Phenotypic control of gap junctional communication by cultured alveolar epithelial cells

VALSAMMA ABRAHAM, MICHAEL L. CHOU, KRISTINE M. DeBOLT, AND MICHAEL KOVAL

Department of Physiology and Institute for Environmental Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

Abraham, Valsamma, Michael L. Chou, Kristine M. DeBolt, and Michael Koval. Phenotypic control of gap junctional communication by cultured alveolar epithelial cells. Am. J. Physiol. 276 (Lung Cell. Mol. Physiol. 20): L825–L834, 1999.—We examined phenotype-specific changes in gap junction protein [connexin (Cx)] expression and function by cultured rat alveolar type II cells. Type II cells cultured on extracellular matrix in medium containing keratinocyte growth factor (KGF) and 2% fetal bovine serum (FBS; KGF/2) retained expression of surfactant protein C and the 180-kDa lamellar body membrane protein (lbm180). These markers were lost when cells were cultured in medium containing 10% FBS (MEM/10). With RT-PCR, cells cultured in MEM/10 showed transient increases in Cx43 and Cx46 mRNA expression, whereas Cx32 and Cx26 decreased and Cx30.3 and Cx37 were unchanged. Transient changes in Cx32, Cx43, and Cx46 protein expression were confirmed by immunoblot. In contrast, cells cultured in KGF/2 retained expression of Cx32 and showed increased expression of Cx30.3 and Cx46 mRNAs, compared with that in day 0 cells. With immunofluorescence microscopy, Cx32 and Cx43 were at the plasma membrane of cells grown in KGF/2, whereas Cx46 was exclusively intracellular. Type II cells cultured in MEM/10 showed ~3- to 4-fold more intercellular transfer of microinjected lucifer yellow through gap junctions than cells grown in 2% FBS. Thus type II cells dynamically alter gap junctional communication, and distinct alveolar epithelial cell phenotypes express different connexins.

connexin; epithelium; intercellular communication; keratinocyte growth factor

Tight junctions are directly responsible for maintaining the permeability barrier within epithelial cell monolayers (17). The junctional complex between epithelial cells is also composed of other elements, which primarily function to mediate intercellular communication rather than directly contribute to the permeability barrier (24, 33, 41). Gap junctions are one such element, which are composed of transmembrane channel-forming proteins in the connexin family (for reviews see Refs. 12, 21, 30, 37). To date, over a dozen mammalian connexins have been identified. Gap junction channels enable the direct transfer of aqueous molecules from the cytoplasm of one cell to a neighboring one. Whereas aqueous molecules move through gap junction channels by passive diffusion, channels formed by different connexins have distinct permeability. Thus cells can use differential connexin expression as one means to regulate gap junctional intercellular communication (6, 10, 11, 28). Channel gating (43), connexin phosphorylation (31, 44), and expression of other factors such as cell adhesion molecules (36) can also contribute to the regulation of gap junction-channel function.

It has been well established that highly purified alveolar type II epithelial cells can be isolated and studied in culture (18, 34). The primary type II cell phenotype is highly sensitive to culture conditions. Under “standard” culture conditions, alveolar epithelial cells rapidly stop expressing type II cell markers such as surfactant proteins (SPs) and no longer form lamellar bodies. However, cultured alveolar epithelial cells are capable of forming tight junctions (8) and show increased expression of type I cell-specific markers such as intercellular adhesion molecule-1 (4, 14) and T1α (16, 39), suggesting that these cells have differentiated to a type I-like cell phenotype.

Lee et al. (33) demonstrated that isolated primary alveolar type II epithelial cells express at least three different connexins, connexin (Cx) 26, Cx32, and Cx43. With increasing time of culture in the presence of fetal bovine serum (FBS), expression of Cx26 and Cx32 decreased, whereas Cx43 expression transiently increased. Consistent with an effect of changing connexin expression on gap junctional intercellular communication, the extent of lucifer yellow (LY) dye transfer between cells increased with increasing time in culture under conditions where the type II cells changed to the type I-like phenotype.

