

# Donor intravascular monocyte trafficking: A potential therapeutic target for primary graft dysfunction following lung transplantation?

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## Editorial:

Ischaemia reperfusion injury at the time of lung transplantation may lead to primary graft dysfunction (PGD).<sup>1</sup> Occurring within the first 72 hours following allograft reperfusion, PGD is characterised by hypoxaemia and alveolar infiltrates in the transplanted organ, and occurs in up to 30% of patients.<sup>2</sup> The development of PGD carries significant early mortality of up to 50% in 30 days.<sup>3</sup> Those who survive the early post-operative period have a higher propensity to develop bronchiolitis obliterans syndrome (BOS),<sup>4</sup> and long term mortality is also higher at 1, 5 and 10 years.<sup>5</sup>

Several risk factors have been identified for the development of PGD including a donor history of smoking, higher FiO<sub>2</sub> at the time of reperfusion, elevated pulmonary arterial pressures, large volume blood transfusion and raised recipient body mass index.<sup>6</sup> Increased length of ischaemic time has also been associated with the condition.<sup>6</sup> This observation has led to the development of *ex vivo* lung perfusion (EVLP). Here lungs are ventilated and perfused with an acellular buffer. Use of this technique has allowed longer total ischaemic time prior to transplantation without an increase in incidence of

PGD.<sup>7</sup> However incomplete understanding of the immune mechanisms leading to ischaemia reperfusion injury is a major limitation to preventing disease onset. There are no disease-specific therapeutics available for the condition, nor to prevent progression to PGD. Improved understanding of the immune responses underlying ischaemia reperfusion injury may allow development of new therapeutic strategies.

Dysregulation of the innate immune response is central to ischaemia reperfusion pathobiology. Neutrophils are a recognised early protagonist of inflammation,<sup>8</sup> with extravasation mediated by preceding monocyte recruitment.<sup>9,10</sup> Monocytes are mononuclear phagocytes, and, in a most simplistic description belong to 2 principle subsets. Classical monocytes (Ly-6C<sup>High</sup>CCR2<sup>+</sup> in mice) are migratory leukocytes in response to injury or inflammation.<sup>11</sup> They may phagocytose pathogens, present antigens via MHCII or secrete chemokines to recruit other cells to the site of inflammation. In lung, classical monocytes may traffic into the interstitium and differentiate to form alveolar macrophages or dendritic cells. Alveolar macrophages are a crucial mediator of the early inflammatory response and deletion of these cells is protective in experimental ischaemia reperfusion injury.<sup>12</sup> Non-classical, endothelial patrolling monocytes (Ly-6C<sup>Low</sup>CX3Cr1<sup>High</sup> in mice) have been shown to adhere to vascular endothelium, slowly crawling via integrin-cell adhesion molecule interactions.<sup>13</sup> These monocytes are also capable of migration and further differentiation.

It is recognised that circulating monocytes can be transferred from donor to recipient during transplantation.<sup>14,15</sup> These cells remain present in lung explants following 10 litres perfusion, and donor monocytes remain detectable in the recipient's circulation many months following transplantation.<sup>14</sup> However the role of these 'intravascular passenger' monocytes in ischemia reperfusion injury has not been previously appreciated. In this issue of *Thorax* Tatham *et al* demonstrate a specific role of donor lung-marginated intravascular monocytes in ischaemia reperfusion injury during lung transplantation.<sup>16</sup>

In a mouse model of *ex vivo* perfusion, a dual intravascular and intra-alveolar antibody delivery technique followed by tissue dissociation and flow cytometry allowed the authors to identify and enumerate intravascular monocytes and interstitial macrophages. Intriguingly despite intravascular perfusion for 15 minutes, approximately half of Ly-6C<sup>High</sup> and Ly-6C<sup>Low</sup> intravascular monocytes were retained within the lung. The activation status of these retained monocytes during ischaemia reperfusion was determined by modelling 2 hours of warm normoxic ischaemia followed by 2 hours of reperfusion incorporating three open-circuit washout periods with comparison to reperfusion only. L-selectin (CD62L) is expressed by Ly-6C<sup>High</sup> monocytes that have recently left bone marrow and is required for trafficking to lymph nodes and tissue during inflammation.<sup>17</sup> On contact with vascular endothelium CD62L is downregulated and as part of monocyte maturation/differentiation markers including the co-stimulatory molecule CD86 are increased. Tatham and colleagues demonstrated that following ischaemia reperfusion there was activation of intravascular Ly-6C<sup>High</sup> monocytes with reduced L-selectin and increased CD86 expression.<sup>16</sup> Ly-6C<sup>Low</sup> monocytes, which do not express high levels of L-selectin also demonstrated increased CD86 expression following ischaemia reperfusion. Together this demonstrated activation of the donor intravascular monocyte pool.

