A Human-Mouse Chimeric Model of Obliterative Bronchiolitis after Lung Transplantation

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Obliterative bronchiolitis is a frequent, morbid, and usually refractory complication of lung transplantation. Mechanistic study of obliterative bronchiolitis would be aided by development of a relevant model that uses human immune effector cells and airway targets. Our objective was to develop a murine chimeric model that mimics obliterative bronchiolitis of lung allograft recipients in human airways in vivo.

Human peripheral blood mononuclear cells were adoptively transferred to immunodeficient mice lacking activity of T, B, and NK cells, with and without concurrent transplantations of human small airways dissected from allogeneic cadaveric lungs. Chimerism with human T cells occurred in the majority of recipient animals. The chimeric T cells became highly activated, rapidly infiltrated into the small human airway grafts, and caused obliterative bronchiolitis. In contrast, airways implanted into control mice that did not also receive human peripheral blood mononuclear cell transfers remained intact. In vitro proliferation assays indicated that the chimeric T cells had enhanced specific proliferative responses to donor airway alloantigens. This model confirms the critical role of T cells in development of obliterative bronchiolitis among human lung allograft recipients and provides a novel and easily implemented mechanism for detailed, reductionist in vivo studies of human T-cell responses to allogeneic human small airways.

Chronic lung allograft rejection typically manifests with obliterative bronchiolitis (OB), a small airway fibroproliferative disease process characterized by varying degrees of airway luminal obliteration with fibrous granulation tissue. Lung transplant recipients with OB develop expiratory airflow obstruction, which is often inexorably progressive, and have increased predilection for infection. Despite extensive investigation, as well as incremental advances in donor organ preservation, surgical techniques, immunosuppressive regimens, and infection prophylaxis, OB remains the single most frequent cause of late graft dysfunction and death after lung transplantation.

Current limitations in prevention and treatment of OB likely reflect incomplete understanding of the responsible immunopathogenic mechanisms. Accordingly, having a model to generate novel insights regarding the disease paradigm, and a ready means to test these hypotheses, could be a boon for the development of more effective modalities to prevent or counter this frequent complication of lung transplantation.

Animal models have been immeasurably important in advancing our understanding of many disease processes, including allograft rejection mechanisms. With particular respect to pulmonary transplantation, heterotopic tracheal allografts in mice undergo histological abnormalities that reproduce the progression of OB in humans, including an initial lymphocytic infiltration, followed by successive stages of epithelial metaplasia and denudation and, ultimately, intraluminal fibroproliferation.

Although this model successfully recapitulates the histological abnormalities of OB, the system is limited to study of nonhuman immune effector cells and airways.

Mice and humans diverged approximately 70 million years ago, however, and have since evolved striking differences of size, reproductive capability, longevity, diet, and habitat, as well as having varied exposures to particular microbial and viral pathogens. All of these factors, and doubtless others, have exerted idiosyncratic selection pressures on the evolution of the respective host defense mechanisms. Even though the immune systems of mice and humans share many common elements and general functions, they also differ in myriad important pathways.
details. Not surprisingly, some immunological thera-
pies that appeared promising in murine subjects have not
translated into comparably effective human treat-
ments. The availability of a facile model of human
immune effector cell and target organ interactions that
mimics the phenotype of clinical disease in situ could be
very useful for fundamental studies, and a unique, poten-
tially more valid tool for early trials of immunosuppressive
agents and biological response modifiers.

We have developed a novel human-murine chimeric
model, in which both the immune effector cells and allograft
tissues are of human derivation. Small airways, the primary
target structure involved in OB, are harvested from human
cadaveric lung explants and are heterotopically implanted
into the dorsal subcutaneous tissue of NOD/SCID mice,
followed by adoptive transfer of human T cells from a donor
allogeneic to the airway donor. In conjunction with anti-
murine NK-cell treatments, the adoptively transferred hu-
man T cells engraft, proliferate, and mediate airway injuries
that mimic OB among lung transplant recipients. This
model provides a ready means to study the immunopatho-
genesis of lung allograft rejection, and may also be espe-
cially useful for early-phase pharmacotherapeutic trials. Mi-
nor modifications of this model may also have utility for in situ
generation of antigen-specific human T cells in large num-
ers for further study or other applications.

