A NOVEL APPROACH WITH MAGNETIC RESONANCE IMAGING USED FOR THE DETECTION OF LUNG ALLOGRAFT REJECTION

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Objective: Although various techniques have been explored for the detection and quantification of allograft transplant rejection, a practical and reliable method that is noninvasive is still elusive.

Methods: For our magnetic resonance imaging experiments, we have developed a new rat model of heterotopic lung transplantation to the inguinal region. Allogeneic transplants (DA to Brown Norway) were performed with and without cyclosporine A (INN: ciclosporin) treatment, with syngeneic transplants (Brown Norway to Brown Norway) serving as controls (n = 6 per group). Magnetic resonance images were obtained with a gradient echo method before and after injection of ultra-small superparamagnetic iron oxide particles.

Results: At day 5, allogeneic transplants without cyclosporine A treatment showed a grade 4 rejection histologically. A significantly lower magnetic resonance signal was seen 24 hours after injection of ultra-small superparamagnetic iron oxide particles compared with the preinjection image (346 ± 7.6 vs 839 ± 43.4 arbitrary units; $P < .05$). Syngeneic transplants showed no evidence of rejection histologically and no differences in magnetic resonance imaging signals between the images before and after injection of ultra-small superparamagnetic iron oxide particles (863 ± 18.8 vs 880 ± 22.5). Allotransplants treated with cyclosporine A showed a grade 2 rejection histologically. The change in magnetic resonance signals in that group was small but showed a significant decrease in signal intensity after injection (646 ± 10.5 vs 889 ± 23.5 , $P < .05$). Immunohistochemistry and iron staining of the allografts indicated that ultra-small superparamagnetic iron oxide particles were taken up by the infiltrating macrophages that accumulated at the rejection site.

Conclusions: We have demonstrated a novel approach for the detection of acute lung allograft rejection using magnetic resonance imaging coupled with injection of ultra-small superparamagnetic iron oxide particles. Despite its limitations, our method might be a first step toward a potential clinical application. (J Thorac Cardiovasc Surg 2000;120:923-34)

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Organ transplantation has now been recognized as a therapeutic procedure for end-stage organ failure. For lung transplantation, the rate of survival and quality of life of the patients have been improving steadily; however, several problems are still to be overcome. Acute and chronic lung rejections continue to be the major reason for graft loss and contribute significantly to the morbidity and mortality after transplantation.

Although various techniques for the detection and quantification of lung allograft rejection have been explored, practical and noninvasive diagnoses of acute rejection after transplantation are still elusive. The technique of biopsy for routine rejection surveillance has contributed to the progressive decline in deaths as a result of acute rejection. However, this technique is invasive, costly, and prone to sampling error. Also, interpretation of biopsy specimens is somewhat subjective and possibly arbitrary. A number of noninvasive approaches for detecting lung transplant rejection have been proposed—an analysis of bronchoalveolar lavage fluid,¹ Doppler measurement of pulmonary circulatory parameters,² radio-spirometry,³ pulmonary lymphoscintigraphy,⁴ high-resolution computed tomography,⁵ and detection with indium 111-labeled lymphocytes.⁶ However, none of these approaches has gained widespread clinical use. A sensitive and noninvasive method of detecting rejection is still being sought.

Ultra-small superparamagnetic iron oxide (USPIO) particles enhance relaxation times in magnetic resonance imaging (MRI). This property has been exploited in the use of USPIO in magnetic resonance lymphography,⁷ where the accumulation of USPIO in the cytoplasm of macrophages within lymph nodes is detected, and also in labeling T cells *in vitro*^{8,9} for tracking purposes *in vivo*.¹⁰ Recently, USPIO has been used for tracking infiltrating renal macrophages in an experimental model of nephrotic syndrome.¹¹ In a preliminary report, we¹² suggested that USPIO particles are useful to detect the accumulation of macrophages in a rat model for renal allograft rejection. Conventional superparamagnetic iron oxide (SPIO) particles, which are 100 to 1000 nm in diameter, are rapidly taken up by the mononuclear phagocytic system and cleared from blood within minutes after intravenous infusion, whereas USPIO particles are not immediately trapped by the mononuclear phagocytic system of the liver and spleen. USPIO particles have a longer half-life of about 2 hours in blood and are found to be present in lymph nodes.¹³

In this article, we present a novel MRI method to detect lung rejection by injection of USPIO particles. It is a simple, noninvasive, highly sensitive, and safe method without the use of radioisotopes.

