Effects of transdifferentiation and EGF on claudin isoform expression in alveolar epithelial cells

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Chen, Stephen P., Beiyun Zhou, Brigham C. Willis, Argelia J. Sandoval, Janice M. Liebler, Kwang-Jin Kim, David K. Ann, Edward D. Crandall, and Zea Borok. Effects of transdifferentiation and EGF on claudin isoform expression in alveolar epithelial cells. J Appl Physiol 98: 322–328, 2005. First published September 10, 2004; doi:10.1152/japplphysiol.00681.2004.—Rat alveolar epithelial type II cells grown on polycarbonate filters form high-resistance monolayers and concurrently acquire many phenotypic properties of type I cells. Treatment with EGF has previously been shown to increase transepithelial resistance across alveolar epithelial cell (AEC) monolayers. We investigated changes in claudin expression in primary cultured AEC during transdifferentiation to the type I cell-like phenotype (days 0, 1, and 8), and on day 5 in culture ± EGF (10 ng/ml) from day 0 or day 4. Claudins 4 and 7 were increased, whereas claudins 3 and 5 were decreased, on later compared with earlier days in culture. Exposure to EGF led to increases in claudins 4 and 7 and decreases in claudins 3 and 5. Claudin 1 was only faintly detectable in freshly isolated type II cells and remained unchanged over time in culture and after exposure to EGF. These results suggest that increases in transepithelial resistance accompanying AEC transdifferentiation and/or EGF exposure are mediated, at least in part, by changes in the pattern of expression of specific claudin isoforms.

Tight junctions (TJ) form circumferential intercellular connections between epithelial cells, separating the apical from the basolateral compartments, thereby contributing to cell polarity (23, 27). In addition, they function as a regulated barrier to the paracellular movement of hydrophilic solutes (including ions) across epithelia, with permeability and ion selectivity of TJ varying among different epithelial cell types (1). A number of integral proteins have been identified as components of TJ strands, including occludins, junctional adhesion molecules, and most recently claudins (1, 11, 17, 26, 29).

Claudins constitute a multi-gene family of ~23-kDa transmembrane TJ proteins that have been implicated as critical determinants of paracellular barrier properties in epithelial cells (11, 18). Overexpression of claudins in normally claudin-deficient L fibroblasts led to TJ establishment, indicating that these proteins are both necessary and sufficient for TJ formation (13, 21). The role of claudins in determining the functional properties of TJ was suggested by diseases caused by mutations of claudin genes (22). Mutation of claudin 14 is associated with hereditary deafness due to an increase in TJ permeability in the organ of Corti in both mice and humans (3, 33). Two different human mutations in claudin 16 are associated with renal magnesium wasting or childhood hypercalciuria, suggesting that claudin 16 plays a crucial role in divalent cation-selective channels in TJ of the thick ascending limb of the loop of Henle (19, 24). Further evidence for the involvement of claudins in the formation of paracellular ion-selective pores or channels came from the demonstration that overexpression of claudin 4 led to a decrease in permeability for Na+ (30), whereas overexpression of claudin 2 in MDCK I cells led to a reduction of transepithelial resistance (TER) without changes in the number of TJ strands (12). These studies led to the suggestion that claudins create paracellular charge-selective channels, as supported by the demonstration of changes in TER associated with changes in extracellular domains of claudins 2 and 4 (9) and alterations in ion selectivity associated with site-directed mutagenesis of selected extracellular amino acids in claudins 4 and 15 (10). Their restricted cell- and tissue-specific patterns of expression and their ability to associate in homotypic and/or heterotypic fashion suggest that the precise combination of claudins contributes to the specific permeability properties of TJ in various epithelia (14, 31, 35).

The normal alveolar epithelium acts as a resistant barrier to the passive flux of water and solutes from the lung interstitium into the alveolar air spaces (2, 15). Disruption of the paracellular permeability barrier in alveolar epithelium from various causes can result in alveolar flooding. Recovery of barrier integrity is essential for restoring the normally dry alveolar airspaces (16). Molecular mechanisms that regulate permeability of this barrier are poorly understood. Several claudin proteins have been identified in lung and, more recently, in alveolar epithelial cell (AEC) (32), but their precise contribution(s) to TJ function remain to be determined.