Materials and Methods

Animals

NOD.CB17-Prkdcscid/J mice (NOD/SCID mice) were ac-
quired from the Jackson Laboratory (Bar Harbor, ME)
and maintained under pathogen-free conditions. In some
cases, a genetically modified mouse strain that is also
deficient in inherent NK-cell activity (NOD/SCID IL-
2Rγc−/−) was similarly maintained and used. This protocol
was approved by the Institutional Animal Care and Use
Committee and the Institutional Review Board of the Uni-
versity of Pittsburgh.

Human Peripheral Blood Mononuclear Cells for
Adoptive Transfers

Blood was obtained from a single healthy volunteer human,
allogeneic to the airway donors, and peripheral blood
mononuclear cells (PBMCs) were isolated by Ficoll den-
sity gradient centrifugation. The cells were washed in com-
plete RPMI 1640 medium (supplemented with 100 U/mL
penicillin, 100 μg/mL streptomycin, 2 mM/L L-glutamine,
50 μM/L 2-mercaptoethanol and 25 mM/L HEPESS) and
then resuspended at 2 × 10^6 cells/mL in Hank’s buffered
saline solution (HBSS). A 0.5-mL volume of this suspension
was adoptively transferred into each recipient animal by
intraperitoneal injection.

In some cases, adoptive transfers consisted of either
CD4 or CD8 T-cell populations that had been individually
isolated from the human PBMCs by negative selection
with immunomagnetic beads (Miltenyi Biotec, Auburn, CA).
Because the numbers of CD8 T cells in the PBMC donor
preparations were often limiting, these adoptive transfers of
isolated CD4 or CD8 T cells consisted of 7 × 10^6 cells/
recipient mouse, to maintain comparable inocula.

Human Airway Grafts

Normal human lung explants, the source for small airway
grafts, were obtained during cadaveric multiorgan harvests;
these explants had been deemed not suitable for therapeu-
tic pulmonary transplantations (eg, due to size or ABO in-
compatibility). The explants were kept on ice and were
processed within 10 hours of harvest. Specimens were ex-
cluded if they had evidence of infection or other underlying
lung disease by clinical history, radiography, gross exami-
nation, or histology. Small airways (2 to 5 mm in diameter,
~6 mm in length) were harvested from these explants by
blunt dissection of peripheral parenchyma. After isolation,
the human airways were flushed with complete RPMI 1640
medium, irradiated at 9 Gy to eliminate residual lympho-
cytes, suspended in freezing medium (10% glycerol:90%
fetal bovine sera), and stored at −80°C.

The cryopreserved airways were later thawed in a
37°C water bath and were successively flushed with
complete RPMI 1640 medium and then sterile HBSS im-
mEDIATELY before subcutaneous dorsal implantation into
recipient animals, using sterile technique.

Hilar Lymph Node Cells

Hilar lymph nodes were dissected from the cadaveric
human lung explants at the time of small airway harvest.
Single-cell suspensions were made by forcing the nodes
through sterile wire mesh. The cells were washed in com-
plete RPMI 1640, resuspended at a concentration of 5 × 10^6
cells/mL in lymphocyte freezing medium (10% dimethyl
sulfoxide, 90% fetal calf serum), and stored at −80°C.

Human-Murine Chimeras

Three treatments were performed in each series of studies:
i) airway control mice had heterotopic airway graft (HAG)
implantations but did not receive human PBMCs; ii) PBMC
control mice received adoptive transfusions of human
PBMCs, but did not have HAG implantations; and iii) ex-
perimental mice had concurrent HAG implantations and
human PBMC adoptive transfers. Within a given experi-
mental series, the HAGs of both airway control and exper-
imental recipients were procured from a single donor lung.
NOD/SCID animals were treated by injections of anti-
murine CD122 antibody (0.5 mg i.p.), purified by protein
G from supernatants of hybridoma TMB1 and 25 μL of
anti-mouse asialo-GM1 antibody (Wako Chemicals, Rich-
mond, VA) twice weekly, beginning on the same day as
the adoptive transfers of human PBMCs or T cells.