Conventional intra-abdominal heterotopic heart and lung transplantation in the rat was described originally by Lee and associates¹⁴ and modified by Fox and Montorsi.¹⁵ This conventional model of transplantation is not suitable for MRI of lungs because of the abdominal and respiratory interference during MR signal acquisition. For *in vivo* experimental study, we have developed a new rat model of heterotopic lung transplantation to the inguinal region so that the MR signal can be detected effectively, independent of the interference of abdominal gas, bowel movement, and respiratory motion.

Materials and methods

Animals. Inbred Brown Norway (BN; RT1ⁿ) and DA (RT1^a) male rats, 2 to 3 months of age, weighing 220 to 250 g each, were obtained from Harlan Sprague-Dawley, Inc (Indianapolis, Ind). Animals were housed individually and provided food and water *ad libitum*. Animal protocols were approved by the Institutional Animal Care and Use Committee of Carnegie Mellon University. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" and the "Guide for the Care and Use of Laboratory Animals," published by the National Institutes of Health (NIH Publication No. 96-03, revised 1996).

Heart and lung transplantation. For the operation, both donor and recipient were anesthetized with an intraperitoneal injection of pentobarbital sodium (35 mg/kg body weight) and heparin (500 U/kg body weight). In the syngeneic group, an en bloc donor heart and lung were taken from a BN rat and transplanted to another BN rat. In the allogeneic group, a graft from a DA rat was transplanted to a BN rat. The graft survival was monitored every day by palpating the transplanted heart. One group of allotransplants (*n* = 6) received subcutaneous injection of cyclosporine A (CsA; 3 mg/kg) from day 0 to day 4, whereas the second group of allotransplants (*n* = 6) and the syngeneic group (*n* = 6) did not receive immunosuppressive therapy.

Donor. The anterior chest and abdomen were prepared in routine surgical fashion with aseptic technique. After the chest wall was opened, the left lung was ligated and excised. The azygos vein with the left and right superior venae cavae were ligated and divided. The descending thoracic aorta was transected, and 10 mL of cold University of Wisconsin solution (Dupont Pharma, Wilmington, Del) was infused into the inferior vena cava until the fluid draining from the aorta was clear. The inferior vena cava was then ligated and divided. The ascending aorta was dissected and transected at the portion between the left common carotid artery and the left subclavian artery, followed by ligation and division of the right brachiocephalic artery and the left common carotid artery. After removal of the heart and lung from the donor, the right lung was washed 3 times through the bronchus with University of Wisconsin solution containing penicillin G. The grafts were then placed in cold University of Wisconsin solu-

Fig 1. Gradient-echo MR images of macrophage samples in vitro. Cells were prepared as described in the “Methods” section. Cell concentration of the samples was 2.0×10^6 cells/mL of 5% gelatin in **A** and **B** and 0.5×10^6 cells/mL gelatin in **C** and **D**. Cells treated with USPIO particles are shown in **A** and **C** and cells not treated with USPIO particles are shown in **B** and **D**.

tion until transplantation. Right before being sutured, the graft was flushed with cold saline solution to wash out the University of Wisconsin solution.

Recipient. The left inguinal portion was opened and dissected to make enough space for the transplanted organs. The left lower part of the abdominal wall was opened in a transverse fashion from the left femoral vessels to the midline. The abdominal organs were retracted to the right and both the aorta and the inferior vena cava just beyond the bifurcation were dissected. The vessels were clamped and an appropriate opening of the aorta was made to receive the aorta of the graft in an end-to-side fashion with 8-0 polypropylene sutures (Ethicon, Inc, Somerville, NJ). Rhythmic heartbeats commenced spontaneously as the heart and the lung regained circulation after removal of the clamp. After hemostasis of the surgical field, the abdominal wall was sutured with 6-0 silk, with care taken to avoid kinking or obstructing the aorta of the graft.

