Adherence of adoptively transferred alloreactive Th1 cells in lung: partial dependence on LFA-1 and ICAM-1

ANNE E. DIXON, JANIS B. MANDAC, PAUL J. MARTIN, ROBERT C. HACKMAN, DAVID K. MADTES, AND JOAN G. CLARK

Division of Pulmonary and Critical Care Medicine, Department of Medicine, University of Washington, Seattle 98195; and Fred Hutchinson Cancer Research Center, Seattle, Washington 98109

Received 14 February 2000; accepted in final form 31 March 2000

Adherence of adoptively transferred alloreactive Th1 cells in lung: partial dependence on LFA-1 and ICAM-1. Am J Physiol Lung Cell Mol Physiol 279: L583–L591, 2000.—T helper type 1 (Th1) cells are important effectors in a number of immune-mediated lung diseases. We recently described a murine model of lung injury induced by adoptive transfer of cloned alloreactive Th1 cells. To investigate mechanisms that result in injury to the lung, we studied the in vivo distribution of radiolabeled Th1 cells. One hour after intravenous administration, >85% of injected radioactivity was left in the lung, and at 24 h, 40% of radioactivity was left in the lung. Adherence of Th1 cells in the lung was significantly inhibited by neutralizing antibody to lymphocyte function-associated antigen-1. Th1 cell adherence also was decreased in lungs of mice deficient in intercellular adhesion molecule-1 (ICAM-1). Th1 cell transfer further induced expression of ICAM-1 and vascular cell adhesion molecule-1 in the lung. Vascular cell adhesion molecule-1-immunoreactive protein was markedly induced in lung endothelium by alloreactive Th1 cells. These findings indicate that Th1 cells localize in normal lung by a mechanism involving lymphocyte function-associated antigen-1 and ICAM-1. Alloreactive cells further induce endothelial adhesion molecules that may facilitate recruitment of inflammatory cells to the lung and amplify Th1 cell-induced lung injury.

lymphocyte function-associated antigen-1; intercellular adhesion molecule-1; T helper type 1 and type 2 cells; homing; cell trafficking

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
molecule-1 (VCAM-1) (5, 35). The selectins have been implicated in lymphocyte migration to the lymph nodes (36), skin (4), and gut (5), and recent evidence suggests that induction of selectins on the pulmonary vascular endothelium may be involved in lymphocyte influx in response to particulate antigen challenge in sensitized mice (37). The α4-integrins are involved in lymphocyte recruitment to the brain (6), skin (14), and pancreas (7), and evidence exists for VLA-4 and VCAM-1 involvement in lymphocyte trafficking to the lung after antigenic challenge (1, 19, 37). LFA-1 and its endothelial counterreceptor have been implicated in lymphocyte recruitment to the skin (32), liver (25), and kidney (11) and also to the lung in models of allergic airway disease (13).

Several studies (3, 13, 38) have documented enhanced expression of adhesion molecules in the pulmonary vasculature during inflammation. The induction of adhesion molecules by cytokines elaborated in response to an initiating inflammatory challenge may amplify the inflammatory response by further facilitating leukocyte migration to the lung. However, the mechanisms by which leukocytes, including memory T cells, initially traffic to the lung are not well understood.

The present study was performed to examine the trafficking of adoptively transferred Th1 cells and its relationship to lung injury. We examined the contribution of L-selectin and VLA-4 and the interaction between LFA-1 and ICAM-1 in the adherence of Th1 cells in the lung. We studied the expression and induction of the vascular adhesion molecules VCAM-1 and ICAM-1 to understand their role in the initial adherence of lymphocytes in the lung and the changes that would facilitate subsequent recruitment of inflammatory cells to the lung.

