Coexpression of RTI\(_{40}\) with alveolar epithelial type II cell proteins in lungs following injury: identification of alveolar intermediate cell types

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The alveolar epithelium in normal lungs is composed of two ultrastructurally distinct cell types, alveolar epithelial type I and II cells (ATI and ATII cells). ATI cells are large, thin cells that together cover 97% of the internal surface area of the lung (34). ATI cells are important for gas exchange, alveolar fluid regulation, and stretch-induced modulation of surfactant secretion (2, 20, 30). Cuboidal ATII cells are located between ATI and II cells (ATI and ATII cells). ATI cells are large, thin cells (34). ATII cells contain characteristic lamellar bodies in their cytoplasm and apical microvilli (35). ATII cells have many known functions, including synthesis and secretion of lung surfactant, fluid transport, and host defense (13).

ATII cells are also required for alveolar epithelial repair (1, 10, 11, 33). After injury, ATII cells proliferate, and a subset of daughter cells transform to new ATII cells (1, 10, 11, 33). Ultrastructural studies demonstrated that the formation of ATII cells is preceded by the presence of intermediate cell types characterized as ATII- or ATI-like (10, 19). However, we know little about the mechanism of ATII formation following injury because of the difficulties in both identifying and quantifying intermediate cell types by electron microscopy.

ATI and ATII cells can be distinguished by the expression of selective proteins (6, 16, 24, 36). These proteins can be used to inform the differences in ATII and ATII cell biology, and as tools to investigate alveolar epithelial injury and repair in response to toxic agents (23). Intermediate cells in the ATII-to-ATI cell transition have been identified based on the coexpression of an ATII cell-associated lectin and the ATII cell-selective protein surfactant protein (SP)-D during the resolution of keratinocyte growth factor (KGF)-induced ATII cell hyperplasia (14).

RTI\(_{40}\) or t\(_{1}\)alpha protein is a membrane protein expressed selectively on the apical surface of ATI cells in the lung (8, 31). The function of RTI\(_{40}\) is not known, although it is required for ATI cell morphogenesis during lung development (29). We hypothesized that alveolar epithelial intermediate cell types coexpress RTI\(_{40}\) and ATII cell-specific or -selective proteins. We tested this hypothesis in a rat model of *Staphylococcus aureus*-induced acute lung injury and a panel of ATI and ATII cell-specific and -selective antibodies. *S. aureus* induced an acute inflammatory reaction that was resolving by day 3 postinoculation. At day 3 postinoculation, the alveolar wall was thickened secondary to ATI cell hyperplasia. With the use of confocal microscopy, there was a fivefold increase in the fractional surface area of alveolar walls stained with ATI cell membrane proteins (RTI\(_{70}\) and MMC4) and a decrease in the fractional surface area associated with ATII-expressing cells. *S. aureus*-treated lungs also contained unique cell types that coexpressed the RTI\(_{40}\) and ATII markers RTI\(_{40}\)/MMC4/RTI\(_{70}\) and RTI\(_{0}\)/MMC4-positive cells. These cells were not observed in control lungs. RTI\(_{40}\)/MMC4-positive cells were also found in cultured ATII cells before they transformed to an ATII-like phenotype. Our data suggest that RTI\(_{40}\)/MMC4/RTI\(_{70}\) and RTI\(_{40}\)/MMC4-positive cells are intermediates in the ATII-to-ATI cell transformation. These data also suggest that the coexpression of RTI\(_{40}\) with ATII cell proteins may be used to identify and investigate ATII cell transdifferentiation to ATII cells following injury.

*Staphylococcus aureus*; aquaporin-5; MMC4 antigen

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Collection of bronchoalveolar lavage fluid. At day 3 postinstillation, PBS-instilled lungs (n = 4) and S. aureus-instilled lungs (n = 4) were lavaged twice with PBS as described previously. Bronchoalveolar lavage (BAL) fluid was centrifuged (1,000 rpm × 10 min), and the supernatant was retained for determination of total protein and RTI40 concentrations. The cell pellets were used to determine the total and differential cell counts as described previously (22). The volume of BAL fluid recovered from S. aureus-treated lungs was not different from PBS-treated lungs (data not shown). Data are presented as the total number of cells, protein, and RTI40 recovered in BAL fluid.