Harvests of Tissues and Cells from Recipient
Animals

Most treated mice were maintained for 4.5 ± 0.5 weeks,
at which time they were euthanized and their spleens and
HAGs were harvested. Single-cell suspensions of mouse splenocytes were made by forcing these organs through sterile wire mesh, followed by erythrocyte lysis with ACK buffer (0.15 mol/L NH₄Cl, 10 mmol/L KHCO₃, and 0.1 mmol/L Na₂EDTA, pH 7.3). Splenocytes were repeatedly washed and resuspended in complete RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) for functional studies, or in staining buffer (HBSS supplemented with 0.1% BSA, pH 7.5) for flow cytometry analyses. Circulating leukocytes from recipient mice were also obtained by tail-vein phlebotomy and density gradient centrifugation of blood using Ficoll specific gravity 1.079.

Human airway grafts were harvested and fixed in 10% neutral buffered formalin, paraffin-embedded, sectioned, and mounted on glass slides.

**Flow Cytometry**

General aspects of these methods and materials have been described previously. In brief, cell-surface molecule expressions of freshly isolated cells were ascertained using anti-human fluorochrome-conjugated monoclonal antibodies (mAb) against the following cell-surface determinants: CD3, CD4, CD8, CD14, CD19, CD28, CD45, and HLA class II (DR) from BD Biosciences (San Jose, CA).

In some cases, cells were stained with anti-human T-cell antigen receptor variable β chain (TCRBV) mAb with specificity for 2S1, 3S1, and 4S1 (Immunotech, Marseilles, France), as well as 5S1 and 8S1 (BD Biosciences). These particular TCRBVs correspond to T-cell subpopulations in high frequencies among healthy humans. Each of the cell-surface anti-human monoclonal antibodies was tested in pilot trials to confirm that it did not cross-react with murine splenocytes (data not shown).

Intracellular cytokine elaborations were determined in PBMNCs stained with anti-human CD4 and were stimulated for 5 hours with phorbol 12-myristate 13-acetate (PMA) (5 ng/mL) and calcium ionomycin (500 ng/mL) in the presence of GolgiStop protein transport inhibitor (BD Biosciences). Cells were then fixed and permeabilized, and stained with fluorochrome-conjugated mAb against selected human cytokines (or CD68). Reagents for these determinations were purchased from BD Biosciences and were used according to the manufacturer’s instructions, as described previously.

Quantitations were performed on ≥10,000 live cells and analyzed using a FACSCalibur system (BD Biosciences).

**Proliferation Assays**

Proliferation assays were established with 2 × 10⁵ splenocytes from recipient animals in 200 μL RPMI-10 medium, supplemented with 10 U/mL IL-2, in individual wells of 96-well plates. Splenocytes from all chimeric populations (PBMNC-only controls, HAG only, and experimental mice that had received concurrent HAG and adoptive transfers of PBMNCs) were individually cultured in triplicate with equal numbers of irradiated (20 Gy) stimulator cells from each of three sources: i) syngeneic control stimulator cells consisting of unmanipulated NOD/SCID mice; ii) allogeneic stimulator cells consisting of cryopreserved pulmonary hilar lymph node cells derived from the same human donor as the HAG; and iii) third-party pulmonary hilar lymph node cells that had been harvested from a human lung explant unrelated to either the HAG or human PBMNC donors. These cocultures were incubated in a 7% CO₂ environment at 37°C for 3 days before addition of [³H]thymidine (1 μCi), and were harvested 16 hours later; counts per minute were determined with a beta counter.

