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Miyamoto, Ei. Association of Local Intrapulmonary Production of Antibodies Specific to Donor Major Histocompatibility Complex Class I With the Progression of Chronic Rejection of Lung Allografts. 京都大学, 2021, 博士(医学)

2021-03-23

https://doi.org/10.14989/doctor.k23100

Association of Local Intrapulmonary Production of Antibodies Specific to Donor Major Histocompatibility Complex Class I With the Progression of Chronic Rejection of Lung Allografts. Transplantation: May 2017 - Volume 101 - Issue 5 - p e156-e165 doi: 10.1097/TP.0000000000001665
Association of Local Intrapulmonary Production of Antibodies Specific to Donor Major Histocompatibility Complex Class I With the Progression of Chronic Rejection of Lung Allografts

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Background. Antibody-mediated rejection may lead to chronic lung allograft dysfunction, but antibody-mediated rejection may develop in the absence of detectable donor-specific antibody (DSA) in recipient serum. This study investigated whether humoral immune responses develop not only systemically but locally within rejected lung allografts, resulting in local production of DSA.

Methods. Lewis rats received orthotopic left lung transplantation from Lewis (syngeneic control) or Brown-Norway (major histocompatibility complex-mismatched allogeneic) donor rats. Rats that underwent allogeneic lung transplantation were subsequently administered cyclosporine until day 14 (short immunosuppression) or day 35 (long immunosuppression). The lung grafts and spleens of recipient animals were tissue cultured for 4 days, and the titer of antibody against donor major histocompatibility complex molecules was assayed by flow cytometry. Explanted lung grafts were also evaluated pathologically.

Results. By day 98, DSA titers in supernatants of lung graft (P = 0.0074) and spleen (P = 0.0167) cultures, but not serum, from the short immunosuppression group were significantly higher than titers in syngeneic controls. Cultures and sera from the long immunosuppression group showed no production of DSA. Microscopically, the lung grafts from the short immunosuppression group showed severe bronchiolitis obliterans and parenchymal fibrosis, along with lymphoid aggregates containing T and B cells, accompanying plasma cells. These findings suggest that local humoral immune responses were not observed by days 28 and 63.

Conclusions. DSA can be locally produced in chronically rejected lung allografts, along with intragraft immunocompetent cells. Clinical testing of DSA in serum samples alone may underestimate lung allograft dysfunction.

(Transplantation 2017;101: e156–e165)

Antibody-mediated rejection (AMR) after lung transplantation (LTx) remains a major cause of graft failure, hampering long-term survival of recipients. Diagnostic criteria for AMR include evidence of donor-specific antibody (DSA) in recipient serum, physiological deterioration, positive histology suggestive of AMR, and positive staining of lung grafts for complement component 4d (C4d). Especially, serum DSAs were reported to have a significant impact on the development of AMR, mixed rejection, and even chronic lung allograft dysfunction. Although the presence of DSAs and humoral rejection after LTx are considered as efficacy endpoints of immunosuppression (IS) drugs, their...
predictive value for long-term outcome is still controversial. The current animal model could be used to better demonstrate the existence of locally produced DSA and/or non-DSA, and the causal relationship between AMR and CLAD. Allograft dysfunction may develop or progress in the absence of detectable serum DSA. These absences have been traditionally interpreted as being due to phasic release of DSA, limitations of the diagnostic test, or absorption of DSA into lung grafts. Paradoxically, false-negative findings of serum DSA remain an important unresolved obstacle that may result in underdiagnosis of subclinical AMR. DSAs were recently identified in eluates from lung graft biopsies, as well as in other solid organ transplant recipients, suggesting that analyzing only serum DSA may underestimate the occurrence of DSA bound to donor tissue. Over the last decade, it has become clear that tertiary lymphoid tissues (TLTs) are associated with allograft rejection after solid organ transplantation. Intragraft TLT, also called de novo lymphoid tissue, contains both T cells and B cells. These cells may generate alloantibodies, suggesting that intragraft lymphoid tissue may be a source of alloantibodies. Intrapulmonary lymphoid neogenesis has been observed in the lung allografts of transplant recipients who developed obliterative bronchiolitis (OB). Moreover, stable homing of recipient-derived T cells and their donor-specific effector function have been demonstrated in orthotopically transplanted lung allografts of major histocompatibility complex (MHC) mismatched rats. Intrapulmonary de novo lymphoid tissue was shown to have the ability to reject allograft airways, even in the absence of secondary lymphoid organs. To date, however, it remains unclear whether humoral alloimmune responses could be elicited locally in lung allografts and could contribute to allograft dysfunction. We hypothesized that humoral immune responses could be activated not only systematically but also locally within rejected lung allografts. Because localized antigen-antibody reactions may be independent of the systemic circulation, this hypothesis may explain the progressive deterioration of lung grafts in the absence of circulating DSA. To test this hypothesis, we used an MHC-mismatched rat orthotopic LTx model to investigate whether antibodies directed against alloantigens are produced locally in lung allografts undergoing rejection, along with intragraft lymphoid neogenesis.

