Transplantation

Tobacco Smoke Exposure in Either the Donor or Recipient Before Transplantation Accelerates Cardiac Allograft Rejection, Vascular Inflammation, and Graft Loss

Ashwani K. Khanna, PhD; Jianping Xu, MD, PhD; Patricia A. Uber, Pharm D; Allen P. Burke, MD; Claudia Baquet, MD, MPH; Mandeep R. Mehra, MBBS

Background—Tobacco exposure in cardiac transplant recipients, before and after transplantation, may increase the risk of cardiac allograft vasculopathy and allograft loss, but no direct evidence for this phenomenon is forthcoming. In this experimental study, we investigated early consequences of tobacco smoke exposure in cardiac transplant donors and recipients with an emphasis on alloinflammatory mediators of graft outcome.

Methods and Results—Using heterotopic rat cardiac transplantation, we tested the effects of donor or recipient tobacco smoke exposure in 6 groups of animals (rat heterotopic cardiac transplantation) as follows: tobacco-naïve allogeneic rejecting controls (n=6), tobacco-naïve nonrejecting controls (n=3; killed on day 5 to simulate survival times of tobacco-treated animals), isografts (n=3), both donor and recipient rats exposed to tobacco smoke (n=4), only donor rats exposed to tobacco smoke (n=7), and only recipient rats exposed to tobacco smoke (n=6). Polymerase chain reaction studies of tissue and peripheral (systemic) protein expression were performed to evaluate inflammatory (tumor necrosis factor-α, interferon-γ, interleukin-6) and alloimmune (interleukin-1 receptor 2, programmed cell death-1, and stromal cell-derived factor-1) pathways, as was histological analysis of the cardiac allografts. Our experiments reveal that pretransplantation tobacco exposure in donors and/or recipients results in heightened systemic inflammation and increased oxidative stress, reduces posttransplantation cardiac allograft survival by 33% to 57%, and increases intragraft inflammation (tumor necrosis factor-α, interferon-γ, interleukin-6) and alloimmune activation (CD3, interleukin-1 receptor 2, programmed cell death-1, and stromal cell-derived factor-1) with consequent myocardial and vascular destruction.

Conclusions—These sentinel findings confirm that tobacco smoke exposure in either donors or recipients leads to accelerated allograft rejection, vascular inflammation, and graft loss. Molecular pathways that intersect as arbiters in this phenomenon include instigation of alloimmune activation associated with tobacco smoke–induced inflammation.

(Circulation. 2009;120:1814-1821.)

Key Words: allograft • inflammation • rejection • surgery • tobacco • transplantation

Several studies have reported a relationship between cigarette smoking and cardiovascular diseases.1-3 Besides other health disorders, cigarette smoke also adversely affects the outcome of renal and cardiac transplantation.4-9 The significance of this is noted in the observation that a pretransplantation history of smoking and recipient recidivism represents a significant risk marker for graft and recipient demise, prompting development of guidelines to avoid transplantation in active tobacco users.5,10 Neither the sentinel contributions of donor or recipient tobacco exposure nor their discriminator molecular pathways involved in these adverse effects are known. Using experimental rat cardiac transplantation, a surgical model in which a “donor” heart is implanted in the abdomen vasculature of a transplant “recipient” animal, we systematically studied the effects of pretransplantation tobacco exposure (in donors, recipients, or both) on the induction of systemic and intragraft specific inflammation, oxidative stress, alloimmune activation, histopathological consequences, and consequently cardiac allograft survival.

Clinical Perspective on p 1821

Methods

Study Design

The effects of donor or recipient tobacco smoke exposure were tested in 6 groups of animals (rat heterotopic cardiac transplantation) as follows: tobacco-naïve allogeneic rejecting controls (n=6), tobacco-naïve nonrejecting controls (n=3; killed on day 5 to simulate survival times of tobacco-treated animals), isografts (n=3), both donor and recipient rats exposed to tobacco smoke (n=4), only donor rats exposed to tobacco smoke (n=7), and only recipient rats exposed to tobacco smoke (n=6). Polymerase chain reaction studies of tissue and peripheral (systemic) protein expression were per-
formed for each animal to evaluate inflammatory and alloimmune pathways; histological analysis of the cardiac allograft was done in at least 2 animals per group.