**Rejection Scoring**

HAG sections recovered from recipient animals were stained with H&E and were histologically evaluated using a scoring system that has been described previously (Table 1). Investigators (M.P.G. and S.R.D.) blinded to treatment assigned the scores based on the most severe histological abnormality present in several sections of each individual HAG.

**Immunohistochemistry**

Paraffin-embedded HAG sections were deparaffinized in three washes of xylene and rehydrated in successively dilute ethanol baths (100%, 95%, 80%, 70%, 50%). Endogenous peroxidase activity was quenched with 0.05% hydrogen peroxide for 10 minutes. Antigen retrieval was performed for 15 minutes in a pressure cooker using citrate buffer (10 mmol/L sodium citrate, 0.05% Tween 20, pH 6). After three washes in PBS, the sections were blocked with 5% goat serum in PBS for 45 minutes. These sections were then incubated overnight at 4°C with murine anti-human CD3 (Dako, Glostrup, Denmark) at 1:200 dilution. Biotinylated anti-mouse secondary antibody (Vector Laboratories, Burlingame, CA), also at a dilution of 1:200, was then incubated for 30 minutes before immunoperoxidase staining with Vectastain ABC (Vector Laboratories) according to the manufacturer’s instructions. After five rinses in PBS, the sections were counterstained for 5 seconds with hematoxylin, and then were mounted with coverslips and mounting medium.

**Table 1.** Histological Scoring of Airway Grafts

<table>
<thead>
<tr>
<th>Score</th>
<th>Histological features</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>Focal squamous metaplasia over ≤10% of luminal circumference</td>
</tr>
<tr>
<td>2</td>
<td>Squamous metaplasia over &gt;10% of luminal circumference</td>
</tr>
<tr>
<td>3</td>
<td>Focal epithelial denudation over ≤10% of luminal circumference</td>
</tr>
<tr>
<td>4</td>
<td>Denudation comprising &gt;10% of luminal circumference</td>
</tr>
<tr>
<td>5</td>
<td>Intraluminal granulation tissue (obliterative bronchiolitis)</td>
</tr>
</tbody>
</table>

Source: Duncan et al.
**Statistical Analyses**

Two-group and three-group comparisons of continuous or ordered data were made by Mann-Whitney U-test or Kruskal-Wallis test, respectively. Replicate measures over time were evaluated by Wilcoxon paired-sample test. Dichotomous variables were analyzed by $\chi^2$ test. Data were analyzed using a StatView software package version 5.0 (SAS Institute, Cary, NC). Data are reported as means ± SEM, and $P$ values of <0.05 were considered significant.

**Results**

**Engraftment of Human T Cells in Immunocompromised Mice**

Initial attempts to engraft human PBMNCs in B6;129S7-Rag1<sup>tm1Mom</sup> or NOD/SCID animals without anti-NK-cell treatments were only sporadically and partially successful. However, use of NOD/SCID recipients, in conjunction with twice weekly anti-murine NK-cell mAb (anti-CD122 and anti-AGM1), resulted in far more frequent engraftment and expansion of adoptively transferred human T cells.

Human T cells were evident in the circulation of most recipient animals by 2 weeks and usually increased further during the next 2 weeks (Figure 1A). More importantly, human T cells comprised a significant proportion of cells in the spleens of recipient animals at 4.5 ± 0.5 weeks after adoptive transfers (Figure 1B). There were no consistent differences in the proportions of chimeric human T cells in the spleens between recipient mice treated only with adoptive PBMNC transfers and those that received both PBMNC and HAG implantations (Figure 1B).

There was a significantly greater proliferation of CD8 T cells relative to the initial adoptive transfer inocula (17.4 ± 3.3 fold increase) than of CD4 T-cells (6.0 ± 1.2 fold increase) ($P = 0.008$). Nonetheless, in the absence of CD4 T cells, adoptive transfers of isolated CD8 T cells alone did not engraft well and/or proliferate (0.3 ± 0.3% of recipient spleen cells, $n = 5$), whereas pure inocula of CD4 T cells underwent substantial (and perhaps enhanced) proliferation after adoptive transfers, constituting 51 ± 13% of recipient spleen cells at harvest ($n = 5$).

