Directed Expression of Cre in Alveolar Epithelial Type 1 Cells

Per Flodby^{1*}, Zea Borok^{1*}, Agnes Banfalvi¹, Beiyun Zhou¹, Danping Gao¹, Parviz Minoo², David K. Ann³, Edward E. Morrisey⁴, and Edward D. Crandall¹

¹Will Rogers Institute Pulmonary Research Center, Division of Pulmonary and Critical Care Medicine, Department of Medicine, and

²Department of Pediatrics, Keck School of Medicine, University of Southern California, Los Angeles, California; ³Department of Clinical and Molecular Pharmacology, City of Hope National Medical Center, Duarte, California; and ⁴Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

Pulmonary alveolar epithelium is comprised of two morphologically and functionally distinct cell types, alveolar epithelial type (AT) I and AT2 cells. Genetically modified mice with cell-specific Cre/loxP-mediated knockouts of relevant genes in each respective cell type would be useful to help elucidate the relative contributions of AT1 versus AT2 cells to alveolar homeostasis. Cre has previously been efficiently expressed in AT2 cells in mouse lung with the surfactant protein (SP)-C promoter; however, no transgenic mouse expressing Cre in AT1 cells has so far been available. To develop an AT1 cell-specific transgenic Cre mouse, we generated a knockin of a Cre-IRES-DsRed cassette into exon 1 of the endogenous aquaporin 5 (*Aqp5*) gene, a gene expressed specifically in AT1 cells in the distal lung epithelium, resulting in the mouse line, *Aqp5*-Cre-IRES-DsRed (ACID). Endogenous *Aqp5* and transgenic Cre in ACID mice showed a very similar pattern of tissue distribution by RT-PCR. To analyze Cre activity, ACID was crossed to two Cre reporter strains, R26LacZ and mT/mG. Double-transgenic offspring demonstrated reporter gene expression in a very high fraction of AT1 cells in the distal lung, whereas AT2 cells were negative. As expected, variable reporter expression was detected in several other tissues where endogenous *Aqp5* is expressed (e.g., submandibular salivary gland and stomach). ACID mice should be of major utility in analyzing the functional contribution of AT1 cells to alveolar epithelial properties *in vivo* with Cre/loxP-mediated gene deletion technology.

Keywords: loxP; aquaporin 5; lung; alveolar epithelium; reporter

Gene knockout with the Cre/loxP DNA recombination system is a widely used strategy to study cell type-specific gene function *in vivo*. In the alveolar epithelium, there are two morphologically and functionally distinct cell types: alveolar epithelial type (AT) I and AT2 cells. Generation of cell-specific knockouts of relevant genes in each of these cell types would greatly enhance our ability to elucidate the relative functional contributions of AT1 and AT2 cells in the lung *in vivo*. Several transgenic mouse strains have previously been generated that direct Cre expression to lung AT2 cells with either the surfactant protein (SP)-C

CLINICAL RELEVANCE

This article describes a new transgenic mouse line expressing the DNA-recombinase Cre specifically in alveolar epithelial type (AT) 1 cells within the distal alveolar epithelium. This new Cre line will enable detailed studies of gene function by gene targeting to modulate gene expression specifically in AT1 cells in the distal mouse lung, thereby advancing our understanding of the functional contribution of this important cell type to alveolar homeostasis at baseline and in disease.

promoter (1–3) or Nkx2.1 (thyroid transcription factor-1 [TTF-1]) regulatory sequences (4), providing valuable tools for studying gene function specifically in this cell type. However, transgenic mice expressing Cre in AT1 cells are not yet available, in part due to lack of reliable AT1 cell-specific genes that could be used to direct Cre expression.

With the aim of expressing Cre in AT1 cells, we focused on using the aquaporin 5 gene (*Aqp5*) to direct Cre expression. *Aqp5*, coding for the water channel protein, AQP5, is expressed predominantly in salivary and lacrimal glands, cornea, trachea, and distal lung (5). In rat and human lung, *Aqp5* has been shown to be expressed specifically in AT1 cells where the protein is localized to the apical membrane (6–10), making *Aqp5* a potential candidate host gene to direct expression of Cre in AT1 cells in transgenic mice. Although *Aqp5* is specifically expressed in AT1 cells in rat and human distal lung epithelium, with no expression in AT2 cells, this specificity in expression may not be consistently present in mouse lung (11). Analysis in our laboratory of several inbred mouse strains has shown differences in cell specificity of AQP5 protein expression. For example, in C57BL/6J mice, many AT2 cells express AQP5, albeit at a low level compared with AT1 cells. On the other hand, because AQP5 expression is entirely AT1 cell-specific in 129S6/SvEvTac mice (unpublished observation), it should be possible to use the *Aqp5* gene to direct expression of Cre specifically in AT1 cells of that mouse strain. Because transgenic mouse lines harboring floxed genes often are generated from embryonic stem (ES) cells derived from strain 129S6/SvEvTac, this issue of genetic background should be easy to resolve by developing new floxed strains on a pure 129S6/SvEvTac background from the start.

