Estimation of paracellular conductance of primary rat alveolar epithelial cell monolayers

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Departments of 1Medicine, 2Physiology and Biophysics, 3Molecular Pharmacology and Toxicology, 4Biomedical Engineering, 5Biochemistry and Molecular Biology, and 6Pathology, 7Will Rogers Institute Pulmonary Research Center, and 8Department of Anesthesiology Critical Care Medicine, Childrens Hospital of Los Angeles, University of Southern California, Los Angeles, California; and 9Department of Biopharmaceutics and Pharmaceutical Technology, Saarland University, Saarbruecken, Germany

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Kim, Kwang-Jin, Zea Borok, Carsten Ehrhardt, Brigham C. Willis, Claus-Michael Lehr, and Edward D. Crandall. Estimation of paracellular conductance of primary rat alveolar epithelial cell monolayers. J Appl Physiol 98: 138–143, 2005. First published July 23, 2004; doi:10.1152/japplphysiol.00478.2004.—Freshly isolated rat type II pneumocytes when grown on permeable tissue culture-treated polycarbonate filters, form confluent alveolar epithelial cell monolayers (RAECM). Cells in RAECM undergo transdifferentiation, exhibiting over time morphological and phenotypic characteristics of type I pneumocytes in vivo. We recently reported that transforming growth factor-β1 (TGF-β1) decreases overall monolayer resistance (Rm) and stimulates short-circuit current in a dose-dependent manner. In this study, we investigated the effects of TGF-β1 (50 pM) or 10% newborn bovine serum (NBS) on modulation of paracellular passive ion conductance and its contribution to total passive ion conductance across RAECM. On days 5–7 in culture, tight-junctional resistance (Rj, kΩ/cm2) of RAECM, cultured in minimally defined serum-free medium (MDSF) with or without TGF-β1 or NBS, was estimated from the relationship between observed transmonolayer voltage and resistance after addition of gramicidin D to apical potassium isethionate Ringer solution under open-circuit conditions. NaCl Ringer solution bathed the basolateral side throughout the experimental period. Results showed that transmonolayer conductance (1/Rm) and tight-junctional conductance (1/Rj) were 0.59 and 0.14 mS/cm2 for control monolayers in MDSF, 1.59 and 0.38 mS/cm2 for monolayers exposed to TGF-β1, and 0.38 and 0.18 mS/cm2 for monolayers grown in the presence of NBS. The contributions to total transepithelial ion conductance by the paracellular pathway are estimated to be 23, 23, and 47% for control, TGF-β1-exposed, and newborn bovine serum (NBS)-treated RAECM, respectively.

air-blood barrier; permselectivity; paracellular resistance; barrier properties

THE ALVEOLAR EPITHELIUM LINING the distal air spaces of the lung provides high resistance to the passive leak of solutes and fluid from the surrounding interstitial and vascular spaces, as suggested by a number of physiological and morphological investigations [see reviews (16, 22, 23)]. Moreover, the alveolar epithelium exhibits many specific solute transport processes, including active Na+ resorption from apical to basolateral fluid (20, 22, 24). The presence of active transport processes for removal of solutes (and water) from the apical compartment is thought to be of major importance for the maintenance of alveolar fluid balance in healthy lungs and for the removal of alveolar fluid during recovery from lung injury (2, 10, 22).

We previously reported that the primary cultured rat alveolar epithelial cell monolayer (RAECM) forms a relatively tight barrier, with overall resistance of ~2 kΩ/cm2, and actively absorbs Na+ from apical fluid (1, 7, 20). These cultured pneumocytes exhibit morphological and phenotypic traits of in vivo type I pneumocytes when grown on permeable supports (6, 9). Transport mechanisms for ions, small solutes (e.g., amino acids), and macromolecules (e.g., peptides and proteins) have been effectively investigated utilizing RAECM (8, 15, 18, 19, 23, 24, 27–29, 39).