Preparation of USPIO particles. Dextran-coated USPIO particles were synthesized in our laboratory according to the method of Palmacci and Josephson¹⁶ with slight modifications. Quantitative determination of iron in the particle suspension was performed for all samples by means of a spectrophotometric method.¹⁷ Iron-core size was measured by transmission electron microscopy (TEM) and found to be in the range 4.0 to 7.5 nm. Mean diameter of whole particles measured by laser light scattering was 29 ± 3 nm. The MR relaxivities, R_1 (spin-lattice relaxation rate constant $1/T_1$ per mole of iron in USPIO) and R_2 (spin-spin relaxation rate constant $1/T_2$ per mole of iron in USPIO), at 4.7 T were $3.8 \times 10^4 \text{ mol}^{-1} \cdot \text{sec}^{-1}$ and $9.1 \times 10^4 \text{ mol}^{-1} \cdot \text{sec}^{-1}$, respectively. Before infusion, a portion of the stock suspension of USPIO particles was dialyzed in phosphate-buffered saline solution, filter sterilized, and assayed for iron content.¹⁷ The solution was diluted with phos-

Fig 2. A new heterotopic lung transplantation model suited for MR signal detection. An en bloc heart and right lung were transplanted to the groin area of the recipient. The gross view of the surgical field is shown in the *left panel*. The transplanted heart is indicated by the *arrow* and the transplanted lung is indicated by the *arrowhead*. The aorta of the graft was connected to the abdominal aorta of the recipient. The transplanted grafts were isolated from abdominal organs by the abdominal wall and put in the subcutaneous tissue. A representative MR image of the allograft on postoperative day 4 in the coronal plane is shown in the *right panel*. The area encompassed by the *dotted line* is the transplanted graft. The lung is indicated by the *arrowhead*.

phate-buffered saline solution to a concentration of 9 μmol iron per milliliter, and 0.8 mL of the suspension was injected intravenously for each study.

In vitro MRI and TEM assessment of the uptake of USPIO particles by macrophages. The ability of macrophages to phagocytose USPIO particles was assessed in vitro by exposing a macrophage cell population to USPIO particles, followed by MRI. Macrophages were isolated from spleens of BN rats according to the method of Coligan and colleagues¹⁸ and cultured in reconstituted RPMI 1640 culture medium (Roswell Park Memorial Institute, Buffalo, NY) under 5% carbon dioxide at 37°C. The cells were then incubated with USPIO (2 mg iron/10 million cells) for 20 hours and recovered with 3 washes of phosphate-buffered saline solution. High-resolution gradient-echo MR images of labeled macrophage cell phantoms (containing 2×10^6 cells/mL and 0.5×10^6 cells/mL) were obtained with a 7-T, 15-cm horizontal bore Bruker AVANCE DRX MR instrument equipped with a 4.3-cm microimaging gradient set.⁸ Acquisition parameters were as follows: repetition time/echo time (TR/TE) = 1000/30 ms; flip angle = 45°; matrix size = 256×256 , giving an in-plane resolution of $50 \times 50 \mu\text{m}$; slice thickness = 0.1 mm; acquisition bandwidth = 25 kHz; number of averages = 4; and scan time = 17 minutes. TEM was performed to determine the location of USPIO particles in the macrophages. The MRI and TEM studies on macrophages were carried out by following the procedure described earlier for the characterization of USPIO-labeled T cells.⁸

In vivo MRI experiments. MRI measurements in vivo were carried out on a 4.7-T Bruker AVANCE DRX MR

instrument equipped with a 40-cm horizontal bore superconducting solenoid. In vivo MR images of transplanted heart-lung tissue were obtained at indicated time points for 24 hours after infusion of USPIO particles. The imaging sequence consisted of a gradient echo sequence, triggered to the electrocardiogram: TR/TE = 1000/10 ms, flip angle = Ernst angle; slice thickness = 1 mm, field of view = 6.0 cm; data matrix size = 256×130 (zero-filled to 256×256); and scan time = 5 minutes. Electrocardiographic gating was used to limit artifacts from the heartbeat. The change of MRI signal intensity was measured in 5 different regions in each transplanted lung.