We have previously generated transgenic mice and rats harboring an enhanced green fluorescent protein (GFP) transgene driven by a 4.3-kb upstream fragment of the *Aqp5* gene (12). Analysis of these animals showed very low expression of GFP in lung tissue, whereas high expression was observed in salivary glands, suggesting that regulatory sequences needed for high expression in lung were lacking. Indeed, we have shown in transfection studies that a conserved part of intron 1 in *Aqp5* is capable of enhancing a luciferase reporter gene specifically in

(Received in original form June 24, 2009 and in final form October 2, 2009)

This work was supported in part by the Hastings Foundation, Whittier Foundation, and research grants HL 38578, HL 38621, HL 62569, HL 64365, and HL 89445 from the National Institutes of Health. E.D.C. is Hastings Professor and Kenneth T. Norris Jr. Chair of Medicine. Z.B. is Ralph Edgington Chair in Medicine. P.M. is Hastings Professor of Pediatrics.

* These authors contributed equally to this work.

Correspondence and requests for reprints should be addressed to Per Flodby, Ph.D., University of Southern California, Keck School of Medicine, Department of Medicine, Division of Pulmonary and Critical Care Medicine, IRD 620, 2020 Zonal Avenue, Los Angeles, 90033 CA. E-mail: flodby@usc.edu

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Cell Mol Biol Vol 43. pp 173–178, 2010

Originally Published in Press as DOI: 10.1165/rcmb.2009-0226OC on September 18, 2009
Internet address: www.atsjournals.org

a lung epithelial cell line (MLE-15), whereas no significant enhancer activity was found in a cell line derived from salivary gland (Pa-4) (13). These transfection studies suggest that sequences in intron 1 of *Aqp5* have important regulatory functions, although it is unknown exactly which regulatory sequences are required for high-level *Aqp5* expression in the lung *in vivo*. Based on this observation, we decided to pursue a knockin strategy for transgenic expression of Cre, the rationale being that all necessary regulatory sequences of the endogenous *Aqp5* gene would then be present to drive Cre expression in an efficient and cell-specific manner. The knockin strategy we used inactivates one copy of the *Aqp5* gene. Based on previous characterization of *Aqp5* knockout mice (14–16), we did not expect noticeable effects of the knockin *per se*, because heterozygous mice have been reported to demonstrate very mild or no phenotypic effects in terms of salivary gland, lung, or kidney function.

The knockin was made by placing a Cre-IRES-DsRed cassette into exon 1 of the *Aqp5* gene with the ES cell line, W2 (derived from strain 129S6/SvEvTac), generating the new Cre line *Aqp5*-Cre-IRES-DsRed (ACID). To test for Cre activity, ACID mice were crossed to the reporter strain, ROSA26^{tm1Sor} (here called R26LacZ) (17), carrying a LacZ reporter transgene that can be activated by Cre/loxP-mediated recombination, and to the reporter strain mT/mG (24), expressing GFP after Cre recombination. Analysis of ACID;R26LacZ and ACID;mT/mG double-transgenic mice demonstrated strong LacZ or GFP reporter expression in a very high fraction of AT1 cells in the distal lung, as well as in acinar cells in the submandibular salivary gland. As expected, reporter expression was also detected in several other tissues where the endogenous *Aqp5* gene is expressed, such as brain (18, 19), tracheal epithelium, stomach (20), and sweat glands (21, 22). ACID mice should be useful for analyzing the functional contributions of AT1 cells to alveolar epithelial homeostasis *in vivo*.

MATERIALS AND METHODS

Knockin Vector Construction and Targeting of ES Cells

The knockin (targeting) vector contained two PCR-amplified genomic fragments surrounding the *Aqp5* translational start site and a Cre-IRES-DsRed cassette inserted in between the fragments. A cassette containing an FRT-flanked PGK-NeoR resistance gene for positive selection was placed immediately downstream of Cre-IRES-DsRed, and a negative HSV-TK selection marker was cloned into a 5'-flanking position (see Figure E1A in the online supplement). For vector construction details and targeting of ES cells, see the MATERIALS AND METHODS in the online supplement.

