. Specifically, the effects of donor alcohol ingestion on the development of OB are unknown. This is an important issue to address, as many lung donors are likely to be young individuals who die in alcohol-related accidents. Importantly, this population is otherwise healthy, and does not display obvious clinical signs of lung dysfunction.

Although there is a lack of published, specific epidemiological data, we predict that conducting prospective (as well as retrospective) clinical studies on the effect of donor alcohol abuse on long-term success of lung transplants will result in the identification of alcohol as a risk factor for the development of OB. In support of our prediction, the now widely known link between alcohol abuse and susceptibility to acute lung injury was unrecognized until clinical studies published 10 years ago established such a link (23). The results presented here may have implications for the consideration of donor characteristics in regard to establishing criteria for determining what is considered "healthy" lung tissue for transplantation.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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