In this study, we estimated electrical resistance (and conductance) contributed by paracellular (vs. transcellular) pathways of alveolar epithelium utilizing RAECM. We also tested the hypothesis that soluble factors [e.g., transforming growth factor β1 (TGF-β1) and NBS] modulate passive ion conductance across RAECM. Observed total transmonolayer conductances (Gt = 1/Rt, where Rt is transmonolayer resistance) are 0.59, 1.59, and 0.38 mS/cm2 for control, TGF-β1-exposed, and NBS-treated monolayers. Estimated tight-junctional conductances (Gj = 1/Rj, where Rj is tight-junctional resistance) are 0.14, 0.38, and 0.18 mS/cm2 for control, TGF-β1-exposed, and NBS-treated monolayers. The resulting contributions to total ion conductance by the paracellular pathway are estimated to be 23, 23, and 47% for control, TGF-β1-exposed, and NBS-treated RAECM, respectively.

MATERIALS AND METHODS

Primary culture of RAECM. Routine generation of RAECM has been reported elsewhere (1, 7, 21). Briefly, type II pneumocytes are freshly isolated from male, specific pathogen-free Sprague-Dawley rats by elastase digestion, followed by differential adhesion-purification with immobilized IgG. These enriched rat type II cells (purity >92% and viability >90%) are plated onto tissue culture-treated polycarbonate filter inserts (1.1 cm2, Corning-Costar, San Francisco, CA) at 1.2 × 104 cells/cm2 (on day 0). The serum-free, defined culture medium (MDSF) consists of a 1:1 mixture of DMEM and Ham’s F-12 nutrient solution, supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 0.1% bovine serum albumin, 10 mM HEPES and nonessential amino acids (0.1 mM final). Some monolayers are

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cultured in the presence of either 50 pM TGF-β1 (R&D Systems, Minneapolis, MN) or 10% NBS from day 0 onward. This soluble factor(s), when used, is present in both apical and basolateral fluids. All materials were purchased from Sigma (St. Louis, MO), unless noted otherwise.

Measurements of bioelectric parameters of RAECM. On days 5 through 7 of primary culture, monolayers are mounted in modified Ussing chambers and bathed on both apical and basolateral sides with NaCl Ringer solution (all in mM: 141 NaCl, 5.4 KCl, 0.78 NaH2PO4, 1.8 CaCl2, 0.81 MgSO4, 5.55 glucose, 15 HEPES, and 0.08 bovine serum albumin). Bathing fluids are continuously agitated by small magnets, which are rotated by an external magnetic stirrer. Transmonolayer potential difference (VTe) is measured with the use of matched AgCl pellet electrodes immersed in 3 M KCl. Agar bridges (PE90, filled with 3% agar in 3 M KCl) connect the 3 M KCl wells and respective bathing fluids, where the tips of the agar bridges in bathing fluids are positioned near the apical and basolateral surfaces of RAECM.

Sensing of the electrical current flowing across RAECM is accomplished by using two matched AgCl pellet electrodes (immersed in 3 M KCl) via conducting agar bridges whose tips are located at the far ends of the Ussing chamber reservoirs, away from the cell surfaces. Overall Rm is estimated from the voltage deflection observed in response to current pulses (5 μA for 5 s, every 1 min) passed across the monolayer. Equivalent short-circuit current is estimated as open-circuit VTe divided by Rm and used as an index of active ion transport rate of the monolayer. After stable VTe and Rm are reached while RAECM are bathed on both sides with NaCl Ringer solution, apical fluid is replaced with potassium isethionate Ringer solution, potassium isethionate Ringer solution is made by substituting NaCl, KCl, and CaCl2 in NaCl Ringer solution with equimolar potassium isethionate (Eastman Kodak, Rochester, NY), sodium isethionate, and calcium-D-glucuronate, respectively. Basolateral fluid remains NaCl Ringer solution during these maneuvers.