Generation of ACID Knockin Mice and Crosses to Reporter Mice

Chimeric males transmitting the knockin allele through the germline were generated and crossed to FLPer females (Jackson Laboratories, Bar Harbor, ME) to remove the PGK-NeoR selection marker (23). Backcrosses to 129S6/SvEvTac mice were then performed before crossing the resulting ACID mice to R26LacZ or mT/mG reporter mice. For analysis, mice harboring one copy of the *Aqp5*-Cre knockin allele and two copies of the R26LacZ reporter allele were generated (ACID;R26LacZ^{+/+}) to obtain a strong reporter signal. For the mT/mG reporter, one transgenic copy was sufficient for detection in ACID;mT/mG mice. Initial assessment of Cre activity in lungs of ACID;R26LacZ double-heterozygous mice was performed by PCR. Further details are provided in the online supplement.

Reporter Expression Analysis of Tissue Sections and Isolated Lung Cells

Analysis of reporter expression in tissues and isolated cells from male ACID;R26LacZ^{+/+} mice was performed by X-gal staining, whereas

reporter expression in male ACID;mT/mG mice was analyzed by fluorescence microscopy as direct detection of Tomato and GFP. Details of tissue preparation, cell isolation, and X-gal staining are provided in the online supplement.

Antibody Staining of Lung Cryosections and Cytospins

To investigate the identity of reporter-positive cells, we performed staining of lung sections and isolated cells with antibodies (Abs) to either AQP5 or pro-SP-C for identification of AT1 or AT2 cells, respectively. For further details, see the online supplement.

Analysis of *Aqp5* and Cre mRNA expression

Expression of *Aqp5*, Cre and 18S rRNA (reference gene) at the mRNA level was analyzed by RT-PCR in 16 tissues from ACID;R26LacZ^{+/+} mice. Total RNA was isolated with the RNeasy Plus Mini Kit (Qiagen, Valencia, CA) and reverse transcribed by random priming with the ThermoScript RT-PCR System (Invitrogen, San Diego, CA). RT reactions without reverse transcriptase were performed for all samples to control for contamination of genomic DNA. HotMaster Taq DNA polymerase (5 PRIME; Thermo Fisher Scientific, Tustin, CA) and the following PCR primers were used for gene-specific amplification: Cre, forward, 5'-TGAGGTTTCGAAGAACCTGATGGA-3', and reverse, 5'-GCCGCATAACCAGTGAAACAGCAT-3'; *Aqp5*, forward, 5'-CGCTCAGCAACAACACAA-3', and reverse, 5'-GACCGACAAGCCAATGGA-3'; and 18S, forward, 5'-CTTTGGT CGCTCGCTCCTC-3', and reverse, 5'-CTGACCGGGTTGGTTTTGAT-3'.

RESULTS

Generation of ACID Knockin Mice

With the goal of generating a mouse expressing Cre specifically in AT1 cells, we constructed a knockin targeting vector by inserting a Cre-IRES-DsRed cassette into the first exon of the *Aqp5* gene (Figure E1A). The targeting construct was linearized with Not I and electroporated into the ES cell line, W2, derived from mouse strain 129S6/SvEvTac. Positive ES cell clones that had incorporated the Cre-IRES-DsRed cassette into the *Aqp5* gene through homologous recombination were identified by Southern blot (Figure E1B). Two karyotypically normal ES cell clones (242 and 329) were used to generate chimeras. We obtained three germline-transmitting chimeric males, from which three parallel lines were established. The NeoR selection marker in the knockin vector was flanked by FRT sites and was removed *in vivo* by crossing the chimeric males to FLPer females to generate mice harboring the final knockin allele, depicted as the ACID allele (Δ NeoR) in Figure E1A. The FLPer mice used were on a mixed C57BL/6;129S6/SvEvTac background, but because we needed to have the ACID mice on a pure 129S6/SvEvTac background to obtain AT1 cell-specific Cre expression, backcrosses of ACID to 129S6/SvEvTac mice were performed (which also served the purpose of segregating out the FLPer transgene after FLP/Frt-mediated NeoR deletion). When comparing the three ACID lines (242.1, 242.3, and 329) for Cre activity and specificity, as described subsequently here, only relatively small differences were observed. Results from the line 242.3, derived from ES cell clone 242, are shown in this study.

Detection of Cre-Dependent LacZ Reporter Activation in Adult Mouse Lung by PCR

To screen for Cre activity, ACID mice were crossed to the reporter strain, R26LacZ (17). This strain harbors a LacZ reporter transgene that is activated by Cre/loxP-mediated deletion of a flox-stop (FS) sequence (see supplemental MATERIALS AND METHODS online and Figure E2). PCR analysis of lung genomic DNA from ACID;R26LacZ double-heterozygous mice

demonstrated an approximately 500-bp product reflecting Cre-dependent deletion of the floxed-stop sequence (Δ FS) in the R26LacZ reporter transgene, as seen in Figure E2. In addition to the Δ FS PCR product, the approximately 725-bp PCR product specific for the undeleted flox-stop (+FS) version of the reporter transgene was detected (Figure E2), presumably from AT2 cells and other lung cells not expressing Cre. In contrast, in R26LacZ lungs, only the +FS PCR product was obtained, whereas no Δ FS PCR product was noted.