Lung homogenization. At day 3 postinstillation, after lavage, S. aureus-instilled lungs were removed, and the inflamed and noninflamed regions were homogenized separately as previously described (n = 4) (22). The PBS-instilled lungs (controls) were normal in appearance and were therefore homogenized whole (n = 4). The protein concentration of homogenized lungs was determined using a Bio-Rad protein assay.

ELISA-based dot blot analysis. The amount of the MMC4 and RTI40 proteins in BAL fluid and lung homogenates was quantified using ELISA-based dot blot assay as previously described (5, 26).

Western blot analysis. Lung proteins were resolved on 12% NuPAGE Bis-Tris ([bis (2-hydroxyethyl) amino] tris (hydroxymethyl) methane) GeI Electrophoresis System (Invitrogen, Paisley, UK) and electrophoretically transferred to polyvinylidene difluoride membrane (Immobilon-P) using the NuPAGE transfer buffer. Membranes were blocked in 2.5% casein in Tris-buffered saline (10 mM Tris (HCl and 0.15 M NaCl, pH 8.2) and developed as for the ELISA-based dot blots. Anti-RTI40 was detected using an anti-mouse IgG horseradish peroxidase (1 in 2,000 in blocking buffer, Rockland Immunocemicals). Aquaporin-5 (Chemicon International, 1 in 100 in DMEM + 10% FCS) was detected with anti-rabbit IgG horseradish peroxidase (either 1 in 1,000 or 2,000 dilution in blocking buffer, Rockland Immunocemicals). The optical density of Western blot bands was determined using a gel documentation and analysis system (UVP GDS7600).

Electron microscopic analysis. At day 3 postinstillation, PBS- (n = 2) and S. aureus-treated lungs (n = 2) were fixed and processed for electron microscopy as described previously (22).

Lung fixation for paraffin sections. At day 3 (n = 4), day 5 (n = 3), day 7 (n = 2), and day 21 (n = 3) postinstillation, PBS- and S. aureus-treated lungs were fixed in formalin. Sections were stained with hematoxylin and eosin.

Lung fixation for confocal microscopy. At day 3 postinstillation, PBS- (n = 3) and S. aureus- (n = 3) treated lungs were fixed with 4% paraformaldehyde for 2 h. Small cubes from the macroscopically inflamed lobes were selected and cryoprotected overnight in 30% (wt/vol) sucrose. Frozen sections were cut the next day as previously described (5, 22).

Immunostaining. Frozen lung sections and cultured alveolar epithelial cells were incubated with various cocktails of primary antibodies followed by isotype- or species-specific secondary antibodies (Molecular Probes, Leiden, Netherlands) as described previously (5, 22) (Table 1). The nucleus was stained with the DNA probe To-Pro-3 (Molecular Probes).

Confocal imaging and analysis. Fluorescent images were acquired using a Zeiss Axiovert LSM 510 confocal microscope. AlexaFluor 488 (green) was imaged with an excitation wavelength of 488 nm. AlexaFluor 546 (red) was imaged at 543 nm. Alexa 647 (blue) was imaged at 633 nm. The sections were sequentially scanned at different excitation wavelengths to ensure discrimination between the overlapping fluorescent spectra of Alexa dyes 488 and 546.

For quantification, a computer-controlled motorized stage was used to obtain tile images consisting of 16 (4 × 4) high-powered (×63 objective) contiguous fields combined into a single red, green, and blue TIFF file. Openlab 3.5.1 analysis software (Improvement) was used to create a binary mask consisting of the positively stained areas in each separate color channel; binary masks were combined to identify areas of colocalization. To determine the length of a positively stained wall for a given color or combination of colors, binary masks were overlaid on the corresponding transmitted light image, and the length of the positively stained alveolar wall was traced and measured using a graphics tablet (WACOM Technology). Data are reported as the percentage of alveolar wall stained with a given antibody.

For three-dimensional viewing of alveolar wall membranes, confocal scanning was carried out using Nyquist settings. The raw data were deconvoluted using Huygens 2 (SVI software) and reconstructed and analyzed for colocalization using Imaris 4.0 software (Bitplane, Zurich, Switzerland) (9).

ATII cell isolations. Type II cells were isolated from the lungs of male Sprague-Dawley specific pathogen-free rats (Harlan) using previously described methods (5, 8) (n = 3 isolations). ATII cells were fixed in 4% paraformaldehyde at days 0, 1, and 5. The ATII cells were 75% pure immediately following isolation; contaminating cells included macrophages, lymphocytes, and ATII cells (RTI40-positive cells accounted for ~1% of the total cells).

