INTRODUCTION

Lung and heart-lung transplantation are now established as therapeutic interventions for many terminal conditions affecting the pulmonary system. Paradoxically, our increasing clinical success exacerbates the donor organ shortage by broadening indications for transplantation and increasing referrals of appropriate patients at an earlier stage in their disease. The most acute need is among patients with either congenital heart disease or cystic fibrosis, for whom heart-lung or double lung transplantation is at present the only viable option. Appropriate organs are often not available for this group of generally young patients; many of those listed for transplant die waiting. Additional patients with other end-stage pulmonary disorders might benefit if more lungs were available.

One potential source of organs is a xenogeneic donor. In choosing a donor species for man one would intuitively choose a donor phylogenetically closely related to man; but while relative physiologic, biochemical, and immunologic similarity might favor primates, there are several important factors militating against this option. Cardiothoracic organs of a size appropriate for adult humans would be available only from large adult male chimpanzees, gorillas, or similar endangered species. These animals harbor epizootic viral infections likely transmissible to man, breed poorly in captivity and slowly in the wild, bear small litters, and take decades to reach usable adult size. Appropriate ethical concerns further weigh compellingly against their use. Several investigators are currently preparing to use baboon hearts clinically for children and small adults. However, even if successful, the use of baboon organs will not address the needs of the vast majority of potential lung recipients.

SPECIFIC ISSUES RELATED TO DISCORDANT LUNG TRANSPLANTATION

Historical background

Campbell et al., in the 1950s, reported using dog lungs as oxygenators for seven patients: high pulmonary vascular resistance limited flow to 400 ml/min. Waldhausen et al. similarly found maximal flows through dog lung of 200 ml/min. Bryant et al. perfused pig lungs with human blood using an ex-vivo perfusion system. They achieved flow rates of less than 10% of normal human levels. Blood retrieved from the cardiopulmonary bypass machine and stored gave better results than fresh blood, suggesting that formed blood elements, which are depleted and dysfunctionalized by storage, contribute to the pace of the rejection response. Rapid elevation of pulmonary vascular resistance and parenchymal edema occurred promptly in all of these situations, and in a number of other experimental models of lung xenotransplantation.

Role of complement

In general, a central role for complement has been shown conclusively in the hyperacute (minutes to hours) dysfunction of vascularized organs transplanted between discordant species; its importance has been confirmed in the pig-to-primate combination. Whether the lung is privileged with respect to complement-mediated damage is the focus of ongoing controversy.

Recently, Kaplon et al. reported short-term (1-3-day) pig lung survival in the baboon, with evidence of only modest levels of antibody deposition and complement activation relative to hyperacutely rejected pig hearts. Flow probes around the main pulmonary artery and the transplanted lung suggested that 10-40% of the cardiac output was perfusing the xenograft. Blood gas samples from the pulmonary vein of the transplant had a high $PO_2$. However, the orthotopic single lung grafts were unable to support the recipient when the contralateral native pulmonary artery was transiently occluded; very high pulmonary vascular resistance in the graft resulted in right heart failure and circulatory collapse.

Similarly, this group recently reported that discordant double lung transplant primate recipients could not be weaned from bypass due to right heart failure. A modest decline in anti-endothelial antibody, coupled with patchy deposition of IgM and complement pathway components, were interpreted as consistent with the absence of hyperacute rejection. However, these observations might also be explained by hypoperfusion of the grafts.
due to lung injury. A high pulmonary venous Po₂ and demonstra-
ble pulmonary artery flow apparently do not correlate with a cli-
nically meaningful level of graft function.

Using an ex-vivo working heart–lung model to address this
issue, we find that pig lung is rapidly damaged by human blood. The
injury is characterized by a rapid, profound rise in pulmonary
vascular resistance (within 5 minutes) and subsequent severe pul­
monary capillary leak. With rare exceptions, oxygen transport
function is lost within 30 minutes. Immunohistochemical staining
shows immunoglobulin deposition (IgM>IgG) as well as deposi­
tion of complement components from both classical and alterna­
tive pathways. Prevention of antibody binding and complement
activation (by antibody absorption combined with heat treatment)
results in graft function similar to that obtained when the graft is
perfused with pig blood. Both features of lung injury (vasocon­
striction and capillary leak) are significantly blunted by strategies
which prevent complement activation, demonstrating that these
phenomena are in large measure complement-mediated.