Histologic analysis and immunohistochemistry. After each MR experiment, the transplanted lung was extirpated, fixed in 4% paraformaldehyde, embedded in paraffin, and processed for 5- μm sections. Hematoxylin-eosin staining and Perls' Prussian blue staining were performed in the Transplantation Pathology Laboratory of the University of Pittsburgh Medical Center. Histologic analysis for pathologic grading of rejection, which is based on the criteria established by the International Society for Heart and Lung Transplantation, was also performed by that laboratory in a blind manner. Monoclonal anti-rat macrophage antibody (ED1; Serotec Ltd, Oxford, United Kingdom) and anti-CD3 antibody (DAKO, Carpinteria, Calif) were used as the primary antibody for macrophage lineage cells and T cells, respectively. Immunohistochemistry was carried out with the ABC staining system (Santa Cruz Biotechnology, Inc, Santa Cruz, Calif) according to the manufacturer's protocol.

Fig 3. MR images showing the effect of USPIO particle infusion in an allotransplant. MR images of the coronal view of the graft are shown in **A** and **B**, and the sagittal view of the graft is shown in **C** and **D**. The MR images of the graft on postoperative day 4 before USPIO infusion are at **A** and **C**, and the images taken 24 hours after infusion are at **B** and **D**. The lung is indicated by the arrow in each panel. Representative images are shown.

Statistical analysis. The results are presented as mean $\pm$ standard deviation. Nonparametric data were analyzed by the Mann-Whitney test, and the remaining data were analyzed by analysis of variance with the use of StatView software (SAS Institute Inc, Cary, NC).

Results

In vitro MR and TEM properties of USPIO-labeled macrophages. An in vitro assessment of macrophage cell phantoms by MRI was performed to investigate the labeling efficiency of USPIO particles.

MR images of USPIO-labeled and unlabeled macrophages in 5% gelatin are shown in Fig 1. In Fig 1, **A**, a phantom consisting of 2×10^6 cells/mL of macrophages labeled with USPIO showed numerous dark spots in MRI, whereas a phantom with the same number of unlabeled macrophages in Fig 1, **B**, did not show any spots. Fig 1, **C** and **D**, showed USPIO-labeled and unlabeled samples, respectively, with a concentration of 0.5×10^6 macrophages/mL. On the basis of our previous USPIO-labeled T-cells result,^{8,12} we believe that each dark spot in the labeled samples represents a sin-

Fig 4. MR images showing the effect of USPIO particle infusion in an isograft. MR images of the coronal view of the graft are shown. The MR images of the isograft on postoperative day 4 before USPIO infusion are at **A** and **C**, and the images taken 24 hours after infusion are at **B** and **D**. The lung is indicated by the *arrow* in each panel. Representative images are shown.

gle USPIO-labeled macrophage (Fig 1, A and C). The density of these dark spots appeared to be dependent on the concentration of macrophages. TEM results indicated that the labeling efficiency of macrophages by USPIO particles in vitro was nearly 80% (results not shown).

In vivo MR experiments on the lung transplantation model. For in vivo MR experiments, we have developed a new rat model of lung transplantation. In this model, an en bloc heart and lung graft was transplanted to the groin area of the recipient to detect MR signals more effectively, shielded from abdominal and respiratory interference. Fig 2 showed a gross view of

the surgical area and a typical horizontal MR image of this model.

