

## REVIEW

# The use of alveolar epithelial type I cell-selective markers to investigate lung injury and repair

M.C. McElroy\*, M. Kasper<sup>#</sup>

*The use of alveolar epithelial type I cell-selective markers to investigate lung injury and repair. M.C. McElroy, M. Kasper. ©ERS Journals Ltd 2004.*

**ABSTRACT:** Alveolar epithelial type I cells cover most of the internal surface area of the lungs.

Ultrastructural studies demonstrate that alveolar epithelial type I cell damage is frequently observed in acute and chronic lung diseases.

This article discusses the use of cell-selective proteins as markers for the investigation of injury and repair of the alveolar epithelium. The utility of proteins specific to alveolar epithelial type I cells as diagnostic markers of alveolar epithelial injury in acute lung injury is considered, and expression of proteins selective for alveolar epithelial type I cells in lungs following injury and in fibrosis are discussed.

*Eur Respir J 2004; 24: 664–673.*

\*MRC Centre for Inflammation Research, School of Molecular and Clinical Medicine, University of Edinburgh, Edinburgh, UK. <sup>#</sup>Dept of Anatomy, Carl Gustav Carus Medical Faculty, Technical University of Dresden, Dresden, Germany.

Correspondence: M.C. McElroy, MRC Centre for Inflammation Research, School of Molecular and Clinical Medicine, University of Edinburgh, Edinburgh, EH8 9AG, UK.

Fax: 44 1316506528

E-mail: mmcelroy@ed.ac.uk

**Keywords:** Alveolar epithelial type I cell, cell differentiation, fibrosis, marker, primary acute respiratory distress syndrome, pulmonary injury

Received: August 21 2003

Accepted after revision: April 29 2004

M.C. McElroy is funded by the Norman Salvesen Emphysema Research Fund (Edinburgh, UK) and M. Kasper by the Deutsche Forschungsgemeinschaft (SFB 13) (Bonn, Germany).

Epithelial damage is a major hallmark of many life-threatening pulmonary diseases, including acute lung injury (ALI) or acute respiratory distress syndrome (ARDS), severe acute respiratory syndrome, cystic fibrosis and idiopathic pulmonary fibrosis (IPF) [1–4]. In addition, alveolar epithelial injury followed by abnormal epithelial repair appears to be a key pathological feature of IPF [4].

Two cell types populate the alveolar epithelium in normal adult lungs, alveolar epithelial type I (ATI) and alveolar epithelial type II (ATII) cells. ATI cells cover ~95% of the internal surface area of the lung. They are branched cells with multiple apical surfaces that extend into adjacent alveoli [5]. The apical surface area of ATI cells is large in comparison with most cells (*i.e.* ~5,000  $\mu\text{m}^2$  for human ATI cells), yet they are very thin cells (*i.e.* 0.2  $\mu\text{m}$  in depth) [5, 6]. The gas exchange barrier is composed of ATI and endothelial cells joined by fused basement membranes. ATI cells are important in the regulation of alveolar fluid balance and surfactant secretion by ATII cells in response to stretch [7–10]. ATII cells cover the remaining 2–5% of the lung's surface area. They are cuboidal cells situated between ATI cells, and contain characteristic lamellar bodies and apical microvilli [5]. ATII cells have many known functions, including the production, secretion and reuptake of pulmonary surfactant [11], regulation of alveolar fluid in normal lungs and during the resolution of pulmonary oedema [12], and synthesis and secretion of immunomodulatory proteins important for host defence, such as surfactant proteins A and D [13, 14].

Damaged ATI cells are replaced by ATII cells [15–19] (reviewed in [19]) (fig. 1). The studies of EVANS and coworkers [15, 20], in particular, provide the foundation of current knowledge concerning the response of the alveolar epithelium to injury. They examined ultrastructural changes to the alveolar epithelium following exposure to the oxidant gas nitrogen dioxide for 48 h. In alveoli adjacent to terminal bronchioles, ATI cells were maximally damaged after 8–12 h [20]. After 48 h of exposure, the denuded basement membranes were repopulated by cuboidal epithelial cells, some of which were ATII cells. EVANS *et al.* [15] followed the fate of proliferating ATII cells (after 48 h of NO<sub>2</sub> exposure) using a combination of autoradiography and electron microscopic analysis. Proliferating cells were labelled with tritiated thymidine, which is incorporated into newly synthesised DNA during the S phase of the cell cycle. After 48 h of NO<sub>2</sub> exposure, most labelled cells were ATII cells; however, following a further 48-h recovery period, the number of labelled ATII cells had decreased, whereas the number of labelled ATI cells had increased. In addition, over the 48-h recovery period, there was a transient increase in label associated with alveolar epithelial cells with characteristics of both ATI and ATII cells (*i.e.* ATII-like cells but spread out over the basement membrane or ATI-like cells containing occasional microvilli and lamellar bodies) [15]. These studies demonstrate three important aspects of alveolar epithelial injury and repair (in response to NO<sub>2</sub>). First, the loss of ATI cells can precede ATII cell proliferation. Secondly, alveolar epithelial repair