MATERIALS AND METHODS

Mice. C57BL/6 (Ly5b), Ly5a, and ICAM-1-deficient mice (40) were obtained from Jackson Laboratory (Bar Harbor, ME). The ICAM-1-deficient mice were on a C57BL/6 background. The mice were housed in microisolator cages under ME). The ICAM-1-deficient mice were on a C57BL/6 back-
ground. The mice were housed in microisolator cages under ME). The ICAM-1-deficient mice were on a C57BL/6 back-
ground. The mice were housed in microisolator cages under ME). The ICAM-1-deficient mice were on a C57BL/6 back-
ground. The mice were housed in microisolator cages under ME). The ICAM-1-deficient mice were on a C57BL/6 back-
ground. The mice were housed in microisolator cages under ME). The ICAM-1-deficient mice were on a C57BL/6 back-
ground. The mice were housed in microisolator cages under ME). The ICAM-1-deficient mice were on a C57BL/6 back-
ground. The mice were housed in microisolator cages under ME). The ICAM-1-deficient mice were on a C57BL/6 back-
ground. The mice were housed in microisolator cages under ME). The ICAM-1-deficient mice were on a C57BL/6 back-
ground. The mice were housed in microisolator cages under ME). The ICAM-1-deficient mice were on a C57BL/6 back-
ground. The mice were housed in microisolator cages under ME). The ICAM-1-deficient mice were on a C57BL/6 back-
ground. The mice were housed in microisolator cages under ME). The ICAM-1-deficient mice were on a C57BL/6 back-
ground. The mice were housed in microisolator cages under ME). The ICAM-1-deficient mice were on a C57BL/6 

**Flow cytometry.** Th1 cells (10^6) were incubated in 50 μl of fluorescence-activated cell sorter (FACS) buffer (PBS with 1% FCS and 0.1% NaN3) with 1 μg of primary antibody for 30 min at 4°C. Primary antibodies included MEL-14 rat monoclonal antibody (Mab) to L-selectin (PharMingen, San Diego, CA), PS/2 rat MAb to the α4-integrin chain of VLA-4 (kindly provided by Dr. W. Henderson, University of Washington, Seattle, WA), M17/4 rat MAb to the αL-integrin chain of LFA-1 (PharMingen), and rat IgG control (PharMingen). Cells were washed three times in FACS buffer and then stained with the secondary antibody, 1 μg of FITC-conjugated mouse anti-rat IgG (Jackson ImmunoResearch, West Grove, PA) in 50 μl of FACS buffer.

Flow cytometry was performed on a Becton Dickinson FACScan, and data were collected on 10^4 light-scatter gated events. The forward- and side-scatter properties of the cells were used to exclude dead cells from the analysis. Data were analyzed with CellQuest software (Becton Dickinson, Palo Alto, CA).

In vivo migration and adhesion of adoptively transferred Th1 cells. Cloned Th1 cells were resuspended at 10^7 cells/ml and labeled with 20 μCi/ml of sodium [51Cr]chromate (NEN, Boston, MA) for 1 h at 37°C. Viable cells were separated from dead cells and free chromium by density centrifugation through Nycodenz (GIBCO BRL, Life Technologies, Gaithersburg, MD) (22). [51Cr]-labeled cells (5 × 10^6/mouse) were administered by tail vein injection. Animals were euthanized, then perfused through the spleen, liver, kidney, thymus, lung, heart, and blood were harvested and weighed. The radioactivity in the organs and an aliquot of the injected cells were measured for 1 min in a gamma scintillation counter (Beckman, Fullerton, CA).

In some experiments, [51Cr]-labeled cells (20 × 10^6) were incubated with either 10 μg of neutralizing MAb to LFA-1 (M17/4; PharMingen), isotype control (Thy-1.2, clone 53-2.1; PharMingen), or 10–40 μg of neutralizing MAb to VLA-4 (PS/2) for 30 min immediately before administration. We generated the F(ab')2 fragment of the LFA-1 and Thy-1.2 antibodies by papain digestion (Sigma, St. Louis, MO) and isolation on a Sephadex G-75 (Pharmacia Biotech, Uppsala, Sweden) column (24). Intact PS/2 antibody was used in this study because the intact antibody and F(ab')2 fragment have been shown to have similar effects in vivo (20). Intact Thy-1.2 antibody was then used as an isotype control.