Representative MR images of each of the experimental groups on postoperative day 4 before injection of USPIO particles and 24 hours after injection are shown in Figs 3, 4, and 5. A clear decrease in MR signal intensity was demonstrated in the image of the allogeneic transplanted lung 24 hours after infusion of USPIO particles compared with the preinfusion signal (Fig 3). In contrast, there was no significant MR signal change in the syngeneic transplanted lung (Fig 4) before and 24 hours after USPIO infusion. In the CsA-treated group, a significant decrease in MR signal was seen in the trans-

Fig 5. MR images showing the effect of USPIO particle infusion in the allotransplant treated with cyclosporine A (CsA). MR images of the coronal view of the graft are shown. The MR images of the transplanted lung on post-operative day 4 before USPIO infusion are at **A** and **C**, and the images taken 24 hours after infusion are at **B** and **D**. The lung is indicated by the arrow in each panel. Representative images are shown.

planted lung 24 hours after USPIO infusion compared with the preinfusion signal (Fig 5). Fig 6 summarizes the MR signals in each group during the experiment. No significant difference of the MR signal intensity among the groups was apparent before USPIO infusion. However, a significant decrease was detected in MR signal intensity in the allograft group without CsA treatment compared with the isograft group (346 ± 18.5 in the allograft group vs 863 ± 46.1 in the isograft group; $P < .001$) 24 hours after infusion of the USPIO particles (Fig 6). In addition, a significant decrease in MR signal intensity was also observed in the group of allografts

treated with CsA compared with the isograft group (646 ± 28.5 vs 863 ± 46.1 , $P < .001$). There is a significant difference in MR signal intensity between allografts with or without CsA treatment as well ($P < .01$).

The in vivo half-life of USPIO in blood after infusion into a rat measured in our laboratory is about 120 minutes, which is consistent with the data previously reported by other laboratories.^{11,13}

Histologic and immunohistochemical analyses of the lung allografts. Histologic analysis of the graft showed a significant difference in rejection grades between the allograft group (all 6 in grade 4) and the

Fig 6. Summary of the measurements of MR signal intensity in transplanted lungs. MR signal intensity was measured at 5 different points of the horizontal image of each transplanted lung. Data are indicated as mean $\pm$ standard deviation of the mean, shown as arbitrary units ($n = 30$, 5 points/rat). Allografts (solid square) showed significantly lower MR intensity compared with isografts (open square) at every time point after USPIO infusion. Allografts with CsA treatment (diamond) also showed significantly lower MR intensity compared with isografts. There was also a significant difference between allografts with or without CsA treatment.

allograft group with CsA treatment (5 in grade 2, 1 in grade 3; $P = .009$).

ED1⁺ cells and CD3⁺ cells were detected immunohistochemically in each allograft from paraffin sections. ED1⁺ and CD3⁺ cells were significantly increased in the allografts without CsA treatment compared with the isograft group (Fig 7), whereas in the allografts with CsA treatment, ED1⁺ cells were also significantly increased compared with the isograft.

Perls' Prussian blue staining in the allografts showed that the distribution of iron particles correlates well with ED1⁺ cells but not with CD3⁺ cells (Fig 8). Perls' Prussian blue staining in a high-power field demonstrated that stained iron particles were observed in the cytoplasm of macrophages (Fig 9). In addition, few iron particles and ED1⁺ cells were found in the parenchyma of native lungs of the animals or in the transplanted lung in the syngeneic group. These results suggest that ED1⁺ cells are actually involved in the rejection process.

Taken together, these results indicate that the iron particles, which are phagocytosed by the macrophages, modulate MR signal intensity at the rejection site of the allograft.

Discussion

In these experiments, we have aimed to establish a new noninvasive method using MRI to detect lung rejection after transplantation. We show here that USPIO particles can be used for in vivo labeling of macrophages, which accumulate at the rejection sites after allogeneic organ transplantation. Macrophages labeled with USPIO particles induce a significant decrease in MR signal intensity in the allograft, and the decrease has an excellent correlation with the pathologic rejection grade.