These findings suggest that the pig lung is susceptible to tradi­
tional hyperacute rejection, and that the process can be modulated
by specific intervention directed at regulation of complement activ­
ation. In our estimation, the claims of Kaplon et al. (that the lung
dysfunction they observe does not represent complement-
mediated hyperacute rejection, and thus that the lung is privileged
with regard to hyperacute rejection) are thus refuted. In fact, their
physiologic and histologic observations are in large measure con­
sistent with our own, and support the conclusion that primate anti-
pig antibody and complement trigger rapid injury to the lung.

Complement-independent mechanisms

Prevention of complement activation alone permits prolonged sur-
vival of discordant heart grafts for days; survival may be extended
to weeks if additional immunosuppression is used. It is possi­
ble, however, that complement-independent mechanisms, driven
either by xenospecific antibody or by other effectors of the immune
response, such as neutrophils and platelets, will render protection of
the lung by complement-directed strategies alone incomplete.

We have attempted to define the role of factors other than com­
plement in discordant lung transplant dysfunction using a tradi­
tional model, depletion of recipient complement with cobra
venom factor (CVF). CVF acts as a C3 convertase, consuming C3,
and thus depleting the complement component common to
both the classical and alternative pathways. Pig lungs were per­
fused with human blood depleted of complement by pretreatment
of plasma with CVF. Neither the elevation of pulmonary vascular
resistance nor capillary leak was prevented. Even when antibody
absorption was added to CVF treatment, hyperacute lung injury
and vasoconstriction occurred.

This result might be taken as evidence for complement-
dependent mechanisms governing hyperacute lung rejection.
However, while CVF depletes C3, in the process it generates high
levels of the neutrophil attractant and anaphylatoxin C3a; in other
models CVF causes neutrophil-mediated, P-selection-dependent
pulmonary capillary leak. We suspect that C3a is responsible for
the vasoconstriction and pulmonary injury observed in these
experiments, thus simulating complement-mediated hyperacute
rejection, and obscuring the role of complement-independent
mechanisms in discordant lung xenograft dysfunction.

Two groups have recently achieved significant prolongation of
pig heart survival in primates using hearts from pigs transgenic
for human complement-regulatory proteins. Parallel experi­
ments used lungs from animals transgenic for human decay acceler­
factor (hDAF), testing for protection from hyperacute rejection by
ex-vivo perfusion with fresh human blood. None of these transgenic lungs was protected from the development of
high pulmonary vascular resistance. Only two of seven lungs ex­
pressed significant levels of hDAF on the pulmonary endothe­
lum; in one of these two cases the rise in vascular resistance
resolved spontaneously, and graft function (as measured by
oxygen transport function) persisted for 90 minutes (vs <20
minutes for controls and other transgenics with low hDAF
expression).

This preliminary experience suggests that strategies directed at
complement regulation may contribute importantly to prolonga­
tion of discordant lung xenografts, but that other factors may also
be crucial to eventual clinical success. Resolution of the relative
importance of complement-dependent and complement-
independent mechanisms to dysfunction of discordant lung
xenografts awaits results of experiments using either soluble com­
plement receptor 1 or pigs with a higher pulmonary endothelial
expression of human complement-regulatory proteins.

COMMENT

In general the lung appears to be more sensitive than the heart to a
variety of systemic insults, as manifested by the pulmonary capil­
lar leak and ARDS syndromes, which sporadically occur during
sepsis or following cardiopulmonary bypass. These insults have
in common not only complement activation but the activation and
intrapulmonary sequestration of neutrophils, platelets, and
macrophages. Supporting this general concept of lung injury are
experiments demonstrating salutary effects for neutrophil deple­
tion, adhesion molecule blockade, and anti-TNF antibody.
These observations suggest that complement-independent mecha­
nisms, driven either by xenospecific antibody or by other effectors
of the immune response such as neutrophils and platelets,
may render protection of the lung incomplete, even with effective
control of complement activation.

Preliminary work identifies thromboxane as the central media­
tor of pulmonary vasoconstriction, and a contributor to capillary
leak, in pig lungs perfused with human blood (Pierson RN III,
Parker RE, unpublished). We believe that platelet or tissue
macrophage activation triggers thromboxane production; whether
thromboxane production occurs consequentially to or indepen­
dently of complement activation is an unresolved question of
major importance to programs hoping to use transgenic lungs for
clinical discordant lung xenotransplantation. Addition of antibody
absorption to thromboxane blockade yields impressive protection
of lung function, approaching 4 hours in some preliminary experi­
ments. Thus, there is reason to suspect that, once the several inter­
connected mechanisms governing hyperacute rejection of the lung
are elucidated, clinically important function across the discordant
species barrier may be achieved.

In summary, discordant pulmonary xenografting is unlikely to
be clinically important for a variety of ethical, infectious disease,
and logistical reasons. Discordant lung xenotransplantation is cur-