Permeabilization of apical cell membranes. After VTe reaches steady state following replacement of apical fluid with potassium isethionate Ringer solution, an aliquot (0.01 ml) of gramicidin D (0.2 M freshly dissolved in methyl alcohol) is instilled into apical fluid to increase cation conductance of apical cellular membranes. The final concentration of gramicidin D is 200 μM, with 0.1% (vol/vol) methyl alcohol. At this concentration of gramicidin, no appreciable effects on any of the bioelectric parameters are noted. Gramicidin D has been reported to increase permeability of apical cell membranes to Na+ and K+ (but not Cl−) (25). Apical potassium isethionate Ringer solution prevents loss of intracellular K+. Moreover, because cellular pores created by gramicidin D are impermeable to Cl−, cell volume changes that might arise due to movement of Cl− across cell membranes are kept to a minimum.

Equivalent circuit analyses and estimation of Rm. On the basis of an equivalent circuit depicting a typical epithelial barrier under open-circuit conditions. Experimentally, transmonolayer potential (VTe) and resistance (Rm) were measured as functions of time after exposure of monolayers to gramicidin D. The linear plot of VTe (on the ordinate) and Rm (on the abscissa) yields y- and x-intercepts of cellular driving force (Ec) and tight-junctional resistance (Rtj), respectively. Rearranging the following equation (Eq. 1 (see MATERIALS AND METHODS)). Similarly, tight-junctional conductance (Gtj) can be estimated as the y-intercept from the linear plot of Gtj (estimated as 1/Rtj) on the ordinate and transmonolayer current (Itj) on the abscissa, using the relation of Eq. 2 (see MATERIALS AND METHODS). By definition, 1/Rm = 1/Rc + 1/Rtj and Gtj = Gc + Gv, where Rc and Gc are total transcellular resistance and conductance, respectively.

Fig. 1. Equivalent circuit depicting a typical epithelial barrier under open-circuit conditions. 

A) Sensing of the electrical current flowing across RAECM is accomplished by using two matched AgCl pellet electrodes (immersed in 3 M KCl) via conducting agar bridges whose tips are located at the far ends of the Ussing chamber reservoirs, away from the cell surfaces. Overall Rm is estimated from the voltage deflection observed in response to current pulses (5 μA for 5 s, every 1 min) passed across the monolayer. Equivalent short-circuit current is estimated as open-circuit VTe divided by Rm and used as an index of active ion transport rate of the monolayer. After stable VTe and Rm are reached while RAECM are bathed on both sides with NaCl Ringer solution, apical fluid is replaced with potassium isethionate Ringer solution, potassium isethionate Ringer solution is made by substituting NaCl, KCl, and CaCl2 in NaCl Ringer solution with equimolar potassium isethionate (Eastman Kodak, Rochester, NY), sodium isethionate, and calcium-D-glucuronate, respectively. Basolateral fluid remains NaCl Ringer solution during these maneuvers.

B) Permeabilization of apical cell membranes. After VTe reaches steady state following replacement of apical fluid with potassium isethionate Ringer solution, an aliquot (0.01 ml) of gramicidin D (0.2 M freshly dissolved in methyl alcohol) is instilled into apical fluid to increase cation conductance of apical cellular membranes. The final concentration of gramicidin D is 200 μM, with 0.1% (vol/vol) methyl alcohol. At this concentration of gramicidin, no appreciable effects on any of the bioelectric parameters are noted. Gramicidin D has been reported to increase permeability of apical cell membranes to Na+ and K+ (but not Cl−) (25). Apical potassium isethionate Ringer solution prevents loss of intracellular K+. Moreover, because cellular pores created by gramicidin D are impermeable to Cl−, cell volume changes that might arise due to movement of Cl− across cell membranes are kept to a minimum.