**Tobacco Smoke Exposure**

This study was conducted according to the National Institutes of Health guidelines for animal experiments. Rats were exposed to cigarette smoke in a specially constructed smoke chamber (Figure 1). The rats were restrained in the chambers and ventilated by the smoking machine. In this machine, smoke is sucked from lit cigarettes by syringes and pumped around the chamber, where rats experience smoke exposure. Rats headed to the smoke compartment are also inhaling the smoke from the tubing triggered by an automatic controlled smoking generator (Figure 1). The smoking machine was set to inhale and exhale at intervals mimicking human smoking, with each cigarette lasting 8 to 10 minutes. Animals were under constant supervision to ensure that they were not distressed. All procedures were approved by the University of Maryland, Baltimore Animal Care and Use Committee (No. 1007009). We used 3R4F research-grade cigarettes with a regular amount of nicotine in this study (KTRDC Tobacco Biotechnology Group, University of Kentucky, Louisville). Animals were exposed to cigarette smoke 5 d/wk for a total of 30 days, with 4 cigarettes given intermittently throughout the day. Control rats were subjected to exactly the same conditions of restraint, caging, and nutrition as the active smoking group. Cardiac transplantation was performed within 3 days of the last exposure to cigarette smoke. The efficacy of cigarette smoke exposure was assessed by quantification of circulating levels of cotinine, the metabolite of nicotine, with a kit from Calbiochem (Spring Valley, Calif.). To further authenticate these levels of tobacco exposure, we determined cotinine levels in nontransplanted rats injected with nicotine.

**Rat Model of Cardiac Transplantation**

Heterotopic heart transplantations as previously described were performed with the Wistar Furth and Lewis rat strains as donors and recipients, respectively. Donor rats were heparinized (1000 U IP) and anesthetized with ketamine (100 mg/kg)/xylazine (10 mg/kg). An abdominal incision was made and extended from the thoracic cavity, and the chest wall was removed. Blunt dissection of the heart, aorta, and pulmonary artery was performed. The pulmonary veins and inferior vena cava were ligated, and the graft was excised and placed in iced physiological saline. Recipient rats were then anesthetized with ketamine (100 mg/kg)/xylazine (10 mg/kg). A midline laparotomy was performed. The abdominal aorta and vena cava were dissected and clamped with straight vascular clamps to prevent bleeding. A longitudinal aortotomy and venotomy ~5 mm in length was performed. Using standard microvascular techniques with 8-0 prolene sutures, we made end-to-side anastomoses from the aorta to the abdominal aorta and from the pulmonary artery to the inferior vena cava. Hemostasis was achieved, and the incision was closed with a 3-0 Vicryl suture. Rats were monitored daily for evidence of allograft slowing and failure. They were anesthetized with ketamine (100 mg/kg)/xylazine (10 mg/kg). Animals were exsanguinated via a native heart intraventricular puncture.

**Detection of mRNA by Reverse Transcription and Polymerase Chain Reaction**

Total RNA was isolated from native and allograft heart tissues (SV RNA isolation kit, Promega, Madison, Wis.), and the quality of RNA was verified with a 260/280 nm ratio. RNA (1 μg) was reverse transcribed to cDNA with the iScript cDNA synthesis kit (BioRad, Hercules, Calif.). We performed real-time quantitative reverse-transcription polymerase chain reaction using a Bio-Rad iCycler system. The primer sequences of interest included those for interleukin (IL) -1 receptor 2 (IL-1R2), β-actin, programmed cell death-1 (PDCD-1), stromal-cell-derived factor-1 (SDF-1), IL-6, IL-10, and tumor necrosis factor-α (TNF-α). To determine the effect of cigarette smoke exposure on oxidative stress, we determined the mRNA expression of heme oxygenase 1 (HO-1), as described previously. The mRNA level of each sample for each gene was normalized to that of the β-actin mRNA. Relative mRNA level was presented as 2^[(cycle threshold/β-actin)−cycle threshold/gene of interest)] as described previously. The results are presented as normalized fold expression with β-actin as a reference gene and the native hearts as a control.