RT-PCR of ICAM-1 and VCAM-1. To induce lung injury, 10^7 Ly5a-specific cells were administered to both Ly5a and Ly5b (control) mice. Mice were euthanized after 1, 24, and 48 h (3 mice/group). The lungs were harvested and snap-frozen in liquid nitrogen. RNA was extracted by the TRIzol method (Life Technologies, Grand Island, NY). RNA samples were treated with DNase to ensure complete removal of contaminating DNA. With a one-tube reaction method (Promega ACCESS system), 1 μg of RNA was reverse transcribed followed by immediate PCR. Primer sequences as previously described (27) were synthesized at the Biotechnology Center of the Fred Hutchinson Cancer Research Center (Seattle, WA): A 50-μl reaction contained 10 μl of the RNA template, 0.2 mM deoxynucleotide triphosphate, 1 mM MgSO4, 0.75 μM each sense and antisense primers, 5 U of avian myeloblastosis virus reverse transcriptase, and 5 U of tfl DNA polymerase in 1× avian myeloblastosis virus-tfl reaction buffer. RT was performed by a 45-min incubation at 48°C followed by an amplification cycle profile of denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min, and elongation at 72°C for 2 min for 30 cycles in a Perkin-Elmer thermal cycler (34). The amplified PCR product was visualized by ethidium bromide-agarose gel electrophoresis with an Eagle Eye II still video system (Stratagene, La Jolla, CA) and quantitated by ImageQuant software (Molecular Dynamics, Sunnyvale, CA). Levels of ICAM-1 and VCAM-1 mRNA were normalized to β-actin mRNA in the same sample.

Immunohistochemistry of ICAM-1 and VCAM-1. VCAM-1 immunohistochemistry was performed on frozen sections of mouse lung obtained from C57BL/6 (control) mice and
C57BL/6 mice euthanized 3 days after receiving $10^7$ anti-Ly5b cells. The excised lungs were inflated and embedded in Tissue-Tek optimum cutting temperature compound (Cryofrm, IFC, Needham Heights, MA), snap-frozen in liquid nitrogen, and stored at $-70^\circ$C. The cryosections were fixed in acetone and stained with an antibody to VCAM-1 (clone 429; PharMingen) or an isotype control. Biotinylated goat anti-rat IgG was used as a secondary antibody and was then visualized by streptavidin-peroxidase followed by diaminobenzidine with NiCl$_2$ enhancement. Sections were counterstained with acridine orange-safranin O.

ICAM-1 immunohistochemistry was performed on sections obtained from untreated control C57BL/6 mice and C57BL/6 mice and ICAM-1-deficient mice 2 days after administration of $10^7$ in vitro activated Th1 cells. Excised lungs were inflated, fixed in Formalin, and embedded in paraffin. Sections were incubated with a MAb to ICAM-1 (clone 3E2; PharMingen) and detected with biotinylated goat anti-hamster IgG (Jackson ImmunoResearch). The biotinylated antibody was then visualized by streptavidin-peroxidase followed by diaminobenzidine with NiCl$_2$ enhancement. Sections were counterstained with acridine orange-safranin O.

RESULTS

Adoptively transferred Th1 cells distributed preferentially to the lung. To determine the in vivo distribution of adoptively transferred Th1 cells, $^{51}$Cr-labeled Ly5a-specific Th1 cells were administered to Ly5a and Ly5b mice. Recipients were euthanized after 1 (A and B), 3 (C and D), or 24 (E and F) h, and organs were harvested for measurement of radioactivity. cpm, Counts/min. Values are means ± SE, representative of 2 experiments; n = 4 mice/group. More than 95% of injected radioactivity was recovered. At 1 h, >85% of radioactivity was in the lung. There was some redistribution over time, but at 24 h, 65% of recovered radioactivity was still in the lung. The distribution was similar in both Ly5a and Ly5b animals.
were preferentially localized in lung tissue (Fig. 1). One hour after instillation, >85% of the counts were in the lung. There was some redistribution over time, but at 24 h, >65% of the recovered radioactivity was still in the lung. The distribution was the same in both Ly5a and Ly5b mice, but only Ly5a mice developed lung injury. In vitro activated $^{51}$Cr-labeled cells had the same in vivo distribution after adoptive transfer (data not shown).