By modification of cell culture conditions, a number of groups have been able to maintain expression of type II cell-specific proteins by alveolar epithelial cells (2, 9, 13, 45). Typically, these systems involve culture of the cells on extracellular matrix-derived material with either serum-free medium or medium containing low levels of serum. Culture of the cells on an air interface or in low volumes of medium may also enhance the expression of SPs (19, 45). Frequently, other factors such as cAMP analogs (2) and keratinocyte growth factor (KGF) (9, 13, 45) are used to increase expression of type II cell markers. The ability of KGF to retain the type II cell phenotype is of particular significance because KGF has also been found to protect alveolar cells from injury caused by a variety of factors, such as bleomycin (46) and irradiation (47).

We examined the effect of culture conditions on gap junction protein expression and function in cultured type II cells. Alveolar epithelial cells cultured on Madin-Darby canine kidney (MDCK)-derived extracellular matrix in medium containing low levels of serum and KGF retained the expression of at least two type II cell markers, SP-C (5) and the 180-kDa lamellar body

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membrane protein (Ibm180) (48). These cells showed differential connexin expression compared with cells cultured in MEM containing 10% FBS (MEM/10). This supports the notion that gap junction protein expression is altered depending on the differentiated state of alveolar epithelial cells. Furthermore, the extent of gap junctional communication correlated with cell phenotype, where cells cultured under conditions in which the type II cell phenotype was retained showed more restricted gap junctional intercellular communication than alveolar epithelial cells cultured in MEM/10, which were highly coupled. This suggests that alveolar epithelial cells may modulate gap junctional intercellular communication as part of the mechanism to control cell phenotype.

**METHODS**

Isolation and culture of type II cells. Sprague-Dawley rat alveolar type II cells were isolated from lavaged, perfused lungs by elastase digestion with the method of Dobbs et al. (18) with modifications. Cells were biopanned with IgG-coated culture dishes to remove alveolar macrophages and other Fc receptor-expressing cells. To obtain type II cells with high purity, we used a second round of immunodepletion by incubating the cells with BioMag beads coated with rabbit IgG (PerSeptive Biosystems, Framingham, MA). Using this approach, we were able to routinely get preparations that contained 90–95% type II cells as assessed by positive phosphe 3R staining (34) or the lamellar body-specific antibody 3C9 (anti-lbm180; kindly provided by Drs. A. B. Fisher and H. Shuman, University of Pennsylvania School of Medicine, Philadelphia, PA) (48).

To allow cells to progress to a type I-like phenotype (7, 8), cells were cultured on standard tissue culture-treated plastic dishes or glass coverslips in MEM/10 composed of MEM (Life Technologies, Gaithersburg, MD) containing 2 mM glutamine, 100 U/ml of penicillin, 100 µg/ml of streptomycin, and 10% FBS. Alternatively, cells were cultured with the use of a modification of protocols used to preserve the type I phenotype in culture (2, 45). Extracellular matrix derived from MDCK cells was deposited onto culture dishes or glass coverslips by cultured MDCK cells. After the cells had grown to confluence, the dishes were incubated with 1% deoxycholate in H2O, pH 8.0, for 3 min at room temperature, washed twice for 5 min with MEM, and then stored at 4°C for up to 1 mo. Just before use, the dishes were incubated in fresh MEM at 37°C for at least 1 h. Type II cells plated on MDCK matrix-coated substrata were cultured overnight in MEM/10, followed by daily medium changes to MEM containing 2% FBS and 5 ng/ml of KGF (KGF/2; Sigma, St. Louis, MO). In some instances, type II cells plated on untreated glass coverslips were treated with KGF. Cells were plated at 3.6 x 10^6 in 1.5 ml medium/35-mm dish or 5 x 10^6 in 3 ml medium/60-mm dish for all culture conditions tested.