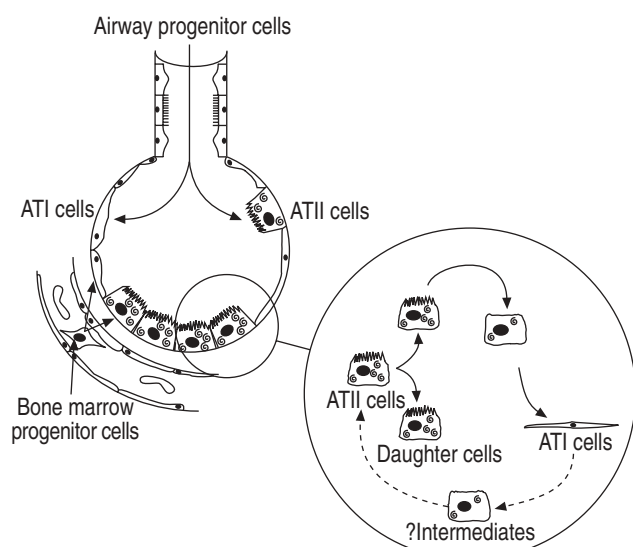


Fig. 1.—Alveolar epithelial repair. Following injury, the alveolar epithelium is repaired by the proliferation and transdifferentiation of alveolar epithelial type II (ATII) cells, *via* intermediate cells, into new alveolar epithelial type I (ATI) cells (solid arrows). Injured alveolar epithelial cells may also be replaced by bone marrow and airway progenitor cells. The role of ATI cells in alveolar epithelial repair is still speculative (dotted arrows).

involves the transformation ATII cells (or a subset thereof) into new ATI cells. Thirdly, ATII cells transforming to new ATI cells do so *via* intermediate cell types.

More recent studies have refined the role of ATII cells in ATI cell repair and raised alternative mechanisms of ATI cell repair (fig. 1) [21–24]. There may be subpopulations of ATII cells with different proliferative capabilities following injury [24]. However, the potential for these putative ATII cell subpopulations to transdifferentiate into ATI cells is unknown. Damaged ATI cells may also be replaced by bone marrow and nonciliated bronchiolar epithelial progenitor cells [21, 22]. It has been assumed for a long time that ATI cells were terminally differentiated. However, experiments designed to investigate the role of mechanical load in foetal lung development suggest that ATI cells have the potential to transdifferentiate into ATII cells [23]. It is not known whether ATI cells are capable of replacing damaged ATII cells in injured adult lungs.

Investigating ATI cell injury and repair is difficult because they are not easily recognised using light microscopy. To date, most studies have relied on visualisation of ATI cells, and, in particular, necrosis of ATI cells, using electron microscopy. A number of proteins have been identified which are expressed by ATI cells but not ATII cells (reviewed in [25]). The present review focuses on the application of ATI-cell-selective proteins as markers for the investigation of injury to and repair of ATI cells in experimental models and human lung diseases. First, known ATI-cell-selective proteins are described (see also [25]). Secondly, the utility of ATI-cell-specific proteins in biochemical assays for quantification of the extent of ATI cell necrosis is discussed. Thirdly, the significance of changes in ATI-cell-selective proteins following injury and in lung fibrosis is evaluated.

### Proteins expressed by alveolar epithelial type I cells

In the following section, the ATI-cell-selective proteins have been categorised into three groups (table 1). Group I contains proteins that are specifically expressed by ATI cells

in the lung (ATI-cell-specific). Group II contains proteins expressed by ATI, and other lung cells, but not ATII cells (ATI-cell-selective), and group III contains proteins either expressed more abundantly by ATI than ATII cells or for which there is insufficient evidence to place them in group II (ATI-cell-selective/associated).

### Group I. alveolar epithelial type I cell-specific proteins

**Rat type I cell 40-kDa protein/type I cell alpha protein.** Rat type I cell 40-kDa protein (RTI<sub>40</sub>)/type I cell alpha (TI $\alpha$ ) protein is an apical membrane protein expressed exclusively by ATI cells in the lung (fig. 2) [26, 52, 53]. A monoclonal antibody directed against RTI<sub>40</sub>/TI $\alpha$  protein was used to identify and characterise the rat gene TI $\alpha$  in a bacterial expression library [53]. The TI $\alpha$  gene encodes a membrane protein predicted to have a large extracellular domain, a transmembrane region and a short cytoplasmic tail [53]. RTI<sub>40</sub>/TI $\alpha$  protein is also detected in nonpulmonary tissues, including the choroid plexus and ciliary epithelium of the eye [54]. RTI<sub>40</sub>/TI $\alpha$  protein is homologous to a number of other proteins, including OTS-8 (mouse phorbol ester-treated osteoblast cell line [55]), 38-kDa glycoprotein (gp38) (mouse thymic epithelial cells [56]), E11 (rat lymphatic endothelial cells and osteoblasts [57]), podoplanin (rat kidney podocytes [58]), 40-kDa glycoprotein (gp40) (Madin-Darby canine kidney cells [59]), PA2.26 antigen (mouse keratinocytes during epidermal carcinogenesis [60]) and Aggrus (human colorectal tumours [27]). Mouse and rat RTI<sub>40</sub>/TI $\alpha$  protein are 78% homologous on the basis of deduced amino acid sequences [53].

Human homologues of RTI<sub>40</sub>/TI $\alpha$  protein have been cloned and are called hTI $\alpha$ -2 and gp36 [61, 62]. hTI $\alpha$ -2 is 50% identical to TI $\alpha$  at the nucleotide level. Northern blot analysis of messenger ribonucleic acid (mRNA) demonstrates that the transcript for hTI $\alpha$ -2 is present in lung and not in other selected tissues (*e.g.* kidney and small intestine) [61]. gp36 is 66% identical to TI $\alpha$  at the nucleotide level. Sequence alignment demonstrates that the greatest variability between RTI<sub>40</sub>/TI $\alpha$  protein and gp36 occurs in the extracellular domain, which might explain why antibodies that recognise rat ATI cells do not also recognise human ATI cells [62]. Although a monoclonal antibody raised against gp36 is reported to recognise the alveolar epithelium, there is insufficient data at present to determine whether gp36 is ATI-cell-specific in human lungs [62].