Statistics. Data are expressed as means (SD). Comparison between samples was analyzed using Student’s t-test. P < 0.05 was considered significant. Tests were performed with GraphPad Instat version 3.0a.

RESULTS

General histology of S. aureus-instilled lungs: day 3 to day 21 postinstillation. At day 3 postinstillation, the alveolar walls from inflamed regions of S. aureus-instilled lungs were thickened compared with the alveolar walls in the noninflamed regions and control lungs (Fig. 1). The number of inflammatory cells present in the air spaces was also increased in S. aureus-instilled lungs (Fig. 1). By day 7 postinstillation, S. aureus-treated lungs were almost normal; the alveolar walls were thinner and there were fewer inflammatory cells in the air spaces (Fig. 1). By day 21, S. aureus-inoculated lungs were indistinguishable from control lungs (data not shown).

Ultrastructure of the alveolar wall at day 3 post-S. aureus instillation. The thickened alveolar walls in S. aureus-instilled lungs were due to increased numbers of ATII cells (i.e., cells containing lamellar bodies and apical microvilli) or cuboidal epithelial cells with, and without, some ATII cell ultrastructural features (that is, ATII-like cells). ATII-like cells included cells with apical microvilli and lamellar bodies but reduced perinuclear cytoplasm, cells with apical microvilli and no lamellar bodies, and cells with no apical microvilli or lamellar bodies (Fig. 2). In addition, ATII cells were also observed overlying ATII cells (Fig. 2). The alveolar epithelium in the noninstilled region from S. aureus-instilled lungs was the same as shown previously for control lungs (data not shown).

Table 1. ATI and ATII cell-specific or selective proteins

<table>
<thead>
<tr>
<th>Name</th>
<th>Cell Specificity</th>
<th>Isotype</th>
<th>Alexa Fluor Anti-Mouse Secondary Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMC4 MAb</td>
<td>ATI and Clara</td>
<td>IgG2a</td>
<td>546 (red)</td>
</tr>
<tr>
<td>Anti-RTI40</td>
<td>ATII</td>
<td>IgG3</td>
<td>647 (blue)</td>
</tr>
<tr>
<td>Anti-RTI40</td>
<td>ATII</td>
<td>IgG3</td>
<td>488 (green)</td>
</tr>
<tr>
<td>Anti-aquaporin-5</td>
<td>ATI and Clara cells</td>
<td>Rabbit polyclonal</td>
<td>Western blot only</td>
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ATI and ATII, alveolar epithelial types I and II, respectively.
Alveolar barrier injury and inflammation on day 3 post-S. aureus instillation. At day 3 post-S. aureus instillation, the total amount of protein recovered in BAL fluid was elevated 1.6-fold above control values (i.e., 3.03 ± 0.89 mg vs. 1.87 ± 0.43 mg, P < 0.05, n = 3). In contrast, our previous study demonstrated that the total amount of protein recovered in BAL fluid was elevated 6.8-fold at day 1 post-S. aureus instillation compared with control values (22).

The total number of BAL leukocytes was elevated 2.5-fold above control values (i.e., 8.33 × 10⁵ ± 1.95 × 10⁵ vs. 3.53 × 10⁵ ± 0.37 × 10⁵, P < 0.05, n = 3) at day 3 post-S. aureus instillation. In contrast, the total number of leukocytes recovered in BAL fluid was elevated 57-fold above control values at day 1 post-S. aureus instillation (22).

The amount of RTI₄₀ recovered in BAL fluid can be used to assess the extent of ATI cell necrosis (25, 26). The amount of RTI₄₀ recovered in BAL fluid at day 3 postinstillation was not different from control values (data not shown).

These protein and inflammatory cell data confirm the acute inflammatory response to S. aureus is resolving by day 3 postinstillation, and there is no evidence of ATI cell necrosis.

Concentration of RTI₄₀, aquaporin-5, and the MMC4 antigen in lung homogenates at day 3 post-S. aureus instillation. The concentration of RTI₄₀ was decreased by >18-fold in lung homogenates from the inflamed regions of S. aureus-inoculated lungs compared with control lungs (Fig. 3). Similarly, the concentration of aquaporin-5, expressed by ATI cells and nonciliated bronchiolar epithelial cells (27), was decreased by fivefold in inflamed regions from S. aureus-inoculated lungs compared with control lungs (Fig. 3). However, in the noninflamed regions, the concentration of RTI₄₀ was not different from control values, whereas the concentration of aquaporin-5 was increased by nearly twofold (Fig. 3).