C) Equivalent circuit analyses and estimation of Rm. On the basis of an equivalent circuit depicting a typical epithelial barrier under open-circuit conditions. Experimentally, transmonolayer potential (VTe) and resistance (Rm) were measured as functions of time after exposure of monolayers to gramicidin D. The linear plot of VTe (on the ordinate) and Rm (on the abscissa) yields y- and x-intercepts of cellular driving force (Ec) and tight-junctional resistance (Rtj), respectively. Rearranging the following equation (Eq. 1 (see MATERIALS AND METHODS)). Similarly, tight-junctional conductance (Gtj) can be estimated as the y-intercept from the linear plot of Gtj (estimated as 1/Rtj) on the ordinate and transmonolayer current (Itj) on the abscissa, using the relation of Eq. 2 (see MATERIALS AND METHODS). By definition, 1/Rm = 1/Rc + 1/Rtj and Gtj = Gc + Gv, where Rc and Gc are total transcellular resistance and conductance, respectively.
Using Eq. 1 in Eq. 2 and rearranging, we obtain Eq. 1:

\[
(Vc/Ec) + (Rte/Rij) = 1
\]  \hspace{1cm} (I)

Experimentally, \(Vc\) and \(Rte\) are measured as functions of time after apical exposure of RAECM to gramicidin D. The linear plot of \(Vc\) (on the ordinate) and \(Rte\) (on the abscissa) yields \(y\)- and \(x\)-intercepts of \(E_c\) and \(R_{ij}\), respectively (25, 37).

We can also derive a relationship between total \(Gc\) and transmonolayer current \(I_{te}\), allowing estimation of \(G_c\), as follows (37, 41). On the basis of the equivalent circuit shown in Fig. 1, total cellular conductance \((G_c)\) comprises the apical \((G_a)\) and basolateral \((G_b)\) membrane conductances arranged in series (i.e., \(1/G_c = 1/G_a + 1/G_b\)). Apical permeabilization of RAECM with gramicidin D is assumed to increase only \(G_a\) (and thus also \(G_c\), with no appreciable changes in \(G_b\), \(G_{ij}\), or \(E_c\). Because \(G_{te}\) comprises \(G_a\) and \(G_{ij}\) in parallel,

\[
G_{te} = G_a + G_{ij}\]  \hspace{1cm} (h)

By definition, \(G_a = I_{te}/E_c\), and by substituting \(G_a\) in Eq. h, we obtain Eq. 2:

\[
G_{te} = I_{te}/E_c + G_{ij}\]  \hspace{1cm} (2)

Experimentally, \(G_{te}\) is estimated as \(1/R_{te}\), and \(I_{te}\) is estimated as \(Vc/R_{te}\), both as functions of time after apical exposure of RAECM to gramicidin D under open-circuit conditions. From the linear plot of \(G_{te}\) (on the ordinate) and \(I_{te}\) (on the abscissa), the \(y\)-intercept is \(G_{ij}\), and the inverse of the linear slope is \(E_c\).

Statistical analyses. Data are presented as means ± SE of \(n\) where \(n\) is the number of observations. Differences among more than two group means are determined by one-way ANOVA with Dunnett’s multiple comparison tests. \(P < 0.05\) was considered statistically significant.

RESULTS

Bioelectric properties of RAECM cultured for 5–7 days under control (MDSF) conditions, exposed to 50 pM TGF-\(\beta_1\), or grown in the presence of 10% NBS, are summarized in Fig. 2. As shown, exposure of RAECM to TGF-\(\beta_1\) caused a decrease (by \(\sim 64\%\)) in \(Rte\) with concomitant increase (by \(\sim 146\%\)) in equivalent short-circuit current. Presence of NBS in culture medium led to increased \(Vc\) (by \(\sim 68\%\)) compared with that observed in control RAECM.

Figure 3 illustrates a representative relationship observed between \(Vc\) and \(Rte\) after exposure of apical cell membranes of control RAECM to gramicidin D. \(R_{ij}\), estimated from the \(x\)-intercept (see Eq. 1) of the linear regression \((r^2 = 0.954)\) of the observed data, is 5.26 k\(\Omega\)cm\(^2\). The corresponding \(E_c\), estimated from the \(y\)-intercept, is 13.2 mV. The correlation coefficient of the linear regression analysis of the observed data is very close to 1.0, indicating that a linear relation between the two variables persists during the experimental period.