Mice with targeted mutations in the TI $\alpha$  locus die at birth due to respiratory failure [63, 64]. Histological analysis of the lungs demonstrates that mice deficient in RTI<sub>40</sub>/TI $\alpha$  protein exhibit fewer ATI cells, narrower and irregular airspaces, and defective formation of alveolar saccules in comparison to wild-type litter mates [63]. In addition, there is abnormally high expression of proliferation markers associated with the alveolar epithelium and mesenchymal cells (*e.g.* fibroblasts) in the distal lung [63]. These data demonstrate that the loss of TI $\alpha$  is important, although not absolutely required, for the formation of ATI cells during the late stages of gestation in the mouse. These data suggest that TI $\alpha$ , ATI cells or both regulate distal airway cell proliferation during lung development.

The role of RTI<sub>40</sub>/TI $\alpha$  protein in normal adult lung is unknown. RTI<sub>40</sub>/TI $\alpha$  protein is a sialoglycoprotein [52, 59, 62]. Highly negatively charged proteins, such as sialoglycoproteins, are relatively resistant to proteases. Therefore, RTI<sub>40</sub>/TI $\alpha$  protein may act as a physical barrier and protect the surface of ATI cells from proteases [62]. The overexpression of TI $\alpha$  in endothelial cells and keratinocytes in culture promotes phenotypic changes (*i.e.* long thin cell extensions) and

Table 1. – Alveolar epithelial type I (ATI) cell-specific and -associated proteins in normal adult lung

	Method <sup>#</sup>	Location in ATI cells	Expression in other cell types <sup>¶</sup>	[Refs.]
Group I. ATI-cell-specific				
RTI <sub>40</sub> /TI $\alpha$ protein	IEM	Apical plasma membrane	None	[26–28]
HTI <sub>56</sub>	IEM	Apical plasma membrane	None	[29]
Group II. ATI-cell-selective				
Caveolin-1/-2	IEM	Microvesicles	Endothelial cells, fibroblasts, smooth muscle cells, bronchial epithelial cells	[30, 31]
Na <sup>+</sup> /K <sup>+</sup> -ATPase $\alpha_2$ -isoform	IF, IEM	Basolateral membrane		[32]
Aquaporin 5	IEM	Apical plasma membrane	Bronchial epithelial cells	[33]
Cytochrome P <sub>450</sub> 2B1	IEM	Cytoplasm	Bronchial epithelial cells	[34]
Carboxypeptidase M	IHC	Apical plasma membrane	Alveolar macrophages	[35]
Group III. ATI-cell-selective/-associated				
ICAM-1	IEM	Apical plasma membrane	Alveolar macrophages, bronchial epithelial cells, endothelial cells, ATII cells?	[36–38]
Connexin 43	IF	Cell membrane, cytoplasm	ATII cells	[39]
P-glycoprotein	IF	Apical plasma membrane	Smooth muscle cells, bronchial epithelium, ATII cells?	[40]
RAGE	IEM	Basal membrane	Some endothelial cells of larger blood vessels, ATII cells (mRNA)	[41–43]
PAI-1	IF	Apical plasma membrane	ATII cells?	[44]
P2X4 purinoceptor	IF	Plasma membrane	ATII cells?	[44]
CDKN2B	IF	Perinuclear	ATII cells?	[44]
ATB <sup>0+</sup>	IF	Apical membrane	Ciliated cells, ATII cells?	[45]
Cytochrome P <sub>450</sub> 1A1	ISH		Bronchiolar epithelium, ATII cells	[46]
Eotaxin	ISH		Airway epithelium, macrophages, lymphocytes, endothelial cells	[47]
GGT	IEM, IF	Apical plasma membrane	Clara cells, ATII cells	[48, 49]
IGFR-2	IHC	Apical plasma membrane		[50]

RTI<sub>40</sub>: rat type I cell 40-kDa protein; TI $\alpha$ : type I cell alpha protein; HTI<sub>56</sub>: human type I cell 56-kDa protein; Na<sup>+</sup>/K<sup>+</sup>-ATPase: sodium/potassium-exchanging adenosine triphosphatase; ICAM-1: intercellular adhesion molecule-1; RAGE: receptor for advanced glycation end-products; PAI-1: plasminogen activator inhibitor; CDKN2B: cyclin-dependent kinase 4 inhibitor 2B; ATB<sup>0+</sup>: Na<sup>+</sup>- and Cl<sup>-</sup>-dependent neutral and cationic amino acid transporter B<sup>0+</sup>; GGT:  $\gamma$ -glutamyltransferase; IGFR-2: insulin-like growth factor receptor 2; IEM: immunoelectron microscopy; IF: immunofluorescence; IHC: immunohistochemistry; ISH: *in situ* hybridisation; mRNA: messenger ribonucleic acid. <sup>#</sup>: ATI specificity assignment; <sup>¶</sup>: in normal adult lungs.

increases the migration rate in wounding experiments [60, 64]. In adult lungs, RTI<sub>40</sub>/TI $\alpha$  protein may, therefore, be important for initiating and maintaining the extended and flattened ATI cell phenotype. A similar role has been proposed for RTI<sub>40</sub>/TI $\alpha$  in protein kidney podocytes [58]. However, other recognised functions of RTI<sub>40</sub>/TI $\alpha$  protein are harder to explain. RTI<sub>40</sub>/TI $\alpha$  protein has also been identified as a platelet aggregation-inducing factor expressed by colorectal tumours [27]. It is not known whether RTI<sub>40</sub>/TI $\alpha$  protein on the surface of ATI cells facilitates platelet aggregation.

**Human type I cell 56-kDa protein.** DOBBS *et al.* [29] have also generated an antibody that specifically reacts with ATI cells in human lungs, human type I cell 56-kDa protein (HTI<sub>56</sub>). This antibody has been used to quantify ATI cell injury in patients with established ARDS (see later). It is not known whether HTI<sub>56</sub> and RTI<sub>40</sub> are homologous. At present, there are no data on the biological or functional properties of HTI<sub>56</sub>.