Human PBMNC leukocyte subpopulations other than T cells did not undergo comparable engraftments or expansions after adoptive transfer, in that ≥98% of human CD45<sup>+</sup> cells within recipient spleens coexpressed the specific T-cell marker CD3, whereas the percentage of CD3<sup>+</sup>/CD45<sup>+</sup> among the initial donor human PBMNC inocula, before adoptive transfers into these mice, was 46.4 ± 5.6%.

**Characterization of Chimeric T Cells**

We hypothesized a priori that adoptively transferred human T cells bearing antigen receptors (TCR) with appropriate avidities would become activated by engagement with recipient xenoantigens, and perhaps also by engagement with human alloantigens among those mice that also received HAG.

Chimeric human T cells within the spleens of murine recipients were highly activated, as evidenced by their increased expressions of HLA class II (DR) compared with the initial donor lymphocytes (Figure 1C). Although a large proportion of circulating CD8 T cells in healthy humans do not express CD28, and many produce granzyme B, analogous findings of CD4 T lymphocytes that lack CD28 expression or make granzyme B are distinctly

![Image](https://via.placeholder.com/150)

**Figure 1.** A: Human T cells (as indicated by CD3 surface marker) were evident in the peripheral blood of most recipient mice by 2 weeks after adoptive transfers, and typically increased over the next 2 weeks. *P = 0.04. Heavy horizontal line indicates mean. B: Human T cells comprised significant proportions of cells within spleens of recipient mice after adoptive transfer. There were no significant differences in the extent of splenic chimerism between recipient animals that received only adoptive transfers of peripheral blood mononuclear cells (PBMNC) ($n = 15$) and those that received concomitant PBMNCs and heterotopic airway grafts (HAG) ($n = 14$). C: The activation marker HLA class II DR was significantly up-regulated on chimeric T cells ($n = 17$), compared with expression on the original, donor lymphocytes ($n = 6$). There were no apparent differences in extent of chimeric T-cell activation between PBMNC only and PBMNC + HAG, so both groups were combined for this analysis. $P$ values are indicated next to brackets. D: A large proportion of chimeric CD4 T cells did not coexpress CD28, but these cells more frequently produced granzyme B, relative to the original donor lymphocytes, which is analogous to findings in human lung allograft recipients with chronic allograft rejection. $P$ values are indicated next to brackets. In box plots (B-D), the lower whisker denotes the 10th percentile; the lower edge of the box, the 25th percentile; the middle line, the median; the square, the mean; the upper edge of box, the 75th percentile; and the upper whisker, 90th percentile.
and the upper whisker, 90th percentile. The median; the square, the mean; the upper edge of box, the 75th percentile; the lower edge of the box, the 25th percentile; the middle line, the chimeric populations. In box plots, the lower whisker denotes the 10th measured are indicated next to brackets.

The cytokine production profile of chimeric T cells was altered, relative to that of the original donor cells, as illustrated here by CD4 T-cell TCRBV expression profiles. *P = 0.036, HAG + PBMNC (n = 6) versus PBMNC only (n = 10); **P = 0.0096, donor T cells (n = 5) versus both chimeric populations. In box plots, the lower whisker denotes the 10th percentile; the lower edge of the box, the 25th percentile; the middle line, the median; the square, the mean; the upper edge of box, the 75th percentile; and the upper whisker, 90th percentile.

abnormal findings that show these cells have undergone multiple prior antigen-driven divisions. Considerable CD28 down-regulation and granzyme B up-regulation were evident among these chimeric CD4 T cells harvested from these animals (Figure 1D), findings that are highly analogous to those seen in human lung transplant recipients with chronic allograft rejection.

The cytokine production profile of chimeric T cells was altered, relative to that of the original donor cells, as illustrated here by CD4 lymphocyte elaborations (Figure 2A). Although there was no clear Th1 versus Th2 bias among these chimeric T cells, and, with the exception of IL-2 and tumor necrosis factor-α, their production of other disease-relevant mediators was increased, and in some cases severalfold (eg, interferon-γ, IL-1β, IL-4) (Figure 2A).