Figure 4 depicts a representative relationship between \(G_{te}\) and \(I_{te}\) observed after exposure of apical cell membranes of control RAECM to gramicidin D. \(G_{ij} (= 1/R_{ij})\), estimated from the \(y\)-intercept (see Eq. 2), estimated by linear regression \((r^2 = 0.996)\) of the observed data, is 0.19 mS/cm\(^2\) (or \(R_{ij}\) of 5.35 k\(\Omega\)cm\(^2\)). \(E_c\), estimated from the inverse of the linear slope, is 13.1 mV. The correlation coefficient of the linear regression analysis of the observed data is again very close to 1.0, in support of a linear relation between \(G_{te}\) and \(I_{te}\) during the experimental period.

As seen in Figs. 3 and 4, linearity observed for both relationships (\(Vc\) vs. \(R_{te}\) and \(G_{te}\) vs. \(I_{te}\)) suggests that permeabilization of apical cell membranes with gramicidin D in potassium isethionate Ringer solution does not appear to significantly alter \(E_c\) or \(R_{ij}\) (or \(G_{ij}\)) during the experimental period. Accordingly, both experimental approaches (\(Vc\) vs. \(R_{te}\) and \(G_{te}\) vs. \(I_{te}\) for estimation of \(R_{ij}\), \(G_{ij}\), and \(E_c\)) yield values not significantly different from each other. Parallel experiments performed on RAECM cultured in the presence of either 50 pM TGF-\(\beta_1\) or 10% NBS also yielded linear relationships for \(Vc\) vs. \(R_{te}\) and \(G_{te}\) vs. \(I_{te}\) after exposure to gramicidin D (data not shown).
A summary of estimated conductances of RAECM grown in MDSF ± 50 pM TGF-β1 or 10% NBS for 5–7 days is shown in Fig. 5. TGF-β1 increases \( G_\text{te} \), \( G_{\text{ij}} \), and \( G_\text{c} \). \( G_{\text{ij}} \) accounts for \( \sim 23\% \) of total conductance under control conditions and in the presence of TGF-β1 (Fig. 6). Interestingly, although \( G_\text{te} \), \( G_{\text{ij}} \), and \( G_\text{c} \) in NBS are each individually not significantly different from control, the contribution of \( G_{\text{ij}} \) to \( G_\text{c} \) in NBS is \( \sim 47\% \), significantly higher than that for control monolayers (Fig. 6). The decrease in \( G_\text{c} \) of NBS-treated monolayers may have caused the increase in \( G_{\text{ij}}/G_\text{c} \) of serum-treated monolayers, although the possibility of cross-modulation of \( G_\text{c} \) and \( G_{\text{ij}} \) remains to be studied. \( G_{\text{ij}}/G_\text{c} \) for both control and TGF-β1-exposed monolayers are estimated to be \( \sim 0.3 \), whereas \( G_{\text{ij}}/G_\text{c} \) is \( \sim 0.9 \) in NBS-grown monolayers.

**DISCUSSION**

In this study, we provide an estimate of tight-junctional passive transport properties of primary cultured RAECM. \( G_{\text{ij}} \) accounts for \( \sim 23\% \) of total passive ion conductance across the control monolayers. TGF-β1 increases \( G_\text{te} \) and \( G_{\text{ij}} \) without appreciably altering the ratio of tight-junctional to total passive ion conductance (\( G_{\text{ij}}/G_\text{te} \)), whereas monolayers maintained in the presence of NBS exhibit increased \( G_{\text{ij}}/G_\text{te} \).

