Understanding Hyperacute Rejection of the Lung: Is This a Special Case?

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The impetus to develop clinical application of lung xenografts is provided by the severe shortage of available donor organs. Despite restrictive recipient criteria and increasing use of "marginal" donor organs, about 20% of lung transplant candidates die while waiting.[1] Available respiratory support methods have limited efficacy in end-stage lung failure (mechanical ventilation) or are probably not suitable for long-term use (ECMO, liquid ventilation), while permanently implantable lung support devices remain prototypic.[1] Lung xenotransplantation represents a potential solution to the clinical need for a readily available supply of lung donors.

The Role of Complement, Antigen and Antibody in Hyperacute Lung Rejection (HALR)

Currently, preclinical xenotransplant efforts are focused primarily on the pig as a potential donor. A pig lung is hyperacutely rejected within minutes when transplanted into primates or perfused ex vivo with human blood. Many groups have shown that expression of human complement regulatory proteins protects a pig lung from complement-mediated injury,[2-4] and that removal of anti-pig antibody also significantly attenuates lung injury.[5-7] However, despite concerted efforts to prevent hyperacute lung rejection (HALR) by adsorbing antibody or inhibiting complement, life-supporting pig lung function in a primate model beyond one day has not yet been achieved.[7-9] This result stands in stark contrast to results obtained in life-supporting renal and heart models using similar interventions.[10,11] Similarly, in an ex vivo perfusion model, even the combination of antibody absorption with local (human decay accelerating factor, hDAF: CD 55) and soluble phase complement inhibition (soluble complement receptor type 1: sCR1, TP-10) does not reliably and completely protect the lung from injury during perfusion with human blood.[12] Thus, either the lung is more vulnerable to injury by low levels of antibody and complement than are other organs, or HALR may be driven by organ-specific mechanisms which are partly or wholly independent of antibody and complement.

The differences in hyperacute rejection (HAR) between different pig organs might reflect qualitative or quantitative differences in antigen targets, or in the level or location of expression of protective molecules. Organs and tissues express proteins which are particular to those organs (e.g., "prostate-specific" antigen, myocardial creatine phosphokinase isoforms, angiotensin II in the lung), and which could by chance act as efficient targets for common human antibody specificities.[13] This seems unlikely, as the molecular weight profile of porcine antigens from porcine microvascular endothelium bound by human anti-pig antibodies does not appear to be different from those expressed on aortic endothelial cells, suggesting that the molecular targets are qualitatively similar, at least between these two cultured endothelial cell populations.[14] It remains possible that binding of relatively sparse tissue-specific antigens could trigger biological reactions particular to the lung. More likely, intense expression of common protein antigens (e.g., histocompatibility antigens) or a particular glycosylation profile (Galα1,3Gal) on the endothelium of a particular organ could increase the density of antibody binding and thus the efficiency of complement activation.

Finally, expression of protective proteins is known to vary markedly between individual transgenic animals, and between tissues and organs from individual donors. For example, membrane cofactor protein

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(MCP, CD46) is not found on smooth muscle cells in porcine lung and vessel walls.[15] Such differences could account for variable ability of organs to modulate the pathogenic inflammatory cascades characteristic of HAR. However, in our experience, even porcine lungs which express high levels of human complement regulatory proteins incur injury during perfusion with human blood or when transplanted into primates. Thus, while quantitative analysis of target or protective protein expression levels or organ- or cell-type-specific antigen distribution patterns may prove a fruitful avenue of investigation, there is currently little evidence to support variability in target antigen expression as the primary explanation for differences between organs in susceptibility to HAR. And while antibody adsorption yields the best published results for both ex vivo and in vivo lung xenografts, the highly efficient depletion accomplished to date is insufficient to reliably protect the lung, suggesting that antibody—and thus antigen—independent mechanisms contribute to HALR.

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The complement inhibitory strategies tested to date in lung models have focused primarily on inhibitors of the common pathway of complement activation (hDAF; hMCP; CD59; sCR1; cobra venom factor). Complement is a pivotal mediator of lung injury in a variety of non-transplant models, and there is considerable evidence to support the hypothesis that the lung is particularly sensitive to proximal complement cascade activation.[16-18] The dramatic lung protection afforded by blocking C5a in these and other models of acute lung injury suggests that the lung's response to C5a may account in part for its particular vulnerability during HAR. Other proteins more proximal in the complement activation cascade, including C1q, C1s, and the anaphylatoxin C4a, may also contribute to HALR.[19] These possibilities have not yet been tested in lung models.

Tissue Monocytes in HALR

Cells specific to the lung may contribute to unique or particularly virulent innate immune system responses, mediated by complement activation or by other pattern recognition receptors expressed on lymphoid lineage cells resident in the lung and other organs.[20-22] We have demonstrated the importance of tissue monocytes to HALR by showing that selective depletion of pulmonary intravascular macrophages, with dichlorobisphosphonate in liposomes,[23] essentially prevents thromboxane release and histamine elaboration associated with perfusion of a pig lung by human blood, and attenuates the associated elevation of pulmonary vascular resistance (PVR) and capillary leak.[24] Depleting or disabling innate immune system cells in the donor is particularly attractive as a strategy to protect porcine organs from putative complement-dependent and complement-independent pro-inflammatory events, and may prove synergistic with complement-inhibitory and antibody-depleting strategies.