Similar to a recent study in which EGF-induced activation of the EGF receptor significantly increased TER in MDCK II cells (25), we previously demonstrated that exposure of AEC monolayers to EGF increases TER (5). To help understand the molecular pathways that regulate such changes in paracellular permeability and to investigate the hypothesis that specific claudin family members contribute to junctional integrity in the alveolar epithelium, we investigated changes in claudin expression in AEC in vitro during transdifferentiation (± EGF) from rat alveolar epithelial type II (AT2) to type I (AT1) cell-like phenotype.
EGF REGULATES CLAUDIN EXPRESSION IN ALVEOLAR EPITHELIUM

METHODS

Materials. Male specific-pathogen-free Sprague-Dawley rats (125–150 g) were obtained from Charles River (Wilmington, MA). Elastase was purchased from Elastin Products (Owensville, MO). Tissue culture-treated polycarbonate filters (0.4-μm pore size; 0.33 and 1.1 cm², Transwell) were from Corning Costar (Cambridge, MA). HEPES, glutamine, and penicillin-streptomycin were obtained from Irvine Scientific (Irvine, CA). Dulbecco’s modified Eagle’s medium and Ham’s F-12 nutrient mixture in a 1:1 ratio (DME-F12), nonessential amino acids, and rat IgG were purchased from Sigma Chemical (St. Louis, MO). BSA and EGF were from BD Bioscience (Bedford, MA). All other chemicals were of the highest commercial quality available and were obtained from Sigma Chemical.

AT2 cell isolation and primary culture of AEC monolayers. Enriched populations of AT2 cells were prepared as previously described under a protocol approved by the Institutional Animal Care and Use Committee of the University of Southern California (4, 6). Briefly, AT2 cells were isolated from rat lungs by elastase (15 U/ml) disaggregation. Elastase was neutralized with 2 mM EDTA, 1% BSA, and 0.1% soybean trypsin inhibitor in a buffered saline solution (in mM: 136 NaCl, 2.2 NaHPO4, 5.3 KCl, 10 HEPES, and 5.6 glucose). Cells were further purified by panning on IgG-coated bacteriologic plates and resuspended directly in a minimal defined serum-free medium (MDSF), consisting of DME-F12 supplemented with 1.25 mg/ml BSA, 10 mM HEPES, 0.1 mM nonessential amino acids, 2.0 mM glutamine, 100 U/ml sodium penicillin G, and 100 μg/ml streptomycin (4, 6). EGF (10 ng/ml) for maximal response (5) was added to both apical and basolateral media from either day 0 (chronic exposure) or day 4 (subacute exposure).

Cells were seeded onto Transwell filter cups at a density of 10⁶ cells/cm² for preparation of high-resistance monolayers. Media were changed on the third day after plating, and the cells were subsequently fed every second day. Cultures were maintained in a humidified 5% CO₂ incubator at 37°C. AT2 cell purity (~90%) was assessed by immunofluorescence staining of freshly isolated cells using a monoclonal antibody (mAb) against pig180 lamellar body protein (Covance, Richmond, CA). Cell viability (~90%) was measured by trypan blue dye exclusion.

RNA isolation and RT-PCR. Total RNA was isolated from rat lung and from AEC on days 0, 1, and 8 by the acid guanidinium thiocyanate-phenol-chloroform extraction method of Chomczynski and Sacchi (8). Primer pairs for amplification of rat claudins 1, 2, 3, 4, 5, and 7 were designed on the basis of previously published mouse claudin sequences (per the National Center for Biotechnology Information website). Total RNA harvested from rat lung was reverse transcribed by use of oligo(dT) primers. cDNA was amplified by use of claudin primer pairs for 35 cycles [denaturation at 95°C for 45 s, annealing at various temperatures (Table 1) for 45 s, and elongation at 72°C for 60 s]. Amplified products were cloned into TOPO cloning vector (Invitrogen, Carlsbad, CA) and sequenced to verify homology to the respective mouse claudin sequences.

Northern analysis. Equal amounts of RNA (5–10 μg) were denatured with formaldehyde, size-fractionated by agarose gel electrophoresis under denaturing conditions, transferred to nylon membranes, and immobilized by UV cross-linking. Blots were probed with claudin-specific probes generated by RT-PCR and labeled with [32P]dCTP by the random primer method. Blots were washed at high stringency and visualized by autoradiography. Autoradiographs were then scanned with a UMAX PowerLook III Scanner (Umax, Fremont, CA) via Adobe Photosop (Adobe Systems, Mountain View, CA) and saved in TIFF file format for further analysis as described below. Level of 18S rRNA was used as loading control.