T-cell antigen receptor variable β chain repertoires were also biased among chimeric T lymphocytes, indicating preferential expansions and contractions of T cells bearing distinct TCRBV families after adoptive transfers by engagement with xenoantigens and/or alloantigens (Figure 2B). There was a prominent increase of TCRBV 8S1 frequency among both populations of chimeric T cells, relative to the original donor lymphocytes, possibly reflecting preferential activation and expansion of one or more founder T cells bearing this TCRBV that have xenoantigen specificity. TCRBV 5S1 frequency was increased among chimeric T cells of recipients with concurrent HAG transplantation, perhaps reflecting particular expansions of T cells in this subpopulation that have alloantigen specificity.

### Alloantigen Avidities of Chimeric Human T Cells

Based on the preceding results, which we interpreted as indicative of cognate in situ T-cell activation and proliferation, we hypothesized that some of the lymphocytes among the recipients with concurrent HAG implantation might represent expansion of daughter progeny with specific avidity for the distinct human alloantigens of the airway allografts.

Splenocytes from all recipient populations had similar baseline thymidine incorporation and de novo responses to irrelevant human antigens of third-party stimulator cells (Figure 3A). However, proliferative responses of the chimeric T cells from recipients with concurrent HAG implantation were significantly increased in cocultures with lymph node cells from the original HAG donor, consistent with a second-set response (Figure 3A).

### Human Airway Heterotopic Grafts as a Model of Obliterative Bronchiolitis

The ultimate aim of these experiments was to develop a model that would enable study of human T-cell responses to allogeneic human airways in vivo.

Pilot studies had shown that human HAGs became heavily enveloped with a richly vascularized fascia originating from the recipient panniculus carnosus within a few days of implantation into these mice, as described previously with murine tracheal transplantsations. Moreover, with only two exceptions (Figure 3B), human HAGs, in animals not reconstituted with human T cells, maintained normal histological appearance and apparent preservation of airway epithelial mucous production for at least 2 months after implantation (Figure 4A).

However, HAGs implanted into mice that also received adoptive transfers of human PBMNCs showed inflammatory infiltrations within 1 to 2 weeks, along with progressive airway epithelial metaplasia and denudation. Granulocyte infiltration into the airway lumen was evident in 3 weeks (Figure 4, B and C), and complete luminal obliteration (along with disappearance of the inflammatory cellular infiltrate) occurred soon thereafter in nearly all HAG transplantations (Figures 3B and 4D). HAGs in the animals that received adoptive transfers of pure human CD4 T cells allogeneic to the graft underwent identical histological abnormalities (data not shown).
Studies in NOD/SCID IL-2Rγnull Mice

Analogous experiments were performed using NOD/SCID IL-2Rγnull animals, except that these animals were not treated with the NK-cell-depleting mAb regimen (ie, anti-CD122 and anti-AGM1). This recently developed strain is reportedly deficient in inherent NK-cell activity, as well as being devoid of T and B lymphocytes.22 Aside from obviating the need for frequent injections of the NK mAb, these animals also support engraftment of other human cell lineages, in addition to T cells.23

Identical adoptive transfers of PBMCNs (from the same healthy human donor as for the previous studies) into the NOD/SCID IL-2Rγnull resulted in enhanced engraftment and propagation of activated human T cells (Figure 5, A and B), compared with the original NOD/SCID animals (Figure 1B). Donor human B cells (CD19+), monocytes (CD14+), and some macrophages (CD68+) were also evident in the spleens of the NOD/SCID IL-2Rγnull recipients.