**Quantification of Circulating Levels of Cytokines**

The Bio-Plex rat cytokine assay was used for simultaneous quantitation of the proinflammatory cytokines IL-1α, IL-2, IL-6, IL-10, interferon-γ (IFN-γ), and TNF-α according to the recommended procedure. In brief, the premixed standards were reconstituted in 0.5 mL sample diluent, generating a stock concentration of 10,000 pg/mL for each cytokine. The standard stock was serially diluted in the same diluent to generate 8 points for the standard curve. Serum samples from rats were diluted 1:10. The assay was performed on a 96-well filtration plate supplied with the assay kit. Premixed beads (50 μL) coated with target capture antibodies were transferred to each well of the filter plate and washed twice with Bio-Plex wash buffer. Premixed standards or samples (50 μL) were added to each well containing washed beads. The plate was shaken for 30 seconds and then incubated at room temperature for 30 minutes with low-speed shaking. After incubation and washing, premixed detection antibodies (50 μL) were added to each well. The incubation was terminated after shaking for 10 minutes at room temperature. After washing 3 times, the beads were resuspended in 125 μL Bio-Plex assay buffer. Beads were read on the Bio-Plex suspension array system, and the data were analyzed with the Bio-Plex Manager software with 5PL curve fitting.

**Tissue Processing and Assessment of Vasculopathy and Cellular Rejection**

On removal, allografts and native hearts were immediately suspended in Tissue Tech and rapidly frozen on dry ice. The Tissue Tech was then stored at −80°C until processing. Two 5-μm sections of the transplanted hearts, one near the base and one at mid ventricle, were stained with hematoxylin and eosin to facilitate determination of the degree of vascular intimal thickening and cellular rejection. Histological rejection was scored in a blinded manner using criteria established by the International Society for Heart and Lung Transplantation (ISHLT) and modified to a linear score system to allow statistical analysis. The worst score per animal was used to define the severity of rejection.
Immunostaining of Cardiac Tissues With Anti-CD3, Anti–SDF-1, and TNF-α Antibodies

Formalin-fixed heart tissues were paraffin embedded, sliced into fine sections, deparaffinized in xylene, and rehydrated in graded ethanol to PBS. After blocking of endogenous peroxidase activity for 30 minutes with methanol/H2O2 (18:1 vol/vol), nonspecific binding was blocked for 1 hour with 1.5% avidin/biotin diluted in PBS supplemented with 10% normal horse serum and 3% BSA. Tissue sections were incubated overnight at 4°C with specific antibodies (50 μg/mL) in the above-mentioned PBS. After extensive washings with PBS, the slides were incubated for 1 hour with 1:1000 diluted biotin-labeled anti-mouse IgG horse anti-serum at room temperature and again washed extensively in PBS and then in ABC solution for 30 minutes. The slides were then developed for 10 minutes in diamobenzidine and rinsed with water for 10 minutes. The slides were mounted with Permount for evaluation. Samples from each group were graded for immunohistochemistry staining. The intensity of immunostaining was graded from 0 (no staining) to 4+/+++ (maximum staining) relative to the staining pattern in cardiac tissues.

Statistical Analysis

We used an actuarial (Kaplan-Meier) analysis to estimate the survival functions for rat cardiac transplant recipients with a combination of donor, recipient, or both exposed to tobacco versus tobacco-naïve untreated allografts. The log-rank test was used to compare the survival distributions. Before conducting the tests to compare gene expression and circulating levels of cytokines, we used F tests to evaluate the homogeneity of variances between groups. Finding significant differences in variance, we used Welch t tests to compare these outcomes between groups. Data were analyzed with GraphPad Prism version 5.0. Results are expressed as mean±SEM, and 2-tailed significance was determined at values of P<0.05.