**Table 1. PCR primers used**

<table>
<thead>
<tr>
<th>Connexin</th>
<th>Primers (Sense/Antisense)</th>
<th>Amplicon Size, bp</th>
<th>Positive Control</th>
</tr>
</thead>
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<tr>
<td>Cx26</td>
<td>5'-CGG AAG TTC ATG AAG GGA GAG AT-3' 5'-GCT TCT TGG GAC TTC CTC GAG CA -3'</td>
<td>365</td>
<td>Liver</td>
</tr>
<tr>
<td>Cx30</td>
<td>5'-GCT GGC GTG AAC AAC CAC TCT-3' 5'-CAC CTT CTC CGG TAT GTG CTC-3'</td>
<td>349</td>
<td>Skin</td>
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<tr>
<td>Cx30.3</td>
<td>5'-ATG AAG TGG GAA TTT CTC CAG-3'</td>
<td>798</td>
<td>Kidney</td>
</tr>
<tr>
<td>Cx31</td>
<td>5'-ATG GAT TGG AAG AAG CTT CAG-3' 5'-TCT TGG ATG GAAG CTC CAG-3'</td>
<td>813</td>
<td>Skin</td>
</tr>
<tr>
<td>Cx31.1</td>
<td>5'-ATA TAC CCT CCC GTC TAT GGT-3' 5'-GAA GAT TTT CAC AGA ATG GTT T-3'</td>
<td>357</td>
<td>Skin</td>
</tr>
<tr>
<td>Cx32</td>
<td>5'-CTG CTC TAC CCG CCG GTG CAT GC-3'</td>
<td>386</td>
<td>Liver</td>
</tr>
<tr>
<td>Cx33</td>
<td>5'-GCG AGT CCG GAG AAG CAC CGG TGG CA-3'</td>
<td>476</td>
<td>Testes</td>
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<td>Cx37</td>
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<td>Cx40</td>
<td>5'-CTG GCC AAC TCA CCC GAG GG-3'</td>
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<tr>
<td>Cx43</td>
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<td>ROS cells</td>
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<tr>
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<td>5'-GCC ACC ACC ACT GCC CCA-3' 5'-ATT CGT GTT GTG GTC GGG GAA ATC-3'</td>
<td>1,218</td>
<td>ROS/Cx45 cells</td>
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<tr>
<td>Cx46</td>
<td>5'-GGA AAG GCC ACA GGG TTT CCT GG-3'</td>
<td>332</td>
<td>HeLa/Cx46 cells</td>
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<tr>
<td>Cx50</td>
<td>5'-CTT TTG ACA GAG GTT GGA ATC GTG-3' 5'-CCG ATT GTC ATC GGG TGT CAG CTC-3'</td>
<td>405</td>
<td>Lens</td>
</tr>
</tbody>
</table>

Cx, connexin.
known to express a given connexin (liver, Cx26 and Cx32; heart, Cx37 and Cx43; ROS cells, Cx46; skin, Cx30.3) while the negative control was done with samples that were not reverse transcribed. Relative amounts of each PCR product were normalized to the amount of β-actin amplified with primers obtained from Clontech and plotted as a function of relative fluorescence units (RFU). β-Actin was a suitable control for the amount of cDNA in each sample because the amount amplified for each treatment was comparable (in RFU/µg RNA: day 0, 0.83 ± 0.17; day 4 MEM/10, 0.87 ± 0.08; day 4 KGF/2, 1.00 ± 0.08; all n = 3 experiments). Data were analyzed with SigmaStat (Jandel), with the Mann-Whitney U-test for significance.

Immunoblot analysis. Cells cultured for varying amounts of time in MEM/10 were harvested in phosphate-buffered saline (PBS) containing 1.0% Triton X-100, 0.2% SDS, 1 mM sodium vanadate, 10 mM sodium fluoride, 1 mM phenylmethylsulfonyl fluoride, 2 µg/ml of leupeptin, and 1 µg/ml of pepstatin. Protein concentration was measured with the bicinchoninic acid reagent (Pierce, Rockford, IL). Samples normalized for total amount of protein were resolved by SDS-PAGE, transferred to polyvinylidene difluoride membrane, and then blocked overnight with 40 mM Tris, pH 7.5, 5% Carnation powdered milk, and 0.1% Tween 20 (Blotto). The blots were incubated with Blotto containing antisera that recognized Cx32 (Zymed, South San Francisco, CA), Cx43 (28), or Cx46 (29), washed, and then further incubated with goat anti-rabbit IgG horseradish peroxidase (Roche Molecular Biochemicals, Indianapolis, IN). In some cases, blots were incubated with a preformed primary-secondary antibody complex. Specific signals corresponding to a given connexin were detected with the enhanced chemiluminescence reagent (Amersham).