MRI is a noninvasive diagnostic modality. However, in the conventional experimental model of heterotopic lung transplantation, abdominal gas, bowel movement, and respiratory motion may interfere with the MR sig-

Fig 7. Immunohistologic counts of CD3⁺ or ED1⁺ cells. The CD3⁺ or ED1⁺ cells were counted in at least 10 fields per section per rat (n = 6 in each group). The fields were randomly chosen and counting was performed in a blinded manner at a magnification of $\times 100$. Data are presented as mean $\pm$ standard deviation of the mean.

nal. Therefore, we developed a new experimental model in which the graft is transplanted to the groin area of the recipient animal. We are able to detect MR signals more effectively in this model, free from any abdominal and respiratory interference.

Cell-specific imaging is an increasingly important field of MR imaging.^{7,9,13} USPIO/SPIO particles have been used clinically for several years as contrast agents in the liver and spleen since they accumulate in the mononuclear phagocyte system of those organs and induce a decrease in MR signal intensity due to iron magnetic susceptibility.⁷ Recently, USPIO particles have been used for lymphography because of their long half-life in vivo.^{11,13} Due to this property in vivo, it is possible for USPIO particles to be trapped by macrophages in the whole body, whereas SPIO particles are mostly trapped by liver and spleen immediately after infusion. Although USPIO particles have been used to detect macrophage infiltration in the nephrotic syndrome model,¹¹ there has been no report applying this technique to the detection of lung rejection.

Acute and chronic organ rejection remain the most critical problems to be overcome in caring for transplant recipients. Frequent episodes of acute rejection are highly associated with the development of chronic rejection,¹⁹ although pathophysiologic mechanisms for this irreversible process remain unclear. An accurate diagnosis of rejection is necessary for appropriate management of patients who have had lung transplants. Currently, biopsy is the most reliable technique in mak-

ing the diagnosis of rejection clinically. A noninvasive, clinically reliable technique for detection of acute and chronic rejection has been sought since none of the previously proposed methods have been widely accepted.

We previously reported that T cells labeled with USPIO particles in vitro^{8,9} could be detected in vivo with MRI,¹⁰ giving rise to the possibility of a noninvasive method to detect rejection. Both T cells and macrophages are involved in the process of acute rejection, as demonstrated immunohistologically. Here, we have shown an approach to detect acute lung rejection with MRI, using macrophages labeled with USPIO particles in vivo. The ability of macrophages to ingest USPIO particles in vitro was 8 to 16 times higher than that of T cells (see Fig 1 and reference 8). The in vivo ability of macrophages to ingest USPIO was also higher than that of T cells, as indicated by the distribution of iron staining in ED1⁺ cells and CD3⁺ cells. Since MR signal changes are detected more effectively when labeled macrophages are used, it is reasonable to use labeled macrophages for tracking cell migration in vivo with MRI for the purpose of detecting rejection. This labeling procedure readily shows a difference in the MR signal intensity between allograft and isograft and appears to have an excellent correlation with the pathologic rejection grade.

Although this noninvasive MRI technique is promising, there are inherent limitations. First, because USPIO particles are primarily ingested by macrophages, it remains unclear whether this tech-

Fig 8. Histologic and immunohistochemical analyses of an allograft. Perls' Prussian blue reaction shows the distribution of the iron-containing cells (**A**). **B** and **C** show ED1⁺ or CD3⁺ cells, respectively. Comparing **B** with **A**, the distribution of the iron-containing cells completely correlates with the presence of ED1⁺ cells, whereas, comparing **C** with **A**, the distribution of CD3⁺ cells does not correlate with that of iron staining. Figures were at a magnification of $\times 100$.

nique can distinguish between infection and rejection processes, both of which involve macrophages. Further investigation is necessary to elucidate and overcome this limitation. Since USPIO particles are primarily taken up by macrophages and much less by T cells (the labeling efficiency for macrophages is ~80% vs ~20% for T cells⁸), the MRI results may not fully reflect the antigen-specific immunologic response of T cells. In addition, it is quite difficult to obtain clear lung MR images with current MR tech-

niques. Imaging needs to be improved before our methods can be applied clinically.

Despite these limitations, *in vivo* labeling of macrophages does offer a potential clinical application for detection of organ rejection. A number of noninvasive approaches for detecting organ transplant rejection have been proposed. However, none of these approaches has been accepted for clinical use. The MRI method that we have demonstrated here may be a first step toward a clinical application.