MATERIALS AND METHODS

Animal Models

Specific pathogen-free inbred male rats weighing 250 to 300 g were obtained from Japan SLC (Hamamatsu, Japan). All animals received adequate care according to the animal protocols approved by the Kyoto University Institutional Animal Care and Use Committee (MedKyo15525). Lewis rats were used as recipients and as syngeneic donors, and Brown-Norway rats were used as MHC fully mismatched donors. Orthotopic left LTx was performed as previously described. Briefly, the donor rat was mechanically ventilated with room air during the procedure. After intravenous administration of 500 IU heparin, the lungs of each rat were flushed with 20 mL of ET-Kyoto solution (Otsuka, Tokushima, Japan). The heart-lung bloc was removed, and the left lung graft was prepared using plastic cuffs. Recipient rats were ventilated using the same settings as for the donors. After intraperitoneal injection of methylprednisolone sodium (10 mg/rat), the graft was orthotopically transplanted using the cuff technique. The graft was reperfused, the chest cavity was closed, and the recipient rat allowed to recover from general anesthesia.

IS Protocols

Allogeneic LTx recipients were treated with cyclosporine A (CsA), administered subcutaneously at a dose of 25 mg/kg per day, on posttransplant days 0, 1, 2, and 4 (induction period) and twice weekly thereafter (maintenance period) until day 14 (short IS protocol) or day 35 (long IS protocol), after which immunosuppressive therapy was discontinued (free period) (Figure 1). Syngeneic recipients were not administered CsA. Each group consisted of 5 animals. Allogeneically transplanted rats administered the short IS protocol were sacrificed on posttransplant days 28, 63, or 98, and those administered the long IS protocol were sacrificed on day 98. Syngeneically transplanted control rats were sacrificed on day 28 or day 98. Briefly, blood samples were obtained from each rat, followed by intravenous administration of 500 IU heparin. After flushing with 10-mL cold saline, the lungs and spleen were removed. The explanted lung graft and the spleen were dissected for tissue culture, and the remaining lung was inflated by injecting 10% formalin into the trachea at a pressure of 30 cm H₂O for fixation.

Tissue Culture

Each explanted lung graft and spleen was placed in 5 mL of cold sterile X-VIVO 15 serum-free medium (Lonza; Chiba, Japan) containing 100 units/mL of penicillin/streptomycin (Thermo Fisher Scientific K.K.; Yokohama, Japan) and 25 μg/mL of amphotericin B (Thermo Fisher Scientific K.K.), as described. The tissue samples were washed 3 times with culture medium, with these washes collected as control samples, and 100 mg of each tissue sample were microdissected with a sterile razor blade in 3 mL of X-VIVO 15 culture medium.

FIGURE 1. Schedule of cyclosporine administration for each IS protocol. Syngeneic control rats did not receive CsA. The IS protocols for allogeneic transplantation consisted of an induction period (CsA administration on days 0, 1, 2, 4, and 7), a maintenance period (CsA twice weekly), and a treatment-free period. Syngeneic recipient rats were sacrificed on day 28 or 63; allogeneic recipient rats that received the short IS protocol were sacrificed on day 28, 63, or 98; and allogeneic recipient rats that received the long IS protocol were sacrificed on day 98. Each group consisted of 5 animals.
medium and cultured in 6-well plates at 37°C under normoxic conditions (20% O₂/0.5% CO₂). After 2 or 4 days, the culture supernatants were recovered.