#### Group II. alveolar epithelial type I cell-selective proteins

Immunoelectron microscopy has been used to demonstrate that caveolin-1 and -2 [30, 31], cytochrome P<sub>450</sub> 2B1 [34], aquaporin (AQP) 5 [33, 65] and the  $\alpha_2$ -isoform of sodium/potassium-exchanging adenosine triphosphatase (Na<sup>+</sup>/K<sup>+</sup>-ATPase) [32] are expressed by ATI but not ATII cells (table 1).

**Caveolin-1 and -2.** Caveolae are small flask-shaped microvesicles found in abundance in ATI and endothelial

cells in the lung. The principle protein component of caveolae is caveolin-1, which is required for the biogenesis of these organelles [66, 67] (reviewed in [68, 69]). In the lung, caveolin-2 is coexpressed with caveolin-1 ([70] and references therein). Caveolae are also enriched with cholesterol and sphingolipids and may serve as specific microdomains or "rafts" in which cell signalling cascades are regulated (reviewed in [69]). In particular, caveolin-1 may negatively regulate proliferation and apoptosis, and may, therefore, act as a tumour suppressor [69, 71]. Mice deficient in either caveolin-1 or -2 are born healthy and are able to reproduce [66, 67, 70]. However, by 4–5 months of age, they develop a lung pathology that resembles fibrosis. Specifically, caveolin-1 and -2 knockout mice show smaller airspaces and thickened alveolar walls [66, 67, 70, 72]. The increased alveolar wall thickness is due to endothelial hyperproliferation and increased deposition of extracellular matrix proteins [69–72]. Both types of knockout mice are also exercise-intolerant in comparison with their wild-type litter mates [66, 67, 70]. The pulmonary defect may be caused solely by caveolin-2 deficiency [70]. Caveolin-1-deficient mice show a dramatic reduction in caveolin-2 levels [66, 67], whereas caveolin-2 null mice show almost normal levels of caveolin-1 [70]. It has been hypothesised that caveolin-2 might be required for the mitigating mechanical forces that stimulate lung cell proliferation [70].

**$\alpha_2$ -Isoform of sodium/potassium-exchanging adenosine triphosphatase.** The alveoli have to be maintained essentially fluid-free in order to ensure efficient gas exchange (reviewed in [12]). In addition, the upregulation of



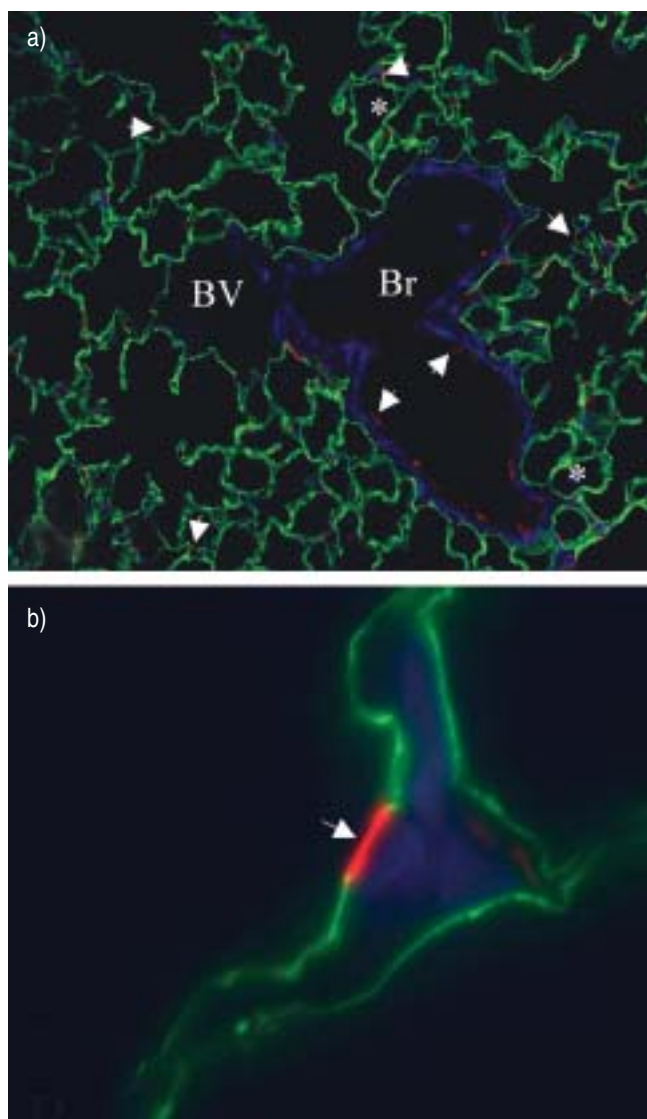


Fig. 2.—Use of alveolar epithelial type I (ATI) cell-specific antibodies to visualise rat ATI cells using immunofluorescence microscopy at a) low and b) high power. ATI cells are stained green with a monoclonal antibody directed against rat type I cell 40-kDa protein (RTI<sub>40</sub>)/type I cell alpha (TI $\alpha$ ) protein. Alveolar epithelial type II cells and Clara cells are stained red with the MMC4 monoclonal antibody (arrow). Br: bronchiole; BV: blood vessel. \*: airspace. (From [51].)

fluid absorption following acute lung injury is an important factor in recovery from ALI or ARDS [12]. Fluid is removed from the airspaces by active transport of sodium across the epithelium by a basolateral Na<sup>+</sup>/K<sup>+</sup>-ATPase. The resulting osmotic gradient promotes reabsorption of water [12]. Until recently, it was believed that ATII cells were the most important cell type in the regulation of alveolar fluid clearance. However, it has now been shown that ATI cells contain apical sodium channels and can actively transport sodium ions [8, 32, 73]. Immunoelectron microscopy suggests that the  $\alpha_2$ -isoform of Na<sup>+</sup>/K<sup>+</sup>-ATPase is expressed in ATI but not ATII cells and accounts for a significant percentage of basal alveolar fluid reabsorption [32].