Western analysis. AEC monolayers on days 0, 1, and 8 in MDSF, or on day 5 in MDSF ± EGF (added on day 0 or day 4), were lysed in 2% SDS sample buffer at 37°C. Equal amounts of protein (5–35 μg) were resolved by SDS-PAGE under reducing conditions and electrophoretically blotted onto Immobilon-P membranes (Millipore, Bedford, MA). After blocking at room temperature with 5% nonfat milk (4 h), membranes were incubated overnight (4°C) with individual anti-claudin antibodies (Abs) (Zymed, South San Francisco, CA) in Tris-buffered saline-0.1% Tween 20. Finally, blots were incubated with horseradish peroxidase-linked anti-primary Ab IgG conjugates (1 h) at room temperature, and complexes were visualized by enhanced chemiluminescence (ECL; Amersham, Piscataway, NJ) and analyzed with an Alpha Ease FC Imaging System (Alpha Innotech, San Leandro, CA).

Immunofluorescence microscopy. AEC monolayers in MDSF ± EGF from day 4 were studied on day 5. Monolayers were washed with PBS (pH 7.2) and consecutively fixed in methanol (5 min) and acetone (1 min). Monolayers were blocked with 5% BSA at room temperature for 1 h, followed by incubation with Abs to claudins 4 and 7 and with biotinylated secondary Abs. Signal was amplified by using an avidin-biotin system with FITC (Avidin-D, Vector Laboratories, Burlingame, CA). Negative controls included substitution of nonspecific rabbit or mouse IgG for primary Abs. Cell monolayers were postfixed in 3.7% formalin and treated with Vectashield antifade mounting medium with propidium iodide to stain nuclei (Vector Laboratories). Slides were viewed with an Olympus BX60 microscope equipped with epifluorescence optics. Images were captured with a cooled charge-coupled device camera (Magnafire; Olympus, Melville, NY) with a barrier filter equipped for simultaneous detection of FITC and propidium iodide. Captured images were imported into Adobe Photoshop (Adobe Systems, Mountain View, CA) as TIFF files.

Statistical analysis. Densitometric analyses were performed using Alpha Ease FC Imaging System. For Northern and Western analyses of claudin expression in AEC undergoing transdifferentiation, densitometric values for day 0 were arbitrarily set to 100%, whereas the others are expressed as a percentage thereof. For Western analysis of claudin expression in AEC after EGF exposure, densitometric values for the lanes from unexposed cells were arbitrarily assigned 100%, whereas those for the lanes from exposed cells are expressed as a percentage thereof. Results of densitometric analyses are expressed as

<table>
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<th>Claudin</th>
<th>Forward Primers (5‘-3’)</th>
<th>Reverse Primers (5‘-3’)</th>
<th>Annealing Temperature</th>
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<td>1</td>
<td>ATCGCAAGTCTTGGTCTGCGATT</td>
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<td>CGIAGACGGAGATGGAAGCTTTG</td>
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<td>CACCAAGGAGCAAGAAGACCTTTG</td>
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<tr>
<td>4</td>
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</tr>
<tr>
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<tr>
<td>6</td>
<td>CATGCTTGGTCTCGTGGTGTACCC</td>
<td>CAGACAGTATTCTGGACAGGAGG</td>
<td>64.2°C</td>
</tr>
</tbody>
</table>

Based on previously published mouse claudin sequences on National Center of Biotechnology Information website, primers for rat claudins 1, 2, 3, 4, 5, and 7 were designed. Rat lung cDNA was amplified by PCR at denaturation conditions, transferred to nylon membranes, and immobilized by UV cross-linking. Blots were probed with claudin-specific probes generated by RT-PCR and labeled with [32P]dCTP by the random primer method.
means ± SE. Significance ($P < 0.05$) of differences between relative densities of more than two data groups was determined by one-way ANOVA with post hoc testing by Student-Newman-Keuls procedure.

**RESULTS**

To identify specific claudin isoforms expressed in rat lung, RT-PCR was performed with gene-specific primers using total RNA isolated from whole rat lung. Bands of appropriate sizes were detected for claudins 1, 3, 4, 5, and 7 (Fig. 1). Consistent with previous reports (29), mRNA for claudin 2 was not detected in whole lung.