Results

Measures of Tobacco Smoke Exposure

Quantification of circulating levels of cotinine, the metabolite of nicotine, demonstrated that these levels were increased in both donor and recipient strains. The levels of cotinine (mean±SEM) were 82.8±4.9 and 84.5±2.5 ng/mL in donor Wistar Furth and recipients Lewis rats, respectively, but undetectable in rats without exposure to cigarette smoke. Cotinine levels in control rats injected with nicotine were 82.3±3.5 ng/mL. Cotinine levels were measured after 30 days of exposure to smoke at the point of cessation of exposure in preparation for transplant surgery.

Effect of Tobacco Smoke Exposure on Allograft Survival

Nonimmunosuppressed, tobacco-naïve allografts were rejected in 7.83±0.16 days (mean±SEM; median, 8 days). Cigarette smoke exposure in the donor (median, 5 days; mean, 4.4±1.2 days; P=0.0039, log-rank test), recipient (median, 4 days; mean, 3.4±1.3 days; P=0.003, log-rank test), or both donor and recipient strains (median, 3 days; mean, 5.25±1.4 days; P=0.0068, log-rank test) shortened allograft survival significantly compared with the tobacco-naïve allografts. There was no statistical difference between the single-exposure (either donor or recipient) compared with the dual-exposure (donor and recipient) animals (P=0.58 and 0.71, respectively, for donor only and recipient only cohorts).

Effect of Tobacco Smoke Exposure on Systemic Inflammation and Oxidative Stress

We quantified mRNA levels of IL-6, IFN-γ, and TNF-α using real-time polymerase chain reaction in splenocytes from rats exposed to cigarette smoke for 30 days and tobacco-naïve control rats. The results are expressed as relative fold expression in 3 rats exposed to cigarette smoke and 3 untreated controls. A summary of these results expressed as mean±SEM is shown in Figure 2A. The results demonstrate a statistically significant increase in mRNA expression of IL-6 (17.82±5.55 versus 1.55±0.37; P=0.008), TNF-α (11.62±2.9 versus 2.33±1.049; P=0.032), and IFN-γ (13.7±4.3 versus 0.37±0.13; P=0.03) in rats exposed to cigarette smoke. The mRNA expression of p27kip1, a significant component of NADPH oxidase, was on average 276±68-fold (P=0.02) increased in rats exposed to cigarette smoke compared with controls (Figure 2B). The Bio-Plex rat cytokine assay was used for simultaneous quantitation of the peripheral blood cytokines IL-2, IL-6, IFN-γ, and TNF-α according to the recommended procedure. The results (Figure 2C) demonstrate that cigarette smoke exposure resulted in significant increases in the levels of circulating levels of the proinflammatory cytokines IL-2 (P=0.0005), IL-6 (P=0.001), IFN-γ (P=0.0025), and TNF-α (P=0.05) in sera of tobacco-exposed compared with tobacco-naïve rats.

Histological Analysis of Cardiac Allografts From Tobacco-Naïve (Untreated Isografts, Rejecting and Nonrejecting) and Tobacco-Exposed (Donor, Recipient, or Both) Animals

Isografts presented normal myocardium, but there was focal mild inflammation without necrosis in allografts obtained from untreated rat transplant recipient with beating allografts, representing ISHLT grade 1R. Myocardium from untreated rejecting allografts showed myocardial inflammation representing ISHLT grade 2R. There was diffuse inflammation without necrosis corresponding to acute rejection of ISHLT grade 1 to 2R in rat cardiac transplant recipients that received donor hearts from rats exposed to cigarette smoke. More severe findings were observed in recipients exposed to cigarette smoke exposure, including more intense inflammation with necrosis indicative of ISHLT grade 2 to 3R. Most interesting, smoke exposure in both the donor and recipient led to ISHLT grade 3R rejection in addition to inflammation with extensive necrosis, interstitial edema, and hemorrhage.

