However, the additional flush after EVLP is expected to clear the medication from the vasculature. Furthermore, the accumulation of the drug in the extravascular space has been shown to be minimal and would have been rapidly dissipated once *in vivo* status had been reestablished (10). In our case there were no signs of fibrinolytic complication such as bleeding.

With the success obtained in the Human Ex vivo Lung Perfusion trial and evidence from research studies demonstrating stable pulmonary function over prolonged normothermic perfusions over 12 hours, we believe that advanced lung preservation techniques should involve EVLP as a platform for accurate diagnosis and directed therapy (16). As opposed to our ongoing preclinical research using advanced molecular therapeutics such as adenoviral IL-10 gene therapy (17), in this case we used readily available clinical diagnostic techniques and a clinically proven, readily available medication to treat donor lungs in an almost guideline-based fashion, but in the *ex vivo* setting. Thus, this case exemplifies how the concept of personalized medicine for the donor organ can be applied in the field of lung transplantation.

Author disclosures are available with the text of this letter at www.atsjournals.org.

Acknowledgment: The authors acknowledge the assistance of Yi-Min Chun with the figures.

TIAGO N. MACHUCA, M.D., PH.D.
MICHAEL K. HSIN, M.D.
HARALD C. OTT, M.D.
MANYIN CHEN, M.D.
DAVID M. HWANG, M.D., PH.D.
MARCELO CYPEL, M.D., M.Sc.
THOMAS K. WADDELL, M.D., PH.D.
SHAF KESHAVJEE, M.D., M.Sc.
University of Toronto
Toronto, Ontario, Canada

References

1. Cypel M, Yeung JC, Liu M, Anraku M, Chen F, Karolak W, Sato M, Laratta J, Azad S, Madonik M, *et al.* Normothermic ex vivo lung perfusion in clinical lung transplantation. *N Engl J Med* 2011;364:1431–1440.
2. Ware LB, Fang X, Wang Y, Babcock WD, Jones K, Matthay MA. High prevalence of pulmonary arterial thrombi in donor lungs rejected for transplantation. *J Heart Lung Transplant* 2005;24:1650–1656.
3. Oto T, Rabinov M, Griffiths AP, Whitford H, Levvey BJ, Esmore DS, Williams TJ, Snell GI. Unexpected donor pulmonary embolism affects early outcomes after lung transplantation: a major mechanism of primary graft failure? *J Thorac Cardiovasc Surg* 2005;130:1446–1452.
4. Fischer S, Gohrbandt B, Meyer A, Simon AR, Haverich A, Strüber M. Should lungs from donors with severe acute pulmonary embolism be accepted for transplantation? The Hannover experience. *J Thorac Cardiovasc Surg* 2003;126:1641–1643.
5. Frenia D, Nathan SD, Ahmad S, Guerrero M, Distefano D, Massamiano P, Lefrak E, Burton N. Successful lung transplantation from a donor with a saddle pulmonary embolus. *J Heart Lung Transplant* 2005;24:1137–1139.
6. Sareyyupoglu B, Shigemura N, Toyoda Y. Utilizing donor lungs after thrombolytic therapy and thrombectomy for acute massive pulmonary embolus. *J Heart Lung Transplant* 2011;30:358.
7. Shihata M, Ghorpade N, Lien D, Modry D. *Ex vivo* bilateral pulmonary embolectomy for donor lungs prior to transplantation. *Ann Thorac Surg* 2008;85:2110–2112.
8. Cypel M, Yeung JC, Hirayama S, Rubacha M, Fischer S, Anraku M, Sato M, Harwood S, Pierre A, Waddell TK, *et al.* Technique for prolonged normothermic *ex vivo* lung perfusion. *J Heart Lung Transplant* 2008;27:1319–1325.
9. Konstantinides S, Geibel A, Heusel G, Heinrich F, Kasper W; Management Strategies and Prognosis of Pulmonary Embolism-3 Trial Investigators. Heparin plus alteplase compared with heparin alone

in patients with submassive pulmonary embolism. *N Engl J Med* 2002;347:1143–1150.