Immunofluorescence. Cells grown on either uncoated or MDCK matrix-coated glass coverslips were washed with PBS, permeabilized with 1:1 methanol-acetone for 2 min at room temperature, and then washed three times in PBS, once PBS plus 0.5% Triton X-100, and then twice in PBS plus 0.5% Triton X-100 plus 2% heat-inactivated goat serum. Samples were incubated with antisera diluted into the PBS-Triton X-100-goat serum mixture for 1 h at room temperature, washed, and then incubated with rhodamine-conjugated goat anti-rabbit Isg horseradish peroxidase (Roche Molecular Biochemicals, Indianapolis, IN). In some cases, blots were incubated with a preformed primary-secondary antibody complex. Specific signals corresponding to a given connexin were detected with the enhanced chemiluminescence reagent (Amersham).

RESULTS

Connexin expression by cultured type II cells. We initially used immunofluorescence to analyze connexin expression by primary type II cells isolated from rat lungs. Cells analyzed after overnight culture in MEM/10 showed expression of Cx32, Cx43, and Cx46 at the cell surface by immunofluorescence (Figs. 1 and 2). These cells also showed prominent lamellar bodies as indicated by immunofluorescence labeling with anti-lbM180 antisera (Fig. 1, c and d).

With increasing time of culture in MEM/10, the cells spread and there was an increase in both Cx32 and Cx46 present at the cell surface in areas where cells were in contact (Fig. 2). In contrast, Cx32 expression became undetectable (see Fig. 6 d). Although Cx43 was predominantly at the cell surface, there was also a considerable intracellular pool of Cx46.

The expression profile for Cx32, Cx43, and Cx46 was confirmed by immunoblot, which is in good agreement with the immunofluorescence images (Fig. 3). Densitometric analysis of immunoblots revealed that the expression of these connexins varied with time in culture. Note that both Cx43 and Cx46 were also phosphorylated as suggested by the presence of multiple molecular-weight isoforms detected by immunoblot.

Effect of culture conditions on connexin expression by type II cells. KGF has been used to allow the culture of alveolar epithelial cells while maintaining some characteristics of the type II phenotype (9, 13, 45). We adapted this approach where type II cells were cultured on surfaces coated with extracellular matrix derived from MDCK cells and in KGF/2. On the basis of morphometric measurement of average cell area, the density of cells cultured in KGF/2 on day 4 (806 ± 71 cells/mm²; n = 26 cells) was −40% greater than the density of cells cultured on uncoated surfaces in MEM/10 (575 ± 47 cells/mm²; n = 57 cells). This was likely due to greater type II cell adherence to the MDCK matrix-coated surface because cells plated on this substratum in MEM/10 also showed a high density on day 4 of culture (723 ± 48 cells/mm²; n = 33 cells). Despite this difference in cell density, the number of nearest neighbors for cells cultured in either the presence or absence of extracellular matrix derived from MDCK cells was comparable (6.6 ± 0.2 neighbors/cell for KGF/2 (n = 14 cells) vs. 6.2 ± 0.3 neighbors/cell for MEM/10 (n = 18 cells)).

Cells cultured in either MEM/10 or KGF/2 showed expression of the tight junction protein ZO-1, indicative that cells cultured under either set of conditions retained an epithelial phenotype (Fig. 4, A and B). Type II cells showed ZO-1 present both at the cell surface and in an intracellular pool. This was consistent with other studies (3, 23, 42) on the intracellular localization of
ZO-1, which indicate that the partitioning of this protein between tight junctions and the cytosol is highly sensitive to the state of the cells. For instance, the presence of intracellular ZO-1 may be due to the culture of these cells on solid substrata as opposed to permeable membranes.

Consistent with maintenance of the type II phenotype, cells cultured in KGF/2 showed expression of SP-C and lbm180, which were absent from alveolar epithelial cells cultured for 4 days in MEM/10. However, the expression of these markers by cells cultured in KGF/2 for 4 days was not uniform. In a typical experiment, roughly 30% (51 of 165) of the cells showed anti-SP-C immunofluorescence and 45% (131 of 281) of the cells had prominent lbm180 expression. In contrast, the pattern of connexin expression was much more uniform.