\( G_{\text{ij}}/G_\text{te} \) is \( \sim 23\% \) for monolayers cultured in serum-free conditions or exposed to 50 pM TGF-β1. By contrast, for monolayers grown in the presence of 10% NBS, \( \sim 47\% \) of \( G_\text{te} \) is attributable to paracellular tight junctions. Epithelial barriers in general can be categorized as leaky vs. tight, based on the ratio \( G_\text{ij}/G_\text{c} \) (or \( R_{\text{ij}}/R_\text{c} \)), where leaky tissues exhibit high \( G_\text{ij}/G_\text{c} \) (33). For example, *Necturus* proximal tubule (\( \sim 260 \Omega \text{cm}^2 \)) and *Necturus* gallbladder (\( \sim 310 \Omega \text{cm}^2 \)) exhibit \( G_\text{ij}/G_\text{c} \) of \( \sim 22–49 \) and 19, respectively, whereas toad and rabbit urinary bladders (3.8–40 kΩcm²) exhibit \( G_\text{ij}/G_\text{c} \) of \( \sim 0.3–1.5 \) (33). Our data show that \( G_\text{ij}/G_\text{c} \) of 0.3, 0.3, and 0.9 can be estimated for control, TGF-β1-exposed, and serum-treated monolayers of primary cultured rat alveolar epithelial cells. These data confirm that the rat alveolar epithelial barrier is appropriately categorized as a tight epithelium.

We note that both relationships of \( V_\text{te} \) vs. \( R_\text{te} \) and \( G_\text{te} \) vs. \( I_\text{te} \) are linear as functions of time after apical glicentin D exposure. Moreover, estimates of \( R_\text{ij} \) (or \( G_\text{ij} \)) resulting from the \( V_\text{te}-R_\text{te} \) relation are not significantly different from those estimated from the \( G_\text{te}-I_\text{te} \) relation. This observation indicates that permeabilization of apical cell membranes with glicentin D in potassium isethionate Ringer solution does not lead to appreciable alterations in \( E_\text{c} \) and \( R_\text{te} \), allowing estimation of \( R_\text{ij} \) (25, 37).

**Fig. 5.** Conductances (\( G_\text{te}, G_\text{ij}, \) and \( G_\text{c} \)) of RAECM grown in MDSF (\( n = 5 \)) with or without 50 pM TGF-β1 (\( n = 6 \)) or 10% NBS (\( n = 4 \)) for 5–7 days. Data shown are means ± SE. *Significantly different from control (MDSF), \( P < 0.05 \).

**Fig. 6.** Transcellular and paracellular components of total ion conductance of RAECM. Fractions (means ± SE, expressed as % of \( G_\text{te} \)) of passive ion movement via transcellular and paracellular routes, estimated as \( G_\text{ij}/G_\text{te} \) and \( G_\text{c}/G_\text{te} \), and the ratio between \( G_\text{te} \) and \( G_\text{ij} \) (converted to %) for RAECM grown in MDSF (\( n = 5 \)) with or without 50 pM TGF-β1 (\( n = 6 \)) or 10% NBS (\( n = 4 \)) for 5–7 days are shown. *Significantly different from control (MDSF), \( P < 0.05 \).

It is important to note that the relationship of \( V_\text{te} \) vs. \( R_\text{te} \) is much more sensitive to alterations in either \( E_\text{te} \) or \( R_\text{ij} \) than that of \( G_\text{te} \) vs. \( I_\text{te} \), since \( V_\text{te} \) is dependent on changes in both transcellular and paracellular resistances (or conductances) (37). When the assumed constancy of \( E_\text{te} \) is violated, the \( V_\text{te}-R_\text{te} \) relation becomes nonlinear (37), preventing estimation of \( R_\text{ij} \). Another limitation of the method employed in this study is that a change in \( R_\text{ij} \) (due, for example, to glicentin D exposure) must result in a measurable change in \( R_\text{te} \) (or \( G_\text{te} \)) to allow reliable estimation of \( R_\text{ij} \) (or \( G_\text{ij} \)). In leaky epithelial barriers (where \( G_\text{ij} \) can be >10-fold larger than \( G_\text{c} \)), permeabilizing agents intended to cause an increase in \( G_\text{ij} \) may not lead to significant increases in \( G_\text{te} \) (which is high to begin with). Thus the present method has been applied for estimation of \( G_\text{ij} \) (or \( G_\text{ij} \)) almost exclusively in tight epithelial barriers (37).