The concentration of the MMC4 antigen was increased in lung homogenates from the inflamed region of S. aureus-treated lungs compared with control lungs, whereas in the noninflamed region of S. aureus-instilled lungs, the concentration of MMC4 antigen was not different from control values (Fig. 3).

Phenotype of the alveolar epithelium in control lungs. The antibodies used here have been shown previously to bind selectively to ATI or ATII cells (Table 1). Here we demon-
strate that these antibodies can be used in combination to investigate the phenotype of ATI and ATII cells simultaneously. RTI40 is expressed on the apical surface of ATI cells, whereas RTII70 and MMC4 are expressed on the apical surface of ATII cells (Fig. 4) (5, 7, 8). The relative proportion of alveolar surface area occupied by ATI to ATII cells, assessed by measuring the fractional surface area of the alveolar wall associated with RTI40 and RTII70/MMC4 staining, was 97.0% vs. 2.5% (a ratio of 39:1) (Fig. 4). This ratio is identical to the

Fig. 2. Electron micrographs of alveolar epithelial cells in S. aureus-injured lungs at day 3 postinstillation are shown. A: flattened epithelial cell containing microvilli (arrowheads) and lamellar bodies (LB) but with reduced perinuclear cytoplasm. Original magnification, ×6,700. B: group of cuboidal epithelial cells with reduced cytoplasm and numbers of LB but with microvilli (arrowheads). Original magnification, ×5,000. C: alveolar epithelial type (AT) I cell overlaying an ATII cell. Inset demonstrates the presence of a membrane between the 2 cell types. Original magnification, ×1,000; for inset, ×125,000.

Fig. 3. Concentration of RTI40, aquaporin-5, and the MMC4 antigen in lung homogenates from control and S. aureus-injured lungs at day 3 postinstillation. Western blots were performed as described in MATERIALS AND METHODS. Protein (10 μg) was loaded per lane. Each lane represents a different lung sample. A: Western blot demonstrates RTI40 is downregulated in lung homogenates from inflamed lungs (Inf) compared with controls (Ctrl) and the noninflamed regions (N-Inf). B: Western blot demonstrates aquaporin-5 is downregulated in lung homogenates from inflamed lungs but upregulated in noninflamed regions compared with control values. C: quantitative changes in the concentration of RTI40 and aquaporin-5. D: concentration of the MMC4 protein was increased in inflamed regions compared with values from controls and from noninflamed regions (data obtained using an ELISA-based dot blot assay). Data in C and D are shown as means (SD). Values significantly different from control values are shown (data analyzed using ANOVA). NS, not significant; RDU, relative densitometry units.
Phenotype of the alveolar epithelium at day 3 post-S. aureus instillation. As in control lungs, the alveolar walls in the inflamed regions were lined by both RTI40-positive cells (ATI cells) and MMC4/RTII70-positive cells (ATII cells) (Fig. 4). However, the fractional surface area covered by MMC4/RTII70-positive cells (ATII cells) was increased by more than fivefold in S. aureus-injured lungs compared with control lungs (Fig. 4). The MMC4 monoclonal antibody also stained ED1-positive cells (a macrophage marker) in the air spaces (data not shown); this is consistent with our observation that the MMC4 monoclonal antibody binds to aminopeptidase N (15). The fractional surface area covered by RTI40 (ATI cells) in S. aureus-injured lungs was significantly decreased compared with control lungs (Fig. 4). The fractional surface area ratio of ATI to ATII cells decreased from 39:1 in control lungs to 7:1 in S. aureus-instilled lungs (Fig. 5).

In regions with thickened alveolar walls and hyperplastic ATII cells, we observed RTI40/RTII70/MMC4-positive and RTI40/MMC4-positive alveolar membranes (Figs. 4 and 6). Three-dimensional analysis of RTI40/MMC4-coexpressing membranes, with deconvolution, confirmed coexpression of these proteins on the same membrane (Fig. 7). Quantitative analysis demonstrated that RTI40/MMC4-positive membranes in S. aureus-injured lungs accounted for ~3% of the fractional alveolar surface area in the inflamed region (Fig. 5). RTI40/MMC4-positive cells, which also expressed RTII70, accounted for one-third of this percentage. The average length of RTI40/MMC4-positive membranes in S. aureus-treated lungs was 13.6 ± 11.4 μm (n = 3) compared with an average length of 7.2 ± 0.7 μm for ATII cells in control lungs.