Vascular inflammation in Tobacco-Naïve (Untreated Isografts, Rejecting and Nonrejecting) and Tobacco-Exposed (Donor, Recipient, or Both) Cardiac Allografts

We compared histological changes in cardiac allograft tissues obtained from untreated isografts, allografts with stable transplantation without rejection, and untreated rejecting allografts. The histopathological analysis demonstrates normal small arteries from isografts with no evidence of vasculopathy. However, small arteries from untreated rat transplant recipients with beating allografts...
showed scattered infiltrating immune cells compared with untreated rejecting allografts that demonstrated small intramural arteries with intimal, medial, and adventitial chronic inflammation with moderate rejection. We compared the vascular inflammation in allografts from transplant combinations in which the donor, recipient, or both donor and recipients were exposed to cigarette smoke. The results show vasculitis with intimal proliferation and edema, almost obliterating the lumen, with evidence of ISHLT grade 2 to 3R rejection. These changes are severe compared with the histology of rejected allografts from tobacco-naïve recipients of cardiac transplantation that did not receive immunosuppressive therapy. There was intimal and adventitial inflammation in rat cardiac transplant recipients that received donor hearts from rats exposed to cigarette smoke. More severe inflammation is observed in rats with recipients exposed to cigarette smoke. Smoke exposure in both the donor and recipient led to dense perivascular infiltrate, typical of severe rejection.

Alloimmune and Inflammation Activation in Cardiac and Splenic Tissue in Tobacco-Naïve (Isografts, Rejecting and Nonrejecting) and Tobacco-Exposed (Donor, Recipient, or Both) Allografts

To determine whether systemic inflammation and oxidative stress associated with cigarette smoke exposure are concurrently associated with heightened alloimmune activation, we evaluated the markers IL-1R2, PDCD-1, and SDF-1 in allograft and spleen tissues. The results from nonrejecting isografts demonstrate no allografts and spleen increase in gene expression for IL-1R2, PDCD-1, and SDF-1 mRNA (Figure 3A). Similar to untreated isografts, no increase in these markers was observed in allograft or spleen tissues (Figure 3B) from tobacco-naïve, nonrejecting recipients with beating hearts on postoperative day 5. We demonstrate 50- to 420-fold increases in myocardial and spleen tissue IL-1R2, PDCD-1, and SDF-1 mRNA in tobacco-naïve rejecting rat cardiac transplant recipients (Figure 3C) with greater in-
creases (up to 3600-fold) in those tissues derived from rejecting tobacco-exposed animals (Figure 3D through 3F).

Myocardial Protein Expression for SDF-1 and TNF-α From Tobacco-Naïve (Untreated Isografts, Rejecting and Nonrejecting) and Tobacco-Exposed (Donor, Recipient, or Both) Allografts

There was no significant staining of TNF-α or SDF-1 protein in tobacco-naïve isografts or allografts, which did not differ in tobacco-naïve nonrejecting allografts in rats killed on day 5 (Figure 4A). In contrast, significant staining for SDF-1 and TNF-α protein was observed in tobacco-exposed recipient rats (Figure 4B). The results of semiquantitative staining for these proteins are shown in the Table.

Discussion

We provide the first direct evidence that pretransplantation cigarette smoke exposure in the donor, recipient, or both accelerates the demise of the cardiac allograft. Importantly, our series of experiments reveal that pretransplantation tobacco exposure results in heightened systemic inflammation and increased oxidative stress, that posttransplantation cardiac survival is reduced by 33% to 57% in smokers, and that this reduced survival occurs in the context of expression of pathways of inflammation, alloimmune activation, and consequent myocardial and vascular destruction. Importantly, this accelerated trajectory of cardiac allograft loss was similar whether tobacco smoke exposure occurred in donors, recipients, or both before cardiac transplantation.