With the use of a similar approach of permeabilizing apical cell membranes with glicentin D in potassium sulfate Ringer solution, electrical properties of the very tight rabbit urinary bladder were studied by Lewis and Wills (25). It was shown that \( R_\text{ij} \) of rabbit urinary bladder epithelium is \( \sim 80–90 \Omega \text{cm}^2 \), with overall \( R_\text{te} \) of \( \sim 10–20 \Omega \text{cm}^2 \). \( G_\text{ij}/G_\text{c} \) for rabbit urinary bladder is thus of the same magnitude as that for RAECM (a moderately tight epithelium) shown in this study.

We and others have shown that TGF-β1 plays important roles in alveolar epithelial homeostasis (11, 31, 35). Willis et al. (35) reported recently that TGF-β1 (50 pM) present in both bathing fluids of RAECM from *day 0* onward decreases overall \( R_\text{te} \) and increases active ion transport rate on *day 6*, with effective half-maximal concentrations of 5.5 and 10 pM, respectively. It was further demonstrated that effects of TGF-β1 on alveolar epithelial barrier properties are associated with increased expression of \( \text{Na}^+-\text{K}^+\text{-ATPase} \) but not of \( \text{Na}^+ \) channels (35). Frank et al. (11) reported that 200–400 pM (and higher) concentrations of TGF-β1, when added to basolateral (but not apical) fluids (that also contained 10% fetal calf serum from *day 0* onward) on *day 4*, significantly decreases \( R_\text{te} \) and equivalent short-circuit current on *day 5*. Pittet et al. (31) showed that TGF-β1 (10–400 pM), when added on *day 2* to bathing fluids of RAECM grown in the presence of 10% fetal calf serum, significantly decreased \( R_\text{te} \) in a dose-dependent manner. TGF-β1 thus clearly increases ion conductance of both transcellular and paracellular pathways, while leaving the relative conductances invariant, suggesting the possibility that there may be coordinate regulation of ion movement via both
pathways, although the downstream signaling mechanisms are not entirely known.

TGF-β1 has been reported to induce conversion of normal thyroid epithelial cells to a mesenchymal phenotype (i.e., attenuation of tight-junctional properties) in the presence of epidermal growth factor (12). TGF-β2 and -β3, but not -β1, have been shown to alter tight-junctional properties of the blood-testis barrier via signaling involving p38 MAP kinase cascades, resulting in loss of ZO-1 and E-cadherins (26). TGF-β and TNF-α are reported to affect tight-junctional function and assembly through activation of inducible nitric oxide synthase activities in testsis, where nitric oxide-soluble guanylate cyclase-cyclic guanylate monophosphate-protein kinase G signaling pathways might be activated to lead to attenuation of tight-junctional properties and/or assembly after the action of these cytokines on cells (26). In proximal tubular epithelial cells, TGF-β1 has been reported to induce phenotypic changes (e.g., loss of cell-cell contact and adherens junction disassembly) that were abrogated by inhibitors of RhoA downstream (e.g., loss of cell-cell contact and adherens junction disassembly) (26).

Our results indicate that RAECM grown in serum exhibit higher $V_{ce}$ and increased short-circuit current compared with those grown in MDSF. As seen in Fig. 6, the effects of NBS led to decreased $G_{j}/G_{Rce}$ and increased $G_{j}/G_{C}$ and $G_{j}/G_{e}$. Many reports (5, 17, 32, 36, 40) have demonstrated that inclusion of serum for culture of epithelial cells increases barrier resistance and decreases leak of hydrophilic solutes through paracellular pathways. Many soluble factors (including TGF-β1) are present in serum, but those that lead to alterations in $G_{j}$ and $G_{j}(G_{j}/G_{e})$ are presently unknown.

In summary, we have demonstrated in this study that paracellular (as well as transcellular) conductances of the alveolar epithelial barrier are regulatable by soluble factors. The mechanisms of such changes remain to be determined. Investigation of modulation and/or regulation of epithelial conductance and its contributing pathways by various soluble factors may lead to improved understanding of tight-junctional biology and/or physiology in relation to pathogenesis and resolution of alveolar pulmonary edema in the mammalian lung.

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