10. Chandler WL, Alessi MC, Aillaud MF, Henderson P, Vague P, Juhan-Vague I. Clearance of tissue plasminogen activator (TPA) and TPA/plasminogen activator inhibitor type 1 (PAI-1) complex: relationship to elevated TPA antigen in patients with high PAI-1 activity levels. *Circulation* 1997;96:761–768.
11. Dalla-Volta S, Palla A, Santolucando A, Giuntini C, Pengo V, Visioli O, Zonzin P, Zanuttini D, Barbaresi F, Agnelli G, *et al.* PAIMS 2: alteplase combined with heparin versus heparin in the treatment of acute pulmonary embolism. Plasminogen activator Italian multicenter study 2. *J Am Coll Cardiol* 1992;20:520–526.
12. Meneveau N, Schiele F, Metz D, Valette B, Attali P, Vuilleminot A, Grollier G, Elaerts J, Mossard JM, Viel JF, *et al.* Comparative efficacy of a two-hour regimen of streptokinase versus alteplase in acute massive pulmonary embolism: immediate clinical and hemodynamic outcome and one-year follow-up. *J Am Coll Cardiol* 1998;31:1057–1063.
13. Knecht MF, Heinrich F, Spanuth E. Evaluation of plasma D-dimer in the diagnosis and in the course of fibrinolytic therapy of deep vein thrombosis and pulmonary embolism. *Thromb Res* 1992;67:213–220.
14. Goldhaber SZ, Haire WD, Feldstein ML, Miller M, Toltzis R, Smith JL, Taveira da Silva AM, Come PC, Lee RT, Parker JA, *et al.* Alteplase versus heparin in acute pulmonary embolism: randomised trial assessing right-ventricular function and pulmonary perfusion. *Lancet* 1993;341:507–511.
15. Inci I, Zhai W, Arni S, Inci D, Hillinger S, Lardinois D, Vogt P, Weder W. Fibrinolytic treatment improves the quality of lungs retrieved from non-heart-beating donors. *J Heart Lung Transplant* 2007;26:1054–1060.
16. Cypel M, Rubacha M, Yeung J, Hirayama S, Torbicki K, Madonik M, Fischer S, Hwang D, Pierre A, Waddell TK, *et al.* Normothermic *ex vivo* perfusion prevents lung injury compared to extended cold preservation for transplantation. *Am J Transplant* 2009;9:2262–2269.
17. Cypel M, Liu M, Rubacha M, *et al.* Functional repair of human donor lungs by IL-10 gene therapy. *Sci Trans Med* 2009;1:4ra9.

Copyright © 2013 by the American Thoracic Society

Use of Technetium-99m-labeled Eosinophils to Detect Active Eosinophilic Inflammation in Humans

To the Editor:

The diagnosis of focal eosinophilic inflammation is notoriously difficult. Here we describe a novel technique using technetium-99m-labeled autologous eosinophils to image eosinophilic vasculitis affecting the lung and skin in a patient with granulomatosis with polyangiitis.

Our patient, a 48-year-old male, presented with cough, purulent sputum, hemoptysis, lethargy, and painful ulcers affecting his hands and feet. His thoracic computed tomography (CT) showed bilateral upper zone nodules with cavity formation. Previously he had experienced recurrent anterior uveitis and small joint polyarthropathy. His medications were hydroxychloroquine and etodolac, which he took intermittently. Examination revealed periungual erythema and multiple deep ulcers affecting his fingers and right ankle (Figure 1A). His blood eosinophil count was $0.76 \times 10^9/L$, with normal renal and liver function

Supported by Asthma-UK [08/11], the Medical Research Council [grant number MR/J00345X/1], the Wellcome Trust [grant number 098351/Z/12/Z], and Cambridge NIHR Biomedical Research Centre.

Author Contributions: N.F. performed experiments and analyzed the data. C.L. performed experiments and analyzed data. A.M.P., A.M.C., and E.R.C. contributed to the design and writing of the study.

Figure 1. Technetium-99m-labeled eosinophil scanning in granulomatosis with polyangiitis. (A) Photograph of vasculitic ulcers affecting the patient's right ankle. (B) The corresponding histology ($\times 20$ magnification) shows active vasculitis with fibrinoid necrosis, red cell extravasation, and surrounding eosinophil infiltration. (C) Planar γ camera images of the legs 5 hours after the injection of technetium-99m-labeled autologous eosinophils shows enhanced uptake corresponding to the area of ulceration on the right ankle. (D) Dynamic imaging of the thorax and abdomen was performed every 20 seconds for 40 minutes after injection of technetium-99m-labeled autologous eosinophils and revealed their rapid transit through both lungs followed by uptake in the liver, spleen, and bone marrow. (E–G) SPECT-CT images at 45 minutes revealed an enhanced signal focally in the lungs, corresponding to the areas of nodularity. (H) A cross section of the surgical lung biopsy ($3.8 \times 2.5 \times 4.5$ cm).