Evaluation of changes in claudin expression during transdifferentiation of AT2 cells toward the AT1 cell-like phenotype in vitro using Northern analysis was performed with gene-specific probes for claudins 1, 3, 4, 5, and 7 that were generated by RT-PCR. As shown in representative blots in Fig. 2A, mRNA for claudins 3, 4, and 7 was detected in AEC by Northern analysis. Both claudin 3 and claudin 4 mRNA were abundant on day 0. Claudin 3 mRNA decreased with increasing time in culture [22 ± 4% by day 1 ($P < 0.05$) and 96 ± 1% by day 8 ($P < 0.05$); Fig. 2B]. Claudin 4 mRNA was decreased on day 8 by 58 ± 1% ($P < 0.05$). Interestingly, claudin 7 mRNA initially increased to 206 ± 38% of control between day 0 and day 1 ($P < 0.05$), subsequently declining on day 8 to 132 ± 37% compared with control ($P > 0.05$) (Fig. 2B). No bands were present by Northern analysis for claudins 1 and 5 (data not shown), indicating that mRNA levels for these claudins are below the level of detection in AEC by this approach. Using these probes, mRNA for claudin 1 and claudin 5 were detected in kidney and whole lung, respectively, as positive controls (data not shown).

To investigate the relationship between levels of claudin mRNA and protein in AEC, expression of claudins 1, 3, 4, 5, and 7 was evaluated in AEC by Western analysis using commercially available Abs (Fig. 3). As shown in these representative Western blots (Fig. 3A) and summarized by densitometric analysis (Fig. 3B), claudin 1 was faintly detected on all days studied and did not change significantly with time in culture. Claudin 3 was abundantly expressed in freshly isolated AT2 cells on day 0, declining by 24 ± 2% on day 1 ($P < 0.05$) and by 83 ± 1% on day 8 ($P < 0.05$). Claudin 5 was also more abundant on day 0 and declined by 82 ± 1% on day 8 ($P < 0.05$), with the majority of the reduction observed on day 1 (65 ± 6%, $P < 0.05$). In contrast, proteins for claudin 4 and claudin 7 were only faintly detected in AEC on day 0 and increased dramatically on later days by 214 ± 13% ($P < 0.05$) and 253 ± 47% ($P < 0.05$) on day 8 compared with day 0, respectively.

We have previously demonstrated that exposure of AEC monolayers to EGF increases TER (5). To evaluate the effects of EGF on claudin expression, AEC maintained in MDSF or treated with EGF from either day 0 (chronic) or day 4 (sub-

![Fig. 1. Reverse transcriptase-polymerase chain reaction (RT-PCR) for claudin mRNA in rat lung. RT-PCR performed using claudin-specific primer pairs with total RNA isolated from rat whole lung resulted in appropriately sized bands for claudin isoforms 1, 3, 4, 5, and 7. Claudin 2 mRNA was not detected. M, 1,000-bp ladder; N, no RT control.](http://jap.physiology.org/)

![Fig. 2. Changes in expression of claudin mRNA in alveolar epithelial cells (AEC) accompanying transdifferentiation. cDNA probes for claudins 1, 3, 4, 5, and 7 generated by RT-PCR were used to evaluate changes in claudin expression by Northern analysis. A: representative Northern blots ($n = 3$) for day 0 (d0), day 1 (d1), and day 8 (d8) are shown with corresponding 18S rRNA. B: scanned blots were analyzed densitometrically to determine band intensities and normalized to the corresponding 18S rRNA loading control. Relative expression on day 0 was set at 100%. *Significant difference from d0, $P < 0.05$.](http://jap.physiology.org/)
acute) were harvested on day 5. Western analysis (Fig. 4A) demonstrated that claudins 4 and 7 increased by 66 ± 15% (P < 0.05) and 101 ± 24% (P < 0.05), respectively, after exposure to EGF from day 4, and by 70 ± 6% (P < 0.05) and 124 ± 32% (P < 0.05) after treatment with EGF from day 0 compared with untreated cells on day 5. In contrast, proteins for claudins 3 and 5 decreased after exposure to EGF. When EGF was added on day 4, expression of claudin 3 decreased by 51 ± 7% (P < 0.05) and that of claudin 5 decreased by 84 ± 1% (P < 0.05) by day 5. With chronic EGF treatment (i.e., from day 0 to day 5), expression of claudin 3 and claudin 5 decreased by 58 ± 3% (P < 0.05) and 86 ± 1% (P < 0.05), respectively. No significant changes were observed in claudin 1 expression after EGF exposure (Fig. 4B).