Analysis of Reporter Expression in ACID;R26LacZ and ACID;mT/mG Mouse Lung

X-gal staining of lung cryosections from ACID;R26LacZ double heterozygotes resulted in a positive signal in many AT1 cells, but staining appeared weak and patchy. To obtain a stronger reporter signal, we generated mice harboring one ACID knockin allele and two copies of the reporter transgene for analysis (ACID;R26LacZ^{+/+}). In these animals, a much stronger and more uniform X-gal staining pattern was observed, demonstrating LacZ reporter gene expression in a very large fraction (>90%) of AT1 cells (Figure 1A). AT2 cells identified by pro-SP-C expression (Figure 1B) were negative for X-gal (Figures 1A and 1B, *red arrows*), indicating that the ACID knockin allele is preferentially expressed in AT1 cells in lung. As expected, homozygous R26LacZ control mice did not show positive X-gal staining (Figure 1C). To further confirm specificity, we crossed ACID mice to the double-fluorescent Cre reporter line, mT/mG, in which tandem dimer Tomato red fluorescent protein is expressed ubiquitously before Cre/loxP recombination, whereas GFP is expressed ubiquitously after Cre/loxP recombination (24). Both proteins are equipped with extra sequence to target their insertion into the cell membrane. As expected, single-transgenic mT/mG mice express strong Tomato (red fluorescent) signal in the alveolar epithelium, whereas no GFP signal is detected (Figure 1D). In contrast, analysis of lung cryosections from double-heterozygous ACID;mT/mG mice revealed distinct GFP expression in AT1 cells, localized to the cell membranes (Figure 1E). Pro-SP-C Ab staining of ACID;mT/mG lung cryosections with Alexa-350–streptavidin detection (Figure 1F, *blue staining*) revealed that the apical surfaces of AT2 cells were GFP negative (indicated by *red arrows*).

To more clearly show cell specificity of Cre-mediated reporter activation in the ACID mouse, we isolated AT2 cells from ACID;R26LacZ^{+/+} lungs and performed X-gal staining, followed by pro-SP-C Ab staining. As shown in Figures 2A and 2B, essentially all pro-SP-C–positive cells were X-gal negative, whereas less than 1% pro-SP-C–positive cells in isolated AT2 cell preparations were X-gal positive, confirming that the ACID knockin allele is preferentially expressed in AT1 cells in alveolar epithelium. Cell specificity and efficiency of Cre-mediated LacZ reporter expression were further evaluated in crude lung cell preparations that are enriched for AT1 cells, followed by X-gal and Ab staining for AT1 and AT2 cell markers. The lung cell preparation protocol is designed to isolate both AT1 and AT2 cells, and usually yields 10–20% AT1 cells and 30–40% AT2 cells. X-gal staining followed by staining for pro-SP-C shows, again, that AT2 cells do not harbor Cre activity capable of switching on the LacZ reporter (Figures 2C and 2D). As demonstrated in Figures 2E and 2F, cells that were X-gal positive were also AQP5 positive. Collectively, the experiments described above show a very high degree of efficiency and AT1 cell specificity of Cre activity in the ACID mouse alveolar epithelium.

Aqp5 and Cre mRNA expression and X-gal staining in adult mouse tissues

To compare the tissue expression profile of endogenous *Aqp5* and transgenic Cre in the ACID mouse, we performed RT-PCR analysis of Cre and its host gene, *Aqp5*, in 16 tissues from ACID;R26LacZ^{+/+} and R26LacZ^{+/+} control mice. As expected, tissue distribution of the Cre transcript mirrored that of endogenous *Aqp5* (Figure 3A). High levels of *Aqp5* mRNA expression were detected in lung and submandibular salivary gland, whereas intermediate levels were found in trachea and stomach, and low but detectable levels were found in testis and skin. The expression pattern of the Cre transgene encoded by the ACID knockin allele was essentially identical to endogenous *Aqp5*, although the expression level of Cre was comparatively higher in testis. To evaluate Cre activity, we performed X-gal staining of tissues from the animals used for RT-PCR analysis. As shown in Figure 3B, reporter expression was detected in all tissues where *Aqp5* and Cre mRNA were found; however, X-gal staining was also