and normal antinuclear antibody and rheumatoid factor. His antineutrophil cytoplasmic antibody (ANCA) immunofluorescence was positive with a (PR3) ANCA of 25 IU/ml (normal range < 0.2 IU/ml). He also had evidence of IgG₄-related disease associated with his ANCA-positive vasculitis with a serum IgG₄ of 4.1 g/L. Surgical lung biopsy revealed dense fibrosis, with scattered noncaseating granulomas, heavy eosinophil infiltration, a medium-small vessel vasculitis, and IgG₄-staining plasma cells. Similar findings were observed in the skin (Figure 1B).

Technetium-99m-labeled eosinophils (98.5% pure) were prepared using clinical grade anti-CD16 immuno-magnetic beads as described (1) and 16×10^6 cells (187 MBq) reinjected. Dynamic images were taken for 40 minutes (1) followed by single-photon emission CT (SPECT-CT) images at 45 minutes and 2, 4, and 6 hours. His legs were imaged using a γ camera (Figure 1C). The intravascular residence time (1.5 h) of the technetium-99m-labeled eosinophils was much shorter than that observed in healthy subjects (1) with only 4.9% of the injected cells remaining in the circulation at 30 minutes. This compares to an intravascular residence time of indium-111-labeled eosinophils in healthy volunteers of 28.1 ± 1.7 hours

(mean $\pm$ SEM), with $14.5 \pm 2.5\%$ of radiolabeled cells remaining in the circulation at 30 minutes (1). Although the technetium-99m-labeled eosinophils accumulated as expected in the liver, spleen, and bone marrow (Figure 1D), SPECT-CT revealed additional uptake in some but not all of his lung nodules (Figures 1E–1G).

Although radiolabeled leukocyte scanning is used routinely for localizing occult infection, this approach has not been used previously to demonstrate focal eosinophilic inflammation. Advances in immuno-magnetic bead technology have permitted the isolation of ultra-pure nonactivated eosinophils from blood, which are amenable to radiolabeling and behave physiologically when reinjected in healthy subjects (1). Alternative, indirect methods of detecting eosinophilic inflammation in humans, such as exhaled nitric oxide and analysis of breath condensates, are nonspecific and can only assess the inflammatory burden in the respiratory tract. In contrast, technetium-99m-labeled eosinophils plus SPECT-CT can localize focal eosinophilic inflammation directly *in vivo*. Furthermore, this technique is less invasive than performing bronchoscopy and transbronchial biopsy, which may be contraindicated in some patients and carry significant risk. This novel approach therefore offers the prospect of

a noninvasive method to aid in the diagnosis, localization, and assessment of disease activity.

Author disclosures are available with the text of this letter at www.atsjournals.org.

Acknowledgment: The authors are grateful to Rosalind Simmonds, the staff within the Nuclear Medicine and Histopathology Department at Addenbrooke's Hospital, and the Wellcome Trust Clinical Research Facility, Cambridge. They acknowledge the help of the Histopathology Departments at the Royal Brompton and Princess Alexandra Hospitals. They also thank the Cambridge Biomedical Research Centre and BRC Core Biochemistry Assay Laboratory and acknowledge the support of the National Institute for Health Research, through the Comprehensive Clinical Research Network. The study was approved by Cambridgeshire Research Ethics Committee (09/H0308/119) and the Administration of Radioactive Substances Advisory Committee of the UK (83/3130/25000).

NEDA FARAH, PH.D.

CHRYSTALLA LOUSIOS, B.Sc., M.B.B.S.

University of Cambridge School of Clinical Medicine
Cambridge, United Kingdom

ADRIEN M. PETERS, B.Sc., M.B.Ch.B., M.A.

Brighton and Sussex Medical School
Brighton, United Kingdom

ALISON M. CONDLIFFE, B.A., M.B.B.S., PH.D.

EDWIN R. CHILVERS, B.M.B.S., PH.D.
University of Cambridge School of Clinical Medicine
Cambridge, United Kingdom

Reference

1. Farah N, Singh NR, Heard S, Loutsios C, Summers C, Solanki CK, Solanki K, Balan KK, Ruparelia P, Peters AM, *et al*. Use of 111-indium-labeled autologous eosinophils to establish the in vivo kinetics of human eosinophils in healthy subjects. *Blood* 2012;120:4068–4071.