Fig 9. A high-power field image of Perls' Prussian blue iron staining of an allograft 5 days after transplantation. The spots of iron particles are found in the cytoplasm of macrophages. Magnification was $\times 400$.

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Discussion

Dr Steven J. Mentzer (*Boston, Mass*). This study extends this group's previous work using iron oxide particles from the labeling of single cells to the labeling of cells infiltrating a rejecting allograft. It is a first step toward a future in which a noninvasive scan can provide both an anatomic and potentially a functional assessment of the inflammatory reaction. How convenient it would be to send our patients for a scan to diagnose acute rejection.

At present, however, there appear to be several obstacles to a noninvasive diagnosis of allograft rejection. I would like Dr Kanno to address 3 of these obstacles. First, the lung is notoriously difficult to image by means of conventional proton MRI, primarily because of the air and the inhomogeneity of the lung parenchyma. MRI scans of the lung frequently look dark, with poor anatomic resolution. In your studies, the iron oxide particles appear to further darken the regions of interest. How limiting are these lung-specific characteristics, and do you think this technique might be better applied to other organs?

Second, T cells are generally thought to play a dominant role in antigen-specific allograft rejection. T cells, as demonstrated by your group's previous work, also take up the iron particles. Macrophages, on the other hand, are intimately involved in many different types of unrelated inflammatory reactions. Do you have any plans to more specifically target the T-cell component of the inflammatory reaction?

Third, an advantage of using an inguinal model of lung transplantation is that there is no movement in the lung, yet you used electrocardiographic gating of the scan. Do you see respiratory movement as a potential limitation of these scans when applied to the diagnosis of acute lung rejection?

The ultimate goal of any diagnostic test of acute transplant rejection is the ability to discriminate between infection and

rejection. The authors appropriately note that this objective remains in the future; nonetheless, I congratulate them for taking a creative and thoughtful step toward this goal.

Dr Thomas M. Egan (*Chapel Hill, NC*). It is an interesting study. Do you know how specific the findings are? Have you done any studies in lungs that are infected rather than lungs that are rejecting? My suspicion is that you would have increased macrophage numbers and therefore a positive scan. From a clinical standpoint, that is usually the problem: differentiating rejection from infection.

Dr Malcolm M. DeCamp (*Cleveland, Ohio*). Addressing the specificity of this technique to look for allograft rejection, could you also comment on how you could use it to discriminate ischemia/reperfusion injury and other global inflammatory changes from specific immune inflammatory changes?

The histologic hallmark of cell-mediated acute rejection is an angiocentric lymphocytic response, yet the macrophages did not appear necessarily to be oriented around the vessels. Could you comment on the dissociation between the T cells that seemed to be infiltrating the biopsy specimens and the iron-labeled macrophages on your photomicrographs?

Dr Kanno. Thank you for your comments.

The conventional MRI technique for lung imaging is very difficult, so in this work we collapsed the lungs. However, now we are trying to do orthotopic lung transplantation in the rat with ventilated lungs, and we are trying to take MR images by using 100% oxygen as a contrast agent. This is very preliminary, but we were successful in distinguishing macrophage infiltration even in the ventilated lung. Of course, we need to improve our MRI technique for the lung.

Regarding T cells and macrophages, the macrophage has a very nonspecific infiltration into the lung, even in infection and rejection. We first used T-cell labeling with USPIO particles, but the labeling efficiency of T cells is very low compared with that of macrophages. That may be the reason that the distribution of T cells is not correlated with that of USPIO particles in the pathologic slides. Therefore, we changed to using macrophages for targeting and tracking by MRI. This is also preliminary, but the distribution of the macrophages in infection and rejection is somewhat different in MR images. The next step for us in differentiating infection from rejection is looking for the distribution changes in MR images, but this is a very challenging task. Our preliminary results indicate that reperfusion injury is different from rejection in the distribution of the macrophages. We still have to do many things to distinguish rejection from infection or reperfusion injury.