We attempted to simulate the human condition as closely as possible by using a unique tobacco smoking chamber and a prolonged 30-day exposure approach and confirming exposure levels by measuring cotinine levels. An alkaloid, cotinine is a principal metabolite of nicotine with a 20-hour half-life. In humans, values ranging from 7 to 100 ng/mL are associated with light to moderate tobacco exposure, and levels >300 ng/mL are seen in heavy smokers. The levels of cotinine in our experiments simulated moderate levels of chronic tobacco exposure (cotinine level, 82.75 ± 4.85 and 84.5 ± 2.5 ng/mL in donor and recipient strains, respectively).
Thus, our exposure levels of tobacco were not toxic or excessive and are well within the range of routine populations of habitual smokers. A second strength of our investigation included the group comparisons that we were able to evaluate, including isografts, tobacco-naive nonrejecting allografts (in mice killed at the accelerated time points of graft loss in the tobacco-exposed groups), tobacco-naive naturally rejecting allografts, and specific groups of equivalent tobacco exposure in donor rats, recipient rats, or both. This allowed us to definitively establish the accelerated contribution of tobacco exposure and to determine the severity of inflammatory and alloimmune pathway activation in allografts and systemic tissues.

We have confirmed that tobacco exposure causes systemic and local tissue inflammation that extends into the immediate posttransplantation period, even after abrupt cessation of tobacco exposure. Thus, our experiments argue that tobacco exposure immediately before transplantation sets up a milieu of graft compromise that is most likely triggered by systemic inflammation in recipients, by intragraft inflammation, and even possibly by immune activation in donor hearts before engraftment. The intragraft and peripheral markers of inflammation (TNF-α, IL-6, and IFN-γ) that we studied have been validated in the context of rejection and adverse cardiac transplant outcomes.16–18 Similarly, the alloimmune biomarkers (IL-1R2, PDCD-1 and SDF-1) that we studied have also been demonstrated to have direct relevance to the paradigm of transplant rejection.19–21 IL-1R2, a cytokine receptor that belongs to the IL-1 receptor family, a member of the immunoglobulin gene superfamily, and one of many corticosteroid responsive genes, is involved in inflammation and apoptosis regulation, and its expression is markedly altered in allogeneic conditions.19,20 The immunoreceptor PDCD-1, an inhibitory costimulatory molecule regulating peripheral tolerance, is a marker of T-cell activation and is increased in expression before acute cardiac allograft rejection.19,20

### Table. Results of Semiquantitative Staining for Proteins

<table>
<thead>
<tr>
<th>Isograft (Tobacco Naive)</th>
<th>Tobacco-Naive Allografts*</th>
<th>Tobacco-Naive Rejecting Allografts</th>
<th>Tobacco-Exposed Donor</th>
<th>Tobacco-Exposed Recipient</th>
<th>Tobacco-Exposed Donor and Recipient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rejection No</td>
<td>0</td>
<td>0.34</td>
<td>327.8</td>
<td>2501.8</td>
<td>2584.6</td>
</tr>
<tr>
<td>Bioassay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1R2 mRNA (spleen)</td>
<td>0.85†</td>
<td>0.34</td>
<td>327.8</td>
<td>2501.8</td>
<td>2.78</td>
</tr>
<tr>
<td>IL-1R2 mRNA (allograft)</td>
<td>0.85</td>
<td>0.84</td>
<td>60.3</td>
<td>3513.5</td>
<td>534.3</td>
</tr>
<tr>
<td>PDCD-1 mRNA (spleen)</td>
<td>0.31</td>
<td>0.25</td>
<td>252.5</td>
<td>1562.7</td>
<td>4.97</td>
</tr>
<tr>
<td>PDCD-1 mRNA (allograft)</td>
<td>0.20</td>
<td>0.26</td>
<td>426.2</td>
<td>956.2</td>
<td>76.9</td>
</tr>
<tr>
<td>SDF-1 mRNA (spleen)</td>
<td>0.93</td>
<td>0.38</td>
<td>62.0</td>
<td>3066.0</td>
<td>1.4</td>
</tr>
<tr>
<td>SDF-1 mRNA (allograft)</td>
<td>0.35</td>
<td>13.0</td>
<td>13.0</td>
<td>1310.8</td>
<td>534.5</td>
</tr>
<tr>
<td>CD3 protein</td>
<td>0‡</td>
<td>+/−</td>
<td>2+</td>
<td>3+</td>
<td>2+</td>
</tr>
<tr>
<td>TNF-α protein</td>
<td>0</td>
<td>+/−</td>
<td>3+</td>
<td>3+</td>
<td>2+</td>
</tr>
<tr>
<td>SDF-1 protein</td>
<td>0</td>
<td>0</td>
<td>3+</td>
<td>2+</td>
<td>4+</td>
</tr>
</tbody>
</table>