We then examined the effect of altering culture conditions on connexin expression by type II cells. As shown in Fig. 5, the profile of connexins expressed by type II cells cultured for 4 days in KGF/2 was distinct from that of cells cultured in MEM/10. Increased Cx43 and Cx46 mRNA expression was detected as early as 1 day after culture (data not shown). The expression of Cx26, Cx32, and Cx43 mRNAs by type II cells cultured in MEM/10 was largely consistent with the observations of Lee et al. (33), who found that type II cells cultured on tissue culture plastic in medium containing 10% FBS showed decreased Cx26 and Cx32 expression and increased Cx43 expression. We also found that type II cells cultured under these conditions expressed Cx30.3, Cx37, and Cx46 mRNAs (Fig. 5). In contrast to cells cultured in MEM/10, type II cells cultured in KGF/2 showed enhanced levels of Cx30.3 and Cx32 mRNAs, as assessed by RT-PCR, compared with alveolar epithelial cells cultured in MEM/10. Cx26, Cx37, Cx43, and Cx46 mRNAs were at comparable levels for alveolar epithelial cells cultured under either set of conditions. We were unable to detect expression of Cx30, Cx31, Cx31.3, Cx33, Cx40, Cx45, or Cx50 mRNAs by RT-PCR in either freshly isolated or cultured type II cells (data not shown).

By the criterion of connexin expression, the phenotype of type II cells cultured for 4 days in KGF/2 was distinct from that of freshly isolated type II cells. In particular, cultured cells had increased expression of Cx43 and Cx46 mRNAs than freshly isolated type II cells (day 0). Cx26 mRNA expression was decreased, and Cx32 mRNA expression was ~30% of the levels shown by day 0 cells.

Immunofluorescence confirmed the expression of Cx32, Cx43, and Cx46 by type II cells cultured in KGF/2 (Fig. 6). Cx32 and Cx43 were present at the cell surface. However, expression of Cx46 was localized to the perinuclear region of the cell and thus not likely to be directly involved in intercellular communication between these cells. On the basis of previous work on Cx46 expression by osteoblastic cells, it is likely that Cx46 is being retained in the trans-Golgi network by type II cells cultured in KGF/2 (29).

FBS increases gap junctional intercellular communication. We then used LY dye transfer to examine gap junctional intercellular communication in cells cultured for 4 days either in MEM/10 or in KGF/2. Day 4 cells cultured in MEM/10 showed extensive transfer of LY (Fig. 7), where dye was transferred to an average of
12.8 ± 6.9 cells/micronjection (n = 29 injections; Fig. 8). Dye transfer was completely inhibited by pretreatment of the cells with the gap junction inhibitor 40 µM 18β-glycyrrhetinic acid. Transfer of LY between alveolar epithelial cells cultured in MEM/10 was rapid, where extensive LY transfer from injected cells past the nearest neighbors was apparent in as little as 30 s.

![Image of immunofluorescence localization](Fig. 2) Immunofluorescence localization of Cx43 and Cx46 in type II cells cultured in MEM/10. Cells were cultured for 1 (a and b), 2 (c and d), or 6 (e and f) days in MEM/10, fixed, permeabilized, and immunostained for Cx43 (a, c, and e) or Cx46 (b, d, and f) expression. In both cases, there was an increase in appearance of connexin immunolabeling at plasma membrane with increasing time in culture. Bar, 10 µm.

![Image of immunoblot analysis](Fig. 3) Immunoblot analysis of Cx32, Cx43, and Cx46 protein expression. A: type II cells cultured for indicated days were solubilized, resolved by SDS-PAGE, transferred to polyvinylidene difluoride, and blotted with either anti-Cx32, anti-Cx43, or anti-Cx46. Control (con) samples were rat liver for Cx32, ROS cells for Cx43, and HeLa/Cx46 cells for Cx46. Duplicate immunoblots of Cx32 (B), Cx43 (C), and Cx46 (D) were scanned, and resulting densitometric data were plotted as function of days in culture.
In contrast, day 4 cells cultured in KGF/2 showed limited transfer of LY (3.8 ± 4.2 cells/microinjection; n = 26 injections). Dye transfer remained limited, even after prolonged incubation at 37°C (>15 min). Similar results were obtained with hydroxyxycoumarin carboxylic acid, dichlorofluorescein, and ethidium bromide, suggesting that limited dye transfer by type II cells cultured in KGF/2 is likely to reflect a difference in the overall extent of coupling. Also, although the average cell perimeter for cells cultured in KGF/2 (134 ± 4 µm; n = 42 cells) was −22% less than the perimeter for cells grown in MEM/10 (163 ± 6 µm; n = 58 cells), the difference in LY dye transfer was too large to be due to a difference in gap junction cross-sectional area at cell contacts.