To assess whether changes in cellular levels of claudin protein reflected changes in membrane-associated claudins, expression of claudins 4 and 7 was evaluated by immunofluorescence microscopy in AEC monolayers (Fig. 5). Representative immunofluorescence images demonstrate localization of claudins 4 and 7 to AEC membranes in monolayers maintained in MDSF on day 5. After treatment with EGF from day 4, there was a marked increase in membrane expression of both claudins 4 and 7 on day 5.

DISCUSSION

We demonstrate expression of claudins 1, 3, 4, 5, and 7 in AEC, suggesting a role for these proteins in TJ formation and regulation of permeability properties of alveolar epithelium. The pattern of claudin expression changes markedly during transdifferentiation of AEC from an AT2 toward an AT1 cell-like phenotype concurrent with monolayer formation and establishment of TJ. Protein for claudins 3 and 5 is greater in abundance in AT2 cells and on earlier days in culture than in AT1-like cells on day 8, whereas claudin 4 and 7 proteins are increased in AT1-like cells. After exposure to EGF, claudins 3 and 5 are reduced, whereas claudins 4 and 7 are increased. Uprogelation of claudins 4 and 7 by EGF is accompanied by increased localization of these proteins to the lateral cell membranes, consistent with increased incorporation into TJ. These findings suggest that the precise combination and/or ratios of claudins expressed by AEC may differentially regulate TJ characteristics between adjacent cells and that the effects of EGF on TER may be mediated by claudin expression.

An intact epithelial barrier is of importance in the maintenance of homeostasis in multicellular organisms. The internal environment must be separated from the external environment, while allowing selective permeability to fluids and solutes, both charged and neutral (1, 23, 27). In alveolar epithelium, as in other epithelia, this task is accomplished by the functionally intact intercellular TJ. Although the protein occludin is structurally associated with TJ and was initially believed to be essential for TJ formation and function, deletion of occludin in transgenic mice was not associated with barrier abnormalities, implying that other proteins must determine TJ properties (20). Increasingly, claudins have become recognized as critical determinants of TJ properties (11, 18, 29). In addition to being responsible for TJ fibril assembly, the particular combination and ratio of specific claudins expressed by an epithelium appear to underlie cell- and tissue-specific permeability properties of TJ (14, 31, 35). In this regard, extracellular domains...
of specific claudins have been shown to affect TER and ion selectivity of some epithelial cell lines (9, 10).

Claudins 3, 4, 5, and 7 were identified as the predominant claudins expressed in lung and AEC. Claudin 1, although detected in whole lung by RT-PCR, was not detected in AEC by Northern analysis and was present at very low levels by Western analysis, suggesting that claudin 1 probably does not play a major role in AEC TJ. Claudin 2 was not detected in whole lung by RT-PCR and was not further evaluated. Several other claudin family members (including claudins 10, 12, 13, 15, and 18) have previously been identified in lung at mRNA and/or protein levels (28, 29). Although these claudins may influence AEC TJ properties, we restricted our studies to those claudins for which Abs were readily available.

Claudins 3 and 5 were more abundant in freshly isolated cells and on earlier days in culture, whereas claudins 4 and 7 were upregulated at later times. Claudin 3 protein decreased over time in parallel with changes in claudin 3 mRNA, but in the case of claudins 4 and 7, changes in protein did not correlate with corresponding mRNA levels, suggesting that these proteins may have variable turnover rates, perhaps because of their homotypic or heterotypic associations. Because claudins can interact in both homotypic and heterotypic fashions with other claudin family members (14), observed differences in relative levels of claudin proteins expressed by AT1 and AT2 cells suggest a mechanism whereby changes in interactions between claudins could determine cell-cell specific differences in barrier properties of the alveolar epithelium, particularly after injury. For example, claudin 4 has previously been shown to confer high TER and lower permeability to Na⁺ in MDCK cells (12, 30), whereas overexpression of other claudin family members (e.g., claudin 2) has been associated with a more leaky phenotype (12, 30). Thus high levels of claudin 4 may be important for establishment of a tight barrier between adjacent AT1 cells or between AT2 and AT1 cells under baseline conditions, whereas permeability between adjacent AT2 cells (e.g., accompanying AT2 cell epithelial proliferation after injury) may be increased in association with lower levels of claudin 4. Whether or not differences in claudin expression between AT2 and AT1 cells translate into differences in barrier properties is presently unknown.