**Aquaporin 5.** AQP5 is a water channel protein located in the apical membrane of ATI cells and bronchiolar epithelial cells in the lung [33, 73]. AQP5-deficient mice demonstrate that AQP5 is important for the movement of water from the airspaces to

the capillary bed in response to an osmotic gradient generated from the vascular bed [74]. However, AQP5 does not appear to be important in water absorption out of the airspaces under normal conditions or under conditions in which alveolar fluid clearance is upregulated by specific agonists [74]. Nor is AQP5 important in lung extravascular fluid accumulation in acute injury [75]. It has been proposed that AQP5 may be important in the regulation of ATI cell volume [76].

**Cytochrome P<sub>450</sub> 2B1 and carboxypeptidase M.** Both cytochrome P<sub>450</sub> 2B1 and carboxypeptidase M are associated with ATI but not ATII cells in the lung [35, 77]. Cytochrome P<sub>450</sub> 2B1 is also present in nonciliated bronchiolar epithelial cells and carboxypeptidase M in macrophages [34, 35]. The specific role of these enzymes in ATI cell function is unknown.

### Group III. Alveolar epithelial type I cell-selective associated proteins

**Intercellular adhesion molecule-1.** Alveolar epithelial intercellular adhesion molecule-1 (ICAM-1) may specifically regulate alveolar macrophage phagocytic activity in the lung [78] or remodelling of the alveolar epithelium following injury [1]. Immunoelectron micrographs demonstrate that ICAM-1 is present on the apical surface of ATI cells, and, in particular, that there is abundant expression on surfaces near cell junctions [36]. ICAM-1 is also detected at lower levels on the surface of capillary endothelial cells [78]. Immunoelectron microscopic studies demonstrate low levels of ICAM-1 on ATII cells [36], but studies using isolated ATII cells have not demonstrated ICAM-1 expression [37]. It is possible that this discrepancy may reflect differences in the methods used to detect ICAM-1, the use of different primary antibodies or loss of ICAM-1 during ATII cell isolation. ICAM-1 is upregulated by ATII cells, but not by ATI cells, in response to hyperoxia and various cytokines [36, 79, 80].

**Receptor for advanced glycation end-products.** The receptor for advanced glycation end-products (RAGE) is a multiligand-binding protein that binds advanced glycation end-products [81]. Advanced glycation end-products are a heterogeneous group of molecules that accumulate in plasma and other body fluids as a consequence of age and certain diseases [81]. The mRNA transcript for RAGE is highly expressed in lung tissue [81]. Immunoelectron microscopic studies demonstrate that RAGE is expressed on the basal surface of ATI cells in the lung [41, 42]. However, mRNA transcripts for RAGE have also been detected in ATII cells in the lung [43]. The role of RAGE in ATI cells is unknown.

**Lectins.** Finally, a group of lectins are able to distinguish between the carbohydrate moieties on the surface of ATI and ATII cells. Lectins such as *Ricinus communis* agglutinin [82], *Lycopersicon esculentum* agglutinin [83], Soybean agglutinin [84] and *Bauhinia purpurea* agglutinin [85] selectively bind to the surface of ATI cells.

### Expression of alveolar epithelial type I cell-selective proteins following injury

Following injury, the amount of ATI-cell-selective proteins in lung tissue may change because of damage to ATI cells. However, ATI-cell-selective proteins may also change because they are also expressed on alveolar epithelial intermediate cell types (*i.e.* morphology-dependent changes in regulation)

or because of changes in regulation by ATI cells (*i.e.* morphology-independent). The use of ATI cell-selective markers in the detection of ATI cell necrosis in both animal and human studies is reviewed below. Data from animal studies that suggest ATI-cell-selective proteins are upregulated by ATI cells in response to toxic agents or expressed by alveolar epithelial intermediate cells are also reviewed.

*Use of rat type I cell 40-kDa protein/type I cell alpha protein and human type I cell 56-kDa protein to quantify ATI cell necrosis in ALI*

Ultrastructural studies on injured lungs demonstrate that necrotic ATI cells are sloughed from their basement membranes into the airspaces [86–88]. Various experimental studies demonstrate that the amount of RTI<sub>40</sub>/TI $\alpha$  protein in distal airway fluid or bronchoalveolar lavage fluid (BALF) can be used to quantify the extent of ATI cell necrosis (table 2). Specifically, in a model of severe ALI, caused by *Pseudomonas aeruginosa*, RTI<sub>40</sub>/TI $\alpha$  protein levels are elevated 80-fold above control values in distal airway fluid 6 h after infection [89]. Morphological analysis of infected lungs at this time point demonstrates widespread ATI cell necrosis and denuded alveolar basement membranes [89]. In addition, the ability of the alveolar epithelium to clear fluid, a functional measurement of epithelial integrity, is completely abolished following *P. aeruginosa*-induced lung injury. In contrast, in a model of focal ATI cell necrosis, induced by nitrogen dioxide exposure, BALF concentrations of RTI<sub>40</sub>/TI $\alpha$  protein are elevated only two-fold above control values and alveolar fluid clearance is stimulated [90]. BALF RTI<sub>40</sub>/TI $\alpha$  protein concentrations are also elevated, to varying degrees, in other acute injury models, including hyperoxia [91], *Staphylococcus aureus*-induced pneumonia [92], acid-instillation followed by ventilator-induced injury [96] and following intratracheal administration of bleomycin (table 2) [94]. In the rat model of acid-instillation, RTI<sub>40</sub>/TI $\alpha$  protein concentrations in oedema fluid and plasma were significantly reduced in animals ventilated at low tidal, or protective, volumes in comparison with those ventilated at higher tidal volumes (*i.e.* 3 versus 6 mL·kg body weight<sup>-1</sup>) [96]. Low tidal volumes were also associated with better alveolar function as measured by the rate of alveolar fluid clearance [96]. Combined, these studies suggest that ATI cell membrane proteins, such as RTI<sub>40</sub>/TI $\alpha$  protein, may be sensitive markers of lung injury resulting from a wide range of different causes. More specifically, however, these studies also suggest that the

extent of ATI cell necrosis depends on the exact nature of the damaging agent.