*Untreated killed on day 5 after transplantation.
†Normalized fold expression.
‡Graded on arbitrary scale from 0 to 4+, with 4+ indicating the highest staining.
SDF-1, the only known biological natural ligand for chemokine CXC receptor-4, is known to regulate hematopoietic stem cell trafficking between the peripheral circulation and bone marrow.\textsuperscript{21} SDF-1 is upregulated in hypoxic tissues and acts as a physiologically relevant chemoattractant for recruiting circulating progenitor cells to sites of ischemic damage.\textsuperscript{22} Its expression has been demonstrated in myocytes, vascular endothelial cells, and fibroblasts and may play a significant role in cardiac allograft rejection and vasculopathy.\textsuperscript{23} A recent study\textsuperscript{24} has also demonstrated increased SDF-1 protein expression in chronic human renal allograft rejection. Thus, we provide confirmation of observational studies\textsuperscript{4,5,25,26} that link tobacco exposure to adverse outcomes after cardiac transplantation, provide direct evidence of this arbitration, and point to pathways involved that may serve as potential therapeutic targets (the Table demonstrates the principle findings by various groups).

It appears plausible to hypothesize that the chain of deleterious events begins with systemic inflammation and oxidative stress in the pretransplantation period of smoking exposure, which results in activation of molecular alloimmune pathways via exposure of “danger signals” after transplantation, subsequently leading to accelerated cellular rejection, vascular inflammation, and graft loss.\textsuperscript{27} Matzinger’s\textsuperscript{27} danger signal hypothesis proposes a model of immune function based on the ability to detect and address dangers rather than self- and non–self-recognition. We conjecture that, in the presence of tobacco-induced inflammatory and pro-oxidant damage, danger signals are created that, in the presence of the allograft, offer resting T lymphocytes both antigen stimulation by an antigen-presenting cell and costimulation with the smoking-related danger signal to then become activated. In the case of donor hearts exposed to tobacco smoke, the allograft itself may produce these danger signals from preexisting tobacco-related cell death, whereas in the case of recipient tobacco exposure, these danger signal peptides are likely circulating, or the milieu causes their rapid development within the allograft as a result of heightened inflammation and oxidative stress. Thus, it is possible to speculate that this cascade of events can be interrupted at the time of transplantation with focused pharmacological intervention in the antiinflammatory and antioxidant pathways. In addition to cessation of tobacco exposure, use of pharmacological agents such as N-acetyl cysteine (an antioxidant) or statin drugs (antiinflammatory drugs) could prove useful in inhibiting these adverse responses. These require independent study and verification and should be considered only hypothesis generating.

There are several limitations of this investigation. We did not study a dose-response relationship to pretransplantation smoking and posttransplantation outcomes. Thus, we cannot be certain whether a dose threshold for these adverse effects exists below which there is little risk or if this relationship is a continuous one. At the doses we studied to simulate mild to moderate tobacco exposure, we can conclude the presence of a threshold maximal risk because we demonstrated no further acceleration of graft loss in those animals with both donor and recipient smoke exposure compared with either donor or recipient smoking. Furthermore, we did not investigate this adverse effect in the presence of immunosuppression because we chose to study natural rejection circumstances owing to a lack of ability to adequately control for similar levels of immunosuppression from a pharmacodynamic standpoint. In addition, we did not evaluate a smoke-exposed isograft control; thus, we cannot definitively distinguish the sequential participation of inflammation and immune stimulation in this particular model.