Figure 1. X-gal and immunofluorescence staining for alveolar epithelial type (AT) 2 cell-specific marker on Aqp5-Cre-IRES-DsRed (ACID);R26LacZ^{+/+} lung cryosections (A–C). (A) X-gal staining of ACID;R26LacZ^{+/+} lung cryosection showing that most AT1 cells (>90%) are positive for LacZ reporter. (B) Pro-SP-C antibody (Ab) staining of the same ACID;R26LacZ^{+/+} lung cryosection demonstrating that AT2 cells (pro-SP-C positive) are X-gal negative

(*red arrows* in A and B). (C) X-gal staining of R26LacZ negative control cryosection demonstrating absence of endogenous β -galactosidase activity. Analysis of Cre activity in mT/mG and ACID;mT/mG reporter mice (D–F). (D) Exclusive Tomato expression in mT/mG single-transgenic mice shows no spontaneous Cre-independent green fluorescent protein (GFP) reporter activation in the lung. (E) AT1 cells in ACID;mT/mG double-transgenic lung show cell membrane expression of GFP. (F) Staining of ACID;mT/mG double-transgenic lung for SP-C demonstrates that the apical surface of AT2 cells is negative for GFP (*red arrows*).

Figure 2. X-gal and immunofluorescence staining for cell-specific markers in cytopins from ACID;R26LacZ^{+/+} lung cells. X-gal staining of purified AT2 cells (A), followed by pro-SP-C Ab staining (B), demonstrates that AT2 cells (pro-SP-C positive) are X-gal negative and that copurified X-gal-positive cells are pro-SP-C negative (indicated by white arrows). X-gal staining of a crude cell preparation (C), followed by pro-SP-C Ab staining (D), also shows that X-gal-positive cells are pro-SP-C negative. X-gal staining of a crude cell preparation (E), followed by AQP5 Ab staining (F), reveals that essentially all X-gal-positive cells are AQP5 positive (AT1 cells), and *vice versa*.

detected in some other tissues to a limited extent. For example, we found reporter expression in parts of the olfactory bulb in the brain, a site where *Aqp5* expression has previously been reported (18). Scattered X-gal staining was also found in cerebellum, whereas the rest of the brain was negative. As expected, very

strong reporter expression was observed in acinar cells in the submandibular salivary gland, and most tracheal epithelial cells were positive. Positive X-gal staining was found in some cells in the heart. In kidney, where only very low *Aqp5* and Cre expression were detected, strong reporter expression was ob-

Figure 3. Evaluation of Cre expression and activity in 16 different tissues in ACID;R26LacZ^{+/+} mice. (A) RT-PCR analysis of endogenous *Aqp5* and Cre mRNA expression. (B) X-gal staining of the corresponding tissues. Staining in brain was limited to olfactory bulb and cerebellum (shown). SMG, submandibular salivary gland; SM, skeletal muscle.

served in collecting ducts, particularly in the medulla. Stomach epithelium (especially near the pylorus) and duodenal glands showed positive staining, consistent with an earlier report (20). Restricted reporter expression was observed in liver and pancreas, probably corresponding to intercalated ducts where *Aqp5* expression has been observed in human pancreas (25). Concordant with our RT-PCR results, strong X-gal staining was observed in testis, where the most intense signal appeared in the interstitium. A distinct reporter signal was seen in sweat glands in skin, which was expected since expression of *Aqp5* has been reported (21, 22). No reporter expression was detected in spleen, ileum, colon, or skeletal muscle, in accordance with our RT-PCR data. Analysis of Cre protein expression in lung and salivary gland by Western analysis did not reveal a clear and specific band in either tissue (data not shown), indicating low protein expression, despite significant activity. Finally, because our ACID knockin construct also included the DsRed gene (coding for a red fluorescent protein), we attempted to detect expression of DsRed in submandibular salivary gland and lung by confocal microscopy, but no specific signal could be detected (data not shown), likely due to low protein expression levels.

DISCUSSION

We have generated and characterized a new transgenic mouse (ACID) expressing DNA recombinase Cre specifically in AT1 cells in the alveolar epithelium. We used a knockin strategy in which the endogenous *Aqp5* gene, coding for the water channel protein, AQP5, was chosen as the host gene to direct Cre expression in AT1 cells. Using this approach, we demonstrated a high degree of cell specificity and efficiency of Cre activity, which were restricted in lungs to AT1 cells of *Aqp5*-Cre;reporter double-positive mice. Cre activity was also prominent in several other tissues, particularly submandibular salivary glands, trachea, stomach, testis, and sweat glands. This AT1 cell-specific Cre-expressing mouse should be of major utility in development of new mutant mice for studies of AT1 cell-dependent properties of alveolar epithelium.