To analyze factors that altered gap junctional communication, we cultured type II cells for 4 days under a variety of conditions (Fig. 8). Increased intercellular communication correlated with high serum content in the medium, whereas the extent of gap junctional dye transfer was independent of KGF or culture of the cells on extracellular matrix-coated surfaces.

**DISCUSSION**

Previous studies (2, 9, 13, 45) have shown that the phenotype of isolated, primary alveolar type II cells can be manipulated depending on culture conditions. With the use of a hybrid approach, type II cells cultured on
MDCK-derived extracellular matrix in medium containing KGF and 2% FBS were found to partially retain markers for the type II cell phenotype, namely expression of SP-C and a lamellar body protein, lbm180. In contrast, the same cells cultured on untreated substrata in medium containing 10% FBS progressed to an alternate phenotype and no longer expressed these type II cell proteins. We then used these culture systems to determine whether alveolar epithelial cells showed differential gap junction protein expression. This was found to be the case, where freshly isolated type II cells and type II cells cultured under different conditions showed expression of distinct subsets of the connexin family (Fig. 5). These results are consistent with the notion that alveolar epithelial cells regulate gap junctional communication as part of the differentiation process.

Differentiation-dependent changes in gap junction protein expression and function have been observed for a number of cultured cell systems. For instance, keratinocytes allowed to differentiate in culture showed selective changes in methionine and cytidine triphosphate permeability through gap junctions as a result of changing connexin expression (11). By the criterion of connexin expression, cells cultured in KGF/2 were in a unique state of differentiation because cultured type II cells showed more Cx30.3, Cx43, and Cx46 expression and less Cx32 expression than freshly isolated type II cells. One possibility is that a monolayer of type II-like cells represents a distinct state as opposed to type II...

Fig. 5. Differential connexin mRNA expression by cultured alveolar epithelial cells. RNA from freshly isolated type II cells (day 0 (d0)) or type II cells cultured for 4 days (d4) in either MEM/10 (MEM) or KGF/2 (KGF) was DNase treated, reverse transcribed, and then amplified by PCR with primers corresponding to specific connexins. Resulting PCR product was resolved by agarose gel electrophoresis, stained with ethidium bromide, and imaged (A). +, Positive control mRNA; −, PCR without prior reverse transcriptase. B: fluorometric analysis of amplicon fluorescence was normalized and plotted as averages ± SE of triplicate determinations. RFU, relative fluorescence units. Significant difference (P < 0.05) from: *d0; †MEM/10 d4.

Fig. 6. Differential connexin protein expression by cultured alveolar epithelial cells. Type II cells cultured for 4 days in either KGF/2 (a–c) or MEM/10 (d–f) were fixed, permeabilized, and immunostained for Cx32 (a and d), Cx43 (b and e), or Cx46 (c and f). Connexins were frequently present at cell surface (arrows), whereas Cx46 was found to be localized to a perinuclear compartment in type II cells cultured in KGF/2 (arrowhead). Bar, 10 μm.
cells in the adult alveolus, which are intermixed with mature type I cells (15, 25). If this is the case, then type II cells in direct contact with other type II cells may reflect the state of alveolar epithelial cells in the developing air spaces (1).

Cx43 and Cx46 protein expression (Fig. 3) did not appear to be proportional to mRNA expression (Fig. 5), indicating that connexin expression by alveolar epithelial cells was likely to be regulated at the posttranslational level. Cx46 expression was also regulated at the level of membrane transport (Fig. 6). The differential localization of Cx46 is intriguing, especially in light of previous work on ROS osteoblastic cells (29). These cells retain Cx46 in the trans-Golgi network as a monomer rather than assembled into hexameric hemichannels. This suggests that the intracellular pool of Cx46 retained by type II cells grown under conditions that preserve the type II cell phenotype will also be in a monomeric form. If so, then cultured type II cells will be useful in determining the relationship between Cx46 transport and oligomerization.