**Histological Examinations**

Formalin-fixed paraffin-embedded lungs cut into 5-μm-thick sections were stained with hematoxylin-eosin (HE) and Elastica-Masson (EM) trichrome stains. Standard HE staining was used to determine whether perivascular mononuclear cell infiltrates (A grade) were present, based on International Society for Heart and Lung Transplantation criteria. Capillaritis was defined as capillary neutrophilic infiltration with karyorrhexis. The positivity rate for each histological characteristic was defined as the percentage of specimens with that characteristic. Graft vascular sclerosis attributable to fibrointimal thickening of pulmonary vessels was defined as described. The numbers of obliterated and nonobliterated bronchioles were counted in 10 randomly selected high-power fields, and the percentage of obliterated bronchioles in each lung specimen was calculated. The percentage of parenchymal fibrosis was quantified in 5 random EM-stained samples at 200× magnification using ImageJ version 1.49 (National Institutes of Health, Bethesda, MD). All pathological grading and semiquantification were performed by a single pathologist (A.Y.) in a blinded manner.

**Immunohistochemical Staining and Immunofluorescence Labeling**

Formalin-fixed 5-μm-thick paraffin sections were used in all immunohistochemical stainings. After deparaffinization and antigen retrieval in boiling 10 mM citrate buffer (Target Retrieval Solution; DAKO; Tokyo, Japan) for 20 minutes, the sections were blocked and incubated overnight at 4°C with a primary antibody. The following anti-rat primary antibodies were used: anti-CD3 (polyclonal rabbit; 1:200; Abcam, Tokyo, Japan), anti-CD8α (monoclonal mouse; 1:200, Abcam), anti-C4d (polyclonal rabbit; 1:800; Abcam), anti-CD79a (monoclonal mouse; 1:300; Abcam), anti-CD20 (monoclonal mouse; 1:300, DAKO), anti-Ki-67 (polyclonal rabbit; 1:800; Abcam), anti-rat IgG (polyclonal rabbit; 1:1000, Vector Laboratories, Burlingame, CA), anti-C4d (polyclonal rabbit; 1:100; Hycult Biotech, Uden, The Netherlands), anti-CD138 (polyclonal rabbit; 1:100; Proteintech, Chicago, IL), and anti-RT1-B (His 19: monoclonal mouse; 1:300; Santa Cruz Biotechnology, Dallas, TX). The sections were washed and incubated with VECTASTAIN Elite ABC Kits (Vector Laboratories), according to the manufacturer’s instructions, followed by counterstaining with hematoxylin.

**DSA Assays**

The reactivity of antibodies against Brown-Norway rat MHC class I molecules (RT1-A1n) was analyzed by flow cytometry using mouse fibroblasts (L cells; negative control) and L cells transfected with RT1-A1n-expressing retroviral vectors (L cells plus A1n), prepared as described. These cells were cultured in Dulbecco modified Eagle medium (Sigma-Aldrich, St. Louis, MO) containing 10% fetal calf serum and 0.5 mg/mL G418 (Nacalai Tesque, Kyoto, Japan). For flow cytometry, 100-μL aliquots of each serum sample or tissue-culture supernatant were incubated with 200 000 L cells plus A1n or control L cells for 30 minutes at 4°C. After washing with phosphate-buffered saline, the cells were incubated with anti-rat IgG heavy chain and light chain APC-conjugated antibodies (R&D Systems, Minneapolis, MN), followed by flow cytometry using a LSRFortessa flow cytometer (BD Biosciences, San Jose, CA). Mean fluorescence intensity (MFI) was assessed using FlowJo software (TOMY Digital Biology, Tokyo, Japan). Positive and negative control cells, consisting of cultured L plus A1n cells and L cells, respectively, were gated by flow cytometry using a fluorescein isothiocyanate (FITC)-conjugated OX27 monoclonal antibody (mouse anti-RT1-A1n; ebioscience, San Diego, CA). The DSA titer was calculated as the MFI of anti-rat IgG binding to RT1-A1n-positive L cells divided by the MFI of anti-rat IgG binding to RT1-A1n-negative L cells. To validate that RT1-A1n-positive L cells have similar surface expression of donor-MHC molecules, the reactivity of antibodies was also analyzed using Brown-Norway rat T cells as the positive control and Lewis rat T cells as the negative control. Spleenocytes isolated from rat spleens using BD Falcon Cell Strainers (BD Biosciences) were incubated with a FITC-conjugated mouse antirat CD3 antibody (ebioscience) for T cell gating by flow cytometry.

**Statistical Analysis**

All data are presented as means ± standard error of the mean. Student t tests were used to determine differences between 2 groups and 1-way analysis of variance followed by post hoc Tukey tests for differences among 3 or more groups. All statistical analyses were performed used JMP version 11.0.0 (SAS Institute Inc., Cary, NC) statistical software, with P values less than 0.05 considered statistically significant.