As expected, we detected endogenous *Aqp5* gene expression in several previously known extrapulmonary locations, and expression of transgenic Cre from the ACID knockin allele essentially mirrored this expression pattern. We detected low-level Cre recombinase activity in cardiac muscle, which correlated with very low expression of *Aqp5* and Cre (although no expression of *Aqp5* had been reported previously in mouse heart [26]) and in kidney (along with prominent LacZ reporter expression in collecting ducts, particularly in the medulla, although it has been reported that the kidney lacks *Aqp5* expression [27]). In the kidney, these results suggest that *Aqp5* and Cre may be expressed during development, but are then turned off in adult tissue, whereas the ROSA promoter-driven LacZ reporter gene continues to be expressed. There are other tissues that express *Aqp5*, such as cornea (5), cochlea (28), vomeronasal organ (29), and tongue (20), but we did not analyze those tissues. Expression in tracheal epithelium is consistent with reported observations (11). Based on knowledge from previous studies that heterozygous mice (*Aqp5*^{+/-}) with one inactivated allele feature no phenotypic effects, we considered the *Aqp5* exon 1 knockin strategy to be the best approach for successful expression of Cre in AT1 cells. Nevertheless, when using the ACID mouse, it is preferable that mice not be bred to homozygosity for the ACID allele to avoid complete inactivation of *Aqp5* and possible unintended effects, and that *Aqp5*^{+/ACID} mice (heterozygotes) are used as experimental control animals. To achieve strict AT1 cell specificity of Cre expression in the alveolar epithelium when using the ACID

mouse, mice must be bred in a 129S6/SvEvTac background. We observed a higher number of X-gal-positive AT2 cells in ACID;LacZ mice that were on a mixed 129S6/SvEvTac;C57BL/6J background (data not shown), but, after several generations of backcrosses onto 129S6/SvEvTac, a high level of AT1 cell specificity was obtained.

Although, ideally, a host gene for transgenic overexpression would be expressed only in the cell/tissue of interest, preferentially expressed AT1 (relative to AT2) cell genes, such as *Aqp5*, podoplanin (*Pdpn*, also called RT140 or T1 α [30–32]), caveolin-1, and RAGE (advanced glycosylation end product-specific receptor) (33), along with other genes identified by microarray with several-fold higher expression levels in AT1 cells (34–38), are also expressed in other tissues. We have developed a monoclonal Ab (VIII B2) that detects an epitope strictly specific to rat AT1 cells (39) and not detected anywhere else in the lung or any extrapulmonary tissues. However, because the gene encoding this protein is still unknown, it is not yet available to drive transgene expression exclusively in AT1 cells. In addition to the current study of AT1 cell-specific Cre expression, a rat podoplanin BAC (bacterial artificial chromosome) has been used for expression of transgenes in AT1 cells in mouse lung epithelium (40), although *Pdpn* expression is found in pulmonary lymphatic endothelium and other tissues. Until exclusive AT1 cell-specific proteins/genes become available, these approaches can be effective for providing new mouse models with which to define the roles of AT1 cells in alveolar function and biology.

In summary, we have shown in this study that the endogenous *Aqp5* gene can be used successfully as a knockin host gene to direct expression of a Cre transgene specifically in AT1 cells within the adult alveolar epithelium, whereas Cre expression in AT2 cells is essentially absent. The availability of this new Cre mouse (ACID) will make it possible to develop mice exhibiting knockout of floxed genes of interest or overexpression of genes using a floxed-stop-transgene strategy specifically in AT1 cells. The fact that Cre is active in tissues other than lung must be taken into consideration when interpreting results from the ACID mouse, but, because the sites of highest extrapulmonary expression are not considered essential for survival, the ACID mouse should be useful for investigating the roles of AT1 cells in function and differentiation of the alveolar epithelium.

Author Disclosure: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

Acknowledgments: The authors thank Drs. Nancy Wu, Linda Liu, and Robert Maxson (University of Southern California [USC] Transgenic Core Facility) for expert advice, and for performance of culture and electroporation of embryonic stem (ES) cells and blastocyst injections, and Dr. Chih-Lin Hsieh (USC Department of Urology) for karyotyping of targeted ES cell clones.