Human small airway grafts did not maintain normal histological appearance after heterotopic transplantation into control NOD/SCID IL-2Rγnull mice (those not receiving PBMCNs) (Figure 5C). No epithelial layer was present in HAGs harvested from these animals 4 weeks after transplantation. In many cases, the airways appeared to be partially collapsed, seemingly due to extrinsic compression and/or loss of structural integrity, but in the absence of dense intraluminal granulation tissue. HAGs harvested from the NOD/SCID IL-2Rγnull that had also been reconstituted with human PBMCNs more closely recapitulated findings of human OB, having dense intraluminal granulation tissue, as well as patchy inflammatory infiltrates (Figure 5D). These experiments were repeated in two experimental trials separated by several weeks, with n ≥ 6 in each experimental arm on both occasions, using airways harvested from two different human cadavers.
Discussion

Our present data show that human airway implantations into NK-cell-depleted NOD/SCID mice that also received adoptive transfers of allogeneic human PBMCs develop progressive histological changes that recapitulate lesions of chronic allograft rejection in human lung transplant recipients. The chimeric human T cells in recipient animals develop the uncharacteristic and potentially highly pathogenic phenotypic and functional differentiation after repetitive cycles of proliferation.9,17

Multiple other species-specific differences in structure and functions of adaptive immune mechanisms have been described.8,9 Unlike the T cells of mice, activated human T lymphocytes express MHC class II, which may in turn may play a role (or multiple roles) in amplification of immune responses and/or in homeostatic regulation of these processes.25,26 Calcium influxes during activation of human T cells can occur through Kv1.3 K+ channels, currently under study as a potential target for therapeutic modulation,27 whereas murine T cells do not express this ion-flux apparatus. interferon-α production by macrophages is an important innate mediator of the response to viral infections in humans, with actions that include induction of T-cell STAT4 activation and Th1 polarization. In mice, however, this cytokine neither activates T-cell STAT4 nor promotes Th1 differentiation.28 Moreover, Th1 polarization of murine T cells generally appears to be relatively more dichotomous and immutable, whereas T cells that cause human diseases may be more plastic or, in any event, pathogenesis can less readily be attributed to distinct Th1 subpopulations.29

Numerous other important elements of target organs or cells that can influence the nature and extent of adaptive immune responses are also species-specific. Chemokine receptors and their ligands mediate important functions, notably including chemotaxis of immune effectors into inflammatory foci, and numerous elements of these processes in humans are not replicated by murine or-
thologies. Species-specific effects of proinflammatory cytokines on expressions of selectins seem likely to have important differential influences on leukocyte adhesion and ingress into target organs. Mice and humans differ broadly in the expression, cellular distribution, and presumably effector functions of their varied Toll-like receptors (TLR), among many other potentially relevant innate immune elements.

The capability of allogeneic human T cells to directly initiate and/or mediate chronic allograft injuries is supported by findings in the present study that show a paucity of other chimeric donor cells among the recipient animals, aside from the expanded T cells, and the proximate localization of T cells at the base of the OB granulation lesions (Figure 4, B and C). Moreover, the generation of OB among recipients of pure CD4 T-cell inocula here, as well as analogous findings in other murine model systems, illustrates the pathogenic potential of isolated T-cell subpopulations. Thus, the present studies corroborate and extend previous experimental findings that implicate the critical dependence of T-cell alloresponses in the development of OB.

Although this human-murine chimeric system appears to have singular relevance for study of OB in lung transplantation recipients, the methodology has some potential limitations (as do most other animal models of human disease). Among other considerations, the apparent inability of this particular mouse strain to support engraftment and expansions of other relevant human immune effector cells (eg, B cells) seems to preclude study of contributions by these other, potentially important elements.

This initial series of studies was focused on development and validation of the model. The operating characteristics of this system were therefore defined in the absence of immunosuppressive treatments, to minimize the number and complexity of experimental variables, with the net result that differences of HAG appearance between the control and immune-reconstituted recipients occurred rapidly and were striking. A similar approach was adopted in development and description of the murine heterotopic airway model, which has since seen wide use, as well as in the development of other animal models used to study the immunobiology of transplantation.