Although type II cells cultured for 4 days in MEM/10 showed extensive intercellular transfer of LY, we found that transfer of LY between alveolar epithelial cells cultured in KGF/2 was limited. Further analysis of culture conditions indicated that high levels of dye transfer correlated with culture in the presence of 10% FBS, whereas cells in 2% FBS showed low levels of dye transfer. The use of either serum-free medium or medium containing low levels of serum is a key factor of culture systems designed to allow alveolar epithelial cells to retain the type II cell phenotype. However, other elements such as the culture of cells on preformed extracellular matrix or stimulation by KGF are also important for determining the phenotype of cultured type II cells. Consistent with this, we found that cells cultured in MEM plus 2% FBS under conditions where one of these elements was missing rapidly lost lamellar bodies (data not shown) despite showing low levels of LY intercellular dye transfer.

Furthermore, as a result of culture in 10% FBS, increased gap junctional communication was correlated with the transition to the type I phenotype. This is consistent with the studies of Paine et al. (38), who found that culture in FBS-containing medium increased loss of some type II cell-specific markers such as SP-A expression and lamellar bodies compared with culture in serum-free medium. This is likely to result from a soluble growth factor unique to FBS because adult rat serum does not have the same effect on type II cells (8).
Despite the difference in gap junctional intercellular communication exhibited by cells cultured in different levels of FBS, Cx43 was expressed at the plasma membrane by cells cultured under both conditions (Fig. 6). One possibility is that differential Cx43 phosphorylation, such as enhanced phosphorytrosine or phosphoserine formation, may occur in type II cells in a FBS-dependent manner that could alter Cx43 function (32, 35). However, in preliminary studies, we did not see culture-specific changes in the pattern of Cx43 phosphorylation based on the presence of multiple Cx43 species by SDS-PAGE (Fig. 3). Alternatively, the presence of Cx46 at the cell surface of cells cultured in MEM/10 may enhance gap junctional communication. Differences in overall cell architecture (24) or in the expression of other cofactors such as cell adhesion molecules (36) may also play a role in altering gap junctional intercellular communication between alveolar epithelial cells.

In lung air spaces, type II cells act as the stem cell precursor for the replacement of type I cells either through normal cell turnover or as a result of injury. Isolated, primary type II cells cultured in medium containing 10% FBS have frequently been used as a model system for the study of changes that occur during replacement of the alveolar epithelium. Along with the observations of Lee et al. (33), our data suggest that changes in gap junction protein expression may play a role in the orderly growth and differentiation of type II cells during lung injury. Consistent with this notion, Kasper et al. (27) found that Cx43 expression by type I and type II cells is upregulated in response to radiation-induced pulmonary fibrosis. Whether the expression of other connexins is altered as a response to lung injury is not known at present.

Despite the fact that the type II-like cells formed limited networks for intercellular communication, in a mixed monolayer, intercellular transport from a type II cell to a single neighboring type I cell may be sufficient to allow diffusion through the remainder of the alveolar epithelium through type I-type I cell gap junctions. This raises the possibility that heterocellular gap junctional communication may be involved in epithelial maintenance in the lung. For instance, disruption of type I-type II cell contacts due to lung injury have been implicated in the initiation of type II cell proliferation and differentiation (26). Because regulated changes in connexin expression have been observed in healing of wounded epidermis (20, 40) as well as in the lung (22, 27), heterocellular type I-type II gap junctional communication may play a role in regulating the proliferation and differentiation of alveolar epithelial cells. To determine whether this is the case, the development of suitable cell culture models or in situ approaches for examining gap junctional communication will be required.

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Address for reprint requests and other correspondence: M. Koval, Univ. of Pennsylvania School of Medicine, Institute for Environmental Medicine, 1 John Morgan Bldg/6068, 3620 Hamilton Walk, Philadelphia, PA 19104-6068 (E-mail: mkoval@mail.med.upenn.edu).

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