ALI and ARDS are life-threatening conditions that may occur as a consequence of either direct (pneumonia and acid aspiration) or indirect (sepsis and trauma) injury to the lungs. As in experimental models of ALI, ultrastructural analysis of the lung in patients who have died due to ARDS demonstrates widespread epithelial damage, including ATI cell necrosis [97]. In addition, clinical studies also demonstrate that preserved alveolar fluid clearance correlates with lower mortality in patients with ARDS [12]. The concentration of various ATI-cell-selective proteins (*e.g.* surfactant protein A and D, and KL-6) in BALF and serum are altered in patients at risk of ARDS and with ARDS [98, 99]. However, since the BALF concentration of these secreted proteins may well change independently of ATI cell necrosis, their usefulness in diagnostic assays may be limited.

At the present time, the antibodies that recognise the human homologue of RTI<sub>40</sub> have not been used to quantify ATI cell injury in patients with ALI or ARDS. However, HTI<sub>56</sub>, a human ATI-cell-specific protein, has been measured in oedema fluid and plasma from patients with established ARDS. The concentration of HTI<sub>56</sub> was significantly elevated in both oedema fluid and plasma from patients with ARDS in comparison to those from hydrostatic pulmonary oedema patients [100]. These data suggest that ATI-cell-specific membrane proteins may be sensitive markers of alveolar epithelial injury in patients with, and at-risk of, ALI or ARDS.

*Specific regulation of alveolar epithelial type I cell-selective proteins by toxic agents*

Hyperoxia is frequently used as a model for the investigation of lung injury and repair. Hyperoxic exposure characteristically causes endothelial damage that is followed by ATI cell damage [101]. CAO *et al.* [102] investigated the lung content of RTI<sub>40</sub>/TI $\alpha$  protein, AQP5 and caveolin-1 following hyperoxic exposure. Instead of a decrease in ATI-cell-selective protein levels (which might be expected if ATI cells were damaged), they found that RTI<sub>40</sub>/TI $\alpha$  protein and AQP5 were both upregulated by approximately five-fold, whereas caveolin-1 levels were unchanged. Immunohistochemical analysis, for RTI<sub>40</sub>/TI $\alpha$  protein, demonstrated that staining remained restricted to ATI cells and that there was no additional staining in other cell types. Moreover, using an alveolar epithelial cell line, CAO *et al.* [102] demonstrated that the

Table 2. – Rat type I cell 40-kDa protein (RTI<sub>40</sub>) in rat lung injury models

Lung injury model	MAB source	Sampling time h	Increase in BALF RTI <sub>40</sub>	Morphology <sup>#</sup>	[Ref.]
<i>Pseudomonas aeruginosa</i> <sup>†</sup>	L. Dobbs <sup>§</sup>	6	80-fold	Necrotic type I cells (EM)	[89]
Nitrogen dioxide (17 ppm)	L. Dobbs <sup>§</sup>	12	2-fold	Necrotic type I cells (EM)	[90]
Hyperoxia (95% oxygen)	L. Dobbs <sup>§</sup>	60	1.7-fold	ND	[91]
<i>Staphylococcus aureus</i> <sup>†</sup>	L. Dobbs <sup>§</sup>	4	3-fold	RTI <sub>40</sub> similar to control <sup>f</sup> (IF)	[92]
<i>S. aureus</i> (repair model)	L. Dobbs <sup>§</sup>	24 and 96	None	RTI <sub>40</sub> similar to control <sup>f</sup> (IF)	[93]
Bleomycin	L. Dobbs <sup>§</sup>	7 days	3-fold <sup>###</sup>	Focal loss of RTI <sub>40</sub> (IHC, IEM) Necrotic type I cells (EM)	[94]
CdCl <sub>2</sub>	E11 [57]	3 days	ND	Focal loss of RTI <sub>40</sub> (IHC)	[95]
Irradiation	MEP-1	8 weeks	ND	Focal loss of RTI <sub>40</sub> (IHC)	[28]
Acid combined with VILI <sup>+</sup>	L. Dobbs <sup>§</sup>	4	2.7-fold	Necrotic type I cells (EM)	[96]

MAB: monoclonal antibody; BALF: bronchoalveolar lavage fluid; ppm: parts per million; VILI: ventilator-induced lung injury; EM: electron microscopy; ND: not determined; IF: immunofluorescence; IHC: immunohistochemistry; IEM: immunoelectron microscopy. <sup>#</sup>: none of the morphological changes were quantified; <sup>†</sup>: anaesthetised and ventilated; <sup>+</sup>: 6 versus 3 mL·kg body weight<sup>-1</sup> tidal volume; <sup>§</sup>: see Acknowledgements section; <sup>f</sup>: lung expression; <sup>###</sup>: relative to total protein.

transcriptional activity of the  $TI\alpha$  promoter was directly regulated by hyperoxia. These data suggest that  $RTI_{40}/TI\alpha$  protein and AQP5 are regulated by ATI cells in response to hyperoxic stress.