**RESULTS**

**Pathological Features of Lung Grafts**

Figure 2 shows a representative explanted lung graft obtained after each transplantation and IS protocol. All syngeneic grafts were well preserved, with slight inflammation, on days 28 and 98. Lung grafts of allogeneically transplanted animals that received the short IS protocol and were sacrificed on days 28 and 63 could be air inflated to a certain degree, with marked inflammatory cell infiltration observed at the circumferences of vessels and Airways. Bronchiole obliteration was often observed in day 63 lung samples. Air inflation was difficult in allogeneic lung grafts of animals that received the short IS protocol and were sacrificed on day 98. These lungs showed intensive spread of fibrotic tissue to the subpleural area, bronchioles and vessels. In contrast, the allogeneic lung grafts in animals that received the long IS protocol and were sacrificed on day 98 were well preserved. Microscopically, perivascular inflammatory cell infiltration was common in these grafts, but fibrotic changes were not apparent.

Figure 3 shows the histological semiquantification of these lung grafts. Grafts of allogeneically transplanted rats that underwent the short IS protocol and were sacrificed on day 28 showed significantly higher A grade than syngeneic controls. Vascular sclerosis scores were significantly higher in the short IS than in the syngeneic control group. There were no significant differences in the percentages of obliterated bronchioles and parenchymal fibrosis. Grafts of genetically transplanted rats that underwent the short IS protocol and were sacrificed on day 98 did not differ significantly in International Society for Heart and Lung Transplantation A grade from syngeneic rats. Vascular sclerosis scores and the percentage of parenchymal fibrosis were significantly higher in the short IS than in the long IS and syngeneic control groups, and the percentage of
obliterated bronchioles was significantly higher in the short IS group than in the syngeneic control group.

Diffuse alveolar damage, alveolar hemorrhage, and neutrophilic capillaritis were observed more frequently in the lung grafts of the short IS group than in grafts of the other 2 groups (Figure 4A). All the explanted lung grafts of the short IS group sacrificed at day 98 showed patchy deposition of C4d in the capillaries (Figure 4A). Alveolar hemorrhage

**FIGURE 2.** Macroscopic and microscopic findings of lung grafts. Macroscopic (upper photographs: arrowheads indicate lung grafts) and microscopic (lower micrographs: EM trichrome staining, scale bars = 50 μm) findings of the lung grafts. The syngeneic lung grafts explanted on days 28 and 98 were well preserved, with infiltration of few inflammatory cells and no obliterated bronchioles. The allogeneic lung grafts of rats that received the short IS protocol and were sacrificed on days 28 and 63 could be air inflated to some degree, with moderate inflammatory cell infiltration around vessels and airways. Some bronchioles were obliterated in grafts obtained on day 63, but not on day 28. The lung grafts of rats that received the short IS protocol and were sacrificed on day 98 were difficult to inflate, with intense collagen fibrosis spreading not only to the subpleural area but throughout the entire lung graft involving the bronchioles and lung parenchyma. The lung grafts of rats that received the long IS protocol were relatively well ventilated, even on day 98. Inflammatory cell infiltration was observed around the vessels and airways, but most of the lung grafts were free of bronchiole obliteration.

**FIGURE 3.** Histological grading and quantification of lung grafts. A, Pathological features of the lung grafts explanted on day 28. A grade and vascular sclerosis were significantly higher in the allogeneic short IS group than in the syngeneic control group. There were no significant differences in the percentages of obliterated bronchioles and parenchymal fibrosis. B, Pathological features of the lung grafts explanted on day 98. Vascular sclerosis grade and the percentage of parenchymal fibrosis were significantly higher in the short IS than in the syngeneic control and long IS groups. The percentage of obliterated bronchioles was significantly higher in the short IS than in the syngeneic control group. ***P < 0.001, **P < 0.01, *P < 0.05. N.S., not significant.
increased over time in the short IS group (Figure 4B; \( P = 0.0236 \), analysis of variance). Neutrophilic capillaritis was observed in all lung grafts of the short IS group sacrificed at day 28, but in only 2 of the 5 lung grafts of the short IS group sacrificed at day 98, with the degree of neutrophilic capillaritis lower in the latter animals.

**Detection of DSA in Supernatants of Lung Graft Culture and Serum**

Figure 5 shows the representative result of DSA analysis using rat T cells. The MFI of APC against Brown-Norway rat T cells was approximately threefold higher than that against Lewis rat T cells.