References

1. Perl AK, Tichelaar JW, Whitsett JA. Conditional gene expression in the respiratory epithelium of the mouse. *Transgenic Res* 2002;11:21–29.
2. Perl AK, Wert SE, Loudy DE, Shan Z, Blair PA, Whitsett JA. Conditional recombination reveals distinct subsets of epithelial cells in trachea, bronchi, and alveoli. *Am J Respir Cell Mol Biol* 2005;33:455–462.
3. Okubo T, Hogan BL. Hyperactive Wnt signaling changes the developmental potential of embryonic lung endoderm. *J Biol* 2004;3:11.
4. Xu Q, Tam M, Anderson SA. Fate mapping Nkx2.1-lineage cells in the mouse telencephalon. *J Comp Neurol* 2008;506:16–29.
5. Raina S, Preston GM, Guggino WB, Agre P. Molecular cloning and characterization of an aquaporin cDNA from salivary, lacrimal, and respiratory tissues. *J Biol Chem* 1995;270:1908–1912.
6. Nielsen S, King LS, Christensen BM, Agre P. Aquaporins in complex tissues. II. Subcellular distribution in respiratory and glandular tissues of rat. *Am J Physiol* 1997;273:C1549–C1561.
7. Funaki H, Yamamoto T, Koyama Y, Kondo D, Yaoita E, Kawasaki K, Kobayashi H, Sawaguchi S, Abe H, Kihara I. Localization and