In a mouse model of viral pneumonia, levels of AQP5 and its mRNA transcripts were both decreased 7 and 14 days following inoculation [103]. Histochemical analysis of the alveolar wall suggested that this decrease in AQP5 expression was not due to ATI cell necrosis or increased numbers of ATII cells. Conversely, immunohistochemical analysis revealed that AQP5 staining was reduced throughout the lung, even in areas without overt inflammation. These data suggest that AQP5 is downregulated by ATI cells following viral pneumonia. AQP5 has also been reported to be downregulated in lung tissue following endotoxin-induced lung injury [104]. The functional significance of AQP5 downregulation is not understood. Although viral and endotoxin-induced lung injury both cause extravascular fluid accumulation, studies in AQP5-deficient mice suggest that AQP5 does not play a role in fluid accumulation following injury [75].

#### *Expression of alveolar epithelial type I cell-selective proteins in alveolar intermediate cells*

Following injury to the lung, the alveolar walls are populated not only by ATI and ATII cells but potentially also by alveolar epithelial intermediate cells and airway or bone-marrow-derived progenitor cells. Very little is known about the proteins expressed by any of these cell types, even ATI and ATII cells, in the lungs following injury. However, data from a small number of *in vivo* studies suggest that alveolar epithelial intermediate cells may express certain ATI-cell-selective proteins.

Like  $NO_2$ , ozone damages epithelial cells located at the junction between the terminal bronchioles and the first alveolar duct [105]. Following ozone exposure, immunoelectron microscopy demonstrates that cytochrome  $P_{450}$  2B1 is located in ATI cells, as observed in noninjured lungs. However,  $P_{450}$  2B1 is also expressed in two other epithelial subtypes, which exhibit ultrastructural characteristics of both ATI and ATII cells [34]. One of these epithelial cell types has microvilli, an extended and thick lateral cytoplasm, and large vacuoles that occasionally contain lamellar bodies. The other type exhibits a smooth surface and ATI-like lateral cytoplasmic extensions that sometimes show focal thickening [34].

Exogenous administration of keratinocyte growth factor stimulates ATII cell proliferation *in vivo*. Following keratinocyte growth factor administration, the number of ATII cells is maximal at 2–3 days; however, by 7 days, alveolar epithelial architecture appears normal [106]. ATII cell numbers are reduced to control levels *via* both apoptosis and transdifferentiation to new ATI cells (interestingly, this process appears to be an example of ATII cell transdifferentiation that is not preceded by ATI cell injury). Prior to the return of the alveolar epithelium to normal, cells have been identified that exhibit both ATII and ATI characteristics. These characteristics include an elongated cytoplasm and expression of an ATI-cell-specific lectin, *L. esculentum* agglutinin, and an ATII-cell-selective cell protein, surfactant protein D [106].

Using confocal microscopy, the phenotype of the lung alveolar epithelium was investigated following intratracheal administration of *S. aureus*. A small population of cells that coexpress  $RTI_{40}/TI\alpha$  protein and an ATII-cell-selective antigen, MMC4, were identified [51, 107].  $RTI_{40}/TI\alpha$  protein/MMC4-positive cultured ATII cells have also been

observed as they become ATI-like [108]. These data suggest that  $RTI_{40}/TI\alpha$  protein/MMC4-positive cells are intermediates in the transition of ATII to new ATI cells (data not shown).

Changes in the surface expression of ICAM-1 have been investigated in a number of animal models using immunogold electron microscopic analysis. ICAM-1 levels on the surface of ATI cells decrease at cell boundaries following hyperoxia but do not change following pneumonia. However, ICAM-1 expression is dramatically increased on the surface of ATII cells following both pneumonia and hyperoxia [80, 109]. Since ICAM-1 expression increases as ATII cells become ATI-cell-like in culture, the upregulation on ATII cells *in vivo* could be an early differentiation event [37]. However, ICAM-1 expression is also upregulated in response to cytokines [79]. Therefore, ICAM-1 expression may also be regulated by ATII cells independently of morphological changes associated with transdifferentiation to ATI cells.

To date, alveolar epithelial intermediate cells have been defined on the basis of their ultrastructural characteristics, and these characteristics appear to include at least two major intermediate cell types [20, 23, 34]. The preliminary studies mentioned above suggest that alveolar epithelial intermediate cells may also be identifiable by investigation of the coexpression of ATI- and ATII-cell-selective markers. However, whether the identified coexpressing cells are ATII cells becoming ATI cells, or *vice versa*, remains unknown. Nor is it known whether different ultrastructural intermediates have different phenotypes. It is well known that the conditions under which isolated ATII cells are cultured influence whether or not they express ATII- or ATI-cell-selective proteins and mRNAs (reviewed in [25]). Moreover, it has also been demonstrated that ATI-like cells can be made to re-express ATII-like phenotypic markers by varying the culture conditions, *e.g.* by going from submerged to air/liquid interface culture [110, 111]. Studies designed to determine coexpression of ATI- and ATII-cell-selective markers in culture may help in the phenotyping of ATII-to-ATI intermediates, and in the determination of whether the same intermediates exist as ATI cells become ATII cells.

#### **Alveolar epithelial type I cell-selective proteins and lung fibrosis**

The term lung fibrosis encompasses a diverse group of lung diseases that share many clinical, radiographic and physiological characteristics. The most common and severe form of fibrosis is IPF. Morphologically, IPF is characterised by regions of fibrosis alternating with regions of normal lung with little evidence of inflammation [112]. At the borders between fibrotic and normal lung are collections of fibroblasts/myofibroblasts, called fibrotic foci. These regions of active fibrosis are also characterised by the presence of different alveolar epithelial phenotypes [4]. Immunohistochemical studies demonstrate that the alveolar epithelium in fibrotic regions expresses a number of profibrotic cytokines and growth factors [18]. Based on these observations, it has been proposed that multiple cycles of alveolar injury and repair lead to aberrant alveolar epithelial cell populations that drive fibrosis [4]. Although there is no disputing that the alveolar epithelium is not normal in IPF, the origin and specific functions of the different epithelial phenotypes remain unknown. It has been proposed that some of the distinct alveolar epithelial cells in IPF are alveolar epithelial intermediate cells [4, 113].