Tatham and colleagues then selectively depleted intravascular monocytes using intravenous liposomal clodronate injection 24 hours prior to experimental lung perfusion in order to determine the effect of these vascular passenger cells on ischaemia reperfusion injury.<sup>16</sup> This method significantly reduced Ly-6C<sup>High</sup> and Ly-6C<sup>Low</sup> monocytes in the vascular compartment, whilst vascular neutrophils, interstitial macrophages and alveolar macrophage frequencies were unaltered. Wet:dry lung ratios and BAL protein concentrations, both biomarkers of pulmonary inflammation, were significantly reduced in liposomal clodronate-treated mice following ischaemic reperfusion. Consistent with this, MIP-2 (CXCL2), a murine functional homologue of the powerful neutrophil chemo-attractant IL-8 (CXCL8),<sup>18</sup>

and shown to recruit neutrophils in experimental PGD,<sup>10</sup> was significantly reduced post reperfusion in the monocyte-depleted group.<sup>16</sup> MCP-1 (CCL1) is an important chemokine for monocyte adhesion to vascular endothelium during trafficking to inflammatory sites.<sup>19</sup> Consistent with lack of consumption by monocytes,<sup>20</sup> this chemokine was significantly increased in the liposomal clodronate group.<sup>16</sup> The authors also demonstrated a reduction in the epithelial cell injury marker RAGE in the monocyte deplete mice, highlighting a potential early role for monocytes in ischaemia reperfusion, and hence PGD development.

Finally, Tatham and colleagues analysed the presence of donor monocytes and granulocytes in lung tissue from a small cohort of 13 human lungs prior to implantation.<sup>16</sup> Consistent with their murine observations, despite anterograde and retrograde perfusion of 5 litres of perfusate, high numbers of donor monocytes and neutrophils were still found in tissue. Electron microscopy confirmed the presence of monocytes and neutrophils in human lung capillaries, with possible cell to cell interactions between leukocytes and endothelial cells. Following transplantation total monocyte numbers and intensity of CD86 staining were shown as inversely correlated with PaO<sub>2</sub>:FiO<sub>2</sub> ratios at 72 hours and 48 hours respectively. Those who went on to develop Grade 2 or 3 PGD at 48 hours had higher expression of the monocyte activation markers CD86 and TREM-1.

The experimental data presented by Tatham *et al*,<sup>16</sup> suggests a previously unappreciated role of 'passenger' intravascular donor monocytes in PGD following lung transplantation. Whilst limited human data is presented in this manuscript, high numbers of monocytes appear to be retained in human lung following standard perfusion protocols. Strengthening the findings of this paper is a recent confirmatory study of the importance of donor intravascular monocytes for neutrophil chemotaxis during PGD.<sup>10</sup> The ability of these cells to withstand perfusion implicates high-affinity adhesive interactions between monocytes and endothelial cells. If mechanical removal of these cells prior to implantation is not clinically feasible, further elucidating the regulation of monocyte adhesion and trafficking, including the role of chemokine ligand/receptor pairs, and integrin/cell adhesion molecule interactions is of high importance in order to identify potential disease-selective therapeutic strategies. Future studies will also be required to examine whether *ex vivo* perfusion may provide a novel means of safely delivering therapy to the donor organ prior to transplantation, whether this approach may avoid systemic toxicity, and if donor intravascular monocyte-targeted therapy may reduce the incidence of PGD or modify the natural history of this devastating condition.