The small area of RTI40/MMC4-coexpressing epithelium in control lungs (0.1%) was limited to areas where ATI cells (RTI40-positive) and the apical membrane of ATII cells (MMC4/RTII70-positive) were adjacent. These areas are likely to represent the interface of separate red- and green-staining membranes within the same voxel rather than coexpression on the same membrane (Fig. 7).

Phenotype of cultured ATII cells. Primary ATII cells rapidly lose ATI characteristics and gain some ATI-like characteristics in culture. Specifically, cuboidal ATII cells spread and

Fig. 4. Expression of ATI and ATII cell-selective markers in control and S. aureus-injured lungs is shown. Lung sections (5–7 μm thick) were incubated with antibodies as described in MATERIALS AND METHODS and imaged using confocal microscopy. A: control lung, individual channels, and merged image demonstrating MMC4 (red) and RTII70 (blue) are coexpressed on an alveolar epithelial cell located between RTI40-positive cells (green). Bar = 10 μm. B: lower magnification of control lung demonstrating MMC4/RTII70 colocalization (pink cell, arrowhead). RTI40 staining is shown in green (arrow). Corresponding transmitted image is also shown. Bar = 25 μm. C: S. aureus-injured lung on day 3 post-instillation. Image demonstrates that the surface area of alveolar walls coexpressing the ATII cell markers MMC4/RTII70 is increased compared with control lungs (purple cells, arrowheads). Whereas the alveolar surface is covered by the ATI cell marker, RTI40 is decreased compared with control lungs (green, thick arrows). Some alveolar membranes are stained with a new combination of MMC4 (red) and RTI40 (green) to generate yellow membranes (thin arrow). Image also demonstrates that monocytes/macrophages in the air spaces express the MMC4 antigen. Bar = 25 μm. D: higher magnification and individual channels of RTI40/MMC4-positive membranes seen in C.
flatten and lose lamellar bodies (4, 8). While accompanying these morphological changes, ATII cells stop expressing ATII proteins (for example, SP and RTII70) and express some ATI cell-associated proteins such as RTI40 and aquaporin-5 (4, 6, 8, 16). Unlike most previous studies, we followed this transition by examining the phenotype of alveolar epithelial cells in culture. We demonstrated that freshly isolated ATII cells coexpressed RTII70 and MMC4 but not RTI40 (Fig. 8). By day 5 in culture, cells expressed RTI40 but not RTII70 or MMC4 (Fig. 8). However, on day 1, although most cells were negative or slightly positive for RTI40, some cells (5.9 ± 1.4%, n = 3) coexpressed RTI40 and MMC4 (Fig. 8). We did not detect RTII70 at day 1 of culture.

**DISCUSSION**

In a previous study we demonstrated that *S. aureus* induced an inflammatory reaction at day 1 postinstillation (22). Here we demonstrate at day 3 post-*S. aureus* instillation that general markers of acute inflammation (i.e., total number of BAL fluid leukocytes) and alveolar wall damage (as assessed by total amount of protein in BAL fluid) are returning to control values. Using morphological methods, we also demonstrate that *S. aureus* instillation induced a localized inflammatory reaction, probably at the initial site of bacterial deposition. By day 3 postinstillation, the lungs are easily separated into noninflamed and inflamed regions. The inflamed regions have a markedly thickened alveolar wall secondary to ATII cell hyperplasia. The noninflamed regions appear normal. After *S. aureus*-induced acute lung injury, our morphological data indicate that the lungs are normal by day 21.

To characterize the regional nature of *S. aureus*-induced ATII cell hyperplasia, we analyzed the concentration of ATI and ATII cell-specific and -selective proteins in inflamed and noninflamed regions at day 3 postinstillation. The concentration of both ATI cell proteins (e.g., RTI40 and aquaporin-5) were specifically decreased in inflamed regions from *S. aureus*-injured lungs. This result is consistent with a decrease in the

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**Fig. 5.** Shown is the percentage of the alveolar wall covered by ATI and ATII cell markers in *S. aureus*-injured and control lungs. Images are captured and analyzed as detailed in MATERIALS AND METHODS. The total epithelial surface measured in control and *S. aureus*-treated lungs was 16,063 ± 3,058 μm² and 15,783 ± 1,203 μm², respectively. A: ATI cells (RTI40-positive membranes). B: ATII cells (RTI70/MMC4-coexpressing membranes). C: alveolar intermediate cells (RTI40/MMC4-coexpressing membranes).