Bleomycin is commonly used to induce pulmonary fibrosis in experimental animals, although, unlike IPF, bleomycin-induced fibrosis is classically associated with inflammation.



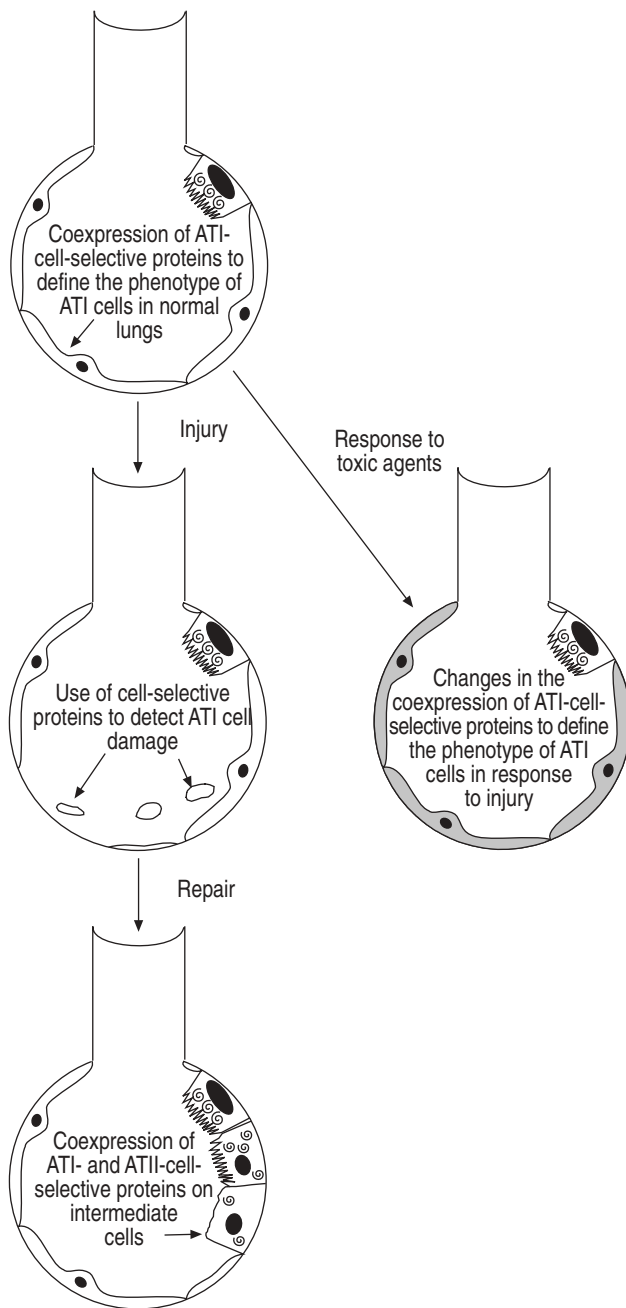


Fig. 3.—Potential applications of cell-selective markers in the investigation of alveolar epithelial type I cell injury and repair. ATI: alveolar epithelial type I.

Four weeks after bleomycin treatment, regions of the alveolar barrier stain positively for some ATI-cell-specific lectins (e.g. *B. purpurea* agglutinin) but demonstrate focal loss of RTI<sub>40</sub>/TI $\alpha$  protein [94]. Other investigations into lung fibrosis also report the loss of ATI-cell-associated proteins, including caveolin-1, RTI<sub>40</sub>/TI $\alpha$  protein, AQP5 and RAGE [30, 95, 114]. More studies are required in order to fully understand to what extent the phenotype of ATI cells change in fibrosis or whether the loss of ATI-cell-selective proteins reflects a decrease in the number of ATI cells. Data from knockout mice, and in particular TI $\alpha$ -deficient mice (see earlier) [63], suggest that TI $\alpha$  (or ATI cells) may play a role in the regulation of distal airway cell proliferation. Therefore, it is possible that loss of ATI cells in fibrosis not only leads to a decrease in surface area for gas exchange but may also

contribute to the increased proliferation of other distal airway cells.

## Conclusions

The present article has reviewed the use of cell-selective proteins as markers for the investigation of ATI cell injury and repair following injury (fig. 3). ATI-cell-selective proteins such as RTI<sub>40</sub>/TI $\alpha$  protein are useful for quantifying the extent of ATI cell necrosis in animal models of lung injury, and human homologues of RTI<sub>40</sub>/TI $\alpha$  protein may form the basis of diagnostic assays in conditions such as ALI or ARDS. The coexpression of ATI-cell-selective markers, such as RTI<sub>40</sub>/TI $\alpha$  protein, AQP5, caveolin-1 and 2, and cytochrome P<sub>450</sub> 2B1, may help identify ATI cells in immunohistochemistry- and immunofluorescence-based studies and facilitate investigation into ATI cell numbers at active sites of injury and repair. The coexpression of ATI cell-selective proteins with AII- selective proteins may help identify alveolar epithelial intermediate cells and their role in lung repair and fibrosis.

A better understanding of the regulation of alveolar epithelial type I cell numbers, and the function of proteins selective for alveolar epithelial type I cells, will lead to a greater understanding of the role of alveolar epithelial type I cells in both normal and diseased lungs.

**Acknowledgements.** The authors kindly thank L. Dobbs (University of California at San Francisco, San Francisco, CA, USA) for the monoclonal antibody directed against rat type I cell 40-kDa protein. They also thank J.V. Gray (University of Glasgow, Glasgow, UK) and S. McKechnie, L. Franklin and K. Tyrrell (all University of Edinburgh, Edinburgh, UK) for comments on the manuscript and G. Clegg (University of Edinburgh, Edinburgh, UK) for assistance in preparing figure 1.

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