Conclusions
These sentinel findings confirm that tobacco smoke exposure in either the donor or recipient leads to accelerated allograft rejection, vascular damage, and graft loss. Our studies also uniquely demonstrate that the adverse effects of cigarette smoking stem not only from the smoke exposure to the recipient smoker but also from donor smoke exposure to the organ transplanted, which maybe equally harmful to eventual allograft survival. Molecular pathways that intersect as arbiters in this phenomenon include instigation of alloimmune activation associated with tobacco smoke–induced inflammation.

Source of Funding
This work was supported by a grant from the University of Maryland Statewide Health Network and a Tobacco-Related Diseases Research Grant through the Maryland Cigarette Restitution Fund Program.

Disclosures
None.

References
Tobacco exposure in cardiac transplant recipients, before and after transplantation, may increase the risk of cardiac allograft vasculopathy and allograft loss. In this experimental study, we provide the first direct evidence that pretransplantation cigarette smoke exposure in the donor, recipient, or both accelerates the demise of the cardiac allograft.

Importantly, our series of experiments reveal that the chain of deleterious events begins with inflammation and oxidative stress in the pretransplantation period of smoking exposure, which results in activation of molecular alloimmune pathways after transplantation, subsequently leading to accelerated cellular rejection, vascular inflammation, and graft loss with shortened allograft survival. Thus, it is possible to speculate that this cascade of events can be interrupted at the time of transplantation with focused pharmacological intervention in the antiinflammatory and antioxidant pathways. Thus, in addition to cessation of tobacco exposure, use of pharmacological agents such as n-acetyl cysteine (an antioxidant) or statin drugs (antiinflammatory drugs) could prove useful in inhibiting these adverse responses. These require independent study and verification and should be considered only hypothesis generating. Our studies also uniquely demonstrate that the adverse effects of cigarette smoking stem not only from the smoke exposure to the recipient smoker but also from donor smoke exposure to the organ transplanted, which maybe equally harmful to eventual allograft survival.

CLINICAL PERSPECTIVE

Tobacco exposure in cardiac transplant recipients, before and after transplantation, may increase the risk of cardiac allograft vasculopathy and allograft loss. In this experimental study, we provide the first direct evidence that pretransplantation cigarette smoke exposure in the donor, recipient, or both accelerates the demise of the cardiac allograft.

Importantly, our series of experiments reveal that the chain of deleterious events begins with inflammation and oxidative stress in the pretransplantation period of smoking exposure, which results in activation of molecular alloimmune pathways after transplantation, subsequently leading to accelerated cellular rejection, vascular inflammation, and graft loss with shortened allograft survival. Thus, it is possible to speculate that this cascade of events can be interrupted at the time of transplantation with focused pharmacological intervention in the antiinflammatory and antioxidant pathways. Thus, in addition to cessation of tobacco exposure, use of pharmacological agents such as n-acetyl cysteine (an antioxidant) or statin drugs (antiinflammatory drugs) could prove useful in inhibiting these adverse responses. These require independent study and verification and should be considered only hypothesis generating. Our studies also uniquely demonstrate that the adverse effects of cigarette smoking stem not only from the smoke exposure to the recipient smoker but also from donor smoke exposure to the organ transplanted, which maybe equally harmful to eventual allograft survival.

Tobacco Smoke Exposure in Either the Donor or Recipient Before Transplantation Accelerates Cardiac Allograft Rejection, Vascular Inflammation, and Graft Loss
Ashwani K. Khanna, Jianping Xu, Patricia A. Uber, Allen P. Burke, Claudia Baquet and Mandeep R. Mehra

_Circulation_. 2009;120:1814-1821; originally published online October 19, 2009; doi: 10.1161/CIRCULATIONAHA.108.840223
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/120/18/1814

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/