To confirm the production of DSA against donor MHC class I, we further used mouse fibroblasts expressing RT1-A1\^n. Figure 6 shows the high reactivity of DSA in the supernatant of a cultured lung graft of an allogeneically transplanted rat treated with the short IS protocol and sacrificed on day 98. The MFI of APC against RT1-A1\^n–expressing mouse fibroblasts was more than fivefold higher than that against RT1-A1\^n–negative mouse fibroblasts.

**Intragraft Lymphoid Aggregates**

Figure 8 shows component analyses of de novo lymphoid tissue in the chronically rejected lung grafts, along with their high reactivity with antibodies against donor-MHC class I molecules. Lymphocyte aggregates were observed in peribronchiolar...
and perivascular areas, which also contained large areas of collagen fibrosis. These de novo lymphoid tissues contained T cells (CD3), B cells (CD79a), plasma cells (CD138), and recipient-derived MHC class II-positive cells (His 19).

Figure 9 shows T cell and B cell aggregates in de novo lymphoid tissue of chronically rejected lung grafts. Immunofluorescence double staining showed the juxtaposition of B cell clusters and T cell aggregation within these de novo lymphoid tissues (Figure 9A). CD8-positive effector T cells were also found in the lung grafts (Figure 9B). Almost all of the B cells in the B cell clusters were positive for rat IgG, suggesting that these cells were producing DSA (Figure 9C). B cells were also positive for the proliferation marker Ki-67 (Figure 9D). A few B cells were sparsely distributed in the stroma apart from intragraft lymphoid aggregation and were not always positive for rat IgG (data not shown).

**DISCUSSION**

This study showed that antibodies against donor MHC class I molecules were locally produced within chronically rejected lung allografts, despite serum DSA being undetectable, suggesting that localized humoral immunity is an aspect of AMR after LTx. Although locally produced alloantibodies and resulting humoral immunity have been associated with solid organ graft rejection, to our knowledge, neither intrapulmonary production of DSA nor local humoral immune response after LTx has been demonstrated to date. Generally, a diagnosis of AMR after LTx is based on allograft dysfunction, the presence of circulating DSA, and suggestive histology such as positive C4d staining and capillaritis in the absence of infectious pneumonia. These indicators, along with the possibility that locally produced antibodies react with lung allografts in situ independent of the systemic circulation, may explain findings of progressive AMR in the absence of detectable serum DSA. Tissue elution assays have shown the significance of intragraft DSA as a risk factor for graft loss in lung and kidney transplant recipients. Moreover, ectopic lymphoid structure have been shown important in grafts with intragraft DSAs, indicating locally activated humoral immunity.

The tissue culture assay results reported in this study showed that DSAs could be produced locally in chronically rejected lung allografts. The finding of parallel longitudinal increases in DSA titers in lung allografts and the spleen suggests that local humoral alloimmune responses in the lung did not develop independent of systemic responses during the progression of chronic lung allograft rejection. Rather, local responses developed in addition or as a complement to systemic responses including those in the spleen. Although primary systemic adaptive immune responses are initiated in secondary lymphoid organs, such as the lymph nodes and spleen, local respiratory immune responses, such as those against viral infection, can be initiated in intrapulmonary lymphoid aggregates, which can be a site of response to antigens. Our results indicate that these intrapulmonary lymphoid aggregates can also contribute to local humoral immunity against alloantigens. Indeed, DSAs were detected only in the lung graft culture assay while not in serum nor even in spleen culture assay in some rats treated with short IS protocol at day 98.
studies should be needed to speculate the determining influencer of the development of locally and systemically activated humoral immune mechanisms.