**Th1 cells expressed LFA-1 and VLA-4.** To identify candidate molecules that might mediate adhesion of Th1 cells in the lung, we analyzed both resting and activated cells for expression of LFA-1, VLA-4, and L-selectin by flow cytometric analysis. Resting cells were positive for the integrins LFA-1 and VLA-4 (Fig. 2). There was no difference in the fluorescence intensity of either LFA-1 or VLA-4 in resting cells compared with that in activated cells. L-selectin expression was not detected in resting or activated cells (data not shown).

**Adherence of Th1 cells in lung was reduced by neutralization of LFA-1 or deficiency of ICAM-1.** To examine the role of LFA-1 in mediating adherence of resting Th1 cells in lung, $^{51}$Cr-labeled Th1 cells preincubated with neutralizing monoclonal antibody to LFA-1 or Thy-1.2 (control) were administered to Ly5a mice. Mice were euthanized at three time points, and the organs were harvested for radioactivity. Neutralizing antibody to LFA-1 did not affect adherence of the cells at 1 or 3 h (Fig. 3A). Neutralizing antibody to LFA-1 significantly reduced adherence of cells in lung at 24 h compared with that in animals injected with untreated cells ($P < 0.0005$ by one-way ANOVA; Fig. 3A). The F(ab')$_2$ fragment of the LFA-1 antibody similarly reduced the binding of resting cells in the lung at 24 h, excluding the possibility that nonspecific binding (of the intact antibody to Fc receptors) was affecting localization.

To determine whether adherence of activated cells in the lung is dependent on LFA-1, in vitro activated $^{51}$Cr-labeled cells were incubated with the F(ab')$_2$ fragment of either LFA-1 or Thy-1.2 (isotype control) and adoptively transferred. Adherence of activated cells in the lung was significantly reduced at 1 h ($P < 0.005$; Fig. 3B).

To further investigate the receptor-ligand interaction utilized by the Th1 cells, we examined Th1 cell adherence in mice deficient in ICAM-1, the major endothelial ligand for LFA-1. $^{51}$Cr-labeled in vitro activated Th1 cells were administered to ICAM-1-deficient and wild-type mice. Adherence of activated cells was significantly reduced in ICAM-1-deficient mice at 1 h ($P < 0.005$; Fig. 4). The histopathological response detected 1–3 days after Th1 cell transfer (i.e., mononuclear cell vasculitis and alveolitis) was only modestly reduced in the ICAM-1-deficient mice, suggesting that other adhesion pathways may compensate or dominate in the evolution of the inflammatory response.

To examine the role of VLA-4 in the adherence of Th1 cells in lung, $^{51}$Cr-labeled cells were incubated with neutralizing antibody to VLA-4 or isotype control before adoptive transfer into mice. Neutralizing MAb to VLA-4 did not affect adherence of resting or activated cells in lung at 1 or 24 h (data not shown).

**Administration of Th1 cell clone induced expression of vascular adhesion molecules.** VCAM-1 and ICAM-1 are the endothelial ligands for VLA-4 and LFA-1, respectively. To assess the induction of VCAM-1 and ICAM-1 expression after Th1 cell transfer, we investigated the expression of endothelial adhesion molecule VCAM-1 and ICAM-1 mRNAs by RT-PCR in the lung of Ly5a and Ly5b mice at intervals after administration of $10^7$ Ly5a-specific Th1 cells. VCAM-1 mRNA levels normalized to $\beta$-actin mRNA were increased at 24 and 48 h in Ly5a mice compared with those in control Ly5b mice ($P < 0.01$ at 24 h; $P < 0.0001$ at 48 h; Fig. 5A). ICAM-1 mRNA was increased 1 h after transfer of cells into Ly5a animals compared with that in