- expression of AQP5 in cornea, serous salivary glands, and pulmonary epithelial cells. *Am J Physiol* 1998;275:C1151–C1157.
8. Borok Z, Lubman RL, Danto SI, Zhang XL, Zabski SM, King LS, Lee DM, Agre P, Crandall ED. Keratinocyte growth factor modulates alveolar epithelial cell phenotype in vitro: expression of aquaporin 5. *Am J Respir Cell Mol Biol* 1998;18:554–561.
 9. Kreda SM, Gynn MC, Fenstermacher DA, Boucher RC, Gabriel SE. Expression and localization of epithelial aquaporins in the adult human lung. *Am J Respir Cell Mol Biol* 2001;24:224–234.
 10. Borok Z, Li X, Fernandes VF, Zhou B, Ann DK, Crandall ED. Differential regulation of rat aquaporin-5 promoter/enhancer activities in lung and salivary epithelial cells. *J Biol Chem* 2000;275:26507–26514.
 11. Krane CM, Fortner CN, Hand AR, McGraw DW, Lorenz JN, Wert SE, Towne JE, Paul RJ, Whitsett JA, Menon AG. Aquaporin 5-deficient mouse lungs are hyperresponsive to cholinergic stimulation. *Proc Natl Acad Sci USA* 2001;98:14114–14119.
 12. Zhou B, Ann DK, Flodby P, Minoo P, Liebler JM, Crandall ED, Borok Z. Rat aquaporin-5 4.3-kb 5'-flanking region differentially regulates expression in salivary gland and lung *in vivo*. *Am J Physiol Cell Physiol* 2008;295:C111–C120.
 13. Flodby P, Zhou B, Ann DK, Kim KJ, Minoo P, Crandall ED, Borok Z. Conserved elements within first intron of aquaporin-5 (Aqp5) function as transcriptional enhancers. *Biochem Biophys Res Commun* 2007;356:26–31.
 14. Ma T, Song Y, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS. Defective secretion of saliva in transgenic mice lacking aquaporin-5 water channels. *J Biol Chem* 1999;274:20071–20074.
 15. Ma T, Fukuda N, Song Y, Matthey MA, Verkman AS. Lung fluid transport in aquaporin-5 knockout mice. *J Clin Invest* 2000;105:93–100.
 16. Kim SW, Gresz V, Rojek A, Wang W, Verkman AS, Frokiaer J, Nielsen S. Decreased expression of AQP2 and AQP4 water channels and Na,K-ATPase in kidney collecting duct in AQP3 null mice. *Biol Cell* 2005;97:765–778.
 17. Soriano P. Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat Genet* 1999;21:70–71.
 18. Ablimit A, Matsuzaki T, Tajika Y, Aoki T, Hagiwara H, Takata K. Immunolocalization of water channel aquaporins in the nasal olfactory mucosa. *Arch Histol Cytol* 2006;69:1–12.
 19. Yamamoto N, Yoneda K, Asai K, Sobue K, Tada T, Fujita Y, Katsuya H, Fujita M, Aihara N, Mase M, *et al*. Alterations in the expression of the AQP family in cultured rat astrocytes during hypoxia and reoxygenation. *Brain Res Mol Brain Res* 2001;90:26–38.
 20. Matsuzaki T, Tajika Y, Suzuki T, Aoki T, Hagiwara H, Takata K. Immunolocalization of the water channel, aquaporin-5 (AQP5), in the rat digestive system. *Arch Histol Cytol* 2003;66:307–315.
 21. Song Y, Sonawane N, Verkman AS. Localization of aquaporin-5 in sweat glands and functional analysis using knockout mice. *J Physiol* 2002;541:561–568.
 22. Nejsum LN, Kwon TH, Jensen UB, Fumagalli O, Frokiaer J, Krane CM, Menon AG, King LS, Agre PC, Nielsen S. Functional requirement of aquaporin-5 in plasma membranes of sweat glands. *Proc Natl Acad Sci USA* 2002;99:511–516.
 23. Farley FW, Soriano P, Steffen LS, Dymecki SM. Widespread recombinase expression using FLP_{re} (flipper) mice. *Genesis* 2000;28:106–110.
 24. Muzumdar MD, Tasic B, Miyamichi K, Li L, Luo L. A global double-fluorescent Cre reporter mouse. *Genesis* 2007;45:593–605.
 25. Burghardt B, Elkaer ML, Kwon TH, Raczy GZ, Varga G, Steward MC, Nielsen S. Distribution of aquaporin water channels AQP1 and AQP5 in the ductal system of the human pancreas. *Gut* 2003;52:1008–1016.
 26. Butler TL, Au CG, Yang B, Egan JR, Tan YM, Hardeman EC, North KN, Verkman AS, Winlaw DS. Cardiac aquaporin expression in humans, rats, and mice. *Am J Physiol Heart Circ Physiol* 2006;291:H705–H713.
 27. Nielsen S, Frokiaer J, Marples D, Kwon TH, Agre P, Knepper MA. Aquaporins in the kidney: from molecules to medicine. *Physiol Rev* 2002;82:205–244.
 28. Mhatre AN, Steinbach S, Hribar K, Hoque AT, Lalwani AK. Identification of aquaporin 5 (AQP5) within the cochlea: cDNA cloning and *in situ* localization. *Biochem Biophys Res Commun* 1999;264:157–162.
 29. Ablimit A, Aoki T, Matsuzaki T, Suzuki T, Hagiwara H, Takami S, Takata K. Immunolocalization of water channel aquaporins in the vomeronasal organ of the rat: expression of AQP4 in neuronal sensory cells. *Chem Senses* 2008;33:481–488.
 30. Rishi AK, Joyce-Brady M, Fisher J, Dobbs LG, Floros J, VanderSpek J, Brody JS, Williams MC. Cloning, characterization, and development expression of a rat lung alveolar type I cell gene in embryonic endodermal and neural derivatives. *Dev Biol* 1995;167:294–306.
 31. Williams MC, Cao Y, Hinds A, Rishi AK, Wetterwald A. T1 alpha protein is developmentally regulated and expressed by alveolar type I cells, choroid plexus, and ciliary epithelia of adult rats. *Am J Respir Cell Mol Biol* 1996;14:577–585.
 32. Ramirez MI, Millien G, Hinds A, Cao Y, Seldin DC, Williams MC. T1alpha, a lung type I cell differentiation gene, is required for normal lung cell proliferation and alveolus formation at birth. *Dev Biol* 2003;256:61–72.
 33. Herzog EL, Brody AR, Colby TV, Mason R, Williams MC. Knowns and unknowns of the alveolus. *Proc Am Thorac Soc* 2008;5:778–782.
 34. Qiao R, Zhou B, Liebler JM, Li X, Crandall ED, Borok Z. Identification of three genes of known function expressed by alveolar epithelial type I cells. *Am J Respir Cell Mol Biol* 2003;29:98–105.
 35. Dahlin K, Mager EM, Allen L, Tighe Z, Goodglick L, Wadehra M, Dobbs L. Identification of genes differentially expressed in rat alveolar type I cells. *Am J Respir Cell Mol Biol* 2004;31:309–316.
 36. Gonzalez R, Yang YH, Griffin C, Allen L, Tighe Z, Dobbs L. Freshly isolated rat alveolar type I cells, type II cells, and cultured type II cells have distinct molecular phenotypes. *Am J Physiol Lung Cell Mol Physiol* 2005;288:L179–L189.
 37. Chen J, Chen Z, Chintagari NR, Bhaskaran M, Jin N, Narasaraaju T, Liu L. Alveolar type I cells protect rat lung epithelium from oxidative injury. *J Physiol* 2006;572:625–638.
 38. Wang J, Wang S, Manzer R, McConville G, Mason RJ. Ozone induces oxidative stress in rat alveolar type II and type I-like cells. *Free Radic Biol Med* 2006;40:1914–1928.
 39. Danto SI, Zabski SM, Crandall ED. Reactivity of alveolar epithelial cells in primary culture with type I cell monoclonal antibodies. *Am J Respir Cell Mol Biol* 1992;6:296–306.
 40. Vanderbilt JN, Allen L, Gonzalez RF, Tighe Z, Edmondson J, Ansaldi D, Gillespie AM, Dobbs LG. Directed expression of transgenes to alveolar type I cells in the mouse. *Am J Respir Cell Mol Biol* 2008;39:253–262.