Stromal lymphoid aggregates that produce DSA locally were observed in chronically rejected lung allografts. These intragraft de novo lymphoid tissues were mainly composed of T cells and B cells, along with plasma cells and recipient-derived MHC class II-positive cells, which act as antigen-presenting cells. B cell clusters positive for anti-rat IgG and proliferation markers were thought to play a role in the local production of DSA. Furthermore, the presence of recipient-derived MHC class II-expressing cells, which are considered essential for interactions with helper T cells, suggest local intralung activation of a recipient’s humoral immune system. B cell clusters within kidney grafts during rejection were reported associated with reduced graft survival and resistance to steroid therapy, independent of C4d deposition or the presence of serum alloantibody.26 In systemic humoral immunity, B cell responses lead to the generation of long-lived plasma cells, which migrate into the bone marrow and continue to produce antibodies indefinitely.27-30 The presence of de novo lymphoid tissue also suggests that the lung itself may become a niche for long-lived plasma cells, in addition to being a site for T cell–B cell interactions.28,31 Intrapulmonary TLT after LTx may act as a locus of activated local humoral immune responses and may therefore be a potential therapeutic target. In addition, humoral responses within intragraft de novo lymphoid tissue may target a more diverse antigen repertoire, including pathogens, auto-antigens, and molecules involved in allograft accommodation.32-34 The results of this study do not exclude the possibility that, in addition to providing optimal protective immunity against respiratory pathogens at local tissue sites,35,36 de novo lymphoid tissue after LTx may play a regulatory role in lung graft tolerance.

Pathological features observed in human lung grafts undergoing AMR were also observed in chronically rejected rat lung allografts. High-grade acute cellular rejection and neutrophilic capillaritis have been reported to be histopathologic indicators of AMR,37 with DSA found to be associated with OB in lung transplant recipients.38-41 The evidence of graft damage by inflammatory cells, such as A grade and DAD/neutrophilic capillaritis, may have become less pronounced because hypocellular collagen fibrosis had partially or extensively replaced the perivascular zone, into which inflammatory cells had infiltrated during early phases. OB has been observed in an animal model of intratracheal administration of antibodies against donor-MHC class I molecules.42 DSA can also affect lung parenchymal cells, perhaps explaining the epithelial damage observed in lung transplants.31 Antibodies directed against donor MHC class I molecules are particularly important in the pathogenesis of chronic vascular rejection.17,44,45 Despite the evidence in the present study suggesting that locally produced alloantibodies contribute to allograft rejection, it remains unclear whether these locally produced antibodies...
FIGURE 8. De novo lymphoid tissues in chronically rejected lung grafts of rats sacrificed on day 98. Representative lesion in a rat receiving the short IS protocol, showing lymphoid aggregation in a chronically rejected lung graft as well as the ability to produce DSA locally. A, Lymphocytic infiltration into collapsed lung parenchyma (dashed line), surrounded by stromal fibrosis spreading between the blood vessels (II) and airways (+) (HE, scale bars in the left and right panels, 100 μm and 50 μm, respectively). Immunohistochemical analyses showed that the de novo lymphoid tissue was mainly composed of T cells (B, anti-CD3, scale bars in the left and right panels, 100 μm and 50 μm, respectively) and B cells (C, anti-CD79a, scale bars in the left and right panels, 100 μm and 50 μm, respectively). Plasma cells (D, anti-CD138, scale bar = 20 μm) and recipient-derived MHC class II positive cells (E, His 19, scale bars in the left and right panels, 100 μm and 50 μm, respectively) were observed around the de novo lymphoid tissue.
Thus, the optimal IS regimen required to re-

Moreover, AMR in humans is likely modified in a

A larger number of animals, sampling at addi-

The authors thank Dr. Etienne Joly for providing L cells

In conclusion, despite serum antibody levels being unde-

We acknowledge that the animal model used in this study

We found that humoral alloimmune responses occur locally

In contrast to the optimal IS regimen, the degree of contribution of locally produced DSA to allo-

The results of this study did not exclude the possibility that cellular immune responses could also cause these lung graft injuries. A larger number of animals, sampling at additional time points, and further mechanistic analyses are required to determine a causal relationship between locally produced DSA and the progression of chronic lung allograft rejection. Furthermore, this model also can be applied for future animal study as to non-DSA and CLAD. Fourth, although this study focused on local DSA production, the details of intrapulmonary production of antibodies in association with lymphoid neogenesis remain to be explored further. For example, it is unclear whether nonspecific antibodies are produced, as suggested by a recent report in cardiac transplantation. Moreover, local immunoglobulin class switch and mutations in the variable domain may occur, suggesting the local evolution of antigen-specific immune responses. Notably, however, this study was designed as a proof-of-concept study, intending to demonstrate the local production of DSA in allograft lungs along with the progression of the rejection process. The finding that humoral alloimmune responses occur locally in lung allografts will lead to efforts to demonstrate a similar phenomenon in human lung allografts, to determine its possible clinical significance, and to develop novel strategies to detect local DSA or local AMR.

ACKNOWLEDGMENTS

The authors thank Dr. Etienne Joly for providing L cells transfected with RT1-Aβ-expressing retroviral vectors.

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