We have previously demonstrated that EGF, a mitogenic polypeptide that exerts a broad range of effects on cell proliferation and tumorigenesis in epithelia, increases TER in AEC monolayers (5). EGF (10–200 ng/ml) added from day 0 or day 4 increased TER on day 5 by ≥60% compared with monolayers maintained in MDSF. Effects of EGF on TER were gradual in onset, occurring 10–12 h after addition of the growth factor, suggesting the requirement for increased cellular protein synthesis. In the present study, we demonstrate that claudins 4 and 7, which increase during monolayer formation and transdifferentiation of AT2 cells to an AT1 cell-like phenotype, are upregulated by EGF. There is increased localization at the lateral cell membrane consistent with accumulation at TJ, suggesting that the increase in TER is being mediated via effects on paracellular pathways. These effects occur with exposure to EGF either from the time of AT2 cell plating or from day 4 in culture, indicating that the effects are independent of both cell adhesion and plating efficiency. Furthermore, the effects appear to be independent of phenotype, because we have previously shown no change in the rate of transition toward the AT1 cell phenotype in the presence of EGF (Z. Borck and E. D. Crandall, unpublished results). Differential changes in patterns of claudin expression after EGF exposure again suggest that certain claudins may confer decreased TJ permeability (e.g., claudins 4 and 7), whereas others may be associated with a more leaky phenotype (e.g., claudins 3 and 5). Changes in the pattern of claudin expression may be cell- and tissue-specific, as shown by the recent findings that MDCK II cell monolayers subjected to EGF-induced EGFR activation

![Image of immunofluorescence microscopy of rat AEC monolayers after exposure to EGF.](https://example.com/image.jpg)

Fig. 5. Immunofluorescence microscopy of rat AEC monolayers after exposure to EGF. Immunofluorescence microscopy of AEC monolayers on day 5 demonstrated increased membrane expression of claudins 4 and 7 after exposure to EGF on day 4 relative to monolayers maintained in MDSF. Absence of staining is seen when primary antibodies are substituted by equivalent concentrations of rabbit or mouse IgG control. Red staining represents propidium iodide-stained nuclei. Photographs are representative of ≥9 monolayers from ≥3 separate preparations. Original magnification ×400.
exhibit a threefold increase in TER, significantly decreased claudin 2 expression, and increased cellular redistribution and expression of claudins 1, 3, and 4 (25).

In the previous study, significant increases in active sodium transport were observed concurrent with effects of EGF on TER (5). These effects appeared to be independently regulated, because effects on TER occurred with a half-time of 12 h, whereas effects on short-circuit current had a half-time of 20 h and were associated with increases in expression of both α1- and β1-subunits of Na+/K+-ATPase. In recent studies to evaluate the effects of transforming growth factor-β1 on barrier properties of AEC monolayers, although we observed an increase in paracellular permeability (decrease in TER), active ion transport increased in association with increased expression of α1- and β1-subunits of Na+/K+-ATPase (34). Similarly, Cheek et al. (7) observed an increase in paracellular permeability (decrease in TER) after nitrogen dioxide exposure, although short-circuit current was unchanged. These observations suggest that the mechanisms regulating paracellular sodium permeability and active ion transport are likely independently regulated.

Our present findings are generally supported by a recent report (32) demonstrating claudin 3, 4, and 5, but little or no claudin 1 or 2, expression in AT2 cells. We confirmed the presence of claudin 5 in freshly isolated AT2 cells, which previously had been described only in endothelial cells. Decreased levels of claudin 3 and claudin 5 expression observed with increasing time in culture accompanying the transition from AT2 to AT1 cell-like phenotype are consistent with the prior study, although we observed increased (rather than decreased) levels of claudin 4 protein. Importantly, decreased TER associated with exposure to methanandamide was accompanied by increases in expression of claudin 3 and claudin 5 (32), whereas we observed decreased expression of these two claudin isoforms when TER was increased because of EGF exposure. These findings support the hypothesis that differential regulation of claudin isoforms including claudin 3 and claudin 5 is important in determination of TER in AEC.

In summary, we demonstrate in the present study expression of several claudin family members in AEC, consistent with a role for these proteins in regulating paracellular permeability of the alveolar epithelial barrier. Changes in specific claudin isoform expression after exposure to EGF provide further evidence supporting a role for claudins in regulating alveolar epithelial barrier properties and suggesting a mechanism to account for increases in TER observed in our previous studies (5). These data suggest a possible novel therapeutic approach for reversing increased AEC permeability after lung injury through over- or underexpression of specific claudin family members to restore barrier function.

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