Although not tested here, we hypothesize that treatment of experimental animals with effective immunosuppressants, to recreate conditions in human allograft recipients, could have a measurable effect on the development of OB (for example, it might slow the progression or extent of airway pathology). Moreover, depending on the agent or agents used, addition of immunosuppressants might also alter specific characteristics of the allogeneic T-cell responses (eg, activation, TCRBV repertoire biases, or antigen-specific proliferation). Indeed, one of the rationales for development of this chimeric model was to provide a unique system that will enable subsequent in situ trials of experimental pharmaceuticals to modify human allograft responses. In this context, the use of humanized mice could add biological credence to initial, murine-based preclinical studies, given the many known species-specific differences between immune systems of mice and humans.

Other potential limitations of heterotopic airway implantations in general have been described, although we think that none of these objections obviate the utility of the model described herein. Heterotopic airway implants are not surgically vascularized at the time of implantation and, indeed the lack of having to perform what would be very intricate microsurgical procedures enhances the feasibility of this model for a wider range of investigators. Moreover, the rapid envelopment of the HAG by a highly vascularized fascia, persistence of normal-appearing allografts in control recipient animals (Figure 4A), and rapid cellular influxes into the implanted airways (Figure 4B), speak, in the absence of compelling contrary data, to the adequacy of graft revascularization and inflammatory cell accessibility. It can also be argued that, unlike human lung allografts, heterotopic airway grafts in mice are somehow deficient because they are not exposed to the environment. A contrary opinion could be advanced, however, that this lack of environmental exposure precludes experimental confounding by idiosyncratic immunological responses to inhaled antigens that likely differ between, and perhaps even temporally within, individual vivaria. Furthermore, the more generally relevant alloantigen-specific responses that lead to fibroproliferative lesions of chronic rejection that are common to all transplanted organs (including those not exposed to the outside environment, such as hearts, kidneys, or livers), can be more readily parsed in the absence of highly variable inhaled dusts, microbes, and the like in a given environment. In any event, it may also be possible to orthotopically implant cadaveric small human airway grafts in humanized, immune-reconstituted mice to specifically and comparatively study effects of environmental exposure.

The finding that HAG in control (no PBMNC transfers) NOD/SCID IL-2Rγnull mice did not maintain a normal phenotypic appearance was unexpected. The choice of NOD/SCID IL-2Rγnull recipients for chimera generation was intended not only to preclude the necessity of repetitive anti-NK-cell mAb treatments, but also to enable concurrent engraftments of other human lineages, more comparable proliferation of both CD4 and CD8 T-cell subpopulations, and generally more consistent and uniform engraftment of the chimeric human T cells (Figure 5A). Although the HAGs implanted into control NOD/SCID IL-2Rγnull mice did not evidence the fibroproliferative luminal obliteration of human OB, we believe the lesser distinction between the HAGs harvested from control and experimental animals (Figure 5, C and D, respectively) currently limits the usefulness of this mouse strain for these applications. For now, we can only speculate that the loss of HAG epithelium in the control NOD/SCID IL-2Rγnull might be due to relative deficiency of some cryptic trophic factor, or to compensatory increased activity of another immune element (eg, macrophages).

In summary, the present data show that small human airways can be harvested from cadaveric normal lungs, cryopreserved until convenient for use, and implanted into a particular strain of immunodeficient mice. In the absence of immune reconstitution, these airway xenografts maintain normal histological appearance and epithelial secretory function for prolonged periods. However,
concomitant adoptive transfers of human T cells, allogeneic to the airway donor, will engraft and propagate in these animals, and mediate injuries that mimick OB of human lung transplant recipients. Current medical practices have not been very effective at prevention or treatment of OB in human lung recipients,2 and there is an obvious need to develop better understanding of the causual immunological processes. Humanized mice models offer an inexpensive, ready means for study of relevant human immune responses to human airway allografts in highly reduced detail, and may be particularly useful for the preclinical development and testing of new therapeutics or other approaches that will be necessary to counter this frequent and morbid disease. Tests of new therapeutics or other approaches that may be particularly useful for the preclinical development and testing of new therapeutics or other approaches that will be necessary to counter this frequent and morbid disease.

References