Coagulation Pathways in HALR

Porcine vonWillebrand Factor (vWF) ligation to platelets occurs through non-physiologic interaction via GP1b and the alpha1 domain of vWF,[25] and human thrombin is constitutively activated by porcine endothelium.[26] Thus, both the porcine endothelium and the human coagulation system tend to be activated even in the absence of other pathogenic interactions. Further, porcine vWF appears to bind human complement even in the absence of anti-pig antibody.[14] This interaction may serve as a nidus for subsequent complement activation and tissue injury on the endothelium in the lung and other organs. In addition, release of vWF multimers from the surface of injured or activated endothelial cells may bind and activate complement in soluble phase, triggering release of anaphylatoxins which are particularly injurious to the lung.

We have recently explored the role of coagulation pathways in HALR, using

- a specific thrombin inhibitor, bivalrudin;
- selective inhibitors of the platelet GPIIbIIIa receptor (Rheopro, SC52012A); and
- aurintricarboxylic acid, which blocks interaction between porcine vWF and the platelet GP1b receptor.

Lung survival was improved significantly by each intervention alone, with blunted PVR elevation and histamine release.[27,28] (Table 1). Thrombin inhibition (bivalrudin) was associated with moderate inhibition of complement activation, whereas combined blockade of both platelet receptors (but not blockade of either receptor alone) essentially prevented complement activation.

Complement inhibition by blocking platelet receptor antagonists was unexpected and is a primary focus of our current investigations. We infer that adhesion of platelets to porcine endothelium, mediated by both thrombin-dependent interactions and by non-physiologic adhesion to porcine vWF,

Table 1 | **Effect of Platelet Receptor and Thrombin Inhibition on Hyperacute Lung Rejection**

Treatment Group	Survival min	F 1+2 nM @10 min	PVR @5 min cmH_2O/ml/m	C3a @1 min g/ml	C3a @10 min g/ml	Histamine @10 min nM
Control (n=7)	8 (1)	5.3 (4)*	0.62 (0.12)	3441 (615)	4096 (702)	106 (12)
ATA (n=5)33 (11)*	2.0 (0.2)*	0.09 (0.02)**	3250 (2898)	4581 (2696)	33 (8)*	
SC52012A (n=5)	31 (22)*	2.1 (0.8)*	0.10 (0.02)**	3265 (1124)	4091 (1275)	32 (15)*
ATA+SC (n=5)	130 (97)*	1.5 (0.2)*	0.11 (0.02) **	113 (68) **	235 (152)*	46 (15)*
Bivalrudin (n=7)	77 (22)**	0.5 (0.8)*	0.16(0.05)**	630 (262)**	1857 (356)*	21 (4)**

Figures in brackets denote standard error. *p<0.05 **p<0.02. Denotes statistically significant change relative to human blood level before initiation of pig lung perfusion. F1+2 = surrogate marker of prothrombin activation. PVR = pulmonary vascular resistance. C = complement fraction. ATA = aurintricarboxylic acid.

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contributes to amplification of the complement cascade during HALR. Blunting of histamine and PVR elevation in all groups, even those in which complement elaboration is not inhibited (aurintricarboxylic acid alone or SC52012A alone), shows that lung macrophage activation is regulated at least in part by factors other than complement activation. Considered in the context of our previous findings with lung macrophage depletion, these observations demonstrate that thrombin/platelet/endothelial interactions play a pivotal role in triggering the lung's innate immune pathways, including amplification of the complement activation cascade.

Is the Lung Unique?

Whether the tissue macrophages and coagulation pathway interactions we have identified are uniquely important to the lung or rather also play a role in the HAR of other organs has not yet been examined. Why might the lung be particularly vulnerable to such pathogenic pathways? Adhesive interactions between neutrophils or platelets and endothelium are known to depend critically on flow conditions (shear stress).[29,30] We hypothesize that shear stress conditions unique to the lung's low-pressure, high capacitance vascular bed may contribute to platelet-dependent complement activation, thrombosis, and inflammation. We speculate that the low shear stress in the pulmonary vascular bed may be a particularly efficient trigger for nonphysiologic interactions between porcine vWF and human GPIb. Thus even if pig endothelium is not activated by antibody or complement, platelet adhesion may occur particularly efficiently in the lung and trigger prothrombotic and pro-inflammatory events. Alternatively, organ-specific receptors may trigger pathogenic reactions preferentially in the lung or be even unique to it. If so, organ-specific drug interventions or donor modifications may be necessary to protect the lung from HAR. Study of common mechanisms that are amplified and thus easier to study in HALR may lead to a better understanding of HAR of other organs, and to development of reagents critical to safe clinical application of organ xenografts.

Conclusion

In summary, previous work by us and by others demonstrates a role for antibody binding and complement activation in HALR, as has also been well demonstrated for other pig organs in primates. However, the weight of evidence supports the conclusion that the pig lung is particularly vulnerable to HAR, in that it is not fully protected by anti-complement strategies or by antibody adsorption alone. This observation raises the possibility that pathways of HAR may prove to be different for various organs, and dependent in variable degree upon complement-driven and complement-independent mechanisms. Because complement activation has not been completely prevented in the lung studies performed to date, it remains possible that more efficient complement control will yield better lung protection. In particular, it seems likely that C5a or other proteins more proximal in the complement activation cascade may prove pivotal in HALR. Alternatively, events triggered by antibody binding, by coagulation system dysregulation, or by other organ-specific cells or pathways may be particularly important to the pathogenesis of HALR relative to HAR of other organs. Defining the mechanisms driving HALR should suggest effective protection strategies, and eventually facilitate successful clinical use of much-needed lung xenografts.

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