## Contributions:

Both authors contributed to writing this editorial and approved the final version

## Competing interests:

None

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## References

1. Christie JD, Carby M, Bag R, et al. Report of the ISHLT Working Group on Primary Lung Graft Dysfunction part II: definition. A consensus statement of the International Society for Heart and Lung Transplantation. *J Heart Lung Transplant*. 2005;24(10):1454-1459.

2. Porteous MK, Diamond JM, Christie JD. Primary graft dysfunction: lessons learned about the first 72 h after lung transplantation. *Curr Opin Organ Transplant*. 2015;20(5):506-514.
3. Lee JC, Christie JD. Primary graft dysfunction. *Proc Am Thorac Soc*. 2009;6(1):39-46.
4. Daud SA, Yusef RD, Meyers BF, et al. Impact of immediate primary lung allograft dysfunction on bronchiolitis obliterans syndrome. *Am J Respir Crit Care Med*. 2007;175(5):507-513.
5. Kreisel D, Krupnick AS, Puri V, et al. Short- and long-term outcomes of 1000 adult lung transplant recipients at a single center. *J Thorac Cardiovasc Surg*. 2011;141(1):215-222.
6. Diamond JM, Lee JC, Kawut SM, et al. Clinical risk factors for primary graft dysfunction after lung transplantation. *Am J Respir Crit Care Med*. 2013;187(5):527-534.
7. Yeung JC, Krueger T, Yasufuku K, et al. Outcomes after transplantation of lungs preserved for more than 12 h: a retrospective study. *Lancet Respir Med*. 2017;5(2):119-124.
8. Sayah DM, Mallavia B, Liu F, et al. Neutrophil extracellular traps are pathogenic in primary graft dysfunction after lung transplantation. *Am J Respir Crit Care Med*. 2015;191(4):455-463.
9. Kreisel D, Nava RG, Li W, et al. In vivo two-photon imaging reveals monocyte-dependent neutrophil extravasation during pulmonary inflammation. *Proc Natl Acad Sci U S A*. 2010;107(42):18073-18078.
10. Zheng Z, Chiu S, Akbarpour M, et al. Donor pulmonary intravascular nonclassical monocytes recruit recipient neutrophils and mediate primary lung allograft dysfunction. *Sci Transl Med*. 2017;9(394).
11. Serbina NV, Pamer EG. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nat Immunol*. 2006;7(3):311-317.
12. Zhao M, Fernandez LG, Doctor A, et al. Alveolar macrophage activation is a key initiation signal for acute lung ischemia-reperfusion injury. *Am J Physiol Lung Cell Mol Physiol*. 2006;291(5):L1018-1026.
13. Collison JL, Carlin LM, Eichmann M, Geissmann F, Peakman M. Heterogeneity in the Locomotory Behavior of Human Monocyte Subsets over Human Vascular Endothelium In Vitro. *J Immunol*. 2015;195(3):1162-1170.
14. Paantjens AW, van de Graaf EA, Heerkens HD, et al. Chimerism of dendritic cell subsets in peripheral blood after lung transplantation. *J Heart Lung Transplant*. 2011;30(6):691-697.
15. Richter N, Raddatz G, Steinhoff G, Schafers HJ, Schlitt HJ. Transmission of donor lymphocytes in clinical lung transplantation. *Transpl Int*. 1994;7(6):414-419.
16. Tatham KC, O'Dea KP, Romano R, et al. Intravascular donor monocytes play a central role in lung transplant ischaemia-reperfusion injury. *Thorax*. 2017.
17. Jakubzick CV, Randolph GJ, Henson PM. Monocyte differentiation and antigen-presenting functions. *Nat Rev Immunol*. 2017;17(6):349-362.
18. Bozic CR, Gerard NP, von Uexkull-Guldenband C, et al. The murine interleukin 8 type B receptor homologue and its ligands. Expression and biological characterization. *J Biol Chem*. 1994;269(47):29355-29358.
19. Gerszten RE, Garcia-Zepeda EA, Lim YC, et al. MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. *Nature*. 1999;398(6729):718-723.
20. Maus UA, Wellmann S, Hampl C, et al. CCR2-positive monocytes recruited to inflamed lungs downregulate local CCL2 chemokine levels. *Am J Physiol Lung Cell Mol Physiol*. 2005;288(2):L350-358.