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**Fig. 6.** Comparison of RTI40/MMC4-positive membranes in control and *S. aureus*-injured lungs following deconvolution is shown. Deconvoluted images were restored in 3 dimensions and then subjected to an object-independent extraction of red (MMC4 expression) and green (RTI40 expression) colors using Colocalization 2.0 (Bitplane) software. Colocalized voxels are shown in yellow. A: RTI40/MMC4 coexpression in the control lung. Colocalization of red and green voxels (yellow) were limited to the junction between RTI40 and MMC4-positive cells in both the x, y, and z, y dimensions. An MMC4-positive cell surrounded by an RTI40-positive cell with colocalization (in yellow) was limited to areas at the junction between RTI40/MMC4-positive cells. B: RTI40/MMC4 coexpression in *S. aureus*-injured lung. In contrast with control lung, colocalization of red and green voxels (yellow) extend across the surface of the alveolar wall in both the x, y, and z, y, dimensions.
number of ATI cells, and/or a decrease in the fractional surface area of ATI cells, secondary to ATII cell hyperplasia. In the noninflamed regions, the concentration of aquaporin-5 was upregulated relative to control values. In contrast, aquaporin-5 is downregulated in regions without overt inflammation (non-inflamed) in an experimental model of viral pneumonia (32).

Our attempts to demonstrate increased numbers of ATII cells by measuring the concentration of ATII cell-specific and -selective proteins were less clear-cut than for the ATI cell proteins. By Western blot analysis, the concentration of pro-SP-C, expressed by ATII cells (3, 21), was not different from control values (data not shown). Potential reasons could include a nonhomogeneous regulation of SP-C expression that could occur with foci of both upregulation and downregulation in different areas of inflammation and of proliferative repair (hyperplasia) known to occur in these lungs. Interestingly, the concentration of SP-D, expressed by ATII cells and Clara cells (26), was elevated in both inflamed and noninflamed regions (data not shown) and was most likely influenced by the complex regulation of this protein by a variety of cytokines and mediators. The concentration of the MMC4 protein, however, was selectively elevated over the concentration of MMC4 protein in noninflamed regions; this may in part be representative of ATII cell hyperplasia. In general, only the concentration of the ATI cell proteins, and the MMC4 antigen, revealed the regional nature of S. aureus-induced injury and supported the observation that ATII cell hyperplasia is accompanied by decreased ATI cell numbers, or fractional surface area, following S. aureus-induced acute lung injury.

Using a panel of ATI and ATII cell-specific and -selective antibodies, we investigated the phenotype of the alveolar epithelium following S. aureus-induced acute lung injury at day 3 postinstillation. As in controls, the alveolar walls in inflamed regions were lined with RTII70/MMC4-positive cells (ATII cells) and RTI40-positive cells (ATI cells). However, the fractional surface area covered by RTII70/MMC4-positive cells was significantly increased compared with control lungs, indicative of ATII cell hyperplasia. RTII70/MMC4-positive cells in S. aureus-injured lungs were not uniformly positive for...
pro-SP-C (data not shown). These data suggest that RTI10/MMC4-positive cells may recognize both the ATI- and ATII-like cells, with few or no lamellar bodies, seen by ultrastructural analysis of the lungs.

In regions associated with increased numbers of RTI10/MMC4-positive cells, we observed membranes that coexpressed ATI and ATII cell proteins, RTI10, MMC4, and RTI10. RTI10/MMC4 cells were the most abundantly mixed phenotype. We did not observe RTI10/MMC4-positive alveolar wall cells in the noninflamed regions from *S. aureus*-instilled lungs. However, RTI10/MMC4-positive cells were observed in vitro as ATII cells transformed to ATI-like cells. These data suggest that the coexpression of RTI10 with ATII cell proteins, such as RTI10 and MMC4, may be used to detect ATI cells in the process of transforming to new ATII cells and therefore provides a means of investigating the mechanism of ATII cell formation following injury.

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