---

**Fig. 2.** Th1 cells express lymphocyte function-associated antigen (LFA)-1 (A) and very late activating antigen (VLA)-4 (B). Resting Th1 cells were incubated with rat monoclonal antibody (MAb) to the integrin $\alpha_L$ chain (for LFA-1; A, solid line), $\alpha_4$ chain (VLA-4; B, solid line), or isotype control (dashed lines). Cells were then stained with FITC-conjugated goat anti-rat IgG.
Ly5b animals and was persistently increased at 24 and 48 h ($P < 0.05$; Fig. 5B).

To examine the potential role of VCAM-1 and ICAM-1 in the initial adherence of Th1 cells as well as the ensuing inflammatory response, we examined VCAM-1 and ICAM-1 by immunohistochemistry. In the normal lung, VCAM-1 was detectable only in rare, isolated endothelial cells. In lung sections obtained 3
days after administration of alloreactive cells, VCAM-1 expression was dramatically increased and specifically localized in the endothelium associated with inflammatory foci (Fig. 6).

ICAM-1 was expressed at high levels in the alveolar structures and vascular endothelium of normal untreated lungs. No ICAM-1 was detected in lung sections from ICAM-1-deficient mice stained in exactly the same manner. We did not detect major changes in the pattern of ICAM-1 expression by this method after Th1 cell administration (Fig. 7).

DISCUSSION

This study was designed to investigate the mechanisms for selective lung injury induced by adoptive transfer of alloreactive Th1 cells. The results demonstrate that Th1 cells distribute preferentially to the lung and that this adherence depends partly on the interactions between adhesion molecules expressed by Th1 cells and by the pulmonary vascular endothelium. The cloned Th1 cells express the integrin adhesion molecules LFA-1 and VLA-4 but not L-selectin. The initial adherence of in vitro activated cells in the lung was reduced by neutralizing antibody to LFA-1 and in ICAM-1-deficient mice. Adherence of Th1 cells in the lung was unaffected by neutralizing VLA-4. Our studies also demonstrated that the endothelial adhesion molecule VCAM-1 was expressed at low levels in the normal lung but was rapidly upregulated after Th1 cell administration. In contrast, ICAM-1 was constitutively expressed at high levels throughout the pulmonary epithelial and endothelial surfaces. ICAM-1 mRNA was further upregulated by administration of Th1 cells.

Other investigators (8, 31) have described similar trafficking of cloned T cells to the lung, but the mechanisms for this have not been previously delineated. Based on the high level of radiolabeled T cells in the lung immediately after intravenous administration, some (9, 28) have suggested that the cells are simply “trapped” in the pulmonary vasculature. However, the Th1 cells in our study were not merely retarded in transit through lung; their retention was stable over time. Twenty-four hours after administration of Th1 cells, 65% of recovered radioactivity was still localized in the lung. In addition, as other investigators (16) have also noted, the same distribution pattern was present after administration of resting or in vitro activated cells despite the much larger size of activated cells.

We found that ICAM-1, the major endothelial ligand for LFA-1, was expressed constitutively on normal pulmonary vascular endothelium and that adherence of activated Th1 cells in the lung is dependent on the interaction between LFA-1 and ICAM-1 as suggested in a previous report (21). The adherence of resting adoptively transferred cells was unaffected by neutralizing LFA-1 until 24 h after transfer. Taken together, the results suggest that LFA-1 on resting Th1 cells is in a low-affinity state and is not utilized for initial adherence but may be involved in later recruitment or retention of Th1 cells in the lung that become activated in vivo. In vitro activated cells utilize LFA-1 for initial adherence to ICAM-1, most likely because activation increases LFA-1 affinity (26). The initial adherence of Th1 cells was only partially dependent on LFA-1 and ICAM-1. Recent research has identified a number of vascular adhesion molecules (e.g., selectins) and T cell ligands (e.g., P-selectin glycoprotein ligand-1, CD44) that might contribute to lymphocyte adherence in tissue (4, 35, 37–39). Ongoing work in our laboratory suggests that both P- and E-selectins may participate...
in the initial adherence of adoptively transferred Th1 cells in our model.