Copyright © 2013 by the American Thoracic Society

Emerging Human Middle East Respiratory Syndrome Coronavirus Causes Widespread Infection and Alveolar Damage in Human Lungs

To the Editor:

Middle East respiratory syndrome coronavirus (MERS-CoV) has alerted the public health systems by causing lethal respiratory disease in 54 of 114 confirmed human cases as of September 7, 2013 (1, 2). Coronaviruses represent a diverse family of enveloped, single-stranded positive-sense RNA viruses. In humans, the four endemic coronaviruses HCoV-229E, -OC43, -NL63, and -HKU1 are known to cause mild respiratory symptoms in the majority of cases (3). In contrast, the phylogenetically distinct severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) caused a global outbreak of severe lower respiratory tract disease (4). The clinical symptoms observed in several MERS-CoV-infected humans were related to pneumonia and severe acute lung injury (2) and closely resembled those observed in individuals suffering from SARS (4). Because autopsy studies have not been reported, essential information is missing about the virus- and host-dependent processes underlying MERS-CoV-related lung damage. Herein, we used an *ex vivo* model of human lung tissue infection (5) and a bronchoalveolar lavage (BAL) sample from a patient with MERS-CoV to describe MERS-CoV replication, tropism, dipeptidyl peptidase 4 (DPP4) receptor expression, and virus-related lung tissue damage.

Ex vivo infection of human lung tissue with MERS-CoV followed by spectral confocal microscopy (methods are available in the online supplement) revealed a widespread cellular distribution of viral antigen in alveolar tissue using antisera

from infected patients (Figure 1A and Figures E1A and E1B in the online supplement), whereas mock-infected tissue or serum from a healthy donor remained negative (Figures E1A and E1B). As expected for coronavirus, antigen was detected exclusively in the cytosol (Figure E2) (6). Growth curve analyses demonstrated a titer increase of infectious MERS-CoV in the supernatants of infected human lung tissue by more than two orders of magnitude within 48 hours (Figure 1B). This was comparable in extent and kinetics to parallel infections of tissue from the same donors with highly pathogenic avian H5N1 influenza A virus known to cause lethal lung disease in humans (5, 7) (Figure 1B). Double infection of lung specimen with both viruses revealed extensive alveolar infection by MERS-CoV, whereas H5N1 influenza A virus infected only type II cells (5, 7) (Figure 1C).

The investigation of MERS-CoV tropism revealed viral antigen in ciliated bronchial epithelium (Figure 2A) as well as in unciliated cuboid cells of terminal bronchi located in the area of bronchial-alveolar transition (Figure 2B). Lung type I cells comprise approximately 95% of the alveolar surface and therefore form the major area of the lung, being critical for gas exchange (8). Type II cells are crucial for basic lung functions like surfactant production and tissue repair (9). Immunofluorescence (Figure 2C) and electron microscopy (Figures E3 and E4) showed strong MERS-CoV antigen expression and identified intra- and extracellular virions in different stages of the replication cycle

Figure 1. Propagation of Middle East respiratory syndrome coronavirus (MERS-CoV) and detection of viral antigen in *ex vivo* infected human lung tissue. (A) Human lung tissue was infected with MERS-CoV for 24 hours and antigen was detected in virus-infected lung tissue using human MERS-CoV antiserum (20) (green). Scale bar = 20 μ m. (B) Human lung explants were infected with either MERS-CoV or H5N1 influenza A/Thailand/1 (Kan-1)/2004 virus (H5N1). Supernatants were collected at the indicated time points and titrated by plaque assay on Madin-Darby canine kidney (MDCK) cells (Thai/04 (H5N1)) or Vero cells (MERS-CoV). Mean values $\pm$ SEM of duplicates of four independent experiments are shown. PFU = plaque-forming units; p.i. = post-infection. (C) Lung tissue specimens were simultaneously infected with MERS-CoV and Thai/04 (H5N1) for 24 hours. Viral antigens were stained with MERS-CoV antiserum (green) and influenza A-specific antibody (red). Scale bar = 5 μ m. All nuclei (blue) were counterstained with 4',6-diamidino-2-phenylindole (DAPI).