We did not demonstrate a role for the VLA-4 integrin in the initial adherence of the Th1 cell clone in the lung. This finding likely is due in part to absent or low-level expression of the endothelial receptor VCAM-1 on normal resting lung endothelium. However, VLA-4 and its endothelial counterreceptor VCAM-1 have been implicated in lymphocyte recruitment to the lung in models of pulmonary inflammation such as intratracheal challenge with sheep red blood cells (37). Intratracheal instillation of the blood cells causes a rapid induction of endothelial VCAM-1 (38) and may then be important in facilitating leukocyte recruitment to the lung. We demonstrated a similar rapid induction of VCAM-1 expression after administration of Th1 cells. Although we were unable to demonstrate a role for VLA-4 and VCAM-1 interactions in the initial adherence of the Th1 cell clone, VCAM-1 may be important in the amplification of Th1 cell-induced inflammation that involves recruitment of recipient mononuclear cells to the lung.

The adherence of Th1 cells in the lung may be necessary for lung injury in our model, but it is not sufficient. Ly5a-specific cells but not Ly5b-specific cells induce lung injury in Ly5a mice. Other models of lung injury induced by adoptive transfer of antigen-specific T cells have been described (18, 23, 33). In these models, the antigen is predominantly or exclusively present in the lung, and homing of transferred cells to the lung could be caused by the presence of antigen. Our results suggest an alternative interpretation. Antigen-specific “memory” T cells may preferentially circulate to the lung by intrinsic mechanisms involving specific adhesion molecules. If antigen is encountered, effector mechanisms come into play. If not, the cells circulate to regional lymph nodes or other sites. Although this pathway may be apparent after adoptive transfer of cells through an intravenous route, it could also be important for recirculation of memory lymphocytes and immune surveillance (30, 31).

Cytotoxic CD8 cells may be similarly classified as type 1 (Tc1) or type 2 (Tc2) according to their cytokine profile. An adoptive transfer study (17) indicated that the preferential adherence of Tc1 cells in the lung may enhance clearance of viral infections and pulmonary metastases (17). This suggests that intrinsic trafficking patterns of lymphocyte subsets may have important functional implications for novel therapeutic approaches using adoptive immunotherapy for treatment of viral infections and malignancies.
T cells play a central role in a number of pulmonary diseases as well as mediating normal host defense. Elucidating the mechanisms by which T cells and T cell subsets incite pulmonary immune responses is essential to understanding these diseases. Th2 cells in the lung have been the subject of much recent interest because these cells may augment airway hyperactivity and inflammation characteristic of asthma. Efforts to ameliorate Th2-induced responses by adoptive transfer of Th1 cells have been thwarted by the finding of exacerbated parenchymal injury induced by Th1 cells (23). The mechanisms underlying this injury are incompletely understood, but trafficking of adoptively transferred Th1 lymphocytes to the lung with subsequent activation and inflammation may be important. A recent study (39) suggested that trafficking patterns of Th1 cells are different from those of Th2 cells, a characteristic that may depend on adhesion molecule and chemokine receptor expression.

In conclusion, we have shown that cloned alloreactive Th1 cells localize in the lung by a mechanism involving LFA-1 and ICAM-1 and then induce expression of vascular adhesion molecules, leading to a progressive mononuclear vasculitis, alveolitis, and interstitial pneumonitis. The further study of adherence of T cell subsets in lung and the mechanisms by which they provoke an immune reaction may provide important insights into the pathogenesis of immune-mediated pulmonary diseases.

We thank Mary Beauchamp, Andrew Elston, and Caroline Sawe for technical advice and assistance and Heather Peake for assistance with preparation of the manuscript.

This work was supported by National Heart, Lung, and Blood Institute Grants R01-HL-55200 and K23